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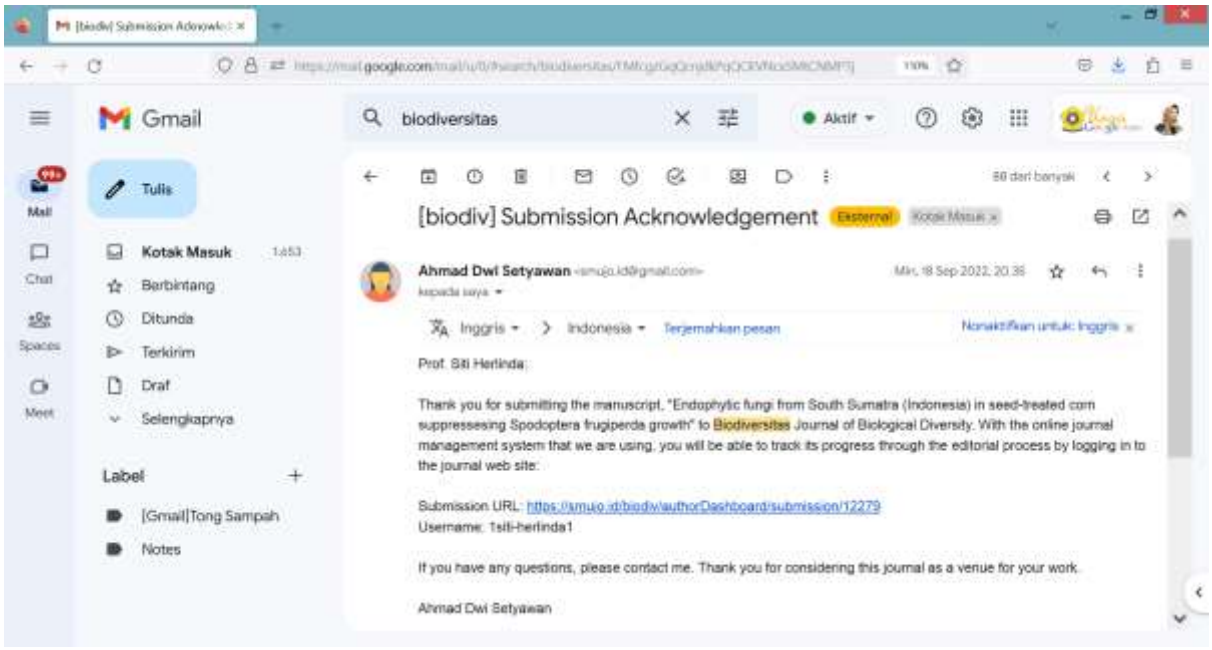

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Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

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The endophytic fungi from South Sumatra have the negative effect on *Spodoptera frugiperda* growth. This study findings highlight that *Beauveria bassiana*, *Metarhizium anisopliae*, and *Curvularia lunata* could inhibit pupal and adult emergence, and decrease the eggs laid and the viable eggs of *S. frugiperda*. The fungi also shorten the adult longevity and increased the the larval mortality. The first report of *C. lunata* was pathogenic against *S. frugiperda*.

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Siti Herlinda

Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

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Abstract. It is necessary to investigate the potential of the endophytic fungi inoculated in seed corn to suppress the growth of *Spodoptera frugiperda*. The aim of the research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. The 20 isolates of the endophytic fungi that have been identified molecularly and used in this study were *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). There were 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and an isolate of *C. lunata* (JaSpkPga(3)), and an isolate of *M anisoplae* (CaTpPga) which were more pathogenic to *S. frugiperda* larvae. The endophytic fungi have the negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased percentage of pupal and adult emergence, and lowered the eggs laid and the viable eggs of *S. frugiperda*. The fungi also shorten the adult longevity and increased the the larval mortality. The first report of *C. lunata* was pathogenic against *S. frugiperda*. These findings highlight the potential of endophytic *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Key words: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

Abbreviations (if any): -

Running title: Endophytic fungi suppressesing *Spodoptera frugiperda* growth

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) began to move into Asia in 2018 (Mahat et al., 2021) and it was first discovered in India (Ganiger et al., 2018), while entering Indonesia for the first time was on 26 March 2019 in West Sumatra (Sartiami et al., 2020). *S. frugiperda* in Indonesia has been found two strains, corn and rice strains (Herlinda et al., 2022). Currently, the FAW began to spread to other provinces and islands in Indonesia, including West Java (Maharani et al., 2019), Lampung (Trisyono et al., 2019), Bengkulu (Ginting et al., 2020), Bali (Supartha et al., 2021), This pest got into South Sumatra in July 2019 (Hutasoit et al., 2020). The FAW damages the maize plant and various other plant species (Montezano et al., 2018) by feeding on leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Montezano et al., 2018; Ginting et al., 2020). The FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al., 2018). In Indonesia, the FAW generally attacks maize with damage ranging from 26.50−70% in Lampung (Lestari et al., 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al., 2021), in Bali reaching 47.84% (Supartha et al., 2021), and in South Sumatra up to 100% (Herlinda et al., 2022).

S. frugiperda larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al., 2021) and after that the larvae hide in the leaf axils or at the base of the developing cob (ear) or in the tip of the cob (Prasanna et al., 2018). Because the FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used (Herlinda et al., 2020; Gustianingtyas et al., 2021; Herlinda et al., 2021; Sari et al., 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et al., 2020). The endophytic fungi that were effective in killing *S. frugiperda*, for example *Beauveria bassiana* and *Metarhizium anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al., 2020). *Metarrhizium robertsii* killed 51.2% of the second instar larvae of *S. frugiperda* (Hernandez-Trejo et al., 2019). The results of previous studies have proven that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra and applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al., 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al., 2021), but it is necessary to investigate the potential of the fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of the research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were from collections of the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from the leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. The 20 fungal isolates have been identified molecularly and confirmed as the endophytic fungi (Herlinda et al., 2021). All isolates have been deposited in the GenBank. The 20 isolates of the endophytic fungi identified molecularly were *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate).

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was carried out in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at room temperature ranging from 27−29 °C and relative humidity ranging from 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, the larvae were brought to the laboratory for mass-rearing following the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because the larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø15 cm, height 25 cm) containing sterile soil. The plastic container was put in a wire mesh cage (30 x 30 x 30 cm3) in which there was a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al., 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while the seeds for control were only immersed in 10 mL of destilled water. Then, the 15 seeds were grown in a sterile glass bottle (250 mL volume) with a sterile filter paper (whatman no. 42) on the bottom which was moistened with 1 mL of destilled water. The seeds were incubated for 10 days. All treatments in this experiment were repeated three times.

The stems and leaves of corn seedling that had been inoculated with the 10 days old endophytic fungus were given to 25 second instars of *S. frugiperda* which had previously been fasted for 1x24 hours. When the maize seedlings were 10 days old, the endophytic fungal isolates had colonized the maize stalks and leaves (Gustianingtyas et al., 2021). The control maize seedlings were also given to 25 second instars of *S. frugiperda*. The larvae were allowed to eat the leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% following the method Russo et al. (2019) Then, the larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days following the method of Herlinda et al. (2020). The dead larvae were cultured in the agar-water medium to confirm the infection by the endophytic fungi or not. The number of dead larvae was calculated daily for getting mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by the female adults were also recorded. The leaf area of maize eaten by the larvae, and the fecal and body weight of the larvae were measured every day from the first to the 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), the percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant

difference between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

The endophytic fungi pathogenecity against *Spodoptera frugiperda* larvae

Of the 20 fungal isolates of endophytic fungi tested in this study, there were 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and an isolate of *C. lunata* (JaSpkPga(3)), and an isolate of *M anisoplae* (CaTpPga) which were more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by *B. bassiana* of JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and *C. lunata* of JaSpkPga(3) isolate ranged from 17−23%. The mortality caused by the six isolates from the beginning of the observation to the last day was always higher, while the larvae control that were only dripped with sterile water did not die. Thus, there were three species of the endophytic fungi that were more pathogenic, they were *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) . The fungi also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the number of eggs laid by the treated female adults decreased significantly compared to the number of eggs laid by the untreated female adults (Table 2).

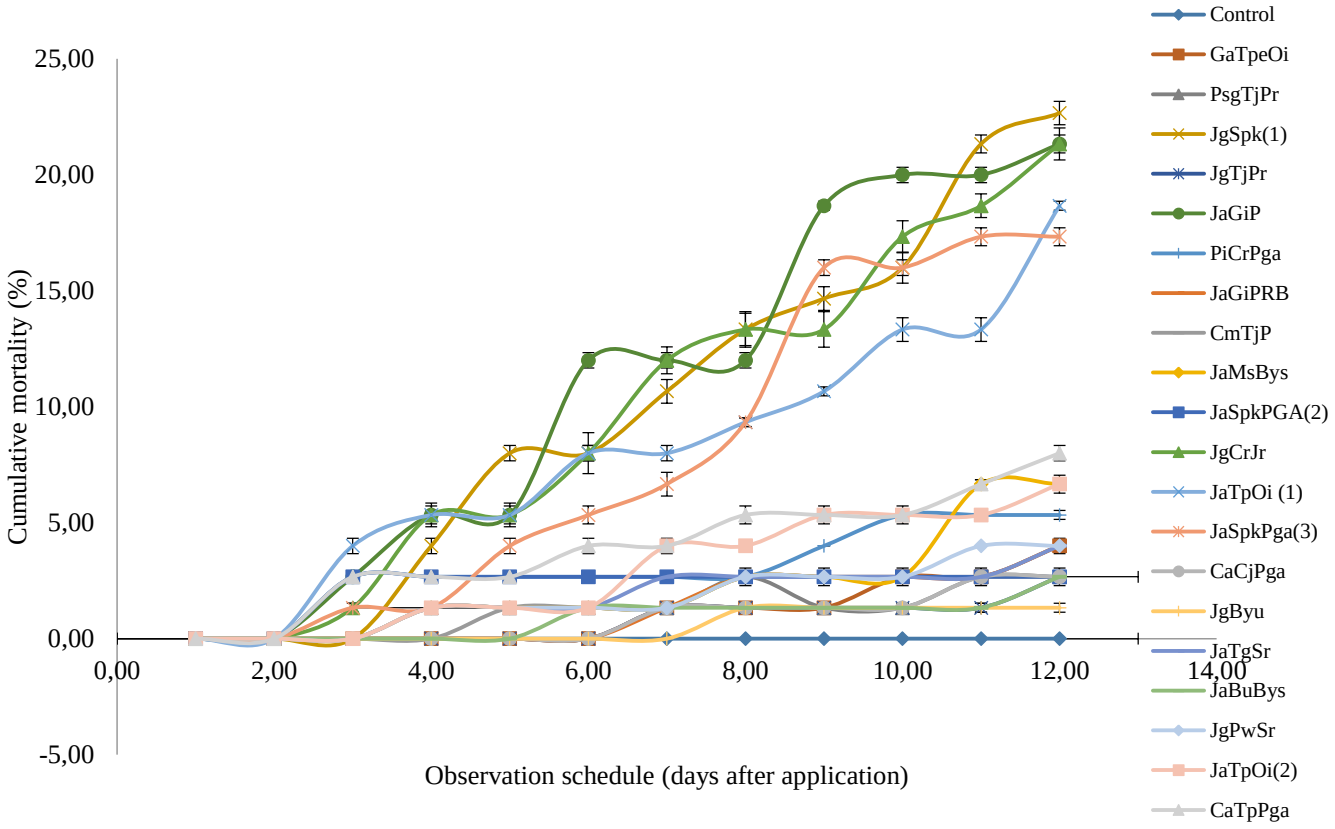

Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

Spodoptera frugiperda growth

The leaf area eaten by the larvae treated with the endophytic fungi (the treated larvae) and the untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by the control larvae was the widest compared to the leaf area eaten by the treated larvae. The weight of the control larvae was also the heaviest compared to the weight of the treated larvae (Table 4). The weight of the control larvae was significantly different from those of the treated larvae (from the second day to the last day of the observation). The larvae weight and leaf area eaten by the treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate corn leaves inoculated with the endophytic fungi significantly decreased appetite and weight compared to control larvae. The weight of feces produced by the larvae treated and control were significant differences, namely the weight of feces produced by the larvae treated was lighter than the weight of feces produced by the untreated (control) larvae (Table 5). Thus, the endophytic fungi have a negative effect on *S. frugiperda* growth.

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a

mummy, darker in color and odorless, while the untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, the endophytic fungi caused the pupae to become shorter and darker, and finally the pupae died, while the control pupae were larger in size and the pupae colors were brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolate	Species	Pupae emergence (%)	Adult emergence (%)
Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef
JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolate	Species	Longevity (days)		Eggs laid/female	Viable eggs (%)
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a

JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde
JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a
JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

	Species	**Leaf area consumed by larvae (cm2 larvae-1 day-1) during 12 days of observation**					
Isolate		**2**	**4**	**6**	**8**	**10**	**12**
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean of weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolate	Species	Larvae weight (mg larvae^{-1}) during 12 days observation					
		2	4	6	8	10	12
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03
GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13
JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80
JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolate	Species	Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b

JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b
JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b
JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

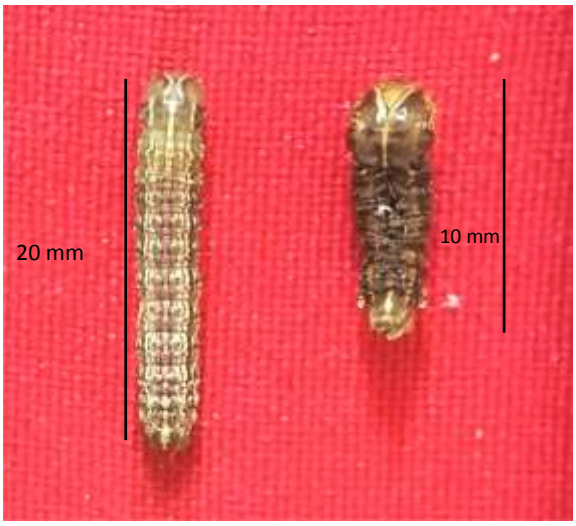

Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

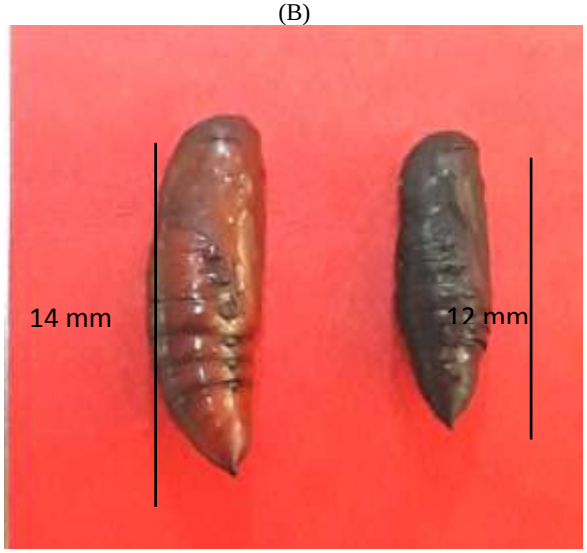

Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

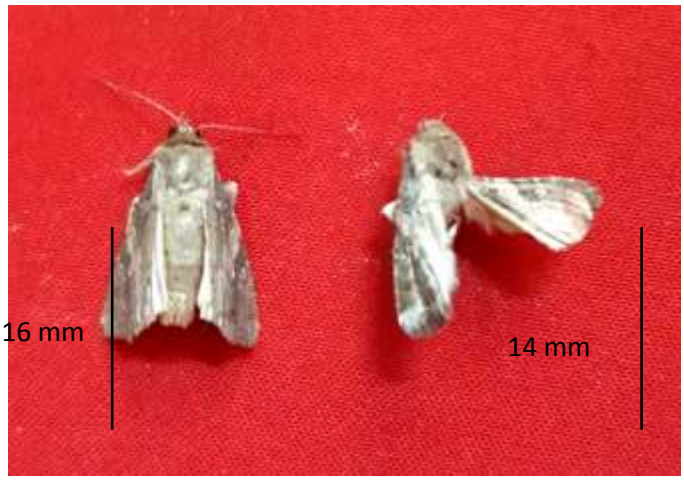

Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Discussion

The obtained research found that three species of the endophytic fungi were more pathogenic were *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate). They caused the higher mortality of the FAW larvae. The fungi also decreased the percentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by the treated female adults. These results showed that the endophytic fungi not only killed the larvae, but also killed the pupae and reduced the adult emergence. The fungi also caused the abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al., 2020; Herlinda et al., 2021). However, the first report of *C. lunata* was pathogenic to *S. frugiperda*. *C. lunata* reported could kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al., 2014).

The obtained study showed that mortality larvae caused by the endophytic fungi were still low because the fungal suspension used were only $1x10^6$ conidia mL^{-1}. If the fungal suspension were increased to $1x10^8$ conidia mL-1 causing higher mortality (41.7–50.0%). In addition, the fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al., 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. However, the advantages of the endophytic fungi of this study not only could kill the larvae, but also kill the pupae and reduce the adult emergence. The fungi also shortened the adult longevity and caused the abnormal adults. Moreover, the ability of the endophytic fungi colonizing the young maize (seedling) via seed treatment could prevent the maize plant from the attack of the hiding *S. frugiperda* larvae in the corn midribs (Herlinda et al., 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al., 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al., 2022).

The endophytic fungi in this current research have negative effect on *S. frugiperda* growth. The endophytic fungi decreased the appetite of larvae so that the leaf area consumed and the fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined. Then, the treated larvae finally could die. The endophytic fungus in seed immersion/treatment caused growth retardation on *S. frugiperda* (Gustianingtyas et al., 2021) and adverse effects on its survival (Russo et al., 2020) because the fungus could produce secondary metabolites and toxic protein or toxins (Vidal and Jaber, 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey, 2004) and beauvericin, a secondary metabolite that is toxic for insects (Safavi, 2012) and *M. anisopliae* produces destruxin, a secondary metabolite that is also toxic for insects (Borisade et al., 2016). The mycelia of endophytic fungi within maize tissue consumed by the larvae of *S. frugiperda* could produce blastospores in the larvae hemolymph (Sari et al., 2022). Then, the blastospores produced the toxic secondary metabolites and the protein toxic with insects (Mancillas-Paredes et al., 2019). The entomopathogenic fungi also could secrete the secondary metabolites in planta that cause antibiosis, antifeedant or deterrent for the *S. frugiperda* larvae (Jaber and Ownley, 2018) and raise the concentrations of terpenoid compound against the FAW larvae (Russo et al., 2020). After consuming the toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al., 2022). The data obtained showed that the mycosis was found only on the *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, the mycosis

was not occurred on control larvae (the untreated larvae). The *S. frugiperda* larvae fed on plants colonized by the endophytic fungi may undergo mycosis (Russo et al., 2020).

Finally, the endophytic fungi have the negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased percentage of pupal and adult emergence, and lowered the eggs laid and the viable eggs of *S. frugiperda*. The fungi also shorten the adult longevity and increased the the larval mortality. The first report of *C. lunata* was pathogenic against *S. frugiperda*. These findings highlight the potential of endophytic *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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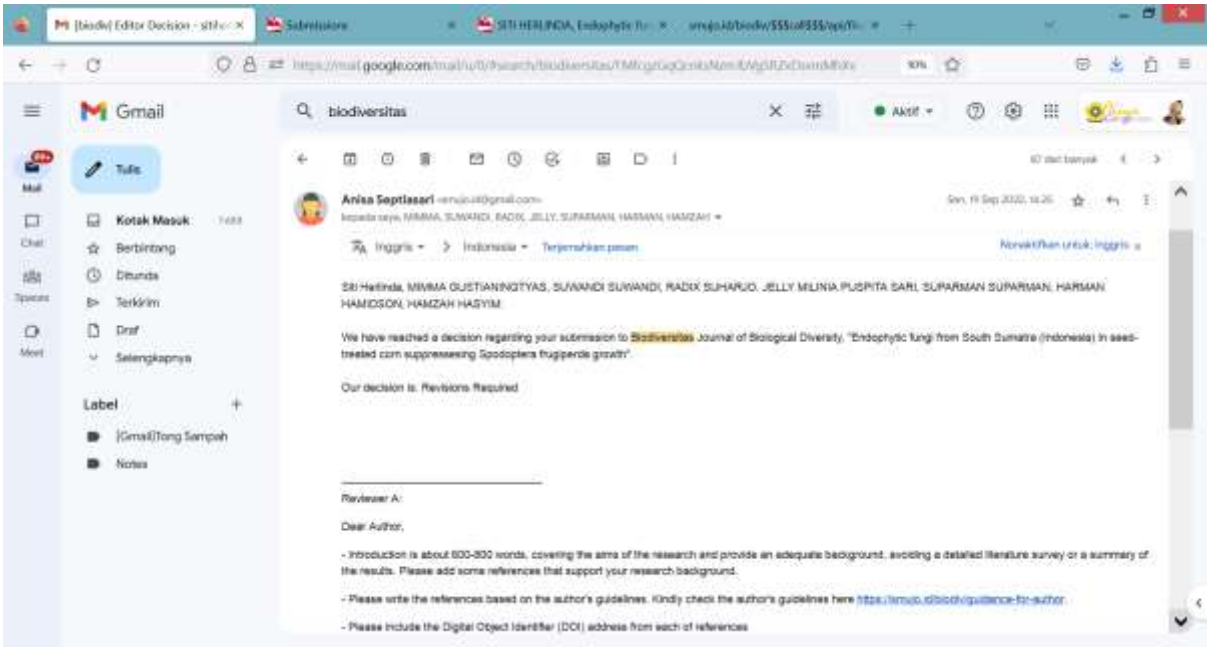

Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

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Abstract. It is necessary to investigate the potential of the endophytic fungi inoculated in seed corn to suppress the growth of *Spodoptera frugiperda*. The aim of the research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. The 20 isolates of the endophytic fungi that have been identified molecularly and used in this study were *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). There were 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and an isolate of *C. lunata* (JaSpkPga(3)), and an isolate of *M anisoplae* (CaTpPga) which were more pathogenic to *S. frugiperda* larvae. The endophytic fungi have the negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased percentage of pupal and adult emergence, and lowered the eggs laid and the viable eggs of *S. frugiperda*. The fungi also shorten the adult longevity and increased the the larval mortality. The first report of *C. lunata* was pathogenic against *S. frugiperda*. These findings highlight the potential of

endophytic *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Key words: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

Abbreviations (if any): -

Running title: Endophytic fungi suppressesing *Spodoptera frugiperda* growth

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) began to move into Asia in 2018 (Mahat et al., 2021) and it was first discovered in India (Ganiger et al., 2018), while entering Indonesia for the first time was on 26 March 2019 in West Sumatra (Sartiami et al., 2020). *S. frugiperda* in Indonesia has been found two strains, corn and rice strains (Herlinda et al., 2022). Currently, the FAW began to spread to other provinces and islands in Indonesia, including West Java (Maharani et al., 2019), Lampung (Trisyono et al., 2019), Bengkulu (Ginting et al., 2020), Bali (Supartha et al., 2021), This pest got into South Sumatra in July 2019 (Hutasoit et al., 2020). The FAW damages the maize plant and various other plant species (Montezano et al., 2018) by feeding on leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Montezano et al., 2018; Ginting et al., 2020). The FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al., 2018). In Indonesia, the FAW generally attacks maize with damage ranging from 26.50−70% in Lampung (Lestari et al., 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al., 2021), in Bali reaching 47.84% (Supartha et al., 2021), and in South Sumatra up to 100% (Herlinda et al., 2022).

The easy method and fast action to control *S. frugiperda* is utilizing the synthetic insecticides (Kumela et al., 2018). However, the insecticide application causes the resistances of the FAW (Zhang et al., 2021). The insecticide kills natural enemies of insect pests, has a negative effect the environment and the human health (Harrison et al., 2019). An alternative more sustainable and eco-friendly control for *S. frugiperda* is urgently needed. Biological control based on utilizing biocontrol agents, such as entomopathogenic fungi is the preferred method control for *S. frugiperda* (Mantzoukas and Eliopoulos 2020). Topical application of the entomopathogenic fungi, such as *Metarhizium anisopliae* killed 75% of *S. frugiperda* larvae (Ramos et al., 2020). *Beauveria bassiana* killed more than 80% of *S. frugiperda* larvae (Ramanujam et al., 2020). However, *S. frugiperda* larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al., 2021) and after that the larvae hide in the leaf axils or at the base of the developing cob (ear) or in the tip of the cob (Prasanna et al., 2018). Because the FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used (Herlinda et al., 2020; Gustianingtyas et al., 2021; Herlinda et al., 2021; Sari et al., 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et a*l*., 2020). The endophytic fungi that were effective in killing *S. frugiperda*, for example *B. bassiana* and *M. anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al., 2020). *Metarrhizium robertsii* killed 51.2% of the second instar larvae of *S. frugiperda* (Hernandez-Trejo et al., 2019). The results of previous studies have proven that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra and applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al., 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al., 2021), but it is necessary to investigate the potential of the fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of the research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were from collections of the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from the leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. The 20 fungal isolates have been identified molecularly and confirmed as the endophytic fungi (Herlinda et al., 2021). All isolates have been deposited in the GenBank. The 20 isolates of the endophytic fungi identified molecularly were *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate).

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was carried out in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at room temperature ranging from 27−29 °C and relative humidity ranging from 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, the larvae were brought to the laboratory for mass-rearing following the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because the larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø15 cm, height 25 cm) containing sterile soil. The plastic container was put in a wire mesh cage (30 x 30 x 30 cm3) in which there was a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al., 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while the seeds for control were only immersed in 10 mL of destilled water. Then, the 15 seeds were grown in a sterile glass bottle (250 mL volume) with a sterile filter paper (whatman no. 42) on the bottom which was moistened with 1 mL of destilled water. The seeds were incubated for 10 days. All treatments in this experiment were repeated three times.

The stems and leaves of corn seedling that had been inoculated with the 10 days old endophytic fungus were given to 25 second instars of *S. frugiperda* which had previously been fasted for 1x24 hours. When the maize seedlings were 10 days old, the endophytic fungal isolates had colonized the maize stalks and leaves (Gustianingtyas et al., 2021). The control maize seedlings were also given to 25 second instars of *S. frugiperda*. The larvae were allowed to eat the leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% following the method Russo et al. (2019) Then, the larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days following the method of Herlinda et al. (2020). The dead larvae were cultured in the agar-water medium to confirm the infection by the endophytic fungi or not. The number of dead larvae was calculated daily for getting mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by the female adults were also recorded. The leaf area of maize eaten by the larvae, and the fecal and body weight of the larvae were measured every day from the first to the 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), the percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant difference between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

The endophytic fungi pathogenecity against *Spodoptera frugiperda* larvae

Of the 20 fungal isolates of endophytic fungi tested in this study, there were 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and an isolate of *C. lunata* (JaSpkPga(3)), and an isolate of *M anisoplae* (CaTpPga) which were more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by *B. bassiana* of JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and *C. lunata* of JaSpkPga(3) isolate ranged from 17−23%. The mortality caused by the six isolates from the beginning of the observation to the last day was always higher, while the larvae control that were only dripped with sterile water did not die. Thus, there were three species of the endophytic fungi that were more pathogenic, they were *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) . The fungi also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the number of eggs laid by the treated female adults decreased significantly compared to the number of eggs laid by the untreated female adults (Table 2).

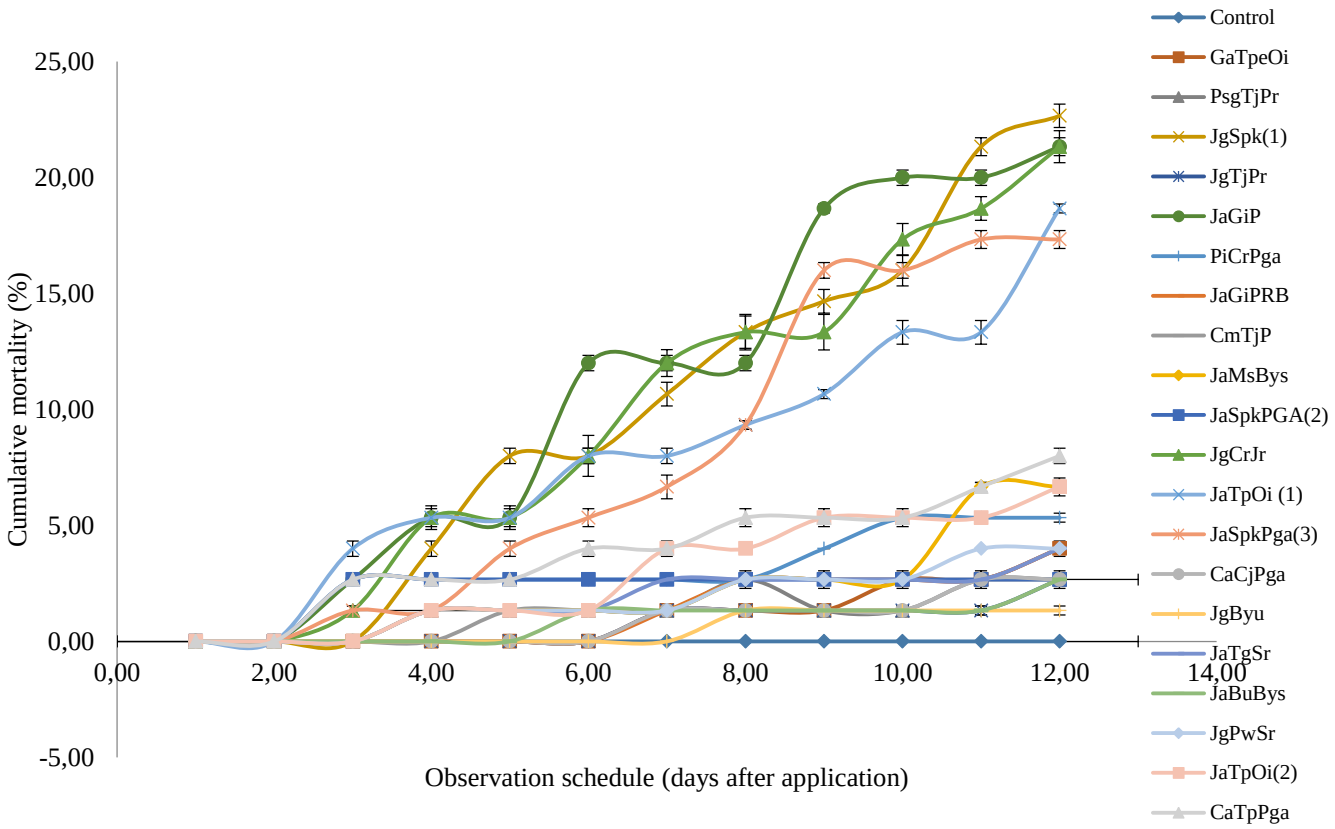

Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

***Spodoptera frugiperda* growth**

The leaf area eaten by the larvae treated with the endophytic fungi (the treated larvae) and the untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by the control larvae was the widest compared to the leaf area eaten by the treated larvae. The weight of the control larvae was also the heaviest compared to the weight of the treated larvae (Table 4). The weight of the control larvae was significantly different from those of the treated larvae (from the second day to the last day of the observation). The larvae weight and leaf area eaten by the treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate corn leaves inoculated with the endophytic fungi significantly decreased appetite and weight compared to control larvae. The weight of feces produced by the larvae treated and control were significant differences, namely the weight of feces produced by the larvae treated was lighter than the weight of feces produced by the untreated (control) larvae (Table 5). Thus, the endophytic fungi have a negative effect on *S. frugiperda* growth.

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a mummy, darker in color and odorless, while the untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, the endophytic fungi caused the pupae to become shorter and darker, and finally the pupae died, while the control pupae were larger in size and the pupae colors were brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolate	Species	Pupae emergence (%)	Adult emergence (%)
Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef

JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolate	Species	Longevity (days)		Eggs laid/female	Viable eggs (%)
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a
JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde
JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a
JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolate	Species	Leaf area consumed by larvae (cm2 larvae-1 day-1) during 12 days of observation					
		2	4	6	8	10	12
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean of weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolate	Species	Larvae weight (mg larvae^{-1}) during 12 days observation					
		2	4	6	8	10	12
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03
GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13
JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80

JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

		Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
Isolate	**Species**	**2**	**4**	**6**	**8**	**10**	**12**
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b
JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b
JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b
JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

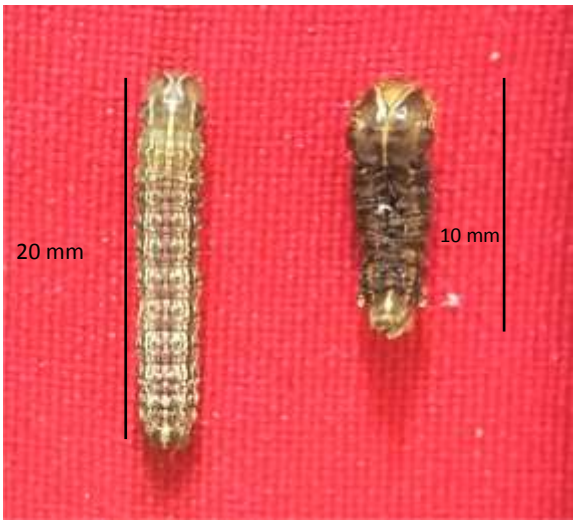

Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

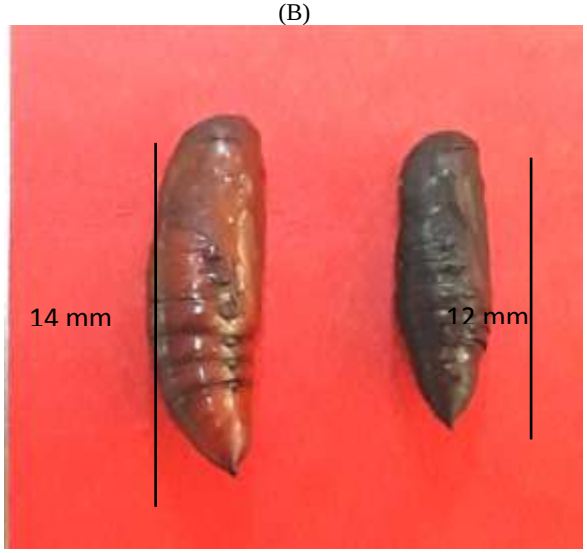

Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Discussion

The obtained research found that three species of the endophytic fungi were more pathogenic were *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate). They caused the higher mortality of the FAW larvae. The fungi also decreased the percentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by the treated female adults. These results showed that the endophytic fungi not only killed the larvae, but also killed the pupae and reduced the adult emergence. The fungi also caused the abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al., 2020; Herlinda et al., 2021). However, the first report of *C. lunata* was pathogenic to *S. frugiperda*. *C. lunata* reported could kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al., 2014).

The obtained study showed that mortality larvae caused by the endophytic fungi were still low because the fungal suspension used were only $1x10^6$ conidia mL^{-1}. If the fungal suspension were increased to $1x10^8$ conidia mL-1 causing higher mortality (41.7–50.0%). In addition, the fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al., 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. However, the advantages of the endophytic fungi of this study not only could kill the larvae, but also kill the pupae and reduce the adult emergence. The fungi also shortened the adult longevity and caused the abnormal adults. Moreover, the ability of the endophytic fungi colonizing the young maize (seedling) via seed treatment could prevent the maize plant from the attack of the hiding *S. frugiperda* larvae in the corn midribs (Herlinda et al., 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al., 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al., 2022).

The endophytic fungi in this current research have negative effect on *S. frugiperda* growth. The endophytic fungi decreased the appetite of larvae so that the leaf area consumed and the fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined. Then, the treated larvae finally could die. The endophytic fungus in seed immersion/treatment caused growth retardation on *S. frugiperda* (Gustianingtyas et al., 2021) and adverse effects on its survival (Russo et al., 2020) because the fungus could produce secondary metabolites and toxic protein or toxins (Vidal and Jaber, 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey, 2004) and beauvericin, a secondary metabolite that is toxic for insects (Safavi, 2012) and *M. anisopliae* produces destruxin, a secondary metabolite that is also toxic for insects (Borisade et al., 2016). The mycelia of endophytic fungi within maize tissue consumed by the larvae of *S. frugiperda* could produce blastospores in the larvae hemolymph (Sari et al., 2022). Then, the blastospores produced the toxic secondary metabolites and the protein toxic with insects (Mancillas-Paredes et al., 2019). The entomopathogenic fungi also could secrete the secondary metabolites in planta that cause antibiosis, antifeedant or deterrent for the *S. frugiperda* larvae (Jaber and Ownley, 2018) and raise the concentrations of terpenoid compound against the FAW larvae (Russo et al., 2020). After consuming the toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al., 2022). The data obtained showed that the mycosis was found only on the *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, the mycosis was not occurred on control larvae (the untreated larvae). The *S. frugiperda* larvae fed on plants colonized by the endophytic fungi may undergo mycosis (Russo et al., 2020).

Finally, the endophytic fungi have the negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased percentage of pupal and adult emergence, and lowered the eggs laid and the viable eggs of *S. frugiperda*. The fungi also shorten the adult longevity and increased the the larval mortality. The first report of *C. lunata* was pathogenic against *S. frugiperda*. These findings highlight the potential of endophytic *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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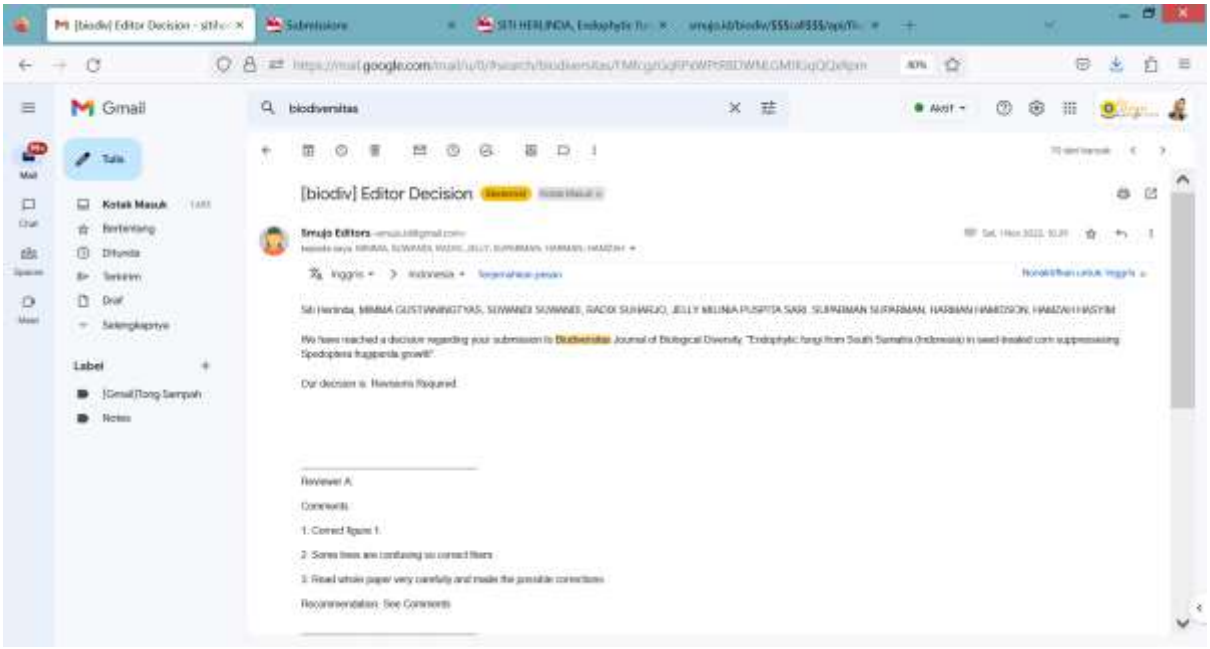
3. Bukti konfirmasi review kedua dan hasil revisi kedua



Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

Abstract. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. A total of 20 isolates of endophytic fungi were molecularly identified, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys isolates), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolates), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). Of the 20 isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga (3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae. The endophytic fungi had negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased the percentage of pupal and adult emergence, and the number of eggs laid by treated female adults. The fungi also shorten the adult longevity and increased the larval mortality. This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. These findings highlight the potential of endophytic fungi, namely *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Keywords: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

Abbreviations (if any):

Running title: Endophytic fungi suppressesing *Spodoptera frugiperda* growth

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) introduced to Asia in 2018 (Mahat et al. 2021) and was first discovered in India (Ganiger et al. 2018), while in Indonesia it was first discovered on 26 March 2019 in West Sumatra (Sartiami et al. 2020). In Indonesia two strains of *S. frugiperda* have been found in corn and rice strains (Herlinda et al. 2022). Currently, FAW has begun to spread to other provinces and islands in Indonesia, including West Java (Maharani et al. 2019), Lampung (Trisyono et al. 2019), Bengkulu (Ginting et al. 2020), Bali (Supartha et al. 2021), This pest entered South Sumatra in July 2019 (Hutasoit et al., 2020). FAW damages maize plant and various other plant species (Montezano et al., 2018), it eat leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Ginting et al. 2020). FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al. 2018). In Indonesia, FAW generally attacks maize with damage ranging of 26.50−70% in Lampung (Lestari et al. 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al. 2021), in Bali reaching 47.84% (Supartha et al. 2021), and in South Sumatra up to 100% (Herlinda et al. 2022).

The easy and fast action to control *S. frugiperda* is the use of synthetic insecticides (Kumela et al. 2018). However, insecticide application causes resistances to FAW (Zhang et al. 2021). Insecticide kills natural enemies of insect pests, negatively effect environment and human health (Harrison et al. 2019). An alternative sustainable and eco-friendly control for *S. frugiperda* is urgently needed. Biological control based on utilizing biocontrol agents, such as entomopathogenic fungi is preferred method to control *S. frugiperda* (Mantzoukas and Eliopoulos 2020). Topical application of entomopathogenic fungi, such as *Metarhizium anisopliae* killed 75% of *S. frugiperda* larvae (Ramos et al. 2020). *Beauveria bassiana* killed more than 80% of *S. frugiperda* larvae (Ramanujam et al. 2020). However, *S. frugiperda* larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al. 2021) and after that larvae hide in the leaf axils or at the base of developing cob (ear) or in the tip of cob (Prasanna et al. 2018). Because FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used (Herlinda et al. 2020; Gustianingtyas et al. 2021; Herlinda et al. 2021; Sari et al. 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et al. 2020). Endophytic fungi that were effective in killing *S. frugiperda*, for example *B. bassiana* and *M. anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al. 2020). *Metarrhizium robertsii* killed 51.2% of the 2nd instar larvae of *S. frugiperda* (Hernandez-Trejo et al. 2019). The results of previous studies have proved that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra, when applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al. 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al. 2021), but it is necessary to investigate the potential of fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were collected from the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. 20 fungal isolates, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolate), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate) were identified at molecularlevel and confirmed as endophytic fungi. All isolates were further deposited in the GenBank.

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was conducted in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at 27−29 °C room temperature and a relative humidity of 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, larvae were brought to the laboratory for mass-rearing according to the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø15 cm, height 25 cm) containing sterile soil. The plastic container

was put in a wire mesh cage (30 x 30 x 30 cm3) containing a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al. 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while seeds for control were only immersed in 10 mL of distilled water. Then, 15 seeds were kept in a sterile glass bottle (250 mL volume) having a sterile filter paper (Whatman No. 42) at the bottom, moistened with 1 mL of distilled water and incubated for 10 days. All treatments were repeated three times.

The stems and leaves of corn seedling that wereinoculated with 10 days old endophytic fungi were given the 25 2nd instars of *S. frugiperda* which were previously been fasted for 1x24 hours. When maize seedlings were 10 days old, endophytic fungal isolates had colonized maize stalks and leaves . The control maize seedlings were also given 25 2nd instars of *S. frugiperda*. The larvae were allowed to eat leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% according to the method of Russo et al. (2019). Then, larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days according to the method of Herlinda et al. (2020). The dead larvae were cultured in agar-water medium to confirm the infection by endophytic fungi or not. The number of dead larvae was calculated daily to observe the mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by female adults was also recorded. The leaf area of maize eaten by larvae, and the fecal and body weight of larvae were measured every day from the first to 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant differences between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Pathogenecity of endophytic fungi against *Spodoptera frugiperda* larvae

Of the 20 endophytic fungal isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga(3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by four isolates of *B. bassiana*, namely JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and one isolate JaSpkPga(3) of *C. lunata* ranged from 17−23%. The mortality caused by six isolates was higher from the beginning of observation to the last day, while control larvae that were only moistened with sterile water did not die. The fungus also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the number of eggs laid by treated female adults significantly decreased as compared to the number of eggs laid by untreated female adults (Table 2).

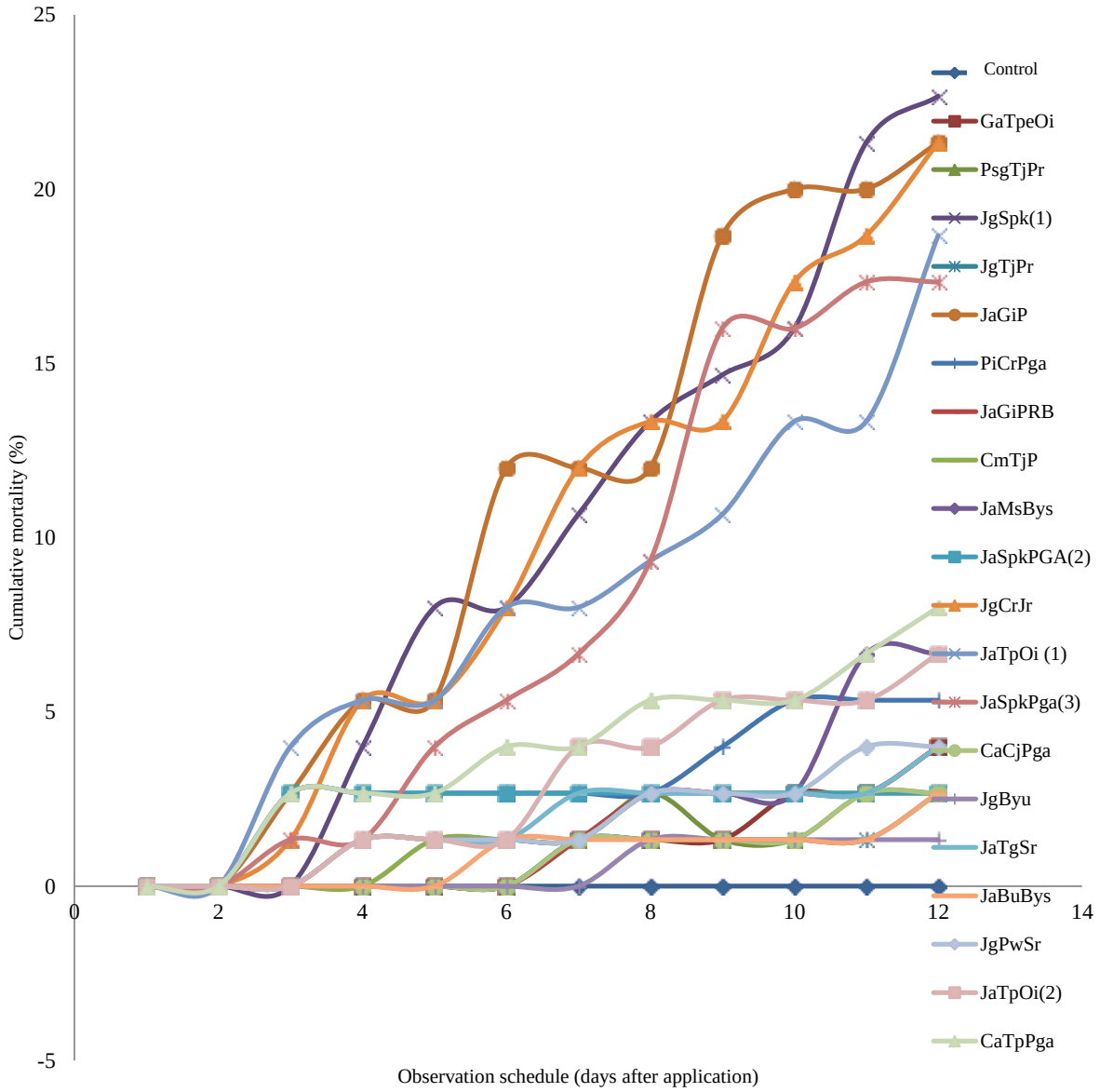


Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

Growth of *Spodoptera frugiperda*

The leaf area eaten by larvae treated with endophytic fungi (treated larvae) and untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by control larvae was widest compared to the leaf area eaten by treated larvae. The weight of control larvae was also heaviest compared to the weight of treated larvae (Table 4). The weight of control larvae was significantly different from those of treated larvae (from the 2nd day to the last day of observation). The larvae weight and leaf area eaten by treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate inoculated corn leaves had a significant reduction in appetite and weight compared to control larvae. The weight of feces produced by treated and control larvae were differed significantly, i.e. the weight of feces produced by treated larvae was lighter than the weight of feces produced by untreated (control) larvae (Table 5). The endophytic fungi had a negative effect on *S. frugiperda* growth.

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a mummy, darker in color and odorless, while untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, endophytic fungi caused the pupae to become shorter and darker, and finally it died, while the control pupae were larger in size, brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolates	Species	Pupae emergence (%)	Adult emergence (%)

Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef
JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolates	**Species**	**Longevity (days)**		**Eggs laid/female**	**Viable eggs (%)**
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a
JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde
JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a

JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

	Species	**Leaf area consumed by larvae (cm2 larvae-1 day-1) during 12 days of observation**					
Isolates		**2**	**4**	**6**	**8**	**10**	**12**
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

		Larvae weight (mg larvae^{-1}) during 12 days observation					
Isolates	**Species**	**2**	**4**	**6**	**8**	**10**	**12**
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03
GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13

JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80
JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates	Species	Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b
JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b
JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b

JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

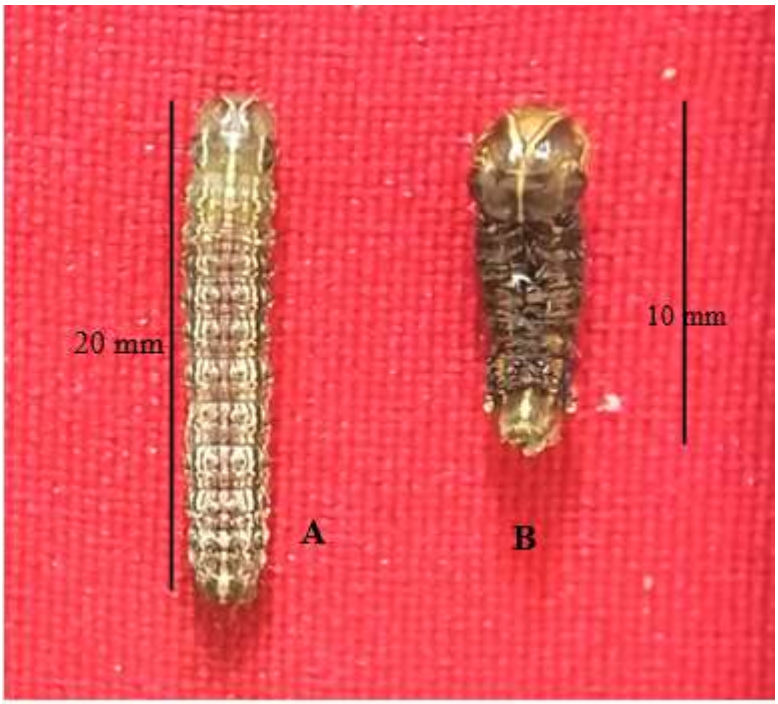


Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

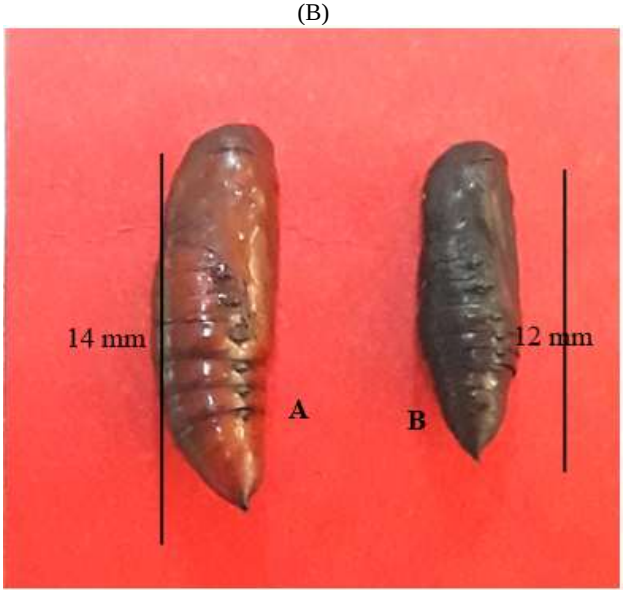


Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

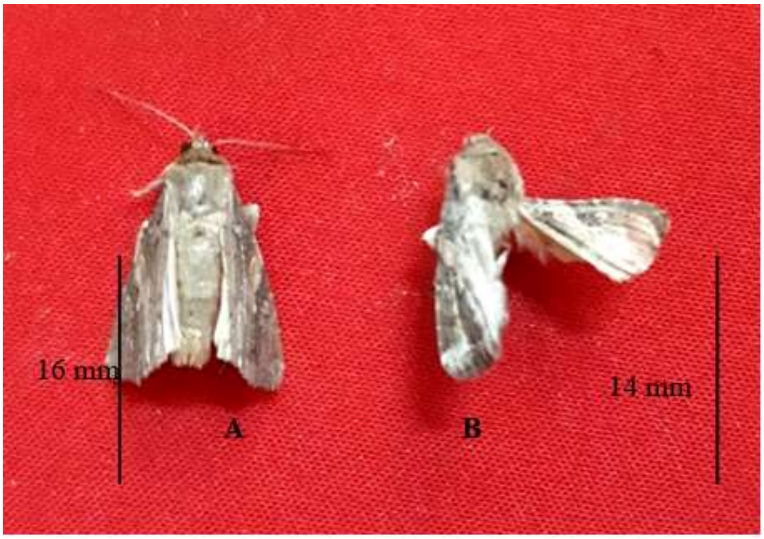


Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Discussion

The results showed that three species of endophytic fungi, namely *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) were more pathogenic . They caused higher mortality of FAW larvae. The fungi also decreased thepercentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by treated female adults. These results showed that endophytic fungi not only killed the larvae, but also killed pupae and reduced the adult emergence. These fungi also produced abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al. 2020; Herlinda et al. 2021). This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. *C. lunata* can kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al. 2014). The present study showed that mortality of larvae was low because the fungal suspension contained only $1x10^6$ conidia mL^{-1} In addition, fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains of *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al. 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. Moreover, the ability of endophytic fungi to colonize young maize (seedling) via seed treatment could prevent the maize plant from the attack of hiding *S. frugiperda* larvae in corn midribs (Herlinda et al. 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al. 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al. 2022).

The endophytic fungi showed negative effect on the growth of *S. frugiperda*. Endophytic fungi decreased the appetite of larvae, so that the leaf area consumed and fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined and finally they died. The endophytic fungus caused growth retardation on *S. frugiperda* (Gustianingtyas et al. 2021) and adverse effects on its survival (Russo et al. 2020) because fungus produce secondary metabolites and toxic protein or toxins (Vidal and Jabe, 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey 2004) and beauvericin, is toxic for insects (Safavi 2012) and *M. anisopliae* produces destruxin, that is also toxic for insects (Borisade et al. 2016). The mycelia of endophytic fungi within maize tissue consumed by larvae of *S. frugiperda* could produce blastospores in larvae hemolymph (Sari et al. 2022). Then, blastospores produced toxic secondary metabolites and proteins which is toxic for insects (Mancillas-Paredes et al. 2019). The entomopathogenic fungi also secrete secondary metabolites in plants that cause antibiosis, antifeedant or deterrent for *S. frugiperda* larvae (Jaber and Ownley 2018) and raise the concentrations of terpenoid compound against FAW larvae (Russo et al. 2020). After consuming toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al. 2022). The data obtained showed that mycosis was found only on *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, mycosis was not occurred on control larvae (untreated larvae). *S. frugiperda* larvae fed on plants colonized by endophytic fungi may undergo mycosis (Russo et al. 2020).

These findings highlight the potential of endophytic fungi, such as *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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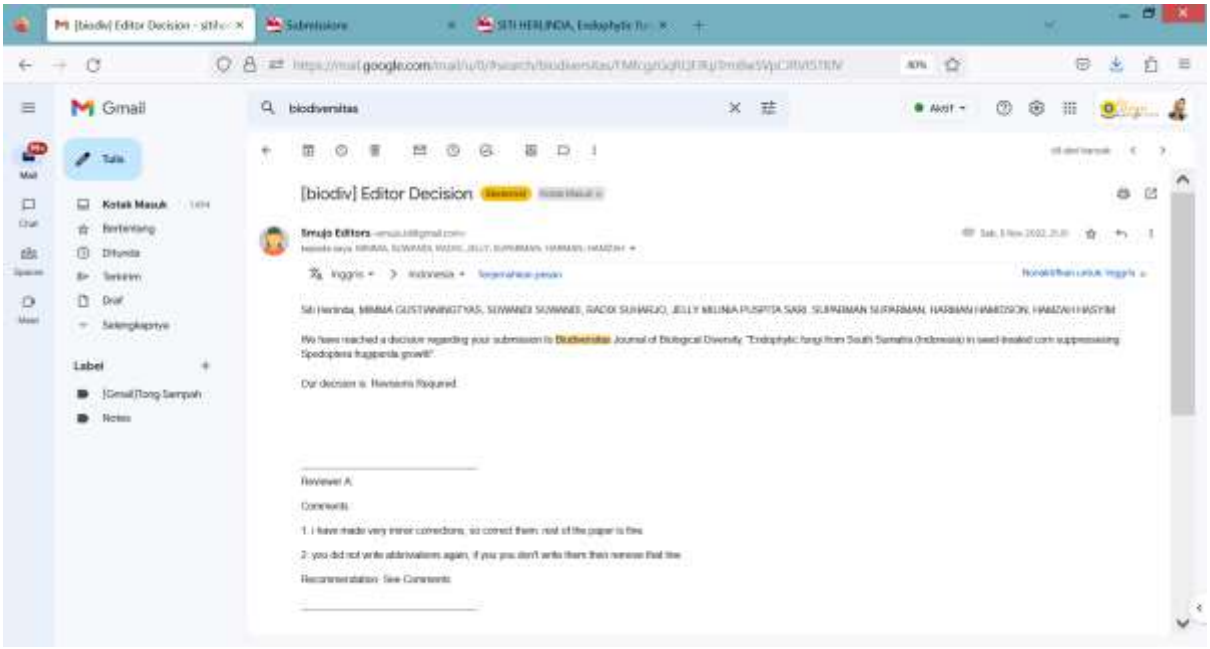
4. Bukti konfirmasi review ketiga dan hasil revisi ketiga



Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

Abstract. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. A total of 20 isolates of endophytic fungi were molecularly identified, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys isolates), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolates), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). Of the 20 isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga (3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae. The endophytic fungi had negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased the percentage of pupal and adult emergence, and the number of eggs laid by treated female adults. The fungi also shorten the adult longevity and increased the larval mortality. This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. These findings highlight the potential of endophytic fungi, namely *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Keywords: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

Running title: Endophytic fungi suppressesing *Spodoptera frugiperda* growth

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) introduced to Asia in 2018 (Mahat et al. 2021) and was first discovered in India (Ganiger et al. 2018), while in Indonesia it was first discovered on 26 March 2019 in West Sumatra (Sartiami et al. 2020). In Indonesia two strains of *S. frugiperda* have been found in corn and rice strains (Herlinda et al. 2022). Currently, FAW has begun to spread to other provinces and islands in Indonesia, including West Java (Maharani et al. 2019), Lampung (Trisyono et al. 2019), Bengkulu (Ginting et al. 2020), Bali (Supartha et al. 2021), This pest entered South Sumatra in July 2019 (Hutasoit et al., 2020). FAW damages maize plant and various other plant species (Montezano et al., 2018), it eat leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Ginting et al. 2020). FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al. 2018). In Indonesia, FAW generally attacks maize with damage ranging of 26.50−70% in Lampung (Lestari et al. 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al. 2021), in Bali reaching 47.84% (Supartha et al. 2021), and in South Sumatra up to 100% (Herlinda et al. 2022).

The easy and fast action to control *S. frugiperda* is the use of synthetic insecticides (Kumela et al. 2018). However, insecticide application causes resistances to FAW (Zhang et al. 2021). Insecticide kills natural enemies of insect pests, negatively effect environment and human health (Harrison et al. 2019). An alternative sustainable and eco-friendly control for *S. frugiperda* is urgently needed. Biological control based on utilizing biocontrol agents, such as entomopathogenic fungi is preferred method to control *S. frugiperda* (Mantzoukas and Eliopoulos 2020). Topical application of entomopathogenic fungi, such as *Metarhizium anisopliae* killed 75% of *S. frugiperda* larvae (Ramos et al. 2020). *Beauveria bassiana* killed more than 80% of *S. frugiperda* larvae (Ramanujam et al. 2020). However, *S. frugiperda* larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al. 2021) and after that larvae hide in the leaf axils or at the base of developing cob (ear) or in the tip of cob (Prasanna et al. 2018). Because FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used (Herlinda et al. 2020; Gustianingtyas et al. 2021; Herlinda et al. 2021; Sari et al. 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et al. 2020). Endophytic fungi that were effective in killing *S. frugiperda*, for example *B. bassiana* and *M. anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al. 2020). *Metarrhizium robertsii* killed 51.2% of the 2nd instar larvae of *S. frugiperda* (Hernandez-Trejo et al. 2019). The results of previous studies have proved that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra, when applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al. 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al. 2021), but it is necessary to investigate the potential of fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were collected from the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. 20 fungal isolates, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolate), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate) were identified at molecular level and confirmed as endophytic fungi. All isolates were further deposited in the GenBank.

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was conducted in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at 27−29 °C room temperature and a relative humidity of 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, larvae were brought to the laboratory for mass-rearing according to the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø15 cm, height 25 cm) containing sterile soil. The plastic container

was put in a wire mesh cage (30 x 30 x 30 cm3) containing a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al. 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while seeds for control were only immersed in 10 mL of distilled water. Then, 15 seeds were kept in a sterile glass bottle (250 mL volume) having a sterile filter paper (Whatman No. 42) at the bottom, moistened with 1 mL of distilled water and incubated for 10 days. All treatments were repeated three times.

The stems and leaves of corn seedling that were inoculated with 10 days old endophytic fungi were given the 25 2nd instars of *S. frugiperda* which were previously been fasted for 1x24 hours. When maize seedlings were 10 days old, endophytic fungal isolates had colonized maize stalks and leaves . The control maize seedlings were also given 25 2nd instars of *S. frugiperda*. The larvae were allowed to eat leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% according to the method of Russo et al. (2019). Then, larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days according to the method of Herlinda et al. (2020). The dead larvae were cultured in agar-water medium to confirm the infection by endophytic fungi or not. The number of dead larvae was calculated daily to observe the mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by female adults was also recorded. The leaf area of maize eaten by larvae, and the fecal and body weight of larvae were measured every day from the first to 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant differences between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Pathogenecity of endophytic fungi against *Spodoptera frugiperda* larvae

Of the 20 endophytic fungal isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga(3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by four isolates of *B. bassiana*, namely JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and one isolate JaSpkPga(3) of *C. lunata* ranged from 17−23%. The mortality caused by six isolates was higher from the beginning of observation to the last day, while control larvae that were only moistened with sterile water did not die. The fungus also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the number of eggs laid by treated female adults significantly decreased as compared to the number of eggs laid by untreated female adults (Table 2).

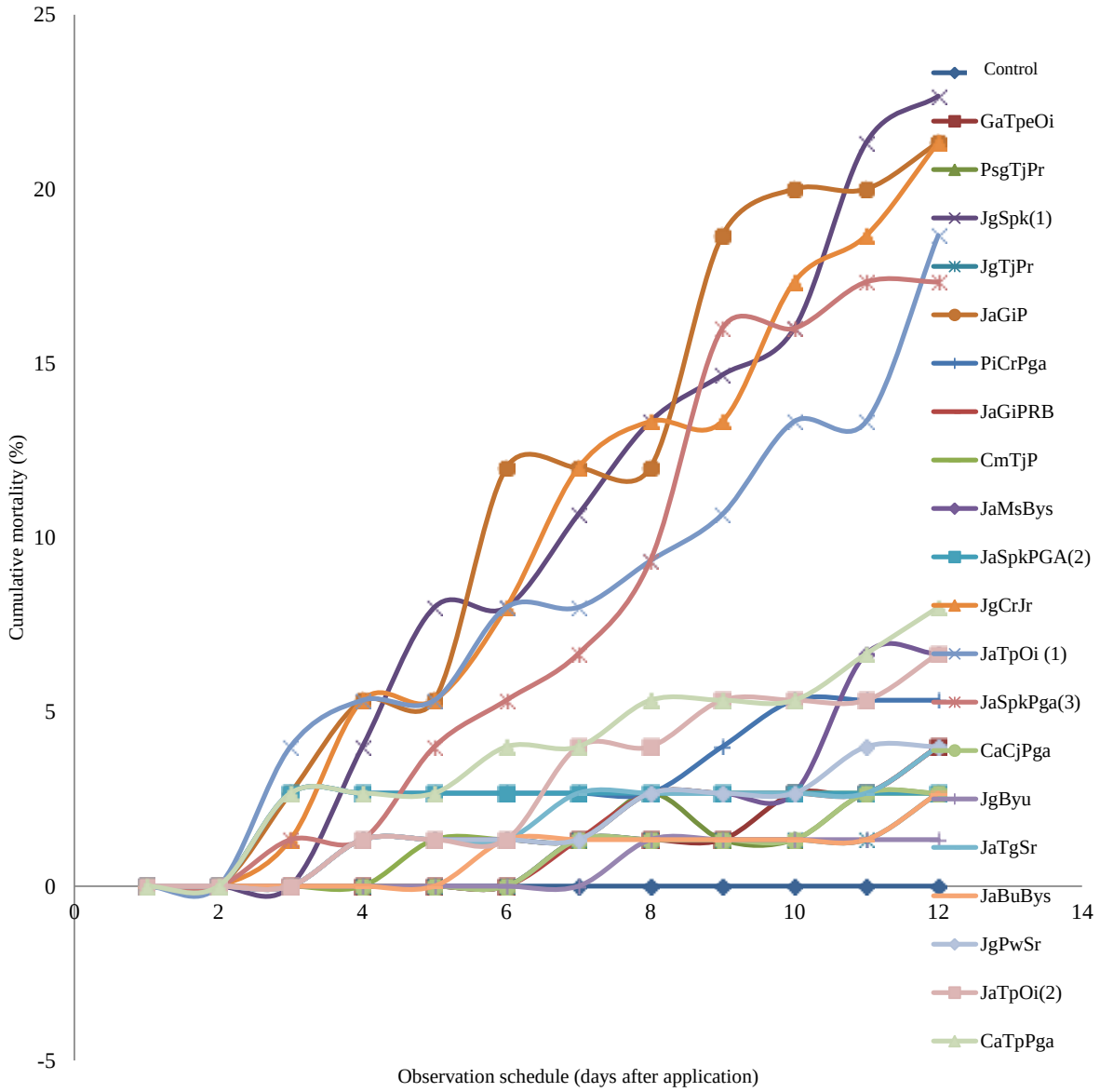


Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

Growth of *Spodoptera frugiperda*

The leaf area eaten by larvae treated with endophytic fungi (treated larvae) and untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by control larvae was widest compared to the leaf area eaten by treated larvae. The weight of control larvae was also heaviest compared to the weight of treated larvae (Table 4). The weight of control larvae was significantly different from those of treated larvae (from the 2nd day to the last day of observation). The larvae weight and leaf area eaten by treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate inoculated corn leaves had a significant reduction in appetite and weight compared to control larvae. The weight of feces produced by treated and control larvae were differed significantly, i.e. the weight of feces produced by treated larvae was lighter than the weight of feces produced by untreated (control) larvae (Table 5). The endophytic fungi had a negative effect on *S. frugiperda* growth.

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a mummy, darker in color and odorless, while untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, endophytic fungi caused the pupae to become shorter and darker, and finally it died, while the control pupae were larger in size, brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolates	Species	Pupae emergence (%)	Adult emergence (%)

Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef
JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolates	**Species**	**Longevity (days)**		**Eggs laid/female**	**Viable eggs (%)**
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a
JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde
JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a

JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

	Species	**Leaf area consumed by larvae (cm2 larvae-1 day-1) during 12 days of observation**					
Isolates		**2**	**4**	**6**	**8**	**10**	**12**
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

		Larvae weight (mg larvae^{-1}) during 12 days observation					
Isolates	**Species**	**2**	**4**	**6**	**8**	**10**	**12**
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03
GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13

JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80
JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates	Species	Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b
JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b
JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b

JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

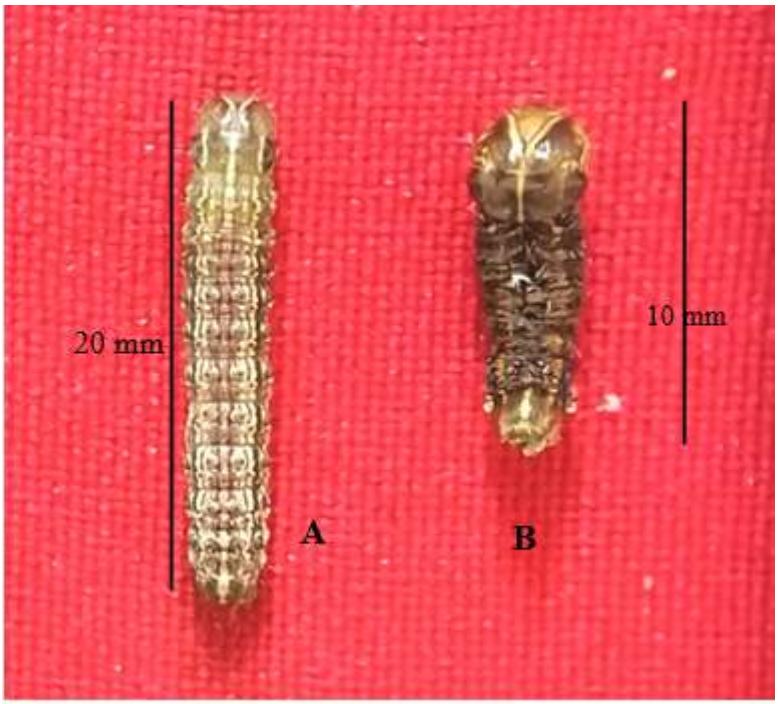


Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

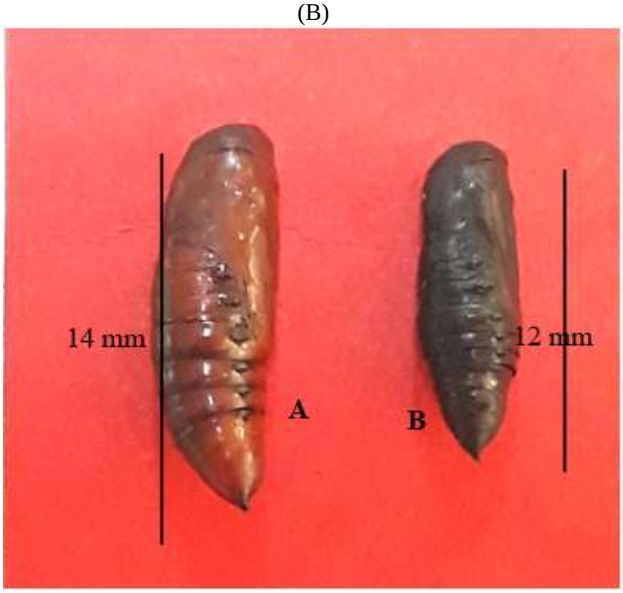


Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

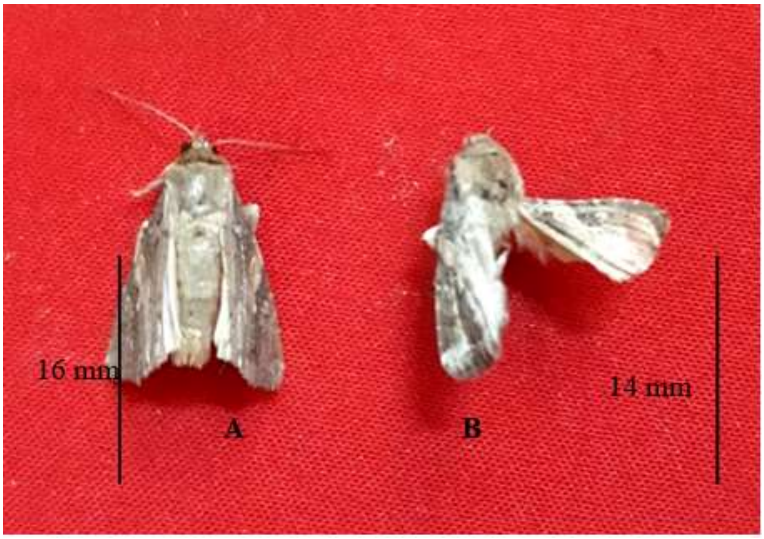


Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Discussion

The results showed that three species of endophytic fungi, namely *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) were more pathogenic . They caused higher mortality of FAW larvae. The fungi also decreased thepercentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by treated female adults. These results showed that endophytic fungi not only killed the larvae, but also killed pupae and reduced the adult emergence. These fungi also produced abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al. 2020; Herlinda et al. 2021). This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. *C. lunata* can kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al. 2014). The present study showed that mortality of larvae was low because the fungal suspension contained only $1x10^6$ conidia mL^{-1}. In addition, fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains of *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al. 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. Moreover, the ability of endophytic fungi to colonize young maize (seedling) via seed treatment could prevent the maize plant from the attack of hiding *S. frugiperda* larvae in corn midribs (Herlinda et al. 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al. 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al. 2022).

The endophytic fungi showed negative effect on the growth of *S. frugiperda*. Endophytic fungi decreased the appetite of larvae, so that the leaf area consumed and fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined and finally they died. The endophytic fungus caused growth retardation on *S. frugiperda* (Gustianingtyas et al. 2021) and adverse effects on its survival (Russo et al. 2020) because fungus produce secondary metabolites and toxic protein or toxins (Vidal and Jabe 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey 2004) and beauvericin, is toxic for insects (Safavi 2012) and *M. anisopliae* produces destruxin, that is also toxic for insects (Borisade et al. 2016). The mycelia of endophytic fungi within maize tissue consumed by larvae of *S. frugiperda* could produce blastospores in larvae hemolymph (Sari et al. 2022). Then, blastospores produced toxic secondary metabolites and proteins which is toxic for insects (Mancillas-Paredes et al. 2019). The entomopathogenic fungi also secrete secondary metabolites in plants that cause antibiosis, antifeedant or deterrent for *S. frugiperda* larvae (Jaber and Ownley 2018) and raise the concentrations of terpenoid compound against FAW larvae (Russo et al. 2020). After consuming toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al. 2022). The data obtained showed that mycosis was found only on *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, mycosis was not occurred on control larvae (untreated larvae). *S. frugiperda* larvae fed on plants colonized by endophytic fungi may undergo mycosis (Russo et al. 2020).

These findings highlight the potential of endophytic fungi, such as *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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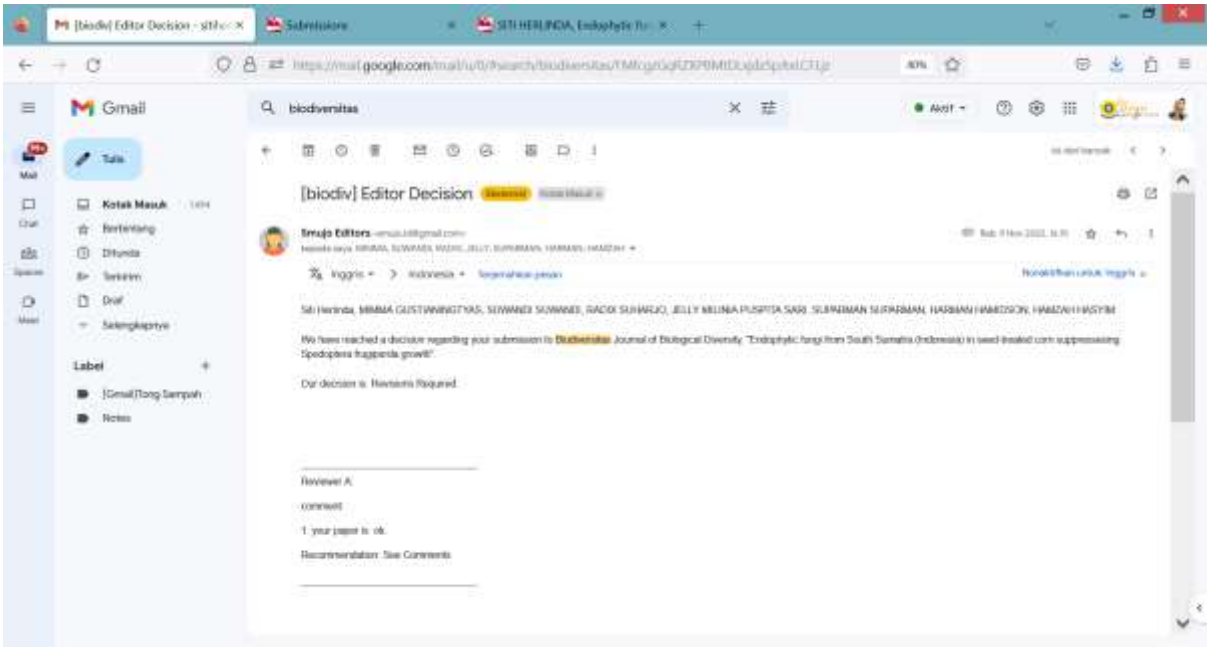
5. Bukti konfirmasi review keempat dan hasil revisi keempat



Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

Abstract. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. A total of 20 isolates of endophytic fungi were molecularly identified, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys isolates), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolates), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). Of the 20 isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga (3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae. The endophytic fungi had negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased the percentage of pupal and adult emergence, and the number of eggs laid by treated female adults. The fungi also shorten the adult longevity and increased the larval mortality. This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. These findings highlight the potential of endophytic fungi, namely *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Keywords: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

Running title: Endophytic fungi suppressesing *Spodoptera frugiperda* growth

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) introduced to Asia in 2018 (Mahat et al. 2021) and was first discovered in India (Ganiger et al. 2018), while in Indonesia it was first discovered on 26 March 2019 in West Sumatra (Sartiami et al. 2020). In Indonesia two strains of *S. frugiperda* have been found in corn and rice strains (Herlinda et al. 2022). Currently, FAW has begun to spread to other provinces and islands in Indonesia, including West Java (Maharani et al. 2019), Lampung (Trisyono et al. 2019), Bengkulu (Ginting et al. 2020), Bali (Supartha et al. 2021), This pest entered South Sumatra in July 2019 (Hutasoit et al., 2020). FAW damages maize plant and various other plant species (Montezano et al., 2018), it eat leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Ginting et al. 2020). FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al. 2018). In Indonesia, FAW generally attacks maize with damage ranging of 26.50−70% in Lampung (Lestari et al. 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al. 2021), in Bali reaching 47.84% (Supartha et al. 2021), and in South Sumatra up to 100% (Herlinda et al. 2022).

The easy and fast action to control *S. frugiperda* is the use of synthetic insecticides (Kumela et al. 2018). However, insecticide application causes resistances to FAW (Zhang et al. 2021). Insecticide kills natural enemies of insect pests, negatively effect environment and human health (Harrison et al. 2019). An alternative sustainable and eco-friendly control for *S. frugiperda* is urgently needed. Biological control based on utilizing biocontrol agents, such as entomopathogenic fungi is preferred method to control *S. frugiperda* (Mantzoukas and Eliopoulos 2020). Topical application of entomopathogenic fungi, such as *Metarhizium anisopliae* killed 75% of *S. frugiperda* larvae (Ramos et al. 2020). *Beauveria bassiana* killed more than 80% of *S. frugiperda* larvae (Ramanujam et al. 2020). However, *S. frugiperda* larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al. 2021) and after that larvae hide in the leaf axils or at the base of developing cob (ear) or in the tip of cob (Prasanna et al. 2018). Because FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used (Herlinda et al. 2020; Gustianingtyas et al. 2021; Herlinda et al. 2021; Sari et al. 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et al. 2020). Endophytic fungi that were effective in killing *S. frugiperda*, for example *B. bassiana* and *M. anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al. 2020). *Metarrhizium robertsii* killed 51.2% of the 2nd instar larvae of *S. frugiperda* (Hernandez-Trejo et al. 2019). The results of previous studies have proved that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra, when applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al. 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al. 2021), but it is necessary to investigate the potential of fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were collected from the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. 20 fungal isolates, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolate), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate) were identified at molecular level and confirmed as endophytic fungi. All isolates were further deposited in the GenBank.

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was conducted in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at 27−29 °C room temperature and a relative humidity of 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, larvae were brought to the laboratory for mass-rearing according to the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø15 cm, height 25 cm) containing sterile soil. The plastic container was put in a wire mesh cage (30 x 30 x 30 cm3) containing a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al. 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while seeds for control were only immersed in 10 mL of distilled water. Then, 15 seeds were kept in a sterile glass bottle (250 mL volume) having a sterile filter paper (Whatman No. 42) at the bottom, moistened with 1 mL of distilled water and incubated for 10 days. All treatments were repeated three times.

The stems and leaves of corn seedling that were inoculated with 10 days old endophytic fungi were given the 25 2nd instars of *S. frugiperda* which were previously been fasted for 1x24 hours. When maize seedlings were 10 days old, endophytic fungal isolates had colonized maize stalks and leaves. The control maize seedlings were also given 25 2nd instars of *S. frugiperda*. The larvae were allowed to eat leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% according to the method of Russo et al. (2019). Then, larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days according to the method of Herlinda et al. (2020). The dead larvae were cultured in agar-water medium to confirm the infection by endophytic fungi or not. The number of dead larvae was calculated daily to observe the mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by female adults was also recorded. The leaf area of maize eaten by larvae, and the fecal and body weight of larvae were measured every day from the first to 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant differences between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Pathogenecity of endophytic fungi against *Spodoptera frugiperda* larvae

Of the 20 endophytic fungal isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga(3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by four isolates of *B. bassiana*, namely JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and one isolate JaSpkPga(3) of *C. lunata* ranged from 17−23%. The mortality caused by six isolates was higher from the beginning of observation to the last day, while control larvae that were only moistened with sterile water did not die. The fungus also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the

number of eggs laid by treated female adults significantly decreased as compared to the number of eggs laid by untreated female adults (Table 2).

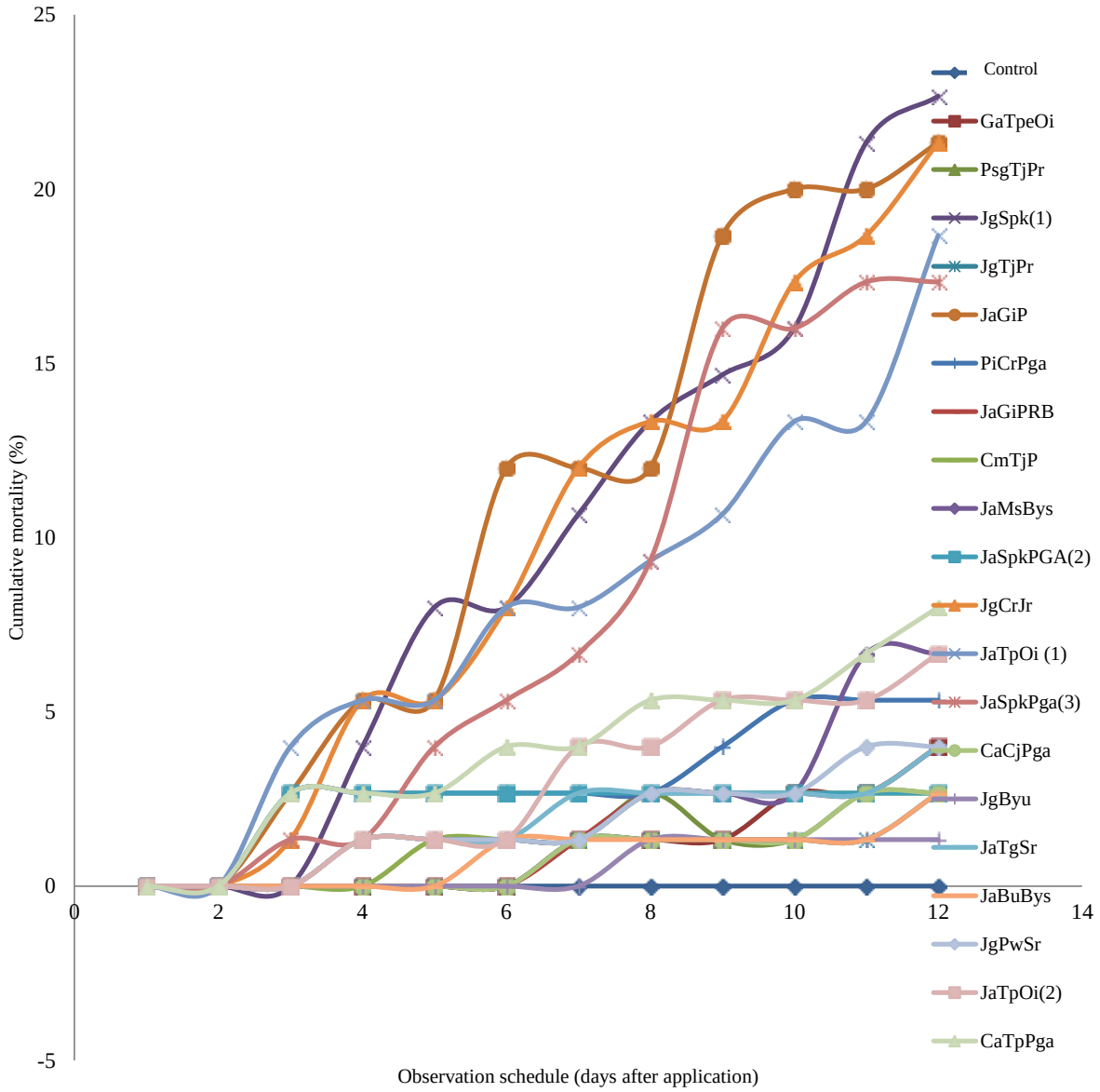


Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

Growth of *Spodoptera frugiperda*

The leaf area eaten by larvae treated with endophytic fungi (treated larvae) and untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by control larvae was widest compared to the leaf area eaten by treated larvae. The weight of control larvae was also heaviest compared to the weight of treated larvae (Table 4). The weight of control larvae was significantly different from those of treated larvae (from the 2nd day to the last day of observation). The larvae weight and leaf area eaten by treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate inoculated corn leaves had a significant reduction in appetite and weight compared to control larvae. The weight of feces produced by treated and control larvae were differed significantly, i.e. the weight of feces produced by treated larvae was lighter than the weight of feces produced by untreated (control) larvae (Table 5). The endophytic fungi had a negative effect on *S. frugiperda* growth.

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a mummy, darker in color and odorless, while untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, endophytic fungi caused the pupae to become shorter and darker, and finally it died, while the control pupae were larger in size, brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolates	Species	Pupae emergence (%)	Adult emergence (%)
Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef
JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolates	Species	Longevity (days)		Eggs laid/female	Viable eggs (%)
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a
JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde

JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a
JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

	Species	**Leaf area consumed by larvae (cm2 larvae-1 day-1) during 12 days of observation**					
Isolates		**2**	**4**	**6**	**8**	**10**	**12**
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

		Larvae weight (mg larvae^{-1}) during 12 days observation					
Isolates	**Species**	**2**	**4**	**6**	**8**	**10**	**12**
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03

GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13
JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80
JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates	Species	Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b
JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b

JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b
JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

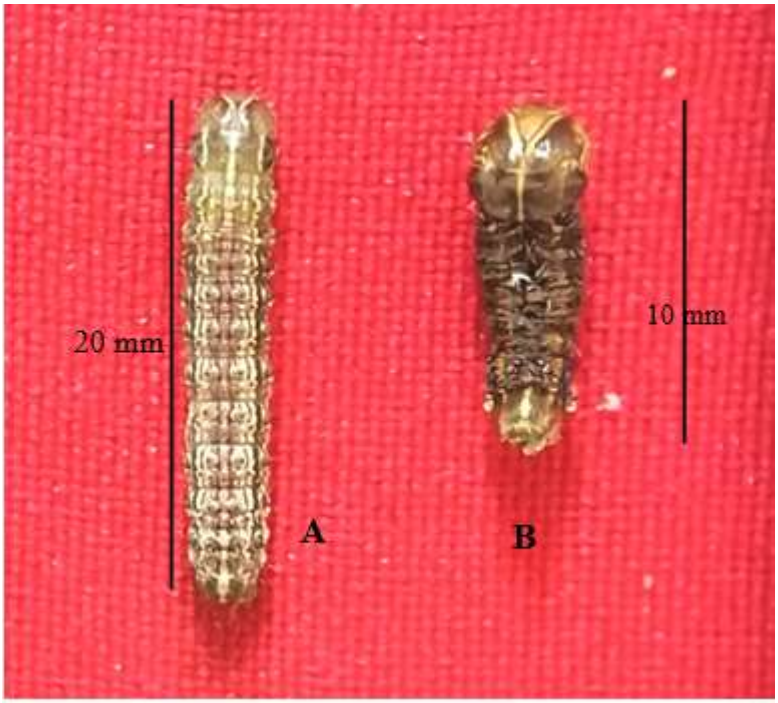


Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

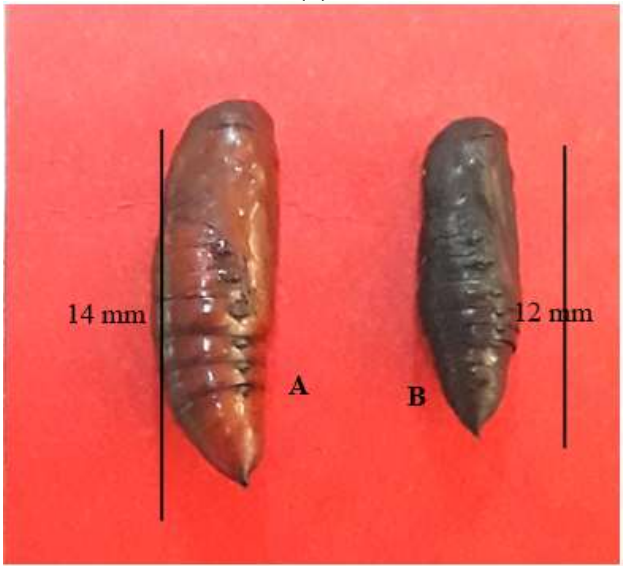


Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

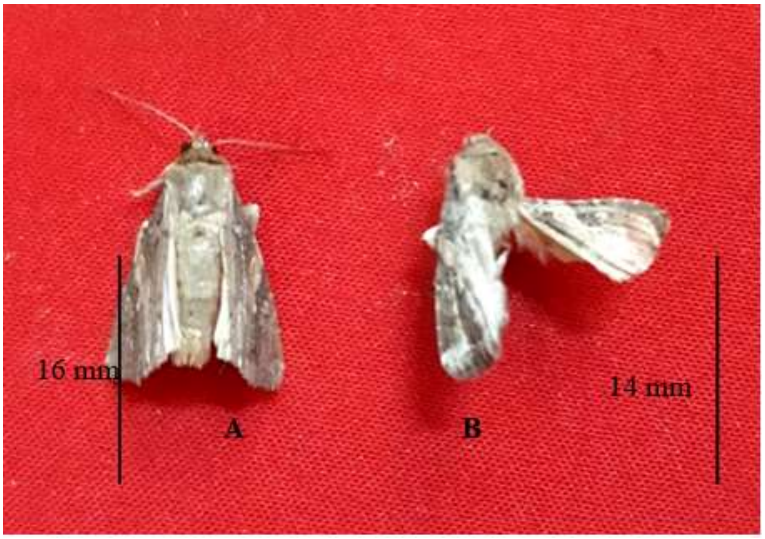


Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Discussion

The results showed that three species of endophytic fungi, namely *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) were more pathogenic . They caused higher mortality of FAW larvae. The fungi also decreased thepercentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by treated female adults. These results showed that endophytic fungi not only killed the larvae, but also killed pupae and reduced the adult emergence. These fungi also produced abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al. 2020; Herlinda et al. 2021). This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. *C. lunata* can kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al. 2014). The present study showed that mortality of larvae was low because the fungal suspension contained only $1x10^6$ conidia mL^{-1}. In addition, fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains of *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al. 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. Moreover, the ability of endophytic fungi to colonize young maize (seedling) via seed treatment could prevent the maize plant from the attack of hiding *S. frugiperda* larvae in corn midribs (Herlinda et al. 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al. 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al. 2022).

The endophytic fungi showed negative effect on the growth of *S. frugiperda*. Endophytic fungi decreased the appetite of larvae, so that the leaf area consumed and fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined and finally they died. The endophytic fungus caused growth retardation on *S. frugiperda* (Gustianingtyas et al. 2021) and adverse effects on its survival (Russo et al. 2020) because fungus produce secondary metabolites and toxic protein or toxins (Vidal and Jabe 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey 2004) and beauvericin, is toxic for insects (Safavi 2012) and *M. anisopliae* produces destruxin, that is also toxic for insects (Borisade et al. 2016). The mycelia of endophytic fungi within maize tissue consumed by larvae of *S. frugiperda* could produce blastospores in larvae hemolymph (Sari et al. 2022). Then, blastospores produced toxic secondary metabolites and proteins which is toxic for insects (Mancillas-Paredes et al. 2019). The entomopathogenic fungi also secrete secondary metabolites in plants that cause antibiosis, antifeedant or deterrent for *S. frugiperda* larvae (Jaber and Ownley 2018) and raise the concentrations of terpenoid compound against FAW larvae (Russo et al. 2020). After consuming toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al. 2022). The data obtained showed that mycosis was found only on *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, mycosis was not occurred on control larvae (untreated larvae). *S. frugiperda* larvae fed on plants colonized by endophytic fungi may undergo mycosis (Russo et al. 2020).

These findings highlight the potential of endophytic fungi, such as *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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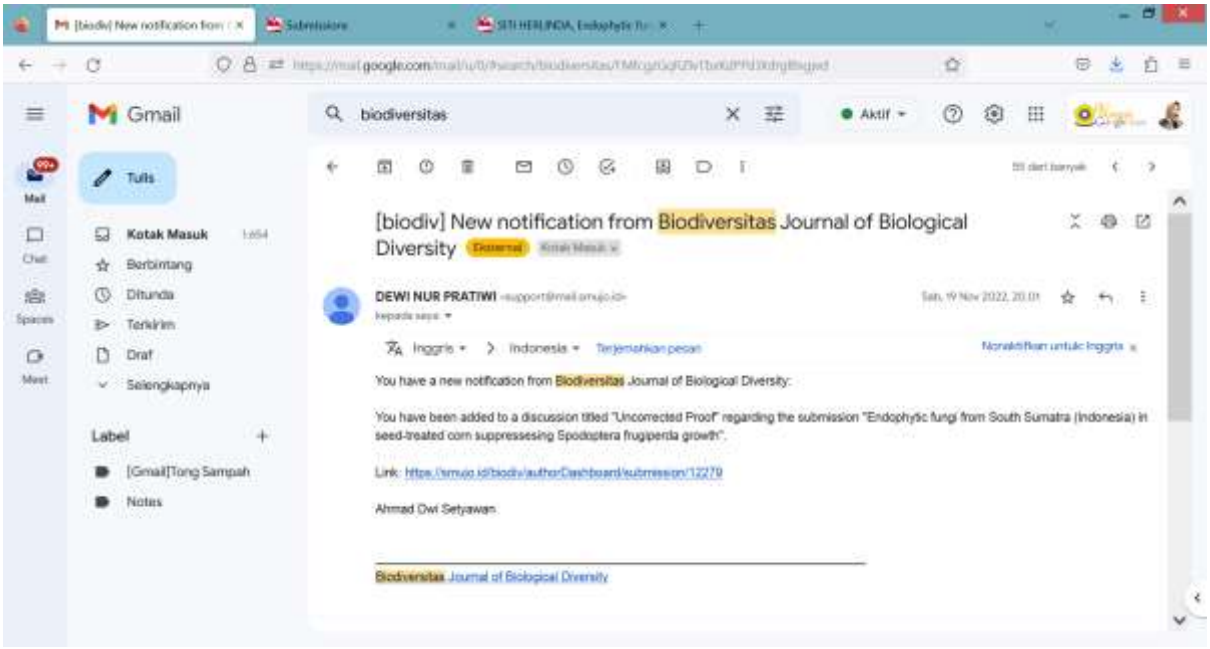

Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing *Spodoptera frugiperda* growth

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Abstract. *Herlinda S, Gustianingtyas M, Suwandi S, Suharjo R, Sari JMP, Suparman, Hamidson H, Hasyim H. 2022. Endophytic fungi from South Sumatra (Indonesia) in seed-treated corn suppressesing* Spodoptera frugiperda *growth. Biodiversitas 23: xxxx.* The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth. A total of 20 isolates of endophytic fungi were molecularly identified, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys isolates), *Beauveria bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolates), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *Metarhizium anisopliae* (CaTpPGA isolate). Of the 20 isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate of each *C. lunata* (JaSpkPga (3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae. The endophytic fungi had negative effect on *S. frugiperda* growth. *B. bassiana*, *M. anisopliae*, and *C. lunata* decreased the percentage of pupal and adult emergence, and the number of eggs laid by treated female adults. The fungi also shorten the adult longevity and increased the larval mortality. This is the first report of pathogenicity of *C. lunata* against *S. frugiperda.* These findings highlight the potential of endophytic fungi, namely *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

Keywords: *Beauveria bassiana*, *Curvularia lunata*, *Metarhizium anisopliae*, seed treatment, *Zea mays*

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest for maize in Indonesia. This pest originating from South America (Otim et al., 2018) introduced to Asia in 2018 (Mahat et al. 2021) and was first discovered in India (Ganiger et al. 2018), while in Indonesia it was first discovered on 26 March 2019 in West Sumatra (Sartiami et al. 2020). In Indonesia two strains of *S. frugiperda* have been found in corn and rice strains (Herlinda et al. 2022). Currently, FAW has begun to spread to other provinces and islands in Indonesia, including West Java (Maharani et al. 2019), Lampung (Trisyono et al. 2019), Bengkulu (Ginting et al. 2020), Bali (Supartha et al. 2021), This pest entered South Sumatra in July 2019 (Hutasoit et al., 2020). FAW damages maize plant and various other plant species (Montezano et al., 2018), it eat leaves, stems, flowers, fruit, growing points, fruit, and whole plant parts (Ginting et al. 2020). FAW causes financial losses of up to 250-630 million US dollars per year in Africa (Bateman et al. 2018). In Indonesia, FAW generally attacks maize with damage ranging of 26.50−70% in Lampung (Lestari et al. 2020), in East Nusa Tenggara around 85 to 100% (Mukkun et al. 2021), in Bali reaching 47.84% (Supartha et al. 2021), and in South Sumatra up to 100% (Herlinda et al. 2022).

The easy and fast action to control *S. frugiperda* is the use of synthetic insecticides (Kumela et al. 2018). However, insecticide application causes resistances to FAW (Zhang et al. 2021). Insecticide kills natural enemies of insect pests, negatively effect environment and human health (Harrison et al. 2019). An alternative sustainable and eco-friendly control for *S. frugiperda* is urgently needed. Biological control based on utilizing biocontrol agents, such as entomopathogenic fungi is preferred method to control *S. frugiperda* (Mantzoukas and Eliopoulos 2020). Topical application of entomopathogenic fungi, such as *Metarhizium anisopliae* killed 75% of *S. frugiperda* larvae (Ramos et al. 2020). *Beauveria bassiana* killed more than 80% of *S. frugiperda* larvae (Ramanujam et al. 2020). However, *S. frugiperda* larvae are generally found on the surface of leaves, flowers, fruit, or corn stalks in the morning around 6.30 to 8.00 a.m. (Gustianingtyas et al. 2021) and after that larvae hide in the leaf axils or at the base of developing cob (ear) or in the tip of cob (Prasanna et al. 2018). Because FAW hides all-day, so they are more difficult to control topically. To control the hidden FAW, many endophytic fungi have been used

(Herlinda et al. 2020; Gustianingtyas et al. 2021; Herlinda et al. 2021; Sari et al. 2022).

The endophytic fungi systemically colonize plant tissues and associate mutually with their host plants (Lira et al. 2020). Endophytic fungi that were effective in killing *S. frugiperda*, for example *B. bassiana* and *M. anisopliae* killed 87 and 75% of the mature instars of *S. frugiperda*, respectively (Ramos et al. 2020). *Metarrhizium robertsii* killed 51.2% of the 2nd instar larvae of *S. frugiperda* (Hernandez-Trejo et al. 2019). The results of previous studies have proved that eight isolates of endophytic entomopathogenic fungi obtained from corn roots in South Sumatra, when applied topically can kill *S. frugiperda* larvae (Gustianingtyas et al. 2021). The endophytic fungi obtained from roots, leaves, and shoots have been found in South Sumatra and identified molecularly (Herlinda et al. 2021), but it is necessary to investigate the potential of fungi inoculated in seed corn to suppress the growth of *S. frugiperda*. The aim of this research was to evaluate the effect of endophytic fungi in seed-treated corn on *S. frugiperda* growth.

MATERIALS AND METHODS

Preparation of fungal isolates

The fungal isolates used in this study were collected from the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya. The fungal isolates were isolated from leaves, shoots, and roots of corn (*Zea mays*), bananas (*Musa* sp.), ridged gourd (*Luffa acutangula*), and red chilies (*Capsicum annuum*) from the lowlands and highlands of South Sumatra. 20 fungal isolates, namely *Chaetomium* sp. (GaTpeOi, JgTjPr, PiCrPga, and CaCjPga isolates), *Aspergillus niger* (PsgTjPr, JgByU, and JaBuBys), *B. bassiana* (JgSPK, JaGiP, JaSpkPGA(2) isolate), JgCrJr, dan JaTpOi (1) isolates), *Curvularia lunata* (JaGiPRB, CMTJP, JaMsBys, JaSpkPga(3), and JgTgSr isolates), *Aspergillus flavus* (JgPWSR isolate), *Penicillium citrinum* (JaTpOi(2) isolate), and *M. anisopliae* (CaTpPGA isolate) were identified at molecular level and confirmed as endophytic fungi. All isolates were further deposited in the GenBank.

Mass-rearing of *Spodoptera frugiperda* for bioassay

Mass-rearing of *S. frugiperda* was conducted in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya at 27−29 °C room temperature and a relative humidity of 76−89%. Larvae of *S. frugiperda* were obtained from maize plants in Indralaya, Ogan Ilir District, South Sumatra, Indonesia. Then, larvae were brought to the laboratory for mass-rearing according to the method of Herlinda et al. (2020). The larvae were reared individually in a porous plastic cup (Ø 6.5 cm, height 4.6 cm) because larvae were cannibals. Larvae were given fresh corn leaves every day (2 cm x 5 cm). The pupae emerged were placed in a plastic container (Ø 15 cm, height 25 cm) containing sterile soil. The plastic container was put in a wire mesh cage (30 x 30 x 30 cm^3) containing a maize plant for adults laying eggs. The mass-rearing was carried out for more than five generations in the laboratory to obtain homogeneous test insects.

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

The bioassay of endophytic fungi against larvae of *S. frugiperda* began with the inoculation of fungi on corn seeds. Fifteen seeds of corn per treatment were surface sterilized using 70% EtOH (Ethyl alcohol) and 1% NaOCl (Sodium hypochlorite) (Gustianingtyas et al. 2021). The seeds were immersed in 10 mL of fungal suspension (1 x 10^6 conidia mL^{-1}) for 6 hours, while seeds for control were only immersed in 10 mL of distilled water. Then, 15 seeds were kept in a sterile glass bottle (250 mL volume) having a sterile filter paper (Whatman No. 42) at the bottom, moistened with 1 mL of distilled water and incubated for 10 days. All treatments were repeated three times.

The stems and leaves of corn seedling that were inoculated with 10 days old endophytic fungi were given the 25 2nd instars of *S. frugiperda* which were previously been fasted for 1x24 hours. When maize seedlings were 10 days old, endophytic fungal isolates had colonized maize stalks and leaves. The control maize seedlings were also given 25 2nd instars of *S. frugiperda*. The larvae were allowed to eat leaves and stems of young maize until they were finished them (~6 hours). The bioassay of endophytic fungi (20 isolates) against larvae of *S. frugiperda* was carried out in an incubator at a constant temperature of 25 °C and a relative humidity of 97% according to the method of Russo et al. (2019). Then, larvae were transferred to a porous plastic cup (Ø 6.5 cm, height 4.6 cm) and fed with fresh corn leaves (2 x 5 cm^2) every day. The dead larvae were recorded daily for 12 days according to the method of Herlinda et al. (2020). The dead larvae were cultured in agar-water medium to confirm the infection by endophytic fungi or not. The number of dead larvae was calculated daily to observe the mortality data. The number of pupae and adults emerging were counted, and the number of eggs laid by female adults was also recorded. The leaf area of maize eaten by larvae, and the fecal and body weight of larvae were measured every day from the first to 12th day.

Data analysis

The differences in body and fecal weight of larvae and the leaf area eaten daily between treatments (20 fungal isolates), percentage of pupae and adult emergence, and the number of eggs laid were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) test (Tukey's test) was employed to test for significant differences between treatments of fungal isolates at P = 0.05. All data were calculated using software of SAS University Edition 2.7 9.4 M5.

RESULTS AND DISCUSSION

Pathogenecity of endophytic fungi against *Spodoptera frugiperda* larvae

Of the 20 endophytic fungal isolates, 4 isolates (JgSPK, JaGiP, JgCrJr, JaTpOi (1)) of *B. bassiana* and one isolate

of each *C. lunata* (JaSpkPga(3)), and *M anisoplae* (CaTpPga) were found to be more pathogenic to *S. frugiperda* larvae (Figure 1). The larvae mortality caused by four isolates of *B. bassiana*, namely JgSPK, JaGiP, JgCrJr, JaTpOi (1) isolates and one isolate JaSpkPga(3) of *C. lunata* ranged from 17−23%. The mortality caused by six isolates was higher from the beginning of observation to the last day, while control larvae that were only moistened with sterile water did not die. The fungus also decreased the percentage of pupae and adult emergence (Table 1). The percentage of eggs hatched and the number of eggs laid by treated female adults significantly decreased as compared to the number of eggs laid by untreated female adults (Table 2).

Growth of Spodoptera frugiperda

The leaf area eaten by larvae treated with endophytic fungi (treated larvae) and untreated larvae (control) showed significant differences (Table 3). The leaf area eaten by control larvae was widest compared to the leaf area eaten by treated larvae. The weight of control larvae was also heaviest compared to the weight of treated larvae (Table 4). The weight of control larvae was significantly different from those of treated larvae (from the 2nd day to the last day of observation). The larvae weight and leaf area eaten by treated larvae compared to the control larvae significantly decreased. Thus, larvae that ate inoculated corn leaves had a significant reduction in appetite and weight compared to control larvae. The weight of feces produced by treated and control larvae were differed significantly, i.e. the weight of feces produced by treated larvae was lighter than the weight of feces produced by untreated (control) larvae (Table 5). The endophytic fungi had a negative effect on *S. frugiperda* growth.

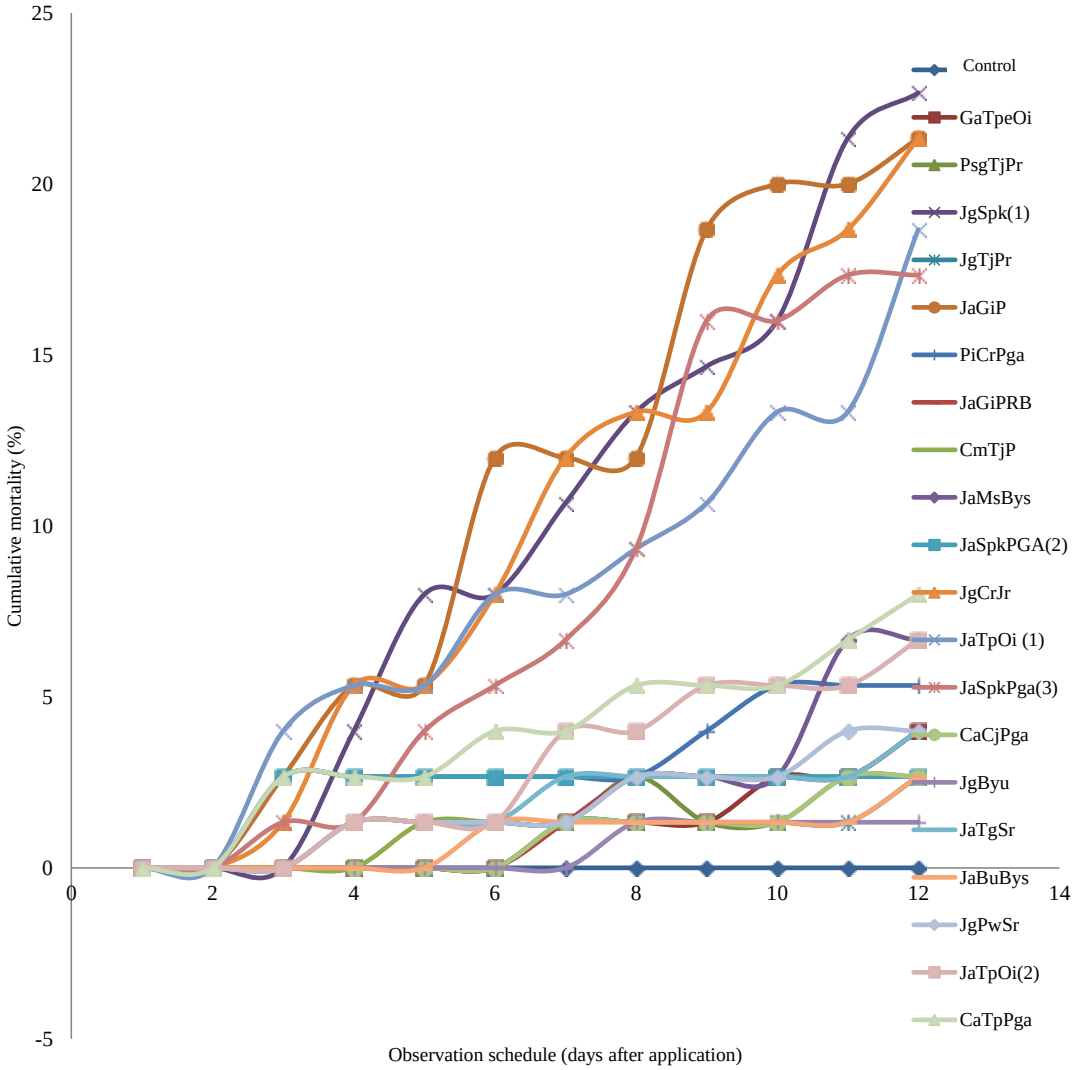

Figure 1. Cumulative mortality of *Spodoptera frugiperda* larvae treated with endophytic fungi during 12 days observation

Table 1. Mean percentage of pupae and adult emergence treated with endophytic fungi

Isolates	Species	Pupae emergence (%)	Adult emergence (%)
Control	-	100.00e	100.00i
GaTpeOi	*Chaetomium* sp.	96.00cd	86.67abcde
PsgTjPr	*Aspergillus niger*	96.00cd	92.00defg
JgSpk(1)	*Beauveria bassiana*	77.33a	73.33a
JgTjPr	*Chaetomium* sp.	97.33cde	89.33cdef
JaGiP	*Beauveria bassiana*	78.67a	76.00ab
PiCrPga	*Chaetomium* sp.	94.67c	90.67cdef
JaGiPRB	*Curvularia lunata*	96.00cd	94.67efgh
CmTjP	*Curvularia lunata*	97.33cde	94.67fgh
JaMsBys	*Curvularia lunata*	93.33c	90.67cdef
JaSpkPGA(2)	*Beauveria bassiana*	97.33cde	96.00efgh
JgCrJr	*Beauveria bassiana*	78.67a	78.67abc
JaTpOi (1)	*Beauveria bassiana*	81.33a	81.33abc
JaSpkPga(3)	*Curvularia lunata*	82.67ab	82.67abcd
CaCjPga	*Chaetomium* sp.	97.33cde	97.33ghi
JgByu	*Aspergillus niger*	98.67de	98.67hi
JaTgSr	*Curvularia lunata*	96.00cd	96.00efgh
JaBuBys	*Aspergillus niger*	97.33cde	90.67efg
JgPwSr	*Aspergillus flavus*	96.00cd	96.00efgh
JaTpOi(2)	*Penicillium citrinum*	93.33c	89.33cdef
CaTpPga	*Metarhizium anisopliae*	92.00bc	82.67abcd
F-value		7.26*	6.14*
P-value		0.00	0.00
HSD value		8.67	9.33

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 2. Mean of adult longevity, eggs laid, and viable eggs of *Spodoptera frugiperda* treated with endophytic fungi

Isolates	Species	Longevity (days)		Eggs laid/female	Viable eggs (%)
		Female	Male		
Control	-	4.33	3.67	143.00h	94.54e
GaTpeOi	*Chaetomium* sp.	3.67	2.67	44.33a	70.92a
PsgTjPr	*Aspergillus niger*	4.00	3.00	96.67defg	70.38a
JgSpk(1)	*Beauveria bassiana*	3.33	3.33	87.00cde	74.86abcd
JgTjPr	*Chaetomium* sp.	3.33	2.67	75.67bcd	83.53abcde
JaGiP	*Beauveria bassiana*	3.67	3.67	95.00defg	77.40abcd
PiCrPga	*Chaetomium* sp.	4.00	2.33	91.33cde	90.08cde
JaGiPRB	*Curvularia lunata*	3.33	2.67	81.33cde	90.71de
CmTjP	*Curvularia lunata*	3.67	3.00	53.00ab	84.45abcde
JaMsBys	*Curvularia lunata*	3.33	2.33	80.00cde	74.36ab
JaSpkPGA(2)	*Beauveria bassiana*	3.33	3.33	135.67h	71.65a
JgCrJr	*Beauveria bassiana*	3.33	2.33	122.67gh	76.56abcd
JaTpOi (1)	*Beauveria bassiana*	2.67	2.67	121.67gh	72.64a
JaSpkPga(3)	*Curvularia lunata*	4.00	3.00	75.00bcd	80.12abcde
CaCjPga	*Chaetomium* sp.	3.00	2.33	82.33cde	89.58bcde
JgByu	*Aspergillus niger*	3.33	3.00	91.67cdef	83.99abcde
JaTgSr	*Curvularia lunata*	3.67	3.00	91.67cdef	73.50a
JaBuBys	*Aspergillus niger*	3.67	2.33	104.33efg	81.41abcde
JgPwSr	*Aspergillus flavus*	3.00	2.33	93.33defg	89.78cde
JaTpOi(2)	*Penicillium citrinum*	4.00	3.67	121.00fgh	82.49abcde
CaTpPga	*Metarhizium anisopliae*	3.33	2.67	68.00bc	74.85abc
F-value		1.10ns	1.33ns	7.05*	1.841*
P-value		0.41	0.31	0.00	0.05
HSD value		-	-	1.42	0.88

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 3. Mean of leaf area consumed by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates	Species	Leaf area consumed by larvae (cm^2 $larvae^{-1}$ day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	5.05df	8.01d	8.97d	9.42g	9.30e	8.67h
GaTpeOi	*Chaetomium* sp.	3.89a	7.33bcd	7.18abc	7.95f	8.06cde	6.46cdef
PsgTjPr	*Aspergillus niger*	4.87cde	6.77abcd	6.75abc	7.48def	8.77de	7.19fgh
JgSpk(1)	*Beauveria bassiana*	4.59bcd	7.21bcd	7.57abcd	7.85ef	7.84cde	6.96efg
JgTjPr	*Chaetomium* sp.	4.33abc	5.17a	6.30a	7.63ef	7.33bcd	7.37fgh
JaGiP	*Beauveria bassiana*	5.35e	5.75abc	6.28a	6.17bc	7.68bcd	5.44bcd
PiCrPga	*Chaetomium* sp.	4.27abc	5.47ab	6.90abc	7.50def	8.10cde	6.83defg
JaGiPRB	*Curvularia lunata*	4.23ab	5.04a	6.46ab	6.31bcd	6.83bc	4.84b
CmTjP	*Curvularia lunata*	4.11ab	8.09d	6.93abc	7.28cdef	7.46bcd	6.60cdefg
JaMsBys	*Curvularia lunata*	4.19ab	5.79abc	7.40abcd	7.75ef	7.14bc	6.18bcdef
JaSpkPGA(2)	*Beauveria bassiana*	4.62bcd	6.73abcd	7.68bcd	7.39cdef	7.53bcd	6.55cdefg
JgCrJr	*Beauveria bassiana*	4.07ab	6.60abcd	8.01cd	7.24bcdef	7.10bc	5.97bcdef
JaTpOi (1)	*Beauveria bassiana*	4.10ab	7.28bcd	6.82abc	6.91bcdef	6.77bc	6.90efg
JaSpkPga(3)	*Curvularia lunata*	4.04ab	7.66cd	6.67abc	6.61bcde	6.33b	6.77defg
CaCjPga	*Chaetomium* sp.	4.25abc	8.00d	7.36abcd	7.25bcdef	7.40bcd	5.96bcdef
JgByu	*Aspergillus niger*	4.18ab	6.45abcd	7.44abcd	7.88ef	7.94cde	8.14gh
JaTgSr	*Curvularia lunata*	3.94a	5.55ab	7.63bcd	8.03fg	7.91cde	7.16fgh
JaBuBys	*Aspergillus niger*	5.40e	6.15abcd	5.97a	4.84a	4.90a	3.51a
JgPwSr	*Aspergillus flavus*	4.91cde	5.17a	6.46ab	6.02b	6.89bc	5.20bc
JaTpOi(2)	*Penicillium citrinum*	5.34e	7.59cd	8.52cd	7.33cdef	7.41bcd	5.30bc
CaTpPga	*Metarhizium anisopliae*	4.50abcd	7.53cd	7.97bcd	7.18bcdef	7.15bc	5.60bcde
F-value		4.43*	1.94*	2.01*	4.39*	3.28*	5.17*
P-value		0	0.04	0.03	0	0	0
HSD value		0.14	0.39	0.28	0.23	0.26	0.29

Note: * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 4. Mean weight of *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates		Larvae weight (mg $larvae^{-1}$) during 12 days observation					
	Species	2	4	6	8	10	12
Control	-	45.17d	54.41	76.06i	115.40h	143.17i	175.03
GaTpeOi	*Chaetomium* sp.	27.47abc	33.60	50.82def	66.40def	92.00fg	117.07
PsgTjPr	*Aspergillus niger*	28.13abc	49.20	64.93hi	74.40efg	85.92efg	104.13
JgSpk(1)	*Beauveria bassiana*	21.60a	26.67	31.43a	35.84a	50.31a	59.20
JgTjPr	*Chaetomium* sp.	26.53abc	36.27	54.82fgh	70.80ef	90.93fg	101.07
JaGiP	*Beauveria bassiana*	30.26c	25.57	40.00abc	49.47bc	56.67ab	60.93
PiCrPga	*Chaetomium* sp.	28.67bc	35.20	48.67cde	64.04def	76.67def	93.60
JaGiPRB	*Curvularia lunata*	28.27bc	40.00	63.59hi	87.33g	111.20h	133.20
CmTjP	*Curvularia lunata*	29.87c	41.88	60.93gh	76.80fg	94.00g	112.80
JaMsBys	*Curvularia lunata*	25.47abc	34.00	60.27fgh	69.60ef	97.98gh	110.80
JaSpkPGA(2)	*Beauveria bassiana*	29.07c	35.07	57.87fgh	75.87fg	92.00fg	115.47
JgCrJr	*Beauveria bassiana*	22.572ab	28.80	35.19ab	45.47abc	60.05abc	74.27
JaTpOi (1)	*Beauveria bassiana*	25.29abc	29.60	37.87ab	50.02bc	60.27abc	71.07
JaSpkPga(3)	*Curvularia lunata*	22.31ab	28.61	34.14ab	44.60ab	60.27abc	72.53
CaCjPga	*Chaetomium* sp.	26.67abc	35.33	54.40fgh	63.87def	73.39cde	92.00
JgByu	*Aspergillus niger*	28.53bc	34.40	53.20fgh	70.80ef	83.47efg	109.33
JaTgSr	*Curvularia lunata*	27.47abc	36.40	51.48def	67.87def	82.67efg	95.20
JaBuBys	*Aspergillus niger*	27.60abc	39.20	52.93fgh	62.13de	72.27cde	83.73
JgPwSr	*Aspergillus flavus*	25.33abc	38.13	50.00cdef	62.40de	77.06def	89.47
JaTpOi(2)	*Penicillium citrinum*	24.67abc	32.40	41.68bcd	56.02cd	67.07bcd	80.00
CaTpPga	*Metarhizium anisopliae*	26.85abc	30.67	36.80ab	49.20bc	61.73abc	61.60
F-value		3.06*	1.76ns	8.89*	14.16*	14.17*	0.95ns
P-value		0.00	0.06	0.00	0.00	0.00	0.53
HSD value		0.65	1.26	0.79	0.79	0.87	15.17

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

Table 5. Mean of fecal weight produced by *Spodoptera frugiperda* larvae treated with endophytic fungi

Isolates	Species	Larvae fecal weight (mg larvae^{-1} day^{-1}) during 12 days of observation					
		2	4	6	8	10	12
Control	-	15.51efgh	21.64def	23.51bcde	27.25bcd	52.29ef	161.33a
GaTpeOi	*Chaetomium* sp.	5.60ab	8.17a	7.97a	7.92a	8.87a	15.31b
PsgTjPr	*Aspergillus niger*	13.77cdefg	15.18bcd	22.86bcde	22.08bc	29.57bcd	39.07b
JgSpk(1)	*Beauveria bassiana*	8.27abc	9.25ab	8.31a	21.61bc	18.38ab	17.28b
JgTjPr	*Chaetomium* sp.	15.13defgh	17.59cde	20.55bc	20.39bc	20.18abc	18.08b
JaGiP	*Beauveria bassiana*	5.02a	13.74abc	21.90bcd	26.87bcd	28.27bcd	22.19b
PiCrPga	*Chaetomium* sp.	11.27cdef	11.68abc	18.78b	15.04ab	15.18ab	23.23b
JaGiPRB	*Curvularia lunata*	12.18cdef	18.99cde	27.45bcdef	25.71bcd	27.63bcd	28.58b
CmTjP	*Curvularia lunata*	8.27abc	9.03ab	8.07a	21.43bc	17.91ab	17.28b
JaMsBys	*Curvularia lunata*	10.02abcde	18.89cde	20.80bc	26.67bcd	34.90cde	28.35b
JaSpkPGA(2)	*Beauveria bassiana*	13.86cdefg	28.30fgh	36.00efg	41.49def	37.77def	30.42b
JgCrJr	*Beauveria bassiana*	9.02abcd	44.93ki	76.51j	72.99i	55.62ef	33.71b
JaTpOi (1)	*Beauveria bassiana*	21.28ghi	29.55fghi	41.51fgh	50.09efgh	41.76def	31.59b
JaSpkPga(3)	*Curvularia lunata*	33.11j	47.26ki	56.88hij	61.39fghi	54.45ef	41.12b
CaCjPga	*Chaetomium* sp.	15.59defg	25.22efg	34.70def	32.90cde	40.71def	37.80b
JgByu	*Aspergillus niger*	10.34bcde	51.90l	55.52hij	68.82hi	60.08f	41.89b
JaTgSr	*Curvularia lunata*	21.39ghi	37.65hijk	52.15ghi	63.66ghi	46.29def	36.27b
JaBuBys	*Aspergillus niger*	17.60fgh	31.53ghij	40.41fgh	47.81efgh	36.83cde	21.57b
JgPwSr	*Aspergillus flavus*	34.39j	40.89jkl	57.52ij	63.46ghi	55.42ef	38.63b
JaTpOi(2)	*Penicillium citrinum*	29.39ij	39.22ijk	56.79hij	62.68ghi	55.00ef	40.77b
CaTpPga	*Metarhizium anisopliae*	23.05hi	24.72efg	32.17cdef	44.81efg	54.32ef	40.24b
F-value		10.14*	18.04*	15.20*	10.86*	6.25*	16.25*
P-value		0.00	0.00	0.00	0.00	0.00	0.03
HSD value		0.91	0.89	1.21	1.43	1.61	3.04

Note: ns = not significantly different * = significantly different; values within a column followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

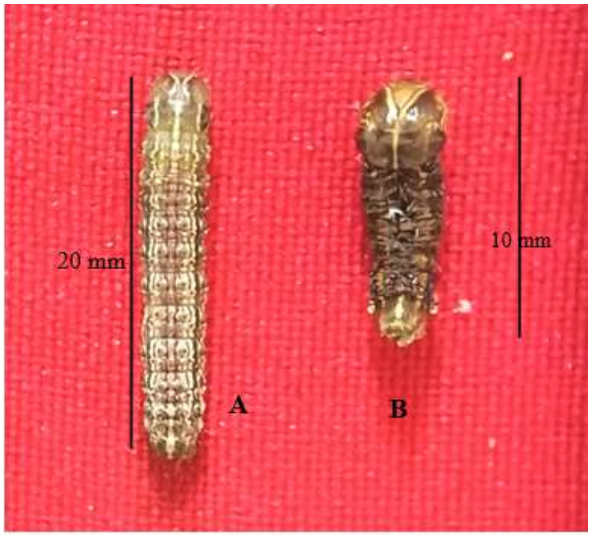

Figure 2. Morphology of *Spodoptera frugiperda* larvae: healthy larvae of control (A) and dead larvae infected by endophytic fungi (B)

Figure 3. Pupal *Spodoptera frugiperda*: healthy pupae of control (A) and malformation (unhealthy) pupae infected by endophytic fungi (B)

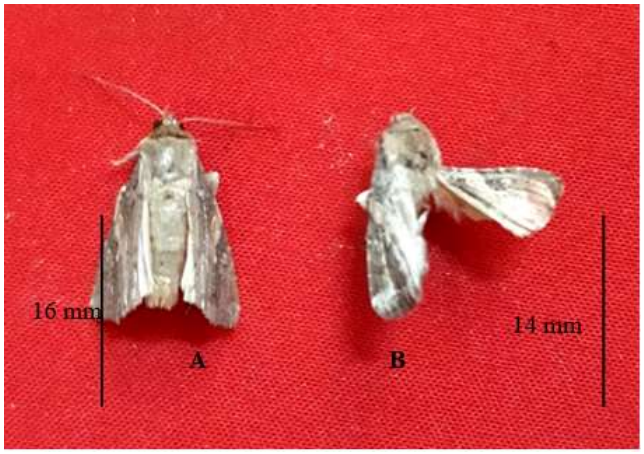

Figure 4. *Spodoptera frugiperda* adults: healthy adults of control (A) and malformation (unhealthy) adults infected by endophytic fungi (B)

Larvae that were sick and died due to eating leaves treated with endophytic fungi showed the typical symptoms. The treated larvae had an abnormal morphology or malformation. The body of sick larvae was shriveled, hard, stiff, dry like a mummy, darker in color and odorless, while untreated larvae had a normal morphology, large size, flexible grip, lighter in color (Figure 2). In addition, endophytic fungi caused the pupae to become shorter and darker, and finally it died, while the control pupae were larger in size, brighter and more vibrant (Figure 3). The abnormal adults produced from the treated larvae had folded wings and were smaller than the normal adults produced from the untreated larvae (Figure 4).

Discussion

The results showed that three species of endophytic fungi, namely *B. bassiana* (JgSPK, JaGiP, JgCrJr, and JaTpOi (1) isolates), *C. lunata* (JaSpkPga(3) isolate), and *M. anisopliae* (CaTpPga isolate) were more pathogenic . They caused higher mortality of FAW larvae. The fungi also decreased thepercentage of pupae and adults emerging, and the percentage of eggs hatched and the number of eggs laid by treated female adults. These results showed that endophytic fungi not only killed the larvae, but also killed pupae and reduced the adult emergence. These fungi also produced abnormal adults of *S. frugiperda*. *B. bassiana* and *M. anisopliae* have been reported to be pathogenic to *S. frugiperda* (Ramos et al. 2020; Herlinda et al. 2021). This is the first report of pathogenicity of *C. lunata* against *S. frugiperda*. *C. lunata* can kill some stored grain insect species, such as *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst.) (Wakil et al. 2014). The present study showed that mortality of larvae was low because the fungal suspension contained only $1x10^6$ conidia mL^{-1}. In addition, fungal strain also affected the mortality of *S. frugiperda* larvae. The commercial strains of *B. bassiana* Bb-18 and *M. anisopliae* Ma-30 at 1×10^8 conidia mL^{-1} applied using the soil drench method could kill 87 and 75% of the fourth larval instars of *S. frugiperda*, respectively (Ramos et al. 2020). For this reason, future research needs to be carried out to increase the pathogenicity of strains/isolates of the endophytic fungi from South Sumatra, Indonesia. Moreover, the ability of endophytic fungi to colonize young maize (seedling) via seed treatment could prevent the maize plant from the attack of hiding *S. frugiperda* larvae in corn midribs (Herlinda et al. 2021). The young maize plant is very susceptible to *S. frugiperda* larvae (Supartha et al. 2021), so the early prevention with seed treatment using the endophytic fungi may increase the maize plant's defense against the FAW larvae (Sari et al. 2022).

The endophytic fungi showed negative effect on the growth of *S. frugiperda*. Endophytic fungi decreased the appetite of larvae, so that the leaf area consumed and fecal weight produced by *S. frugiperda* larvae also decreased. In addition, the body weight of *S. frugiperda* larvae treated with endophytic fungi also declined and finally they died. The endophytic fungus caused growth retardation on *S. frugiperda* (Gustianingtyas et al. 2021) and adverse effects on its survival (Russo et al. 2020) because fungus produce secondary metabolites and toxic protein or toxins (Vidal and Jabe 2015). For example, *B. bassiana* secretes bassiacridin, a protein toxic for insects (Quesada-moraga and Vey 2004) and beauvericin, is toxic for insects (Safavi 2012) and *M. anisopliae* produces destruxin, that is also toxic for insects (Borisade et al. 2016). The mycelia of endophytic fungi within maize tissue consumed by larvae of *S. frugiperda* could produce blastospores in larvae hemolymph (Sari et al. 2022). Then, blastospores produced toxic secondary metabolites and proteins which is toxic for insects (Mancillas-Paredes et al. 2019). The entomopathogenic fungi also secrete secondary metabolites in plants that cause antibiosis, antifeedant or deterrent for *S. frugiperda* larvae (Jaber and Ownley 2018) and raise the concentrations of terpenoid compound against FAW larvae (Russo et al. 2020). After consuming toxic metabolites or protein, the insects died, then the fungi keep growing with the result that their mycelia and spores cover over the cadaver body causing mycosis (Sari et al. 2022). The data obtained showed that mycosis was found only on *S. frugiperda* larvae consuming the fungal-endophytically colonized leaves. However, mycosis was not occurred on control larvae (untreated larvae). *S. frugiperda* larvae fed on plants colonized by endophytic fungi may undergo mycosis (Russo et al. 2020).

These findings highlight the potential of endophytic fungi, such as *B. bassiana*, *M. anisopliae*, and *C. lunata* from South Sumatra to protect young maize plant against *S. frugiperda* by seed treatment.

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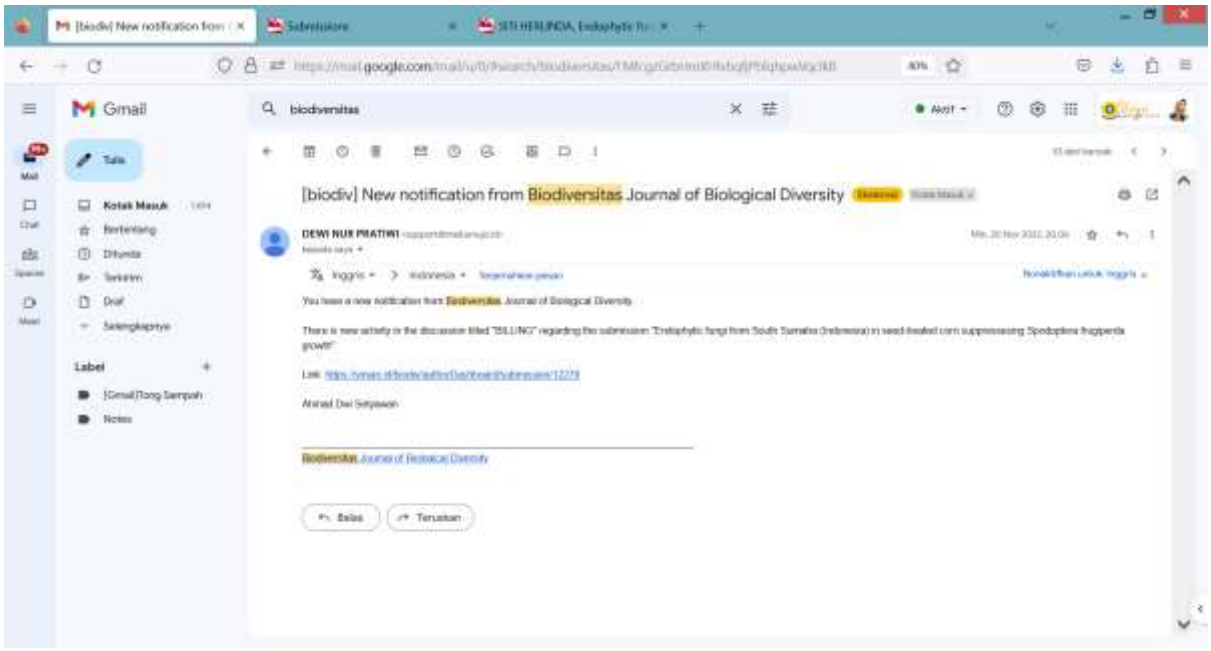

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