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Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the growth of *Spodoptera frugiperda* by seed inoculation

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Abstract. *Sari JMP, Herlinda S, Suwandi S, Elfita. 2023. Effect of* Beauveria bassiana *and* Metarhizium anisopliae *on the growth of* Spodoptera frugiperda *by seed inoculation. Biodiversitas 24: 2350-2357. Spodoptera frugiperda* J.E.Smith 1797 larvae are more difficult to control because they hide in the leaf sheaths. Therefore, it is necessary to use endophytic entomopathogenic fungi to control *S. frugiperda* larva. The aim of this research was to observe the effect of endophytic *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorokīn on the growth of *S. frugiperda* larvae by seed inoculation. A total of six isolates of *B. bassiana* (5 isolates) and *M. anisopliae* (1 isolate) were isolated from infected-host insect corpses and identified molecularly. The corn seeds were immersed using a fungal suspension of 1 x 10^{10} conidia mL^{-1}. The results showed that feeding on maize leaves colonized with *B. bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *M. anisopliae* (WTTJC260521B isolate) significantly reduced the larval and pupal weight. The highest mortality (above 50%) of *S. frugiperda* larvae was recorded by *B. bassiana* (JGTP240521A isolate). The treated larvae could produce abnormal and malformation pupae and adults, increasing mortality. Both fungi killed the larvae and induced disruption of normal growth larvae, pupae, and adults of *S. frugiperda*. *B. bassiana* was more pathogenic than *M. anisopliae* in killing the larvae of *S. frugiperda.* Results revealed that endophytic fungi *B. bassiana* and *M. anisopliae* inoculated through seed treatment had a negative impact on the growth of *S. frugiperda* larvae. Further studies are needed on the effect of *B. bassiana* and *M. anisopliae* by foliar spraying or seed treatment on maize plant to control *S. frugiperda* in the field.

Keywords: Endophytic fungi, entomopathogens, fall armyworm**,** *Spodoptera frugiperda, Zea mays*

INTRODUCTION

Fall armyworm (FAW) or *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera: Noctuidae) is an important insect pest species in the world today because this pest can spread and invade quickly to various locations. This species is also very destructive because it is voracious and has many host plants (polyphagous) (Montezano et al. 2018). It is originated in South America (Otim et al. 2018) and has spread all over the world; in 2016, it was found in Africa (Goergen et al. 2016), 2017 in Europe (Early et al. 2018), and in 2018 it has entered Asia, especially India (Ganiger et al. 2018; Mahat et al. 2021). On 26 March 2019, this pest was reported to be found in West Sumatra (Sartiami et al. 2020). In Indonesia, it can be found in almost all provinces; for example, Bengkulu (Ginting et al. 2020), Lampung (Lestari et al. 2020), and South Sumatra (Herlinda et al. 2020). It has attacked more than 353 species from 76 families of host plants worldwide (Montezano et al. 2018). Pest attacks caused 80-100% damage in East Africa (Ethiopia and Kenya) (Sisay et al. 2019). *Spodoptera frugiperda* eat corn leaves and their attack reaches 100% (Mukkun et al. 2021). In addition, two pest strains were found in Indonesia, namely rice and corn strains (Herlinda et al. 2022b). The financial losses due to this pest attack reached US $ 13 million per year (Harrison et al. 2019). In addition, *S. frugiperda* could damage during the larval stage, and larvae bored the leaves, stems, flowers, fruits, and growing points. (Herlinda et al. 2022b).

The FAW are generally sprayed with synthetic contact insecticides (Kumela et al. 2018; Zhang et al. 2021). However, the negative impact of spraying synthetic insecticides is greater than the benefits of reducing insect-pest populations because it can induce their resistance (Zhang et al. 2021). The insecticides can pollute the environment and cause toxic residues in agricultural products that are hazardous to human health (Harrison et al. 2019). Alternative safer environmental and human health controls include biological controls, such as entomopathogenic fungi (Mantzoukas and Eliopoulos 2020). Furthermore, several species of entomopathogenic fungi could kill *S. frugiperda* larvae by topical spraying, including *Metarhizium* spp. (78% mortality) (Herlinda et al. 2020) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (80% mortality) (Ramanujam et al. 2020).

The larvae were hidden on the leaf surface at sunrise until 9 AM (Herlinda et al. 2022b). This larval behavior caused the topical application of entomopathogenic fungi less effective (Gustianingtyas et al. 2021). In addition, fungal conidia applied to the surface plants are more difficult to survive in extreme weather (sunlight, wind, rain) (Boomsma et al. 2014). Therefore, it is necessary to use entomopathogenic fungi that can comprehensively live within plant tissues, they are the endophytic entomopathogenic fungi (Herlinda et al. 2022a; Sari et al. 2022). The endophytic fungi can colonize plant tissues either intracellularly or intercellularly. That could benefit their host plants (Lira et al. 2020). The fungi could increase plant growth and suppress the growth of insect pests (Russo et al. 2020). Furthermore, the endophytic fungi are protected from the adverse effects of extreme weather because of their position within plant tissue (Lira et al. 2020). Therefore, the endophytic fungi isolated from plants in South Sumatra had 20 isolates, and the fungal bioassay on *S. frugiperda* larvae showed that larval mortality was still low, less than 30% (Herlinda et al. 2021; 2022a). Six isolates of *B. bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokīn isolates from larvae of *S. frugiperda* was confirmed to be endophytic and pathogenic. In addition, other isolates from lepidopteran larvae from South Sumatra highlands were also confirmed to be endophytic and pathogenic (Sari et al. 2022). However, six isolates of the endophytic *B. bassiana* and *M. anisopliae* isolated from *S. frugiperda* and the lepidopteran larvae have never been reported to have a negative impact on the growth of *S. frugiperda* larvae. The aim of this research was to observe the effect of endophytic *B. bassiana* and *M. anisopliae* on the growth of *S. frugiperda* larvae by seed-inoculation.

MATERIALS AND METHODS

Preparation of *B. bassiana* and *M. anisopliae* isolates

A total of six isolates of *B. bassiana* (5 isolates) and *M. anisopliae* (1 isolate) used were isolated from infected-host insect corpses from crops in Tanjung Pering, Ogan Ilir (3°13′23″S104°38′27″E), Tanjung Cermin, Pagar Alam (4°02′23″S103°13″14″E), and Nendagung, Pagar Alam (3°56′22″S103°12′15″E) in South Sumatra in 2021. The isolates were stored in the Laboratory of Entomology, Faculty of Agriculture, Universitas Sriwijaya, Indonesia. Isolates of *B. bassiana* and *M. anisopliae* were identified molecularly and confirmed as endophytic fungi (Sari et al. 2022). The isolates were further deposited in GenBank. *B. bassiana* consists of five isolates (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521), and *M. anisopliae* consists of one isolate (WTTJC260521B).

Mass-rearing of *Spodoptera frugiperda*

Spodoptera frugiperda was mass-reared in the Laboratory of Entomology following the method of Herlinda et al. (2020). They were maintained in the laboratory for more than five generations. The temperature and relative humidity during mass-rearing was 28-29°C and 82–83%, respectively. The lighting was set to photoperiod 12:12 (L:D) hours. The larvae of *S. frugiperda* were kept individual because they had cannibalistic behavior. The larvae were fed fresh maize leaves and replaced daily. The prepupae and pupae emerging were placed in a wire mesh cage (30x30x30 cm^3) containing sterile soil and corn seedling for adults to laying their eggs. The FAW mass-rearing was conducted for over five generations to obtain homogeneous test insects for bioassays.

Assessing the effect of fungi inoculated within seed corn on *Spodoptera frugiperda* growth

Fungal inoculation in corn by seed treatment was conducted following the method of Herlinda et al. (2021). In this experiment, seeds were immersed in a fungal suspension of $1x10^{10}$ conidia mL^{-1}. All the six isolates were first cultured in Sabouraud Dextrose Agar (SDA) medium and incubated for 14 days. Then, SDA fungal cultures were transferred to the Sabouraud Dextrose Broth (SDB), following the method of Gustianingtyas et al. (2020). Forty-five corn seeds for treatment were surface sterilized according to the method of Russo et al. (2020). Seeds were soaked in 10 mL of fungal suspension ($1x10^{10}$ conidia mL^{-1}) for 24 hours, whereas untreated seeds (the control) were immersed in 10 mL of sterilized water. Finally, seeds were grown in the hydroponic medium according to the method of Novianti et al. (2020) and incubated for 14 days.

For bioassay, leaves of 14-day-old maize inoculated with the fungi were provided to 50 first instar neonate (hatching within 24 hours) of *S. frugiperda* larvae, whereas untreated larvae (control) were provided non-inoculated maize leaves. First, neonate larvae were allowed to feed on fungal inoculated and non-inoculated maize (control) for 6 hours. Then, treated and control larvae were individually kept in a porous plastic cup (Ø 6.5 cm, height 4.6 cm). Finally, they were fed on fresh, healthy, non-inoculated leaves (2x5 cm per day per larvae) and replaced daily with a fresh new one. This treatment was repeated three times for each isolate and designed using completely randomized block designs. Variables were recorded every two days.

Data analysis

The differences in the leaf area eaten by larvae, the larval body and fecal weight, the larval body length, the larval head width of each instar, and the pupae weight of each treatment were analyzed by analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) or Tukey's test was applied to determine the significant differences among the treatments (isolates) at p=5%. All the data were calculated using SAS University Edition 2.7 9.4 M5 software. In addition, the data on cumulative larval mortality were presented in a graph.

RESULTS AND DISCUSSION

Growth of *S. frugiperda* treated with *B. bassiana* and *M. anisopliae*

The results showed that *B. bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *M. anisopliae* (WTTJC260521B isolate) were able to colonize maize leaves after inoculated by seed immersion treatment ($1x10^{10}$ conidia mL^{-1}) for 24 hours. Furthermore, fungi could colonize all leaves, and the percentage of leaves colonized by the fungi was 100% at 14 days after inoculation. Therefore, all isolates of *B. bassiana* and *M. anisopliae* used were confirmed as endophytic fungi. However, no fungal mycelia were found within the maize leaves of control and on the last rinsed water.

Leaf area eaten by *S. frugiperda* larvae fed on maize leaves colonized with *B. bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *M. anisopliae* (WTTJC260521B isolate) were significantly lower than those of control (Table 1). The lowest leaf area eaten by the sixth instar larvae was treated with JGNT300521 isolate of *B. bassiana* (17.88 cm^2 $larvae^{-1}$ day^{-1}), which was significantly (p<0.0001) different from other treatments. In comparison, the highest leaf area eaten was recorded by control larvae (7.45 cm^2 $larvae^{-1}$ day^{-1}), which was significantly (p<0.0001) different from all fungal treatments. Thus, the neonate larvae feeding on leaves colonized by *B. bassiana* and *M. anisopliae* could decrease their appetite to consume new fresh leaves.

The body weight of *S. frugiperda* larvae treated with endophytic *B. bassiana* and *M. anisopliae* was significantly lower than that of the control (Table 2). The lowest weight was recorded in sixth instar larvae treated with WTTJC290521B isolate of *B. bassiana* (79.53 mg), which was not significantly different from WTTJC260521A and JGNT300521 isolates. In comparison, the highest weight found in control larvae (270.53 mg) was significantly (p<0.0001) different from all fungal treatments. The fecal weight of *S. frugiperda* larvae treated with *B. bassiana* and *M. anisopliae* was also significantly lower than that of the control (Table 3). The body length of *S. frugiperda* larvae treated with endophytic *B. bassiana* and *M. anisopliae* was significantly (p<0.0001) lower than that of the control, except for the first instar (Table 4). The head width of all instar larvae treated with *B. bassiana* and *M. anisopliae* was significantly (p<0.0001) lower than that of the control (Table 5). Thus, feeding on leaves colonized by *B. bassiana* and *M. anisopliae* could also decrease larval body weight and size.

The pupal weight of *S. frugiperda* of control was the highest (308.67 mg) (p<0.0001) among the fungal treatments (Table 6). The lightest pupae were found on those treated with JGTP240521 isolate of *B. bassiana* (110.67 mg) and were not significantly different from those treated with JGNT300521 isolate (154.67 mg). The pupal length of *S. frugiperda* of the control was also the highest among the fungal treatments. Therefore, feeding on leaves colonized by *B. bassiana* and *M. anisopliae* can reduce the pupal weight of *S. frugiperda*.

Table 1. Leaf area eaten by *Spodoptera frugiperda* larvae fed on maize leaves colonized with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	**Species**	**Mean of leaf-eaten larvae (cm^2 $larvae^{-1}$ day^{-1})**					
		1st larvae	**2nd larvae**	**3rd larvae**	**4th larvae**	**5th larvae**	**6th larvae**
Control	-	1.63a	7.16a	8.28a	12.58a	13.56a	17.88a
JGTP240521A	*Beauveria bassiana*	1.34b	2.29de	2.55c	6.90cd	8.19c	13.95bc
WTTJC260521A	*Beauveria bassiana*	1.37ab	2.85cde	3.06c	5.77d	6.52d	11.00d
WTTJC260521B	*Metarhizium anisopliae*	1.59ab	5.40b	7.33a	8.23b	11.70cd	15.41ab
WTTJC290521A	*Beauveria bassiana*	1.45ab	4.12bc	5.68b	6.23cd	7.95c	10.23d
WTTJC290521B	*Beauveria bassiana*	1.39ab	3.13cd	4.53b	7.04bc	9.15e	12.19cd
JGNT300521	*Beauveria bassiana*	1.35b	1.78e	2.87c	3.16e	5.19e	7.45e
F-value		4.67*	41.26*	71.73*	123.56*	80.63*	45.41*
P-value		0.01	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD value		0.10	0.34	0.28	0.23	0.26	0.36

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

Table 2. Body weight of *Spodoptera frugiperda* larvae fed on maize leaves colonized with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	**Species**	**Mean of larvae body weight (mg)**					
		1st larvae	**2nd larvae**	**3rd larvae**	**4th larvae**	**5th larvae**	**6th larvae**
Control	-	7.03a	11.82a	25.83a	65.17a	115.40a	270.53a
JGTP240521A	*Beauveria bassiana*	5.42ab	7.43b	21.82b	31.29c	61.80b	132.35bc
WTTJC260521A	*Beauveria bassiana*	4.88ab	6.52b	14.26b	16.59ef	49.66b	78.78e
WTTJC260521B	*Metarhizium anisopliae*	4.96ab	6.58b	21.82a	38.75f	92.74a	172.61b
WTTJC290521A	*Beauveria bassiana*	4.77b	7.96b	12.53b	14.68b	28.01c	123.78cd
WTTJC290521B	*Beauveria bassiana*	5.09ab	5.81b	12.96b	22.39d	23.42c	79.53e
JGNT300521	*Beauveria bassiana*	4.58b	5.72b	11.76b	21.05de	34.42c	86.56de
F-value		3.43*	15.5*	32.73*	178.65*	89.52*	46.77*
P-value		0.32	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD value		0.42	0.44	0.55	0.54	1.16	1.99

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

Table 3. Fecal weight of *Spodoptera frugiperda* larvae fed on maize leaves colonized with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	Species	Mean of larva fecal weight (mg day^{-1})					
		1 st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	0.78a	6.71a	17.94a	27.52a	51.48a	61.41a
JGTP240521A	*Beauveria bassiana*	0.54bc	2.44b	10.82d	15.52d	30.84c	33.35c
WTTJC260521A	*Beauveria bassiana*	0.43c	2.35b	9.21d	16.29cd	30.60c	32.38c
WTTJC260521B	*Metarhizium anisopliae*	0.66ab	5.29a	13.60ab	25.27ab	43.62ab	47.88b
WTTJC290521A	*Beauveria bassiana*	0.43c	1.64b	7.30cd	17.56cd	31.75c	35.41c
WTTJC290521B	*Beauveria bassiana*	0.42c	2.44b	10.82bc	20.47bc	34.22bc	35.82c
JGNT300521	*Beauveria bassiana*	0.37c	1.93b	9.21bcd	17.70cd	29.73c	32.50c
F-value		13.06*	13.90*	22.25*	20.94*	17.68*	43.67*
P-value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD value		0.10	0.62	0.72	0.54	0.77	0.60

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

Table 4. Body length of *Spodoptera frugiperda* larvae fed on maize leaves colonized with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	Species	Mean of larva length (mm)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	2.52	6.82a	11.61a	14.99a	24.89a	26.10a
JGTP240521A	*Beauveria bassiana*	2.07	4.84d	10.71b	12.83bc	17.97bc	20.26c
WTTJC260521A	*Beauveria bassiana*	2.55	6.37bc	10.75ab	13.17bc	17.92bc	21.42bc
WTTJC260521B	*Metarhizium anisopliae*	2.47	7.39ab	12.00ab	14.96a	20.52b	23.73ab
WTTJC290521A	*Beauveria bassiana*	2.45	5.51cd	10.66ab	12.58c	16.94bc	19.20c
WTTJC290521B	*Beauveria bassiana*	3.05	7.92ab	11.87ab	13.16bc	17.88bc	19.83c
JGNT300521	*Beauveria bassiana*	2.89	8.13a	12.05a	14.31ab	19.42bc	21.14bc
F-value		1.83ns	14.19*	4.09*	8.25*	21.27*	13.08*
P-value		0.18	<0.0001	0.02	0.0011	<0.0001	0.0001
HSD value		-	0.31	0.36	0.23	0.31	0.35

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

Table 5. Head width of *Spodoptera frugiperda* larvae fed on maize leaves colonized with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	Species	Head width (mm)					
		1st larvae	2nd larvae	3rd larvae	4th larvae	5th larvae	6th larvae
Control	-	1.05	1.31a	1.50a	1.49a	1.67a	1.80a
JGTP240521A	*Beauveria bassiana*	1.01	1.12b	1.21d	1.32cd	1.39b	1.54c
WTTJC260521A	*Beauveria bassiana*	1.00	1.11b	1.21d	1.28d	1.41b	1.54c
WTTJC260521B	*Metarhizium anisopliae*	1.03	1.31a	1.44ab	1.47bc	1.62a	1.65b
WTTJC290521A	*Beauveria bassiana*	1.00	1.15b	1.33bc	1.39bc	1.50b	1.56bc
WTTJC290521B	*Beauveria bassiana*	1.01	1.12b	1.21d	1.32cd	1.45b	1.60bc
JGNT300521	*Beauveria bassiana*	1.01	1.08b	1.24cd	1.30cd	1.41b	1.57bc
F-value		2.45ns	14.27*	27.69*	18.40*	23.97*	27.46*
P-value		0.09	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
HSD value		-	0.12	0.11	0.10	0.11	0.08

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

Table 6. Pupal weight and length of *Spodoptera frugiperda* treated with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

Isolates	Species	Pupal weight (mg)	Pupal length (mm)
Control	-	308.67a	16.00[a]
JGTP240521A	*Beauveria bassiana*	110.67[e]	11.67[c]
WTTJC260521A	*Beauveria bassiana*	237.67[abc]	12.33[bc]
WTTJC260521B	*Metarhizium anisopliae*	254.00[ab]	13.67[b]
WTTJC290521A	*Beauveria bassiana*	178.33[cd]	11.67[e]
WTTJC290521B	*Beauveria bassiana*	199.00[bcd]	11.33[c]
JGNT300521	*Beauveria bassiana*	154.67de	11.33[c]
F-value		23.91*	17.91*
P-value		<0.0001	<0.0001
HSD value		2.36	0.26

Notes: 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

Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* against Spodoptera frugiperda larvae

The instar neonate larvae that consumed maize leaves colonized by endophytic *B. bassiana* and *M. anisopliae* had their appetite and body weight of larvae and pupae decreasing significantly. Two days after application, the fungal-treated larvae movement was slower than that of the control ones. Three days after application, larvae began to die (Figure 1). The cumulative mortality of *S. frugiperda* larvae treated with *B. bassiana* (JGTP240521A isolate) on the last observation day was highest (above 50%). The cumulative larval mortality of other fungal isolate treatments was higher (20-51%) compated to the control. The control mortality was below 10%. In addition, larvae mortality caused by fungal treatments was higher than the control.

The larvae treated with endophytic *B. bassiana* on 4th-day observation (3x24 hours after application) began to die, and larval body color became darker. However, the corpse was not covered with fungal hyphae. The corpse's body began to harden, stiffen, dry, and shrivel on 5th and 6th days; fungal mycelia began to grow and cover the corpse's body. On 7th and 8th day, corpse was completely covered with white mycelia and spores of *B. bassiana* (Figure 2). The larvae treated with endophytic *M. anisopliae* also showed similar symptoms, but the mycelia and spores that covered the corpse's body were whitish green. Re-isolation of the fungal spores from the corpses found that fungal spores and mycelium were similar with the given seed treatment.

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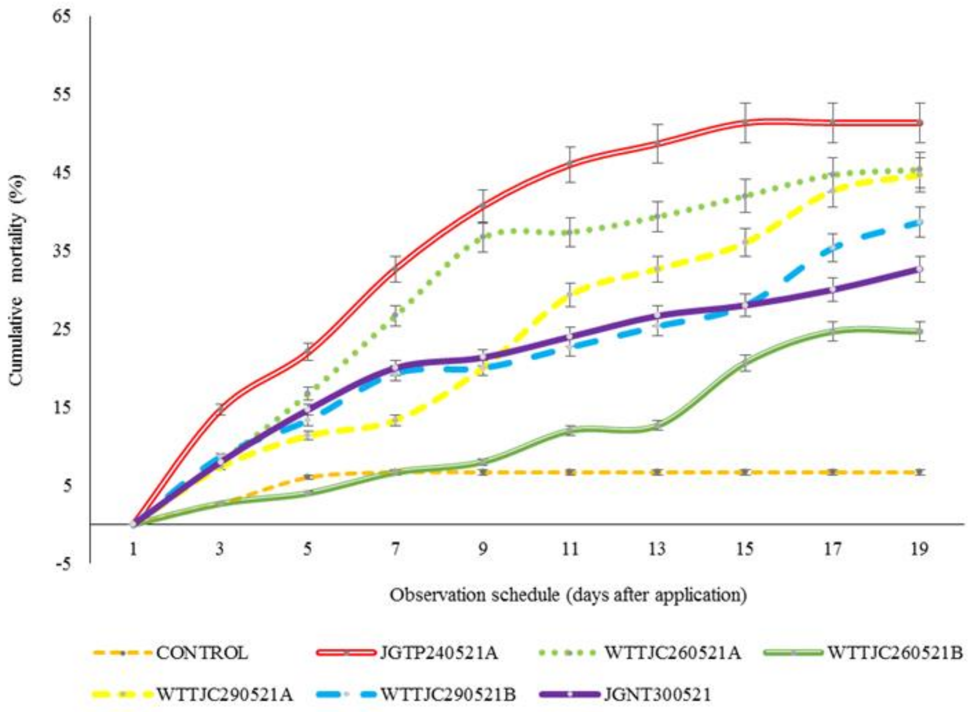

Figure 1. Cumulative larva mortality of *Spodoptera frugiperda* treated with *Beauveria bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *Metarhizium anisopliae* (WTTJC260521B isolate)

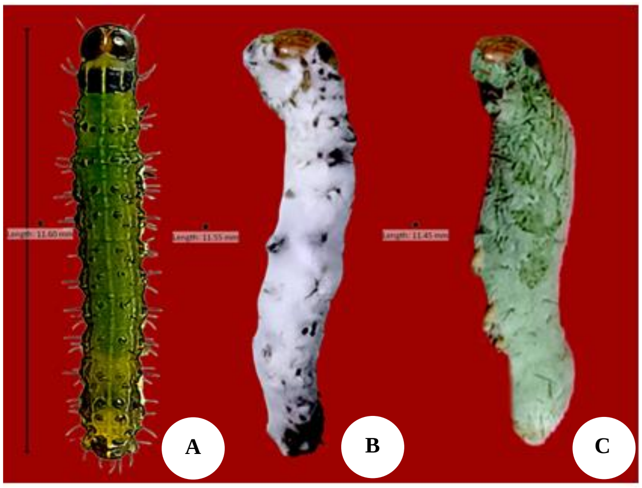

Figure 2. Morphology of healthy larvae (control) (A) and corpses from larvae feeding on leaves colonized by *Beauveria bassiana* (B) and *Metarhizium anisopliae* (C)

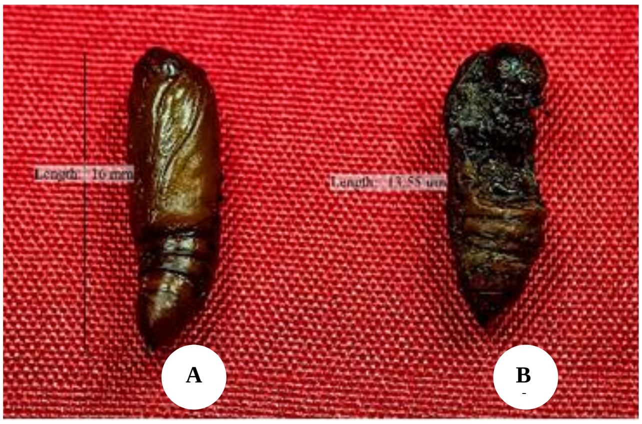

Figure 3. A. Morphology of healthy pupae; B. Abnormal unhealthy pupae

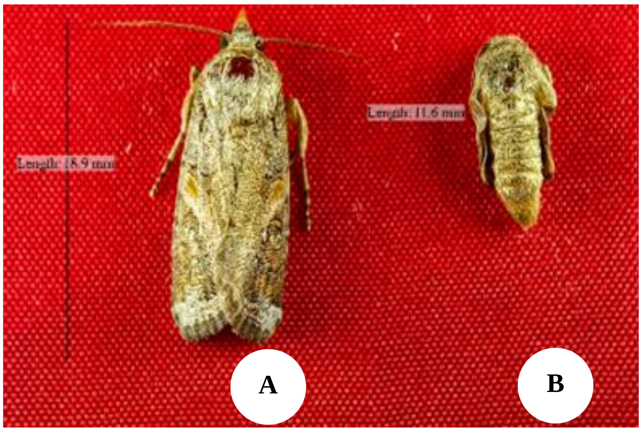

Figure 4. A. Morphology of healthy adults; B. Abnormal, unhealthy adults

The fungal-treated larvae also successfully become pupae, but they were generally small, abnormal, and malformed (Figure 3). Some pupae could successfully become adults. However, the adults were generally small and malformed. In addition, their wings could not stretch (Figure 4), and they died soon. The malformation of pupae and adults resulted in increasing insect mortality; therefore, mortality occur not only in larvae, pupae, or adults but also in all stages.

Discussion

In the present study, neonate larvae feeding on leaves colonized by *B. bassiana* and *M. anisopliae* could decrease their appetite and larval body weight and size. Furthermore, both fungi could reduce the pupal weight of *S. frugiperda* and kill larvae. In three stages, endophytic fungi infect insect hosts (Boomsma et al. 2014); first infection stage is fungal spores, or hypha, that enter orally and infect through the gut epithelium. Then, in the growth stage, it proliferates through hyphal growth. Finally, in the reproduction stage, sexual ascospores of the Hypocreales (*B. bassiana* and *M. anisopliae*) are forcibly discharged from the corpse's body, while the asexual conidia of Hypocreales are passively released (Boomsma et al. 2014).

The results clearly showed that larvae treated with endophytic *B. bassiana* and *M. anisopliae* could slowly kill larvae, pupae, and adults. Three or four days after the neonate larvae were treated with the fungi, some began to die. The fungal spores cause dead larvae or hypha of endophytic fungus to enter orally and infecting through the gut epithelium (Boomsma et al. 2014). Then, the hyphae and spore grow and produce blastospores in the hemolymph; the blastospores could produce secondary metabolites that could kill larvae (Mancillas-Paredes et al. 2019). Then, fungi keep growing saprophytically by absorbing the corpse's body fluids (Gabarty et al. 2014). Moreover, the sexual ascospores and asexual conidia are produced from the corpse's body (Boomsma et al. 2014), and they induce mycosis (Russo et al. 2020). The symptoms of mycosis in corpse's body was hardened, stiffened, dry, shriveled, and covered with mycelium and spores of fungus. The treated live larvae could produce abnormal size and malformation pupae and adults. The adult wings became asymmetrical, and mycosis occurred on the corpse's body. The abnormal pupae and adults resulted in increasing insect mortality. The malformed adults with asymmetrical wings cannot spread their wings and copulate. Therefore, it could contribute to reducing the insect population density in the field. These findings highlighted that endophytic *B. bassiana* and *M. anisopliae* could protect maize plants against *S. frugiperda* by seed treatment.

The mortality of larvae treated with the *B. bassiana* and *M. anisopliae* in the present study was higher (the highest mortality was more than 50%) than in previous studies, which only reached less than 25% (Herlinda et al. 2021; 2022a). *Beauveria bassiana* and *M. anisopliae* isolates caused increase in mortality, isolated from different sources and cultured within different media. However, all the isolates in the present study were confirmed as endophytes. Therefore, this is the new report of *B. bassiana* and *M. anisopliae* isolated from the larval corpse that were confirmed as endophytes. Fungal culture media and isolate sources affect the fungi's pathogenicity against *S. frugiperda*. The ability of *B. bassiana* and *M. anisopliae* to colonize corn leaves by seed treatment could protect the maize plant from *S. frugiperda* larvae hiding within the corn midribs (Herlinda et al. 2021). The endophytic fungi began to protect corn during the seedling period, which was very susceptible to *S. frugiperda* larvae (Supartha et al. 2021). Therefore, early prevention by seed-treated endophytic fungi could increase the maize plant's defense against *S. frugiperda* larvae (Sari et al. 2022).

The present study also found that fungal-treated larvae which left alive resulted in abnormal and malformation pupae and adults. The malformations on pupae and adults also occurred in *S. frugiperda* larvae treated with endophytic *B. bassiana* (Faddilah et al. 2022) and *M. anisopliae* (Lestari et al. 2022) by seed treatment and foliar spraying (Gustianingtyas et al. 2021). Another study examined the adverse effects of *B. bassiana* treatment on the parent generation of *H. armigera* carried over to the next generation (Kalvnadi et al. 2018). Furthermore, eggs exposed to *B. bassiana* and *M. anisopliae* killed eggs directly. They could continue to kill the emerging larvae, pupae, and adults of *Culex quinquefasciatus* Say 1823 (Ramayanti et al. 2022) and *Aedes aegypti* Linnaeus 1762 (Ramayanti et al. 2023).

In conclusion, the finding revealed that feeding on maize leaves colonized with *B. bassiana* (JGTP240521A, WTTJC260521A, WTTJC290521A, WTTJC290521B, and JGNT300521 isolates) and *M. anisopliae* (WTTJC260521B isolate) significantly reduced the larval and pupal weight. Furthermore, both fungi killed the larvae (20-51% of mortality) and also induced disruption of normal growth larvae, pupae, and adults of *S. frugiperda*. However, *B. bassiana* was more pathogenic than *M. anisopliae* in killing the larvae of *S. frugiperda*. Therefore, endophytic *B. bassiana* and *M. anisopliae* inoculated through seed treatment had a negative impact on the growth of *S. frugiperda* larvae. Further studies are needed to observe the effect of *B. bassiana* and *M. anisopliae* by foliar spraying or seed treatment to the maize plant for controlling *S. frugiperda* in the field.

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