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Population and attacks of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) on corn inoculated with endophytic entomopathogenic fungi from South Sumatra, Indonesia

Populasi dan serangan Spodoptera frugiperda J.E. Smith (*Lepidoptera: Noctuidae*) *pada jagung yang diinokulasikan jamur endofit entomopatogen asal Sumatera Selatan, Indonesia*

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ABSTRAK

Kerusakan akibat serangan *Spodoptera frugiperda* pada jagung di Sumatera Selatan mencapai 100%. Pengendalian hayati *S. frugiperda* dengan menggunakan jamur entomopatogen telah banyak dilakukan. Penelitian ini bertujuan untuk menentukan populasi telur dan larva dan insidensi dan tingkat keparahan serangan oleh *S. frugiperda* pada jagung yang diinokulasi dengan jamur endofit entomopatogen. Tiga isolat jamur yang digunakan dalam penelitian ini terdiri atas *Beauveria bassiana* (isolat JgSPK), *Penicillium citrinum* (isolat JaTpOi (2)) dan *Metarhizium anisopliae* (isolat CaTpPga). Semua isolat jamur endofit entomopatogen secara signifikan dapat menurunkan insidensi dan tingkat keparahan serangan yang disebabkan oleh larva *S. frugiperda*. Telur dan larva, kejadian dan tingkat keparahan serangan *S. frugiferda* diamati. Benih jagung yang diberi perlakuan dengan jamur endofit entomopatogen tidak mempengaruhi populasi telur dan larva *S. frugiferda*. Fluktuasi populasi telur dan larva cenderung dipengaruhi oleh umur tanaman jagung. Jamur endofit entomopatogen berpotensi dikembangkan sebagai alternatif pengendalian hayati *S. frugiferda* pada jagung

Kata Kunci: *Beauveria bassiana*, *Penicillium citrinum*, *Metarhizium anisopliae*, ulat grayak jagung, *Zea mays*

ABSTRACT

Spodoptera frugiperda damage on corn in South Sumatra reached 100%. Biological control of *S. frugiperda* using entomopathogenic fungi has been widely practiced. This study aimed to determine *S. frugiperda* larval abundance and infestation in maize inoculated with endophytic entomopathogenic fungi. The three fungal isolates used in the current study consisted of *Beauveria bassiana* (JgSPK isolate), *Penicillium citrinum* (JaTpOi (2) isolate) and *Metarhizium anisopliae* (CaTpPga isolate). All endophytic entomopathogenic fungal isolates could siginifcantly decrease the incidence and severity caused by *S. frugiperda* larvae. Egg and larval, incidence and severity of *S. frugiferda* infestation were observed. Corn seeds treated with endophytic entomopathogenic fungi did not affect the population of eggs and larvae. Fluctuations of the egg and larval population tended to be influenced by corn age. Entomopathogenic endophytic fungi could potentially be developed as an alternative to biological control of S. frugiferda in corn.

Keywords: *Beauveria bassiana*, fall armyworm, *Penicillium citrinum*, *Metarhizium anisopliae*, *Zea mays*

INTRODUCTION

Corn (*Zea mays* L.) in Indonesia is currently experiencing a significant decline in production due to insect pests, namely *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) atau fall armyworm (FAW) (Mahat et al., 2021). In March 2019, this pest was found attacking corn in West Sumatra (Sartiami et al., 2020) and now the FAW has spread to other provinces and islands in Indonesia, such as South Sumatra (Hutasoit et al., 2020). According to Herlinda et al. (2022a) *S. frugiperda* damage in South Sumatra reached 100%. In addition, damage to corn in Lampung ranges from 26.50-70% (Lestari et al., 2020). *S. frugiperda* is very dangerous and can reduce corn production because this pest attacks the growing point, leaves, stems, flowers and even fruit. (Herlinda et al., 2021b) and is polyphagous with 353 host plant species from 76 families (Montezano et al., 2018), and reported in South Sumatra to have 28 species of host plants (Herlinda et al., 2022b).

Biological control of *S. frugiperda* using entomopathogenic fungi has been widely practiced. Research conducted by Ramanujam et al. (2020), *Beauveria bassiana* was applied topically and caused 80% mortality of *S. frugiperda* larvae. In addition, in the present study Ramos et al. (2020), reported that *Metarhizium anisopliae* was able to kill 75% of the larvae. However, *S. frugiperda* larvae have the behavior of coming out on the leaf surface in the morning and hiding in the midrib of corn during the day to night (Herlinda et al., 2021a). This behavior may cause topical control using entomopathogenic fungi to be less effective in suppressing *S. frugiperda* infestation in the field. (Sari et al., 2022). The application of entomopathogenic fungi that are endophytic (within plant tissues) is a better alternative in suppressing populations and reducing the infestation of *S. frugiperda* larvae. The endophytic fungus *B. bassiana* can be inoculated into plants by seed treatment, sprayed on the leaves and watered on the roots to colonize the leaves, stems, or roots of plants (Bamisile et al., 2018). In the seed treatment, colonization of endophytic fungi occurred 7 to 14 days after inoculation and could colonize 80-100% of young leaf tissue (Faddilah et al., 2022). Endophytic fungi can survive within plant tissues for more than 14 days after inoculation on corn leaves, stems, and roots (Russo et al., 2021). *S. frugiperda* is infected by endophytic fungus through digestion when they are eaten. This results in decreased appetite, weight loss, stiffness, shrinking, shriveling, and stiffening like a mummy. Eventually, the body turns black but does not smell (Gustianingtyas et al., 2021). Furthermore, abnormal or malformed pupae and adults can also be caused by endophytic fungus (Herlinda et al., 2022a). Efficacy testing of endophytic fungi against *S. frugiperda* larvae was conducted in the laboratory (Faddilah et al., 2022; Herlinda et al., 2022a; Lestari et al., 2022). Endophytic fungi not only inhibit insect pests but also promote plant growth (Liu et al., 2022). There is currently limited information available regarding the impact of applying endophytic entomopathogenic fungi (EPF) from South Sumatra on the *S. frugiperda* population and attack in corn. Thus, the purpose of this study was to determine the population and infestation of *S. frugiperda* larvae in maize inoculated with endophytic entomopathogenic fungi.

MATERIALS AND METHODS

Preparation of Fungi

This research was conducted from August to November 2023 in corn field and Entomology Laboratory, Department of Plant Protection, Faculty of Agriculture, Sriwijaya University. Endophytic fungal species have been identified molecularly. Bioassays were conducted in a room with a temperature of 25 °C and 90% relative humidity. This study used a Randomized Block Design with one control and three seed treatments repeated four times.

Reproduction of Entomopathogenic Endophytic Fungus Isolates

The endophytic EPF used in this study was a collection of the Entomology Laboratory of Sriwijaya University which was explored in 2020 by Gustianingtyas et al. (2021). The three isolates consisted of one species of *Beauveria bassiana* (isolate code: JgSPK), one species of *Penicillium citrinum* (isolate code: JaTpOi (2)) and one

species of *Metarhizium anisopliae* (isolate code: CaTpPga) (Table 1). The fungal species were obtained from South Sumatra with corn and red chili plants. The fungi were recultured following the method of Sari et al. (2022). The fungi were cultured aseptically onto Sabouraud Dextrose Agar (SDA) medium, then they were recultured onto Sabouraud Dextrose Broth (SDB).

Corn inoculated with Fungi

Inoculation of endophytic fungi into plant tissues was carried out through seed inoculation or seed treatment. Before inoculation, the seeds were surface sterilized using the method of Elfita et al. (2019) which has been modified, namely by submerging the seeds in 70% ethanol for 2 minutes then dipped in 1% sodium hypochlorite for 2 minutes and finally rinsed with sterile distilled water 3 times for 1 minute each. The fungal cultures from SDB medium were incubated for 14 days and harvested for seed treatments. Maize seeds were soaked in a fungal suspension of 1×10^{10} conidia/mL for 1 × 24 hours. (Gustianingtyas et al., 2021; Russo et al., 2021), then dried on sterile tissue paper under aseptic conditions in laminar air flow for ± 15 minutes. For the control, the seeds were only soaked with sterile water for 1 × 24 hours. Then, the seeds were planted in the corn field.

Preparation of Corn Field

Fungus-treated corn seeds and control were planted following a spacing of 50 x 30 cm with each hole containing 2 seeds. Maize maintenance was carried out by weeding, watering, and fertilizing the soil. Weeding was done before the seeds were planted and twice a week when the seeds were planted. Watering was conducted twice a day, in the morning at 6 a.m. and in the afternoon at 5 p.m. Fertilization was conducted once a week to prevent the corn stalks from falling down.

Observation of *Spodoptera frugiperda* Population and Attacks

The scouting system was used to calculate incidence of damage or the percentage of infested corn and to estimate the attack intensity or severity caused by *S. frugiperda* larvae (Kuate et al., 2019). The corn fields were scouted using a "W" pattern approach and the total sample observed was 30 plants (10 consecutive plants at three different spots along the "W" transect), this treatment was replicated four times. The degree of wounds, tattering, dead hearts, pinholes, and other damage to the plants varied. A rating system for grading the severity of damage to whorl-stage plants was used to determine the attack intensity or severity (Kuate et al., 2019).

According to Kuate et al. (2019), the percentage of severity was determined by dividing the sum of scores (apart from score 1) by the number of damaged plants. The percentage of infested plants, also known as an incidence, was determined by dividing the total number of infested plants by the total number of plants observed and multiplying by 100%.

The following was carried out using the visual damage severity rating scale, which was scored from 1 to 5. According to Kuate et al. (2019), there were five possible outcomes: 1) no destruction; 2) 1−10% damage to the leaves or only the destruction of the leaf cuticle; 3) 11−25% leaf destruction with the presence of chewed areas > 5 mm, funnel leaves healthy; 4) 26−50% leaf destruction with the presence of chewed areas > 1 cm, the funnel less severely harmed; and 5) > 50% leaf destruction, plant stunting and highly damaged funnel (Figure 1).

Data Analysis

Differences in data on egg and larval population, incidence and severity of *S. frugiferda* infestation were analyzed using ANOVA (analysis of variance). A difference of means were compared using Tukey's honestly significant test (HSD).

Table 1. Isolates of endophytic entomopathogenic fungi from South Sumatra, Indonesia used in this research

Isolate origin	Fungal species	Isolate code	GenBank Acc. No
Maize	*Beauveria bassiana*	JgSPK	MZ356494
Maize	*Penicillium citrinum*	JaTpOi (2)	MZ359812
Red chilies	*Metarhizium anisopliae*	CaTpPga	MZ242073

Source: Herlinda et al. (2021a)

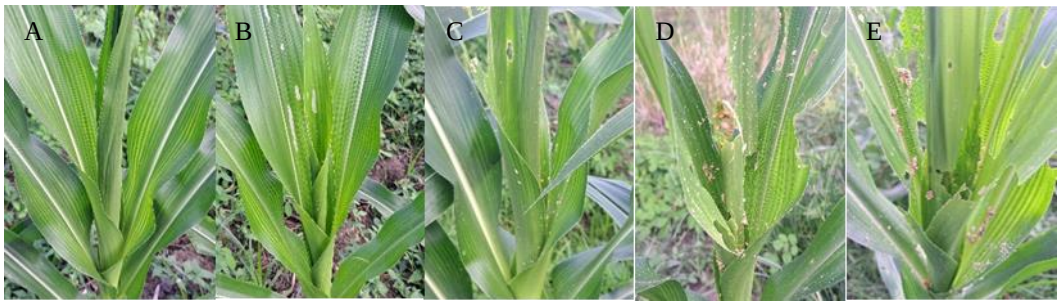

Figure 1. Symptoms damage by *Spodoptera frugiperda* larvae in corn plant: no damage (A), 1−10% leaf damage (B), 11−25% leaf damage (C), 26−50% leaf damage (D), more than 50% leaf damage (E)

RESULTS

Damage by *Spodoptera frugiperda* on Corn Field

The current research was conducted at corn field for a season. Mean incidence (% of infested plant) by *Spodoptera frugiperda* during a season of corn were not significantly different (P >0.05) (Table 1). However, the severity caused by *S. frugiperda* larvae treated with the endophytic EPF were significantly different from control (untreated with fungal) (P < 0.001) (Table 2 and 3). The highest severity occorred when the corn was 56 day old, and the the severity decreased slowly. All isolates of endophytic EPF could siginifcantly decrease the incidence and severity caused by *S. frugiperda* larvae.

The female adults laid the egg mass on the top or below leaf surface (Figure 2). The current study found that whorl and leaves of corn were attacked by the larvae. The larvae's assault symptoms displayed recognizable traits, such as holes they made in the leaves and transparent bite marks on the leaves. There was dark, sawdust-like larval frass on the stems or leaves. Because the leaf rolls formed the holes, the larvae could even attack young leaves that were still curled up. Additionally, the larvae damaged the funnel and punctured the cobs, flower, and stalks of corn. The larval attack was harmless during the generative stage and intense during the vegetative stage.

Population of *Spodoptera frugiperda*

Eggs of *S. frugiperda* have been detected in corn ranging in age from seven days to seventy-one days. Egg population of *S. frugiperda* was generally not influenced by the application of the fungus to corn but was more influenced by the age of the corn. The egg population reached its peak when the corn was 42 days old (Table 4). Larval population of *S. frugiperda* was found starting from seven day-old corn up to 70 day-old corn. Furthermore, the larval population of *S. frugiperda* was also not influenced by the application of the fungus to corn but was more influenced by the age of the corn. When the corn was 49 days old, the larval population peaked (Table 5).

Table 1. Mean incidence (% of infested plant) by *Spodoptera frugiperda* larvae during a season of corn

Fungal isolates	Mean incidence (% of infested plant)									
	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days
Control	27.50	54.17	71.67	80.83	90.00	92.50	94.17	94.17	94.17	94.17
JgSPK	20.00	43.33	68.33	80.00	90.83	90.83	92.50	93.33	93.33	95.00
JaTpOi (2)	26.67	36.67	67.50	86.67	92.50	92.50	96.67	96.67	96.67	96.67
CaTpPga	25.00	45.83	62.50	66.67	81.67	85.00	85.83	86.67	88.33	89.17
F-value	0.73ns	1.19ns	1.5ns	1.93ns	1.83ns	0.83ns	1.48ns	1.67ns	1.33ns	1.27ns
p-value	0.56	0.35	0.27	0.18	0.2	0.5	0.27	0.23	0.31	0.33
HSD value	-	-	-	-	-	-	-	-	-	-

ns = not significantly different from each other at (alpha 0.05) based on Tukey's test (HSD)

Table 2. Mean severity by *Spodoptera frugiperda* larvae on 7 days up to 35 day-old corn

Fungal isolates	Mean severity by *Spodoptera frugiperda* (%)				
	7 days	14 days	21 days	28 days	35 days
Control	7.58a	19.85a	26.64a	32.82ab	52.06a
JgSPK	5.62c	16.62b	19.18b	36.72a	49.69b
JaTpOi (2)	7.43ab	11.67c	23.99a	33.68ab	53.41a
CaTpPga	6.72b	20.42a	22.11ab	29.75b	46.05c
F-value	25.92*	66.82*	8.05*	6.63*	43.23*
p-value	1.58×10^{-5}	9.29×10^{-8}	3.20×10^{-3}	6.80×10^{-3}	1.04×10^{-6}
HSD value	0.86	1.62	3.21	2.85	1.18

* = significantly different; data labeled by the different letters in a column were significantly at (alpha 0.05) based on Tukey's test (HSD)

Table 3. Mean severity by *Spodoptera frugiperda* larvae on 42 days up to 70 day-old corn

Fungal isolates	Mean severity by *Spodoptera frugiperda* (%)				
	42 days	49 days	56 days	63 days	70 days
Control	57.08a	59.67a	61.59a	54.10b	53.41a
JgSPK	54.13b	54.82bc	61.01ab	53.88b	51.67ab
JaTpOi (2)	56.90a	58.03ab	60.76ab	58.10b	50.15b
CaTpPga	51.05c	53.17c	55.84b	54.27b	49.58b
F-value	21.03*	10.43*	4.47*	10.15*	6.51*
p-value	4.54×10^{-5}	1.20×10^{-3}	3.00×10^{-2}	1.30×10^{-3}	7.20×10^{-3}
HSD value	1.49	2.23	3.08	1.53	1.62

* = significantly different; data labeled by the different letters in a column were significantly at (alpha 0.05) based on Tukey's test (HSD)

Table 4. Egg population of *Spodoptera frugiperda* during a season of corn

Fungal isolates	Egg population of *Spodoptera frugiperda* (eggs/30 plants)									
	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days
Control	246.25	327.00a	446.25	186.50	166.75ab	208.25	179.50	98.25	267.50a	26.75
JgSPK	146.25	120.75ab	266.75	135.00	94.75b	190.75	112.75	47.75	79.50b	38.25
JaTpOi (2)	140.00	206.25ab	348.50	191.50	208.50a	203.25	150.00	62.75	143.00ab	13.00
CaTpPga	170.25	83.50b	344.50	198.25	148.50ab	169.50	170.75	59.75	83.50b	25.50
F-value	0.14ns	4.44*	0.77ns	0.61ns	5.59*	0.41ns	1.30ns	0.59ns	6.73*	2.6ns
p-value	0.94	2.50×10^{-2}	0.53	0.62	1.20×10^{-2}	0.75	0.32	0.64	6.40×10^{-3}	0.1
HSD value	-	0.61	-	-	0.24	-	-	-	0.44	-

ns = not significantly different; * = significantly different; data labeled by the different letters in a column were significantly different at (alpha 0.05) based on Tukey's test (HSD)

Table 5. Larval population of *Spodoptera frugiperda* during a season of corn

Fungal isolates	Larval population of *Spodoptera frugiperda* (larvae/30 plants)									
	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days
Control	6.25	8.25a	5.50	6.50	13.75	14.50	16.50	4.25	4.50	2.00
JgSPK	3.50	8.00a	6.25	8.75	10.50	15.50	17.25	4.75	3.25	2.50
JaTpOi (2)	5.00	14.25b	5.25	8.75	12.00	15.00	18.50	4.00	3.50	3.50
CaTpPga	4.75	8.25a	5.25	8.25	9.50	14.50	17.00	3.75	3.25	1.75
F-value	0.65ns	3.59*	0.13ns	0.73ns	3.25ns	0.47ns	0.2ns	0.13ns	0.41ns	0.82ns
p-value	0.6	0.04	0.94	0.56	0.06	0.71	0.89	0.94	0.75	0.51
HSD value	-	1.02	-	-	-	-	-	-	-	-

ns = not significantly different; * = significantly different; data labeled by the different letters in a column were significantly different at (alpha 0.05) based on Tukey's test (HSD)

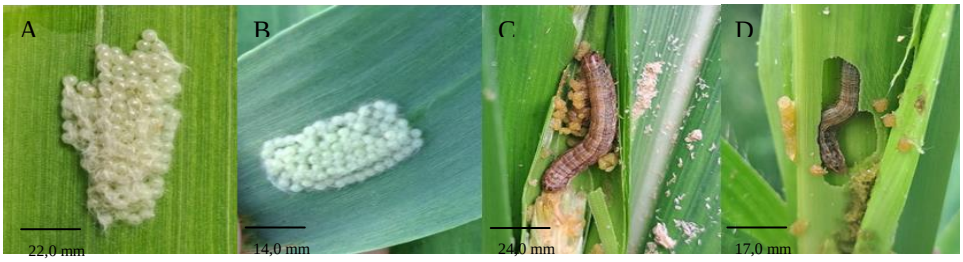

Figure 2. *Spodoptera frugiperda* eggs and larvae in corn plant: egg mass on the top leaf surface (A), egg mass on the below leaf surface (B), larvae feeding on leaves (C), larvae feeding on leaf whorl (D)

DISCUSSION

Seed treatment using endophytic EPF before planting affected the severity caused by *S. frugiperda* larvae in corn fields. However, the incidence (% of infested plant) by *Spodoptera frugiperda* almost found on the corn. The severity caused by *S. frugiperda* larvae was higher on vegetatif stage and gradually declined on generative stage. Every isolate of endophytic EPF has the potential to significantly reduce the frequency and intensity of *S. frugiperda* larval infections. Corn seeds treated with the endophytic EPF significantly increase mortality *S. frugiperda* larvae in the laboratory Sari et al. (2022). The fungi could cause the larvae's digestive organs to deteriorate and malfunction (Mondal et al., 2016). Corn seeds treated with endophytic EPF did not show a tendency to decrease or increase the population of eggs and larvae, but the tendency was more due to the influence of corn age. The trend of population data is similar to previous research by Herlinda et al. (2022a)

Larvae found in fields attack leaves and whorls with symptoms of larval infestation characterized by larval bite holes in the leaves and leaves with transparent bite marks. Young leaves that are still rolling can also be attacked by larvae due to hollow leaf rolls. Larvae also perforate corn stalks, flowers and cobs. The symptoms found in this study are similar to the observations made in this study Herlinda et al. (2022a).

CONCLUSSION

All isolates of endophytic EPF could siginifcantly decrease the incidence and severity caused by *S. frugiperda* larvae. The egg and larval population of *S. frugiperda* was also not influenced by the application of the fungus to corn but was more influenced by the age of the corn.

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