INCIDENCE OF *SPODOPTERA FRUGIPERDA* J.E SMITH AND ENTOMOPATHOGENIC FUNGI IN CORN PLANTS IN THREE DISTRICTS OF BENGKULU PROVINCE (REJANG LEBONG, KEPAHIANG, AND BENGKULU UTARA)

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Abstract

Spodoptera frugiperda is an important pest that attacks corn plants and causes economic losses. This study aims to evaluate the incidence of attacks S. frugiperda and entomopathogenic fungi on corn plants in three districts in Bengkulu (Rejang Lebong, Kepahiang, and Bengkulu Utara). Observations of sample plants were carried out using purposive sampling. The total sample of corn plants at each research location was 30 plants with an area of 500 m^2 for each corn plant. Observations of corn plant pests were carried out on the plants 30 days after planting. The highest incidence of S. frugiperda occurred in Rejang Lebong district (Talang Rimbo 4 was 75.83%), then Kepahiang, and the lowest attack was in North Bengkulu (Tanjung Agung: 31.67%). The highest incidence of entomopathogenic fungi-infected larvae in the field was also found in Talang Rimbo 4 was 33.3%. The identification results showed that the entomopathogenic fungus found in the field was Metarhizium sp. and laboratory testing on S. frugiperda larvae was able to resulted in mortality was 95% at a spore density of 10^7 conidia/ml.

Keywords: Corn, Entomopathogenic fungi, Mortality, Spodoptera frugiperda, Pests

1. INTRODUCTION

Corn is one of the important commodities in Indonesia because it is the staple food of some of the population in Madura and East Nusa Tenggara, besides corn is also used as feed for the poultry industry, and corn flour (Center for Socio-Economic and Agricultural Policy, 2014). Global corn production continues to increase along with the increasing need for corn in the future. Corn plant productivity in Indonesia is still relatively low at only 5.33 tons/ha, when compared to corn plant productivity in the world (FAO, 2020).

One of the obstacles to increasing corn plant productivity is pest attacks. Pests that usually attack corn plants include earthworms (*Agrotis ipsilon*), seed flies (*Atherigona* sp.), stem borers (*Ostrinia furnacalis*), cob borers (*Helicoverpa armigera*), armyworms (*Spodoptera litura*), aphids (*Aphis* sp.), and grasshoppers (*Locusta* sp.) (Subandi, 2004). Pests found in recent years was *S. frugiperda* (Herlinda et al., 2022, Ginting et al., 2020). Corn crop yield losses due to *S. frugiperda* attacks are 4 to 8 million tons per year with nominal losses of between US$ 1 - 4.6 million per year in African countries. Infestation of *S. frugiperda* larvae on corn plants when young leaves are still rolled up causes yield losses of 15-73%, with the incidence of attacked plants reaching 55-100% (Nonci, 2019).Controlling plant pests is very important for success in corn farming, farmers are generally still fixated on the use of chemical pesticides that hurt the environment. Entomopathogenic fungi have the potential to control pests. These fungi can attack larvae and imago from various orders of Coleoptera, Lepidoptera, Homoptera, Hemiptera and Isoptera (Sallam, et al., 2007). However, in Bengkulu province, farmers are not yet widely aware of the existence of *S. frugiperda*, therefore it is necessary to obtain information about the incidence of S. frugiperda on corn plants in various regions in

Bengkulu. Based on this, this study needs to be conducted. This study aims to evaluate the incidence of *S. frugiperda* and *Metarhizium* sp. attacks on corn plants in three districts in Bengkulu (Rejang Lebong, Kepahiang, and Bengkulu Utara).

2. MATERIALS AND METHODS

This research was conducted in Rejang Lebong, Kepahiang, and Bengkulu Utara, and the Plant Protection Laboratory, Department of Plant Protection, Faculty of Agriculture, University of Bengkulu. This research was conducted from March to August 2022.

2.1. Survei Insidensi *Spodoptera frugiperda*

The survey was conducted on corn plants that were 30 days old after planting, during the survey the incidence of *S. frugiperda* attacks and entomopathogenic fungi that infect *S. frugiperda* larvae in the field was observed, observations of sample plants were carried out by purposive sampling. The total sample of corn plants in each research location was 30 plants with an area of 500 m^2 for each plant. Observations of the incidence of attacks were carried out by calculating the intensity of attacks carried out by observing the symptoms of damage to plants. The scale of damage to plants is determined based on scoring. The intensity of attacks is calculated using the formula:

$$IS = \frac{\sum(ni \times vi)}{NxZ} \times 100\ \%$$

Description:
IS = intensity of pest attack
ni = number of leaves attacked with scale i
vi = value of scale damage i
Z = highest scale of damage
N = total number of leaves observed

Scale of Damage

Scoring	Description
0	No damaged plant parts
1	Damaged plants $\leq$ 0-25%
2	Damaged plants $\geq$ 25-50%
3	Damaged plants $\geq$ 50-75%
4	Damaged plants $\geq$ 75% -100%

2.2. Identification of Entomopathogenic Fungi

The larvae infected by entomopathogenic fungi in the field were taken to the laboratory for isolation. The infected larvae were sterilized first, then the conidia or mycelium were inoculated on Sabouraud Maltose Agar yeast (SMAY) media and incubated at 25oC for 14 days. The pure cultures obtained were morphologically identified using an Olympus CX-21 microscope at 40x magnification, morphological observations refer to Lubilosa (2005). The pure cultures obtained were used as a source of inoculum for further propagation and testing.Uji Patogenisitas Cendawan Entomopatogen terhadap Larva *Spodoptera frugiperda.*

The test was conducted using a completely randomized design with the treatment of *Metarhizium* sp. conidia suspension at a concentration of 10^3, 10^4, 10^5, 10^6, 10^7 conidia/ml and control (sterile water). The pathogenicity test of entomopathogenic fungi was carried out referring to the method of Dutta et al., (2014). The third instar larvae of *S. frugiperda* maintained in the laboratory were applied with *Metarhizium* sp. conidia by spraying *S. frugiperda* larvae containing conidia suspension according to the treatment. Each treatment was repeated four times, each repetition consisting of 10 larvae. Observations of the mortality of the test larvae were carried out every day starting from the first day after application until four weeks. Observation variables include: time of larval death, percentage of *S. frugiperda* larval mortality, percentage of pupa and imago formation, percentage of deformed pupa and imago.

2.3. Data Analysis

Larval mortality data were analyzed using Anova, and if there were differences between treatments, further testing was carried out using BNT at 5% level, the effect of treatment application on pupa and imago formation was carried out using Chi-Square analysis.

3. RESULTS AND DISCUSSION

3.1. Incidence of *Spodoptera frugiperda*

Spodoptera frugiperda attacks were found in all research locations, marked by symptoms of pest attacks, the presence of larval movement marks, and the presence of coarse powder resembling sawdust on the leaf surface, or around the tops of corn plants. Larvae also attack plant growth points. Larvae are cannibalistic so that 1-2 larvae are found on one corn plant (Figure 1), this cannibalistic behavior occurs in instars 2 and 3. Advanced instar larvae can cause severe damage which often only leaves the leaf veins and stems of corn plants (Nonci, et al., 2019).

Attack intensity

The results showed that the highest attack intensity was in the Rejang Lebong Regency area (Talang Rimbo 4) on the Bisi 18 variety reaching 75.83%, then kepahiang and the lowest attack in Bengkulu Utara (Table 1.)

Table 1. The intensity of *Spodoptera frugiperda* attacks on various varieties at the observation location.

Districts	Location	Varieties	Intensity (%)	Altitude (m asl)
Rejang Lebong	Sumber Bening	Bonanza	31.6	10
	Curup Selatan	Bonanza	50	0
	Sambil Rejo	Bonanza	41.67	6.6
	Talang Rimbo 1	Bonanza	54.17	20
	Talang Rimbo 2	Pioner 27	60	16.6
	Talang Rimbo 3	Bisi 18	61.67	23.3
	Talang Rimbo 4	Bisi 18	75.83	33.3
	Sumber Urib	Pioner 18	41.67	0
Kepahiang	Merigi	Pioner 18	42.50	0
	Tabat Manok	Pioner 18	55	0
	Kabawetan	Pioner 18	31.67	0
Bengkulu Utara	Tanjung Agung 1	Pioner 27	35.83	0
	Tanjung Agung 1	Pioner 18	31.67	16.6

The high intensity of attacks that occurred in Rejang Lebong was because corn planting was not carried out simultaneously so there was a source of imago invasion originating from corn plantations around the research location. Supartha et al. (2021a) reported that the source of moth invasion could come from host plants around the planting location. The intensity of *S. frugiperda* attacks on corn plants was also greatly supported by the availability of host plants as a food source. Lepidoptera larvae prefer young plants to older plants (Speight et al., 2008), so that *S. frugiperda* attacks in the vegetative phase cause more severe damage in the field (Trisyono et al., 2019). In addition to food availability factors, altitude and temperature also affect the physiology, abundance, and distribution of insects (Shi et al., 2011).

The intensity of *S. frugiperda* attacks was generally related to its population level, the higher the population of S. frugiperda, the higher the percentage of plants attacked, as well as the intensity of the attack (Wilyus et al., 2021). In addition, damage due to *S. frugiperda* larvae attacks will also expand along with the development of the larvae, marked by several symptoms of attack, such as the presence of holes in the leaves, up to the growing points (Nonci et al., 2019). The intensity of *S. frugiperda* shows different variations at each altitude (Table 1). The results of the study showed that pest attacks had occurred in lowlands to highlands.

The distribution area in the highlands was found at an altitude of >1000 masl, namely in Rejang Lebong and Kepahiang Regencies, while in the medium plains <700-500 m asl, namely in Bengkulu Utara. According to Syarkawi et al. (2015) altitude is also one of the factors that influences the spread of insects. The highest intensity of *S. frugiperda* larvae was found at plant age 4-5 (MST) at an altitude of >1000 masl with an attack intensity of 38.92% and the lowest in plants aged 6-7 (MST) with an altitude of <700 masl, namely 11.78% (Putu et al., 2022). According to Nonci et al. (2019), the lower the intensity of *S. frugiperda* larvae attacks in higher areas due to the influence of the temperature which ranges between 19°C - 27°C in the field so that it is less suitable.

The corn cultivation system in Rejang Lebong and Kepahiang uses a monoculture planting pattern for corn plants, while in North Bengkulu it uses an intercropping pattern of corn and papaya plants. Zulza (2020) reported that the intercropping pattern of corn with soybeans increases corn yields and can reduce pest attacks. Supartha et al., (2021b) reported that environmental factors such as temperature, food sufficiency, and natural enemies were extrinsic factors that greatly influenced the development of pest populations in the field, in addition to intrinsic factors such as high fecundity and short life cycles (Plessis, 2020).



Figure 1. Symptoms of *S. frugiperda* attack in the field, (A) Bonanza variety in South Curup, (B) Pioneer 18 variety in Kepahiang, and (C) Pioneer 27 variety in Bengkulu Utara.

Farmers also use chemical pesticides to control larvae that attack corn plants. Various brands of pesticides are used, including Prevathon 50 SC (Rejang Lebong), Jayam 50 EC (Kepahiang), and Lannate 40 SP (Bengkulu Utara). These chemical pesticides are applied by spraying. The application is carried out twice a week. Farmers use pesticides to reduce pest populations, so the use of pesticides is increasingly widespread and farmers become dependent. Yatim (2018) stated that the use of chemical pesticides makes farmers dependent and has bad effects on humans because it can kill natural enemies that should be useful for biological control and also pollute the surrounding environment.

3.2. Incidence of Entomopathogenic Fungi

The percentage of larvae infected with entomopathogenic fungi in the field was highest in Rejang Lebong Regency at Talang rimbo 1-4 locations, while in Rejang Lebong (South Curup and Sumber Urip), Kepahiang (Merigi and Kabawetan), North Bengkulu (Tanjung Agung 1) there were no infected larvae (Table 2). This is caused by environmental factors that do not support the development of entomopathogenic fungi in the field. Geographical location, climate, habitat, altitude, soil pH and organic matter affect the presence of fungal species (Abdel-Raheem, 2020). In this study, *Metarhizium* sp. infection in *S. frugiperda* varied in various regions, because it was influenced by environmental conditions. Manjula et al. (2003) stated that temperature, rainfall, and humidity affect the incidence of disease in insects. Choudhary et al. (2012) reported that temperature, humidity, and rainfall play an important role in the incidence, distribution, prevalence, and effectiveness of entomopathogenic fungi.

Table 2. Larvae infected with entomopathogenic fungi at the research location

Districts	Location	Infected larvae	Healthy larvae	Infected larvae(%)
Rejang Lebong	Sumber Bening	3	30	10
	Curup Selatan	0	30	0
	Sambil Rejo	2	30	6.6
	Talang Rimbo 1	6	30	20

	Talang Rimbo 2	5	30	16.6
	Talang Rimbo 3	7	30	23.3
	Talang Rimbo 4	10	30	33.3
	Sumber Urib	0	30	0
Kepahiang	Merigi	0	30	0
	Tabat Manok	0	30	0
	Kabawetan	0	30	0
Bengkulu Utara	Tanjung Agung 1	0	30	0
	Tanjung Agung 1	0	30	16.6

Entomopathogenic fungi found in the field have characteristics of a slightly green mycelium color and cover the entire body of the insect. The larvae are found under and above the leaf surface and at the base of the leaf. Lubis (2020) reported that *S. frugiperda* larvae in corn plants infected with *M. rileyi* fungi in the field, with characteristics of mummification (stiff body), the formation of white mycelium that covers the entire surface of the larval integument, and finally changes in color to green. According to Arsi et al. (2020) spores will fly blown by the wind, then the spores will stick to the leaves or parts of the plant so that insect pests that come to the plant and eat parts of the plant will be infected by the fungus that sticks to the plant. These spores can also stick directly to larvae or insects that are spread by the wind.

Based on the results of observations of the morphological characteristics of fungal isolates that have been obtained from the field. Macroscopic morphological characteristics, the color of the colony in the early growth phase is white and changes color to green. The lower surface of the colony in the center of the colony is yellow and the edges are white. Alvarez et al., (2018) stated that at the beginning of the growth of the *M. rileyi* fungus, the colony is white and changes to pale green and dark green. The results of microscopic morphological identification of the fungus *Metharizium* sp. show that the fungus has a slightly elliptical conidia shape, straight conidiophores, septate hyphae, and hyaline. Alvarez, et al., (2018) reported that *Metarhizium* has the characteristics of septate and hyaline hyphae.

3.3. Mortality and time of death of larvae

Mortality percentage is one of the important indicators in testing the effectiveness of insecticides against test insects. Laboratory test results showed that different concentrations of conidia produced different mortality responses. The higher the concentration of conidia, the higher the insect mortality. For all treatments tested, the mortality of *S. frugiperda* larvae increased with increasing concentration of conidia. This means that the higher the number of conidia, the greater the chance of the target pest being infected or dying (Shanthakumar et al., 2010).

Table 3. Mortality of *Spodoptera frugiperda* at various concentrations

No	Concentration (Conidia/ml)	Mortality (%)	Time of death (Days)
1	10^7	95a	4.53
2	10^6	55b	5.44
3	10^5	45bc	5.80
4	10^4	30bc	5.82
5	10^3	25c	6
6	Control	0d	0

The results of the larval mortality test after the application of the tested *Metarhizium* sp. fungus resulted in mortality in *S. frugiperda* larvae (Table 3). The average percentage of *S. frugiperda* larval mortality was the lowest and highest at concentrations of 10^3 and 10^7 conidia/ml with mortality reaching 25% and 95%. Mortality values differed between each treatment, these results indicate that local isolates

of this fungus can infect and kill *S. frugiperda* larvae even with the lowest concentration treatment, namely a conidia density of 10^3 conidia/ml. The results of this study indicate that the higher the concentration of conidia, the higher the mortality. The success of entomopathogenic fungi in controlling target pests is highly dependent on conidia density, growth media quality, type of target pest, pest stage, application method, and environmental factors including ultraviolet light, rainfall, and humidity (Junianto 2000). *B. bassiana* applied to *S. litura* mortality reached 82.5%, 7 days after application with a conidial density of 10^{10} conidia/ml (Rosmiati et al., 2019).

Increasing the concentration of conidia from 10^2 conidia/ml to 10^8 conidia/ml caused an increase in the mortality of *H. armigera* instar 2 larvae from 12.5% to 92.50% (Gundannavar et al., 2005). The increase in the percentage of mortality of *H. armigera* instar 2 larvae occurred at a concentration of 1.25×10^5 conidia/ml to 1.25×10^7 conidia/ml with mortality reaching 45% to 85.2% (Tang et al., 1999).

Larvae infected by the entomopathogenic fungus *Metarhizium* sp. show symptoms such as slow movement, not wanting to eat, and discoloration, then stiff death. Uge et al. (2021) stated that the process of fungal infection in the body of the larvae begins when the conidia attach to the host cell, which the fungus uses to penetrate the insect's body with the help of enzymes (protease, chitinase, quitobiase, upase, and lipoxygenase) and spreads to the body tissue of the larvae and causes death in *S. frugiperda* larvae.

The effectiveness of this fungus can be seen from the time required to kill *S. frugiperda* larvae, there is no significant difference in time because each treatment has almost the same value, in the range of five to six days after application (Table 3). Montecalvo and Navasero (2020) stated that larval death began to occur significantly on the fourth to fifth day. Mortality of *S. frugiperda* larvae reached 95% within 4.53 days. Fronza et al. (2017) reported that the process of entomopathogenic fungal infection to the death of the host insect took an average of six to eight days and varied according to the strain and species of the host.

Percentage of deformed pupae and imago

The results of the application of conidial suspension did not kill all larvae, but showed a further effect on the larvae. The difference in each suspension concentration resulted in a different percentage of pupae and imago formed, there were also deformed pupae due to the further effect of the application of entomopathogenic fungi applied to *S. frugiperda* larvae and caused the pupae to fail to become imago (Table 4). The higher the concentration of *Metarhizium* sp. given to *S. frugiperda* larvae, the percentage of the number of imago formed also decreased.

Table 4. Percentage of healthy and defective pupae and imago at various concentrations.

No	Perlakuan	Pupa Terbentuk	Pupa Cacat	Imago Terbentuk
1	Kontrol	100	0	100
2	10^3	75	25	50
3	10^4	70	25	45
4	10^5	55	15	40
5	10^6	45	20	25
6	10^7	5	5	0

Table 5. Chi-Square analysis of the effect of conidia application on pupa and imago formation

Quadrant Unit	Value	Conclusion
X^2	28.992	X^2 hitung > X^2 tabel
X^2	24.995	
Degrees of freedom		

Based on the table above, entomopathogenic fungi can cause a decrease in the number of pupae formed and cause infection in the pupae, the higher the concentration applied tends to decrease the percentage of the number of pupae formed. Nelly (2022) reported that larvae that were applied with *B. bassiana* fungi experienced mortality, larvae that were still alive would still become pupae, and some pupae that were formed experienced imperfect organ damage (defects). Chi-Square analysis was carried out to determine the significance of deformed pupae in larvae due to the application of entomopathogenic fungi. The results of the Chi-Square analysis showed that the calculated χ^2 results were greater than the χ^2 table (Table 5), so it can be concluded that the deformed pupae formed were due to the treatment given. Indrayani et al. (2011) *M. rileyi* can interfere with the development process of *H. armigera* larvae which results in abnormalities in the pupa and imago stages. Nelly (2022), reported that entomopathogenic fungi produce toxins that are toxic and inhibit the metabolism of insects.

4. CONCLUSION

The highest incidence of *S. frugiperda* occurred in Rejang Lebong district (Talang Rimbo 4: 75.83%), then Kepahiang, and the lowest attack in Bengkulu Utara (Tanjung Agung: 31.67%). The highest incidence of entomopathogenic fungi in infected larvae in the field was also found in Talang Rimbo 4 at 33.3%. The identification results showed that the entomopathogenic fungi found in the field were *Metarhizium* sp. and laboratory testing of *S. frugiperda* larvae was able to cause mortality of 95% at a spore density of 107 conidia/ml.

5. ACKNOWLEDGEMENTS

Thank you to the Laboratory of the Plant Protection Department, Faculty of Agriculture, UNIB for facilitating this research.

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