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Pathogenicity Of Endophytic Bacteria As Entomopathogens Against Spodoptera Litura Fabricius. (Lepidoptera: Noctuidae)

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ABSTRACT

One of the biological controls of pest and plant disease is the use of enophytic bacteria. This study aimed to obtain endophytic bacterial from the root tissue of the shallot plant, which is potential as entomopathogens against the larvae of *Spodoptera litura*. This research was arranged in a completely randomized design (CRD) with nine treatments consisting of eight endophytic bacteria (isolation from shallot root tissue) and one control with three replications. Parameters observed were larval mortality, percentage of normal and abnormal pupae formed, percentage of normal and abnormal imago formed. The results showed that the bacteria of *Serratia marcescens* JB1E3 and *Bacillus cereus* P.14 caused the highest mortality in the larval phase, while *Serratia marcescens* ULG1E4 and *Bacillus* sp. SJI showed a long-term (latent) effect, resulting in no formation of pupa and imago of *Spodoptera litura*.

Keywords: Endophytic bacteria, entomopathogens, mortality, pathogenicity, *Spodoptera litura*

Patogenisitas Bakteri Endophitik Sebagai Entomopatogen Terhadap *Spodoptera Litura* Fabricius. (Lepidoptera: Noctuidae)

ABSTRAK

Salah satu pengendalian hayati hama dan penyakit tanaman adalah penggunaan bakteri endofit. Penelitian ini bertujuan untuk mendapatkan bakteri endofit dari jaringan akar tanaman bawang merah yang berpotensi sebagai entomopatogen terhadap larva *Spodoptera litura*. Penelitian ini disusun dalam Rancangan Acak Lengkap (RAL) dengan sembilan perlakuan yang terdiri dari delapan galur bakteri endofit (yang diisolasi dari jaringan akar bawang merah) dan satu kontrol dengan tiga ulangan. Parameter yang diamati adalah mortalitas larva, persentase pupa normal dan abnormal yang terbentuk, serta persentase imoga normal dan abnormal yang terbentuk. Hasil penelitian menunjukkan bahwa bakteri *Serratia marcescens* JB1E3 dan *Bacillus cereus* P.14 menyebabkan kematian tertinggi pada fase larva, sedangkan *Serratia marcescens* ULG1E4 dan *Bacillus* sp. SJI menunjukkan efek jangka panjang (laten), mengakibatkan tidak terbentuknya pupa dan imago *Spodoptera litura*.

Kata Kunci: bakteri endofit, entomopatogen, mortalitas, patogenisitas, *Spodoptera litura*

INTRODUCTION

Tobacco cutworm (*Spodoptera litura*) is one of the important pests that attack vegetable and fruit crops in Indonesia. This pest can damage leaves and fruit and, if not controlled, will cause economic losses (Ahmad et al, 2013). *S. litura* has many host plants, including chili, cabbage, rice, corn, tomatoes, sugarcane, beans, oranges, tobacco, shallots, eggplant, potatoes, beans, kale, spinach, bananas, and ornamental plants (Marwoto and Suharsono, 2008).

The attack of *S. litura* larvae on plants can occur in the vegetative and generative phases. In the vegetative phase, the larvae eat young leaves until only the leaf bones are left, and in the generative phase, they eat young pods. The damage caused by *S. litura* larvae is about 12.5% on young plants and more than 20% on older plants. Heavy attacks can cause plants to die (Moekasan et al, 2000).

The control of *S. litura* larvae has been performed using synthetic insecticides. Insecticide application is carried out intensively with an interval of 2-3 days, and the insecticide sprayed is a mixture of various types of active ingredients. Insecticidal active ingredients used to control *S. litura* larvae are Cypermethrin 110 gr/l, Chlorpyrifos 200 gr/l, and Deltamethrin 25 gr/l (Susila, 2006). The use of insecticides, in addition to suppressing the population of *S. litura* larvae, also has negative effects on other organisms and the environment, including resistance, resurgence, accumulation of residues in crop yields, killing of natural enemies, and health problems in humans and animals (Directorate of Horticultural Plant

Protection, 2008). For this reason, research on alternative control that is more environmentally friendly is necessary. The technology of using biocontrol agents to control pests is one alternative control that needs to be developed. Research on the use of bacteria as biological control of insect pests is still being carried out, one of which is using bacteria derived from plant tissue known as endophytic bacteria (Strobel et al., 2003). Endophytic bacteria are able to suppress the population of various types of insect pests on various host plants. Screening endophytic bacteria from corn root tissue, 29 isolates were obtained, and six of them had the potential as entomopathogens with a mortality rate >50%, the highest percentage of larval mortality was 66.663%, with the fastest LT50 value of 2.257 days (Arizona, 2018). Christina (2013) reported that *B. thuringiensis* could cause mortality in larvae of *C. binotalis, P. xylostella*, and *S. litura* up to more than 50%. At a concentration of 1.5 x 10^7 spores/ml, it could kill up to 100% after 96 hours, making it potential as an environmentally friendly biopesticide.

The endophytic bacteria used in this study were derived from the roots of shallots, consisting of *Bacillus cereus* P.14, *Bacillus* sp. HI, *Bacillus cereus* Se 07, *Bacillus* sp. SJI, *Serratia marcescens* ULG1E2, and *Serratia marcescens* JB1E3. The bacteria had been selected and tested for their ability to suppress bacterial leaf blight (BLB) and increase the yield of shallots (Resti et al., 2013). They were also tested for their antagonistic effect on the fungi causing bacterial leaf blight in rice plants, including *Colletotrichum capsici*, *Colletotrichum gloesporioides*, *Ralstonia solanacearum* (Resti et al., 2017), and *Xanthomonas oryzae pv oryzae* (Resti et al., 2018). However, basic information about their pathogenicity has not been widely reported. Therefore, their ability to control pests will be tested, especially for controlling *S. litura* larvae. The study aimed to obtain potential endophytic bacterial as entomopathogens against *S. litura* larvae.

MATERIALS AND METHOD

This research was arranged in a completely randomized design (CRD) consisting of nine treatments, including eight endophytic bacterial isolates (Table 1) and one control, with three replications, each consisted of 15 larvae of *S. litura*.

Table 1. Endophytic bacteria from shallots root tissue used as treatments

Treatment	Gram	Hypersensitive Reaction
S. marcescens JB1E3	-	-
S. marcescens JB1E2	-	-
S. marcescens ULG1E4	-	-
S. marcescens ULG1E2	-	-
B. cereus P.14	+	-
Bacillus sp. *SJI*	+	-
B. cereus Se 07	+	-
Bacillus sp. HI	+	-

Rearing of *S. litura* larvae

S. litura eggs taken from the field were reared and fed 24 hours with fresh leaves. After the larvae entered the prepupa stage, they were transferred to a plastic box containing sawdust. Pupae were placed in a container lined with filter paper and covered with insect cages, and white paper was placed inside as a place for laying eggs. The imago was fed with honey that was dipped in cotton and hung above the cage. Groups of eggs laid by imago were taken and transferred into boxes lined with filter paper and kept until the eggs hatched. The hatched larvae were reared until the second instar and used as test insects in this study. The larval feed used in this study was fresh long bean leaves that had been washed whose plants were planted and maintained without insecticide treatment at the greenhouse of the Faculty of Agriculture, Andalas University, Padang.

Rejuvenation and Propagation of the Endophytic Bacteria

The endophytic bacterial isolates stored in microtubes were rejuvenated on NA medium using the scratch method and incubated for 2 x 24 hours. The pure cultures then were propagated for entomopathogenic testing. The endophytic bacteria were propagated by mixing the culture with sterile distilled water. The bacterial suspension was then transferred into a test tube, homogenized with a vortex, and the population density was 10^8 cells/ml (Klement *et al*., 1990).

Endophytic Bacterial Pathogenicity Test

The method used is leaf dipping (Balfas and Wilis, 2009). The tested insects used were second instar larvae. Fresh long bean leaves as feed for larvae were soaked in a suspension of endophytic bacteria with a population density of 10^8 cells/ml and inverted using tweezers for 10 minutes, then air-dried.

In this test, fifteen larvae of the second instar were placed in a petri dish lined with filter paper, and the larvae were fed with long bean leaves, which had been treated with a suspension of endophytic bacteria. For control, the long bean leaves were soaked with sterile distilled water. Endophytic bacterial suspension treatment was carried out for two days (twice change of feed). On the third and subsequent days, the larvae were fed using long bean leaves without endophytic bacterial suspension treatment.

The larval mortality was observed from the first day of application until the formation of pupa and imago. Koch's postulate test was carried out in each dead larva through the fluid released by the infected larvae.

Koch's Postulate Test

Koch's postulate test was conducted to prove that the larvae of *S. litura* died due to the activity of the endophytic bacteria. Larvae that died in each treatment were surface sterilized with 70% alcohol and placed in

a petri dish containing NA media, then incubated for 2x24 hours. After incubation, the growing bacteria were compared with the endophytic bacteria applied at the beginning.

Observation Variables

Larval Mortality

The observation was made by counting the number of dead larvae every day starting from the first day after the application of endophytic bacteria until the fifth day. Mortality was calculated using the following formulae:

$$M = \frac{n}{N} \text{ X } 100\ \% \quad \ldots (1)$$

Remarks:
M : larval mortality (%)
n : number of dead larvae
N : total number of larvae

The percentage of larval mortality obtained was then corrected using the Abbots formula:

$$P = \frac{po-pc}{100-pc}\ x\ 100\ \% \quad (2)$$

Remarks:
P : percentage of dead insects after correction (%)
Po : percentage of dead insects due to the treatments
Pc : percentage of dead insects in control treatment

In this observation, the LT_{50} value was also determined to obtain the time required for endophytic bacteria to kill 50% of the tested insects using the SPSS application probit analysis.

Percentage of Pupae Formed

Percentage of the pupae formed was calculated using the following formula:

$$P = \frac{b}{N} \text{ x } 100\ \% \quad \ldots (3)$$

Remarks:
P : percentage of pupae formed
b : number of pupae formed
N : total number of larvae

Percentage of Normal and Abnormal Pupae

Percentage of normal and abnormal pupae was calculated using the following formula:

$$P = \frac{\text{number of normal/abnormal pupae}}{\text{Number of the pupae formed}} \text{ x } 100\ \% \quad \ldots(4)$$

Percentage of Imago Formed

The observation was made by counting the number of imago from each treatment when the imago was formed. The percentage of imago formed was calculated using the following formula:

$$I = \frac{d}{N} \text{ x } 100\ \% \quad \ldots (5)$$

Remarks:
I : percentage of imago formed
d : number of imago formed
N: total number of larvae

Percentage of Normal and Abnormal Imago

Percentage of normal and abnormal imago was calculated using the following formula:

$$P = \frac{\text{number of normal/abnormal imago}}{\text{Total Imago Formed}} \text{ x } 100\ \% \text{ .(F 6)}$$

RESULTS AND DISCUSSION

T Endophytic Bacterial Pathogenicity on *S. litura* larvae

Larval Mortality

The results of the analysis of variance on the mortality of *S. litura* larvae showed that the seven treatments of endophytic bacteria were significantly different from the control. The mortality percentage of *S. litura* larvae treated with endophytic bacteria can be seen in Table 2.

Table 2. Percentage of mortality of *S. litura* larvae 5 days after being treated with 8 endophytic bacteria along with their LT_{50} values

Treatment	Mortality (%)		LT_{50} (Days)
S. marcescens JB1E3	70.27	a	2.204
B. cereus P.14	56.75	ab	3.467
S. marcescens ULG1E4	45.94	abc	4.628
S. marcescens ULG1E2	43.23	abc	4.443
S. marcescens JB1E2	37.83	bc	5.037
Bacillus spSJI	37.83	bc	5.717
B. cereus Se 07	37.83	bc	5.181
Bacillus sp HI	21.62	cd	7.300
Control	0.00	d	-
CV = 17.28			

*Means followed by the same letters in the same column are not significantly different based on the LSD test at 5%.

The eight endophytic bacteria are pathogenic for *S. litura* larvae, increasing their mortality rate. The mortality of *S. litura* larvae treated with endophytic bacteria ranged from 21.62% to 70.27%. The highest was in the treatment of *S. marcescens* JB1E3, which was 70.27%, while the lowest was in the treatment of *Bacillus* sp. HI, which was 21.62%.

The lowest LT_{50} value was found in the treatment of *S. marcescens* JB1E3 bacteria, which was 2.204 days, meaning that the endophytic bacteria could cause the death of 50% of *S. litura* larvae within two

days. Meanwhile, the treatment of *Bacillus* sp. HI bacteria required the longest time to cause the death of 50% of *S. litura* larvae, which was 7.30 days.

This graph shows the mortality rate of *S. litura* larvae every day during the observation. The cumulative mortality rate of *S. litura* larvae after the application of endophytic bacteria varied depending on the isolates. On the first day, the highest mortality percentage was in the treatment of *S. marcescens* JB1E3, reaching 15.5%. In the treatment of *B. cereus* P.14, there was no larval death, but on the second day, this treatment had the second-highest mortality percentage compared to other bacteria until the fifth day. In control treatment, there was no significant and stable increase in mortality until the fifth day. The treatment of *S. marcescens* JB1E3 was the treatment with the highest larval mortality and the shortest period to kill 50% of the larvae, which was two days.

The difference between normal larvae and symptoms of infected *S. litura* larvae can be seen in Figure 2.

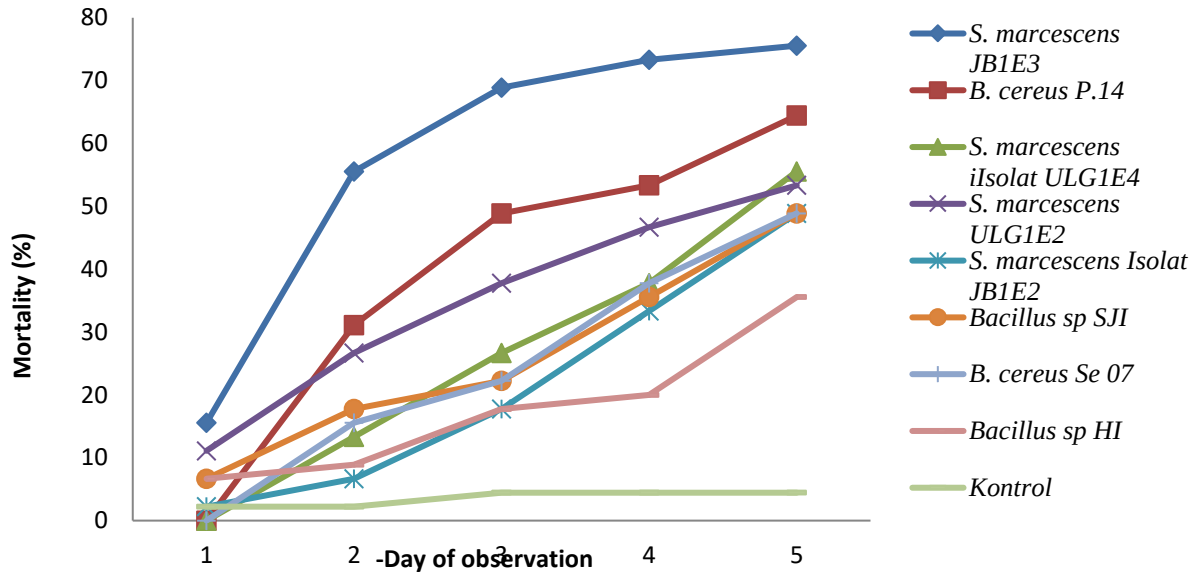

Figure 1. The cumulative mortality rate of *S. litura* larvae from the first day to the fifth day after the application of endophytic bacteria

Figure 2. Normal *S. litura* larvae and those infected with endophytic bacteria; A. Normal larvae; B1. slow larval growth (14 days after application); B2. shrunk larval body (40x magnification); B3. Blackened larvae

The symptoms of the endophytic bacterial infection in S. litura larvae included reduced feeding activity, slower movement and less sensitivity to touch, and slower growth of larvae than normal larvae. Larvae that died due to the endophytic bacterial infection experienced changes in color and shape. The larvae body turned its color to reddish, dark brown to blackish, shriveled, and the larval body discharged fluid and smelled bad. The larvae then dried and shrank with whole integuments (Figure 2 B2).

Larvae infected with endophytic bacteria experienced diarrhea, indicated by the symptoms of the melting of the larvae's feces, vomiting a blackish green liquid from the mouth, and releasing a red liquid according to the bacteria infecting them, as evidenced by the results of Koch's postulate.

Koch's postulate test

Koch's postulate test was performed on infected larvae in each treatment of endophytic bacteria using the direct planting method on NA media. The test results showed the same bacterial morphology as endophytic bacteria applied after being incubated for 2 x 24 hours.

Percentage of Pupa and Imago Formed

The treatment of eight endophytic bacteria had significantly different effects on the formation of pupae and imago of S. litura. The results of the LSD test at the 5% level can be seen in Table 3.

Table 3. Percentage of the normal and abnormal pupae dan imago of *S. litura* formed after treated with eight endophytic bacteria

Treatment	Number of larvae	Total Pupae	% Pupae formed	Normal (%)		Abnormal (%)		Total Imago	% Imago formed	Normal (%)		Abnormal (%)	
Control	45	37	82.22	94.60	a	5.40	a	35	77.78	97.15	a	2.85	a
S. marcescens ULG1E2	45	18	40.00	61.12	b	38.88	b	11	24.44	72.73	b	27.27	b
B. cereus P.14	45	16	35.56	50.00	bc	50.00	c	8	17.78	75.00	bc	25.00	bc
Bacillus sp. HI	45	15	33.33	40.00	bcd	60.00	cd	6	13.33	83.34	bcd	16.66	d
S. marcescens JB1E3	45	9	20.00	55.56	cde	44.44	de	5	11.11	100.00	cde	0.00	e
S. marcescens B1E2	45	4	8.89	25.00	de	75.00	def	1	2.22	0.00	f	100.00	f
B. cereus Se 07	45	4	8.89	25.00	de	75.00	def	1	2.22	100.00	g	0.00	g
Bacillus sp. SJI	45	2	6.67	0.00	f	100.00	g	0	0.00	0.00	g	0.00	g
S. marcescens ULG1E4	45	3	4.44	0.00	f	100.00	g	0	0.00	0.00	h	0.00	g

*Means followed by the same letters in the same column are not significantly different based on the LSD test at 5%.

The larval mortality rate affected the number of pupae formed. The higher the larval mortality rate, the fewer pupae are formed, thereby affecting the number of imago formed. Table 3 shows that the highest percentage of abnormal pupae formed was in the treatment of *Bacillus* sp. SJI and *S. marcescens* ULG1E4, which was 100% and no normal pupae formed, respectively. Meanwhile, in the treatment of *B. cereus* P.14, the percentage of normal and abnormal pupae was the same, namely 50%.

The normal pupae were reddish-brown, and the tail made a move when touched, while the abnormal pupae were imperfectly shaped, small in size, wrinkled on the body surface, flattened in half, did not make a move when touched, had fluid, and the body was hollow with a foul smell (Figure 3).

Based on the analysis of variance in the percentage of imago formed, the treatment of endophytic bacteria showed a significantly different effect. Table 3 shows that the treatment of *S. marcescens* JB1E2p had the highest percentage of abnormal imago, which was 100% of the total imago formed, thus causing no imago to be formed. Meanwhile, in the treatment of *Bacillus* sp. SJI and *S. marcescens* ULG1E4, there was no imago formed.

Imago formed from larvae of *S. litura* treated with endophytic bacteria were normal, and some were abnormal (Figure 4). The normal imago has the characteristics of a perfectly formed silvery brown wing color without the slightest flaw, while the abnormal imago is not perfectly formed with curled wings.

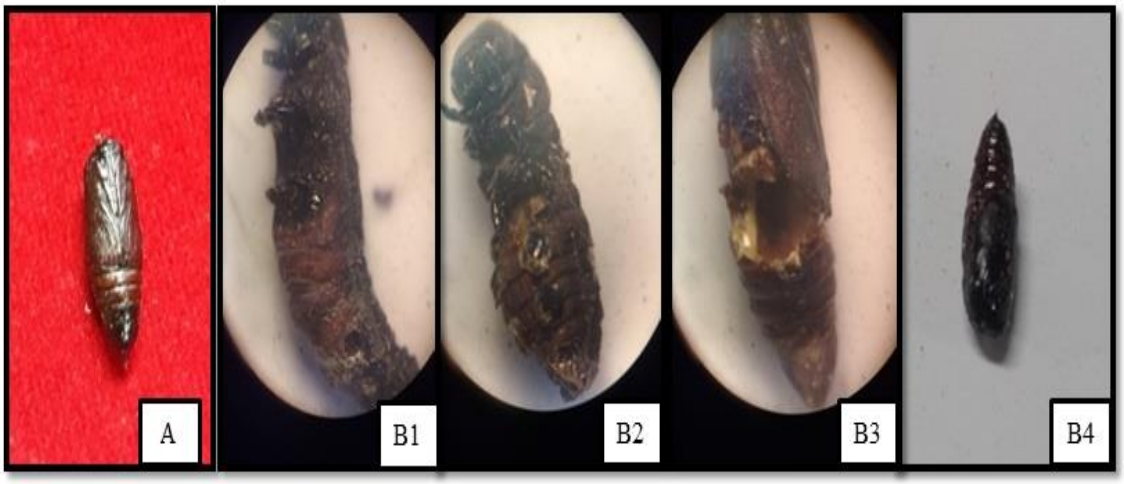

Figure 3. Normal pupae of *S. litura* and those infected with endophytic bacteria; A. Normal pupae; B. Abnormal Pupae; B1. Formation from pre-pupa to imperfect pupa at four days of age (40x magnification); B2. Pupa body shrinking at a week of age (40x magnification); B3. Pupa body excreting fluid and forming hollow at ten days of age (40x magnification); B4. Pupae blackened at two weeks of age

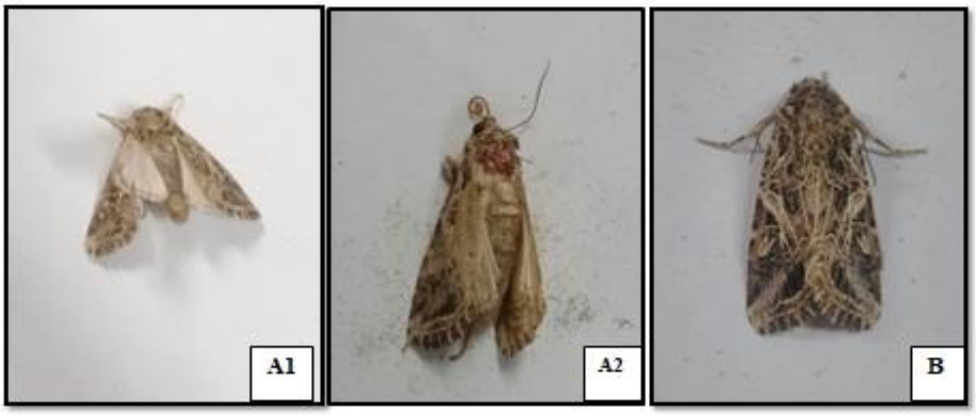

Figure 4. Normal imago of *S. litura* and those infected with endophytic bacteria; A1 and A2. Abnormal Imago abnormal (wing curls) and B. Normal Imago

DISCUSSION

The eight endophytic bacteria used in this study were capable of infecting (pathogenic) *S. litura* larvae, thus causing mortality. Each treatment of endophytic bacteria had different levels of pathogenicity and different incubation times. The difference was indicated by the difference in the percentage of larval mortality ranging from 21.62 to 70.27%, the percentage of normal pupae formation between 25 to 61.11%, and the percentage of normal imago formation from 72 to 100%.

The difference is due to the different pathogenicity among these endophytic bacteria. Bacterial pathogenicity is influenced by the ability of bacteria to produce toxins and enzymes. According to Tanada and Kaya (1993), in general, bacteria infect insects as toxemia, septicemia, and bacteremia. Toxemia occurs when bacteria produce toxins, and bacteria normally enter the digestive tract of insects. Septicemia often occurs in pathogenic bacteria that attack the hemocoel, multiply, produce toxins and kill insects. Bacteremia occurs when bacteria multiply in the hemolymph of insects.

In the life cycle *of S. litura*, the long-term (latent) effect of entomopathogenic bacteria greatly affects the population of this pest in the field. In this study, there were two bacteria (*Bacillus* sp. SJI and *S. marcescens* ULG1E4) causing no pupa and imago formed and even abnormalities in the pupa and imago stages.

Physiological effects due to toxins produced by endophytic bacteria have not been ascertained. However, through the symptoms of infection that occurred in the third instar of *S. litura* larvae, it is known that there is an interaction between bacterial toxins and the symptoms caused. Symptoms that arise are slower larval growth, larval bodies shrinking, blackened larvae (Fig. 2), larvae secreting fluid, and diarrhea. The symptoms of the endophytic bacterial infection are the same as those due to *Bacillus thuringiensis* infection, as reported by Trizelia (2001). The symptoms of a bacterial attack on insect pests begin with signs of inactivity, decreased appetite, weakness, diarrhea, and discharge from several parts of the body, as well as suffocation. After the insect dies, the insect appears dark brown or black. The insect's body then dries and shrivels. This is because the bacterial toxins damage the digestive system of the larvae, causing death. Then according to Bravo et al. (2007) the infected larva shrink, the color of the body increasingly blackened and shrunk. This is caused by these bacterial toxins damage the digestive system of the larvae causing death.

The larvae infected and died his body became soft and when we touched skin will break and body fluids out lackish red. This softened body It can be caused by th inning of the insect cuticle due to enzymatic processes by bacteria residing in in the body of the test insect. One of the enzymes that plays a very important role in the process by which the insect cell wall is destroyed chitinase enzymes. Chitinolytic activity produced *by S. marcescens* is proven capable of disrupting the process the formation of larvae and pupae were observed through reduction in larval and pupal weight (Anggarwal et al., 2015).

Two endophytic bacteria causing a mortality rate of >50% are thought to have an ability to grow and develop rapidly in the larval body and produce high levels of toxins and enzymes in the digestive tract compared to other bacteria. Gilbert et al. (2005) reported that the chitinase enzyme produced by *S. marcescens* could synthesize the intestinal cell wall (peritrophic) of insects by hydrolyzing β-1,4 glycosidic bonds in the structure of chitin, which is the main component of insect body tissues. Chitinase will induce damage to the peritrophic membrane in the digestive tract of insects, causing cell reduction and lysis. Besides being able to grow in the body of insects rapidly, *S. marcescens* can also produce toxins or Lipid A (endotoxin) in certain lethal doses that can kill insects in a short time (Lauzon et al., 2003).

The formation of abnormal pupae and imago is presumably due to nutrient deficiency and the toxins secreted by endophytic bacteria, then tissue damage occurs. This is in accordance with Sari's research (2016), stating that the disruption of larval body metabolism due to bacterial toxins produced causes the larvae to lack energy to enter the pupa stage, if the larvae do not die by the toxin, the pupae will form abnormally (defective).

CONCLUSION

Eight endophytic bacteria tested were pathogenic for *S. litura* larvae. *S. marcescens* JB1E3 and *B. cereus* P.14 caused the highest larval mortality. Meanwhile, *S. marcescens* ULG1E4 and *Bacillus* sp. SJI had long-term effects (latent) and could cause no pupae and imago formed in the life cycle of *S. litura*.

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REFERENCES

Ahmad M, Gaffar A & Rafiq M. 2013. Host Plants of leaf worm Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. Asian J Agri Biol. 1(1): 23-28, https://www.researchgate.net/publication/257262786_Host_plants_of_leafworm_Spodoptera_litura_Fabricius_Lepidoptera_Noctuidae_in_Pakistan

Agastya MI., Afandhi A, & Aini LQ. 2017. Efektifitas pestisida biologis *Bacillus cereus* dan *Bacillus megaterium* sebagai pengendali *Spodoptera litura* fabr (Lepidoptera: noctuidae). Jurnal Penelitian Pertanian Terapan. 17(2): 145-148, https://doi.org/10.25181/jppt.v17i2.294

Anggarwal C, Paul S, Tripathi V, Paul B & Khan MA. 2015. Chitinolytic activity in serratia marcescens (strain sen) and potency against different larval instars of Spodoptera litura with effect of sublethal doses on Insect Development. Bio Control 60(5), 631-640, https://doi.org/10.1007/s10526-015-9674-3

Balfas R & Wilis M. 2009. Pengaruh ekstrak tanaman obat terhadap mortalitas dan kelangsungan hidup *Spodoptera litura* F. (Lepidoptera: noctuidae). buletin penelitian tanaman rempah dan obat. 2 :148-156, https://repository.pertanian.go.id/handle/123456789/3829

Bravo A, Gill SS & Soberon M. 2007. Mode of action of bacillus thuringiensis cry and cry toxins and their potential for insect control. Toxicon 49(4): 423-435, 10.1016/j.toxicon.2006.11.022

Christina LS, Tarore D, & Manengkey G. 2013. Prospek pemanfaatan biopestisida bakteri entomopatogenik isolat lokal sebagai agen pengendali hayati hama tanaman sayuran. Eugenia: 19(1): 21-25. https://doi.org/10.35791/eug.19.1.2013.8375

Dara SK.2017.IPM solutions for insect pests in California strawberries: efficacy of botanical, chemical, mechanical, and microbial options. CAPCA Adviser 19(2): 40-46.

Direktorat Perlindungan Tanaman Hortikultura. 2008. Pengenalan dan Pengendalian Hama Tanaman Sayuran Prioritas. Jakarta: Direktorat Perlindungan Tanaman Hortikultura.

Fan YH, Fang WG, Guo SJ, Pei XQ, Zhang YG, Xiao YH, Bidochka MJ & Pei Y. 2007. Increased insect virulencein *Beauveria bassiana* strains over expressing anengineered chitinase. Applied Environmental Microbiology 73(1) :295–302, https://doi.org/10.1128/aem.01974-06

Gilbert GI, Iatrou K & Gill SS. 2005. Biochemistry of Digestion, in: Comprehensive Molecular Insect Science Biochemical and Molecular Biology. Elsevier Press, Oxford, UK. 25 : 171-224.

Hajek A. 2004. Natural Enemeis: An Introduction to Biological Control. Cambbridge : Cambridge University Press.

Higuchi H, Yamamoto H & Suzuki Y. 1994. Analysis of Damage to Soybeans Infested by the Common Cutworm, *Spodoptera litura* Fabricius: II. Estimation of Leaf Area Damaged by Young Larvae using Spectral Reflectivity. Japanese Journal of Applied Entomology and Zoology 38 : 297-300.

Hungl PQ, & Annapurna K. 2004. Isolation and Characterization of Endophytic Bacteria in Soybean (*Glycine* sp.). Omonrice, 12: 92-101.

Hofte H, & Whitely HR. 1989. Insecticidalcrystal protein of *Bacillus thuringiensis*. Microbiology. Rev.53:242–255.

Ilyas M. 2006. Isolasi dan identifikasi pada relung rizosfir tanaman di kawasan cagar alam gunung mutis, nusa tenggara timur. Jurnal Biodiversitas 7(3): 216 – 220, http://dx.doi.org/10.13057/biodiv/d070304

James EK, & Olivares FL. 1996. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. Critical Reviews in Plant Sciences 17(1) : 77–119, http://dx.doi.org/10.1080/07352689891304195

Kalshoven LGE. 1981. The Pests of Crops in Indonesia. Revised and Translated by P.A. Van der Laan. Jakarta. PT. Ikhtiar Baru Van Hoeve.

Khairani G. 2010. Isolasi dan Uji Kemampuan Bakteri Endofit Penghasil Hormon IAA (*Indole Acetic Acid*) Dari Akar Tanaman Jagung (*Zea mays*L). [Skripsi]. Medan. FMIPA. Univeritas Sumatera Utara. 50 hal.

Klement Z Rudolph K, & Sand DC. 1990. Methods in Phytobacteriology. Budapest. Academia Kiado.

Lauzon CR, Bussert TG, Sjogren RE & Prokopy RJ. 2003.*Serratia marcescens* as a Bacterial Pathogen of*Rhagoletis pomonella* Flies (Diptera: Tephritidae). Europe Journal of Entomology 100: 87-92, doi:10.14411/eje. 2003.017

Lembaga Pertanian Sehat Develop Useful Innovation for Farmer. 2008. Virus Patogen Serangga: Bio insectisida Ramah Lingkungan http://www. pertaniansehat.or.id.Diaksestanggal11Oktober 2018.

Lelo,T. 2013. Standar Operasional Prosedur (SOP) Bawang Merah Kalimantan Tengah. Pemerintah Provinsi Kalimantan Tengah, Dinas Pertanian dan Peternakan: Palangka Raya.

Margulis L, Jorgensen J, Dolan S, Kolchinsky R, Rainey F, & Ching S. The Arthromitus stageof *Bacillus cereus*: Intestinal symbionts ofanimals." *Proceedings of the National Science Academy US A*. 1998. 95(3) :1236–1241, https://doi.org/10.1073/pnas.95.3.1236

Marwan H, Meity SS, & Abdjad AN. 2011. Isolasi dan seleksi bakteriendofit untuk pengendalian penyakit darah pada tanaman pisang. J HPTTrop 11(2): 113-121, https://dx.doi.org/10.23960/j.hptt.211113-121

Marwoto & Suharsono. 2008. Strategi dan komponen teknologi pengendalian ulat grayak (*Spodoptera litura* Fabricius) pada Tanaman kedelai. Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian. Jurnal Litbang Pertanian 27: (4).

Moekasan, TK, Prabaningrum L, & Ratnawati ML, 2000. Penerapan PHT pada sistem tanaman tumpang gilir bawang merah dan cabai. Balai Penelitian Tanaman Sayuran. Pusat Penelitian dan Pengembangan Hortikultura. Badan Penelitian dan Pengembangan Pertanian, Jakarta. Hlm. 4-5, 30

Nusyirwan. 2017. Studi musuh alami *Spodoptera litura* Hbn pada agroekosistem tanaman bawang merah. Balai Pengkajian Teknologi Pertanian (BPTP) Sumatera Barat. Jurnal Penelitian Pertanian Terapan 13(1): 33 – 37.

Pracaya. 2007.Hama dan Penyakit Tanaman. Penebar Swadaya. Jakarta. 427 hal.

Prayitno, AA. 1983. Biologis *S. litura* (Lepidoptera: noctuidae) pada daun bawang merah (*A. Ascalonicum*), Kedelai *(G. Max*), Kacang Tanah *(A.Hypogeal).*Departemen Hama dan Penyakit Tumbuhan Bogor. Fakultas Pertanian.

Prinsloo HE. 1960. Parasitiese mikroorganismes by die Bruinsprinkaan Locustana pardalina(Walk.).S. Afr. Tydskr Landbouwet 3: 551-560.

Purwantisari. 2008. Pengendalian hayati penyakit lodoh (busuk umbi kentang) dengan agens hayati jamur-jamur antagonis isolat lokal. J. BIOMA. 10(2) : 13-19.

Radji M. 2005. Peranan bioteknologi dan mikroba endofit dalam pengembangan obat herbal. Majalah ilmu kefarmasian. 2(3): 113-126

Resti Z, Habazar T, & Putra DP. 2013, Skrining dan identifikasi isolat bakteri endofit untuk mengendalikan penyakit hawar daun bakteri pada bawang merah.J. HPT Tropika 13(2) : 167 –178.

Resti Z, Yanti Y, & H. Rahma. 2007. Distribusi penyakithawar daun bakteri pada tanaman bawang(*Xanthomonas axonopodis pv allii*) sebagaipenyakit baru di Sumatera Barat. LaporanPenelitian DIPA Unand. Padang. Universitas Andalas.

Sari TA. 2016. Uji Tingkat kerapatan populasi bakteri merah *Serratia* Sp.terhadap hama *Spodoptera litura* F (Lepidoptera: noctuidae). [Skripsi]. Padang. Fakultas Pertanian. Universitas Andalas.

Schulze B, Boyle C, & Sieber TN, Editor. Microbial root endophytes. Berlin: Springer. http://dx.doi.org/10.1007/3-540-33526-9_1.

Strobel GA, & Daisy B. 2003. bioprospecting for microbial endophytes an their natural product. Microbiol and Mol. Biology Rev 67(4): 63-68.

Susila AD. 2006. Panduan budidaya tanaman sayuran. departement agronomi dan hortikultura. institut pertanian bogor. Di Akses Tanggal 21 April 2018. 115 hal.

Tanada Y, & Kaya HK. 1993. Insect Pathology. San Diego : Academic Press, INC. Harcourt Brace Jovanovich, Publisher. 62 hal.

Tarabily K, Nassar AH, & Sivasithamparam K. 2003. Promotion of Plant Growth By An Auxin-Producing Isolate of The Yeast Williopsis Saturnus Endophytic In Maize Roots. The Sixth U.A.E University Research Conference. Plant Growth Regul 40 : 97-106.

Trizelia. 2001. Pemanfaatan *Bacillus thuringiensis* Untuk Pengendalian Hama *Croccidolomia binotalis*. Makalah Falsafah Sains Program Pasca Sarjana IPB. Diakses dari http://rudyct.250x.tripod.com/sem1_012/trizelia.htm.

Trizelia, Syahrawati MY, & Mardiah A.2011. PatogenesitasBeberapa Isolat Cendawan Entomopatogen *Metarhizium*spp. terhadap Telur *Spodoptera litura* Fabriscus(Lepidoptera : Noctuidae). Jurnal Entomologi Indonesia 8(1) : 45-54.

Udiarto BK, Setiawati W, & Suryaningsih E. 2005. Pengenalan Hama dan Penyakit pada Tanaman Bawang Merah dan Pengendaliannya. Panduan Teknis PTT Bawang Merah No.2. Bandung: Balai Penelitian Tanaman Sayuran. Pusat Penelitian Dan Pengembangan Hortikultura. Badan Penelitian Dan Pengembangan Pertanian.

Utami KP. 1997. Virus SENPV atasiulat grayak pada bawangmerah. Trubus 337- tahun 2018-Desember :74-76.

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