Anjoro: International Journal of Agriculture and Business
Vol. 5 Issue 1, April 2024
p-ISSN: 2721-8678 | e-ISSN: 2721-7914. **DOI: 10.31605/anjoro.v5i1.3504**

Effectiveness of *Bacillus thuringiensis* (Bt) application in controlling mortality of *Spodoptera litura* on rice plants (*Oryza sativa* L.)

Jefri Sembiring*, Johana Mendes, Rangga Kusumah, Diana Sri Susanti, Anwar, Abdullah Sarijan, Mani Yusuf

Department of Agriculture, Musamus University, Merauke, Indonesia

**Corresponding author's e-mail: jsembiring@unmus.ac.id*

Received December 23rd, 2023; revised April 19th, 2024; accepted April 26th, 2024

ABSTRACT

Rice (Oryza sativa L.) is the primary commodity for the Indonesian people because most of the Indonesian population depends on rice. The armyworm pests Spodoptera litura causes irregular holes in the leaves, thus disrupting the photosynthesis process and reducing rice production, so it is necessary to treat the pest. Bacillus thuringiensis is a pathogenic bacterium for insects, such as S. litura. This study aimed to look at the effectiveness of B. thuringiensis on the mortality of S. litura on a laboratory and field scale. The research was conducted in the Agrotechnology Laboratory, Faculty of Agriculture, Musamus University. The research design was a complete randomized design (RAL). The research study was conducted on five treatment doses of B. thuringiensis, which were 20 g/500 ml of water; 40g/500 ml of water; 60 g/500 ml of water; 80 g/500 ml of water; 100 g/500 ml of water. Each treatment was repeated five times. The results showed that, applying B. thuringiensis can significantly decrease the population and intensity of S. litura. The highest mortality rate in laboratory trials was observed at a dose of 100 grams/500 ml of water, with 50 dead individuals. In contrast, the lowest mortality rate was recorded at a dose of 20 gram of material/500ml of water, with only 12 death S. litura larvae. In field testing, the best dose was 50 gram/14 liters of water, and it caused the death of 50 S. litura larvae. Spraying B. thuringiensis on rice plants can reduce the population and intensity of S. litura.

Keywords:
Bacillus thuringiensis, Biological control, Merauke, Rice, Spodoptera litura

1. Introduction

Rice (*Oryza sativa* L.) is a staple food for millions of people worldwide and is particularly important in Indonesia, where it is a crucial commodity. As the population grows, the demand for rice also increases, which puts pressure on rice production and productivity [1]. It is, therefore, imperative to ensure that rice production keeps pace with population growth. The latest report shows that the total rice harvest area in 2022 is estimated to be 10.45 million hectares, with the highest harvest area in March at 1.76 million hectares and the lowest in December at around 0.36 million hectares. The 2022 rice harvest area has increased by 40.87 thousand hectares compared to last year.

This increase is a positive sign that rice production is on the rise, but there is still much work to ensure enough rice to feed the growing population. Investing in and supporting rice production ensures that everyone has access to this essential food source [2]. Population growth can also reduce planting area due to land use conversion, thus affecting productivity. In addition, efforts to increase rice production have experienced various obstacles caused by the environment and plant-disturbing organisms (OPT). Many types of pests attack rice plants and cause damage in terms of quality and quantity [3]. Rice plants are ideal hosts for many stem-boring, grain-

eating, root-eating, and leaf-eating insect pests. Pests found on rice plants consist of the order Diptera (Tephritidae), Hemiptera (Cicadellidae and Coreidae), Lepidoptera (Crambidae) [4], and Orthoptera (Acrididae, Gryllidae, and Pyrgomorphidae). Insect pests that attack rice plants are identified based on family abundance [5]. In addition, the insect *S. litura* Fabricius (Lepidoptera: Noctuidae) [6], or armyworm, is an important pest in rice plants [7]. This armyworm attacks rice at night and hides at the base of the plant, in the soil, and hidden places during the day. The caterpillars eat the rice leaves starting from the tip of the leaf and leaving the leaf veins so that what is left is just a rice plant without leaves. This armyworm attack occurred at the same time as water drought in the land, so the potential population of armyworms is predicted to increase if control measures are not taken quickly. Economic losses can occur because farmers can experience crop failure [8,9].

In Merauke District, pest control still relies on synthetic chemical control. This can impact non-target organisms and agricultural products' food safety [10,11]. For this reason, it is necessary to develop environmentally friendly control. Biological control is the control of OPT by utilizing living things [12]. This is also inseparable from several obstacles, namely the limited type of bacteria and the low adaptability of bacteria to the environment [11,13]. According to [14], location microbes as biological agents are more adaptive in the atmosphere than microbes from other regions. In addition, it is necessary to test the effectiveness of each exploration isolate before it is developed and propagated for its use as a control or material for developing entomopathogens [15,16]. Biological control aims to utilize microorganisms in plant pest control. The provision of microorganisms is carried out to suppress targeted pests or, in this case, disturb plants (problems).

For this reason, it is necessary to develop bioinsecticides to control plant pests, especially in Merauke Regency. Insect groups based on the diversity of their functions in rice agroecosystems include insect pests, natural enemies, and neutral insects such as pollinators. The biotic component that controls insect populations of pests in agroecosystems is a natural enemy. Natural enemies of rice wetland agroecosystems include predators, parasitoids, and entomopathogens [5].

B. thuringiensis is one of Indonesia's most dominant bacterial species controlling various arthropod species indicated as pests. *B. thuringiensis* is widespread in soil, is pathogenic in various orders, and become the most widely used biological control agent. Subspecies *B thuringiensis* becomes a microorganism that can be added to the ecosystem to control insects naturally [17,18]. Entomopathogenic bacteria from the species *B. thuringiensis* can be used as natural insecticides on plants, soil, aquatic environments and even facilities for food storage. After the application of *B. thuringiensis* to nature, bacteria can survive as components of natural microflora. Bacterial group This entomopathogen can be found in most ecological niches[18][19]. Insecticidal Crystal greatly influences its toxicity activity. These bacteria produce protein (ICP) during sporulation. Spread The environment strongly influences entomopathogenic bacteria, plant varieties, and vegetation in its natural habitat. Seasons play a major role in the diversity number and subspecies of populations of entomopathogenic bacteria [20,21]

B. thuringiensis is a type of bacteria that causes insect death or is an entomopathogen. This is one alternative that has begun to be done to control essential pests of rice plants and other crops [3,21]. The use of these bacteria has the hope of being developed

because it is easy and cheap to apply and does not damage the environmental ecosystem [22]. To date, crystalline proteins toxic to larvae of various orders of insects that are pests on food crops and horticulture have been identified. Most of these crystalline proteins are more environmentally friendly because they have a specific target so that they do not kill insects, are not targets and are easily decomposed so that they do not accumulate and pollute the environment [11]. This study aimed to see the effectiveness of *B. thuringiensis* on the mortality of *S. litura* on a laboratory and field scale.

2. Methods

The research was conducted in the Agrotechnology Laboratory, Faculty of Agriculture, Musamus University.

2.1. Laboratory Tests

2.1.1 Propagation of Test S. litura

The test insects used are *S. litura*, which are uniform or have the same instars. For this reason, imago insects or larvae are collected from agricultural land to be reared to obtain instar 3, which will be used as test insects. *S. litura* larvae are obtained from cabbage vegetable plants and kept in plastic boxes with gauze windows. Feed larvae in the form of Collard leaves are changed daily. The larvae that will form pupae will be given sterile sawdust instead of soil to form pupae. The pupa that has developed is then transferred to a plastic jar of a larger size and left until the image appears. Imago will be fed 10% honey, absorbed on cotton, and hung in a pot. Imago that will lay eggs are prepared pieces of Collard leaves covered with wet cotton to keep the leaves fresh. The eggs laid are then taken, transferred to plastic boxes, and left until larvae appear. The larvae of *S. litura* used are instar three larvae.

2.1.2 Experimental Design

The study design was a complete randomized design (RAL). The study consisted of five treatment doses of *B. thuringiensis* as follows: 20 g/500 ml water; 40g/500 ml water; 60 g/500 ml water; 80 g/500 ml water; 100 g/500 ml water, and each treatment was repeated five times. The test insects used as many as ten individuals of *S. litura* instar three larvae. The method of leaf dyeing is based on Prijanto [23]; pieces of Collard leaves are dipped one at a time into a pre-made suspension. The leaf pieces are then drained to reduce excess fluid. The treated leaves are then inserted into each petri dish covered with tissues. Then, ten larvae of instar three pests of *S. litura* are introduced into the saucer. Observations were made 24, 48, 72 and 96 hours after treatment (HAT) by counting the number of dead larvae.

2.2. Second Testing and Field Application

The 2nd test method uses *B. thuringiensis* at the recommended dose based on the 1st (*First*) test larval mortality probit analysis results. The dosage of *B. thuringiensis* is 6 g/500 ml of water, 13 g/500 ml of water, 25 g/500 ml of water, 31 g/500 ml of water and 50 g/500 ml of water, and each treatment is repeated 10 times. The test insects used as many as 10 individuals of *S. litura* instar 3 larvae. Leaf dyeing method based on Widjayanti et al. [7]: Pieces of pet leaves are dipped one by one into the suspension that has been made. The leaf pieces are then drained to reduce excess fluid. The treated leaves are then inserted into each petri dish covered with tissues. Then,

10 individuals of larvae of instar 3 pests of *S. litura* are introduced into the saucer. Observations were made 24, 48, 72 and 96 hours after treatment (HAT) by counting the number of dead larvae. In field tests, insecticide 90.03 g or LD 90% was used. The insecticide preparation *B. thuringiensis* was then applied evenly on the surface of each rice plot with an area of 3 m × 50 m. The treatment was repeated 8 times, spraying for 7 HAT, 14 HAT, 21 HAT, 28 HAT, 35 HAT, 42 HAT, 49 HAT and 56 HAT. Calculate population density and intensity of *Spodoptera* sp. attacks at 5 diagonal points of 1 × 1 meter for 7 observations.

2.3. Data Analysis

Cumulative mortality data of *S. litura* larvae were processed by probit analysis using the POLO PC program.

3. Results and Discussion

3.1. Laboratory Testing

Mortality of *S. litura* larvae at 24 HAT of *B. thuringiensis* with residual method showed mortality from low to high doses. The highest number of mortality was found at a dose of 100 g/500 mL of water in as many as 26 individuals, and the lowest number of mortality in as many as three individuals was found at a dose of 20 g/500 mL of water, while in the control treatment showed mortality of 1 individual. At the observation of 48 HAT, the mortality of *S. litura* larvae increased with each treatment dose of 8, 10, 18, 35, and 37 individuals with no mortality in controls. In subsequent observations of 72 HAT, the mortality of *S. litura* larvae increased with the number of individuals who died, as many as 45 individuals at a dose of 80 g/500 mL of water while at treatment doses of 20 g/500 mL of water, 40 g/500 mL of water, 60 g/500 mL of water and 100 g/500 mL of water the number of mortality successively became 12, 15, 25, and 44 individuals. Observations of 96 HAT showed increased mortality at all treatment doses and doses of 100 g/500 ml of water mortality to 50 individuals (Figure 1).

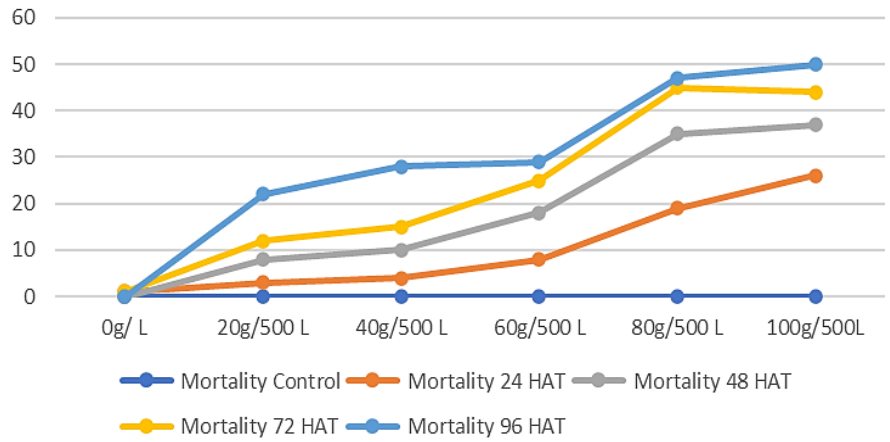

Figure 1. Development of larval mortality of *S. litura* due to treatment of *B. thuringiensis*. HAT (Hours After Treatment)

Based on the results of probity analysis, the treatment of *B. thuringiensis* was more toxic at the observation of 24 HAT and 48 HAT. This is indicated by LC50 values of 97.74 g and 70.32 g, respectively, while LC90, 147.60 g and 122.21 g, respectively, are suspected of causing test insects' death (Table 1). According to Zulfiana et al. and Devi et al. [6,24], *B. thuringiensis* given to *S. litura* larvae can cause mortality and increase mortality by 100% at 10 HSA doses of 30 g.mL$^{-1.}$ The mechanism of action of *B. thuringiensis* when entering the body of pests is to produce proteins that will interfere with/neutralize the digestive system of insects so that they cannot process food in the stomach. Protein crystals produced by *B. thuringiensis* also cause swelling, peeling, and damage to the epithelial cells of the armyworm's mid-intestine. Fundamental changes in infected mid-intestinal cells, namely the enlargement of the nucleus, changes in the endoplasmic reticulum to its configuration resembling a vacuole, and the decay or unification of microcephaly. Some strains can be used for bioinsecticides in larvae of the Order Lepidoptera [25,26].

Table 1. Estimator of toxicity of *B. thuringiensis* to mortality of *S. litura*

Time Observation (HAT)	a ± GB	b ± GB	LD_{50} (SK 90%)(%)	LD_{90} (SK 90%)(%)
24	-2.51 ± 0.49	0.03 ± 0.006	97.74 (89.70 – 110.41)	147.60 (127 – 186.30)
48	-1.74 ± 0.27	0.03 ± 0.004	70.32 (59.04 – 082.38)	122.21 (104.38 – 159.87)
72	-1.97 ± 0.36	0.04 ± 0.005	-	-
96	- 0.91 ± 0.23	0.03 ± 0.004	-	-

Notes: a: intercept, b: slope, GB: error, LD: lethal doses, SK: confidence interval

In Table 1, LC50 values at 24 and 48 HAT were 97.74 g and 70.32 g, respectively, while LC 90 was 147.60 g and 122.21 g, respectively. LC50 and LC 90 values are more specific estimators of concentration limits that can kill 50–90% of test insects within a certain period; if using concentration values below these values, it is estimated that it will not cause the death of test insects. Everyone in the population will respond if the stimulus has a more excellent value. The fastest mortality time was at 24 and 48 HAT, whereas, at observation time, 72 and 96 HAT did not appear at LC 50 and LC90 values, allegedly due to the addition of low and slow mortality of test insects.

S. litura is a pest that actively [27] damages crops and causes economic [22] losses for rice farmers because it is a limiting factor that reduces production in terms of quality and quantity. This loss is one of the reasons farmers use chemical insecticides to control the problem of plant-disturbing organisms (OPT) [28]. Attacks by *S. litura* larvae on plants can occur in the vegetative and generative phases [25]. *B. thuringiensis* is one of the microorganisms [29] that can control important pests on rice plants [30]. Naturally, *B. thuringiensis* exists in nature or agricultural lands to create control without human intervention. The balance of ecosystems in nature is essential, for biological control is one technique to maintain the balance of nature. So, the increase in pests will be linear with the development of natural enemies of the organism. The lack of larval mortality at the concentrations is likely due to the bacteria used, whose pathogenicity has been reduced [6], thus affecting mortality from test insects [8,9].

3.2. *Advanced Testing and Field Applications*

The caterpillar *S. litura* (Lepidoptera: Noctuidae) is a polyphage pest with high mobility [31] and reproductive capacity widespread throughout tropical and temperate Asia, Australia and the Pacific islands [32]. In the 2nd test, the dose of B. thuringiensis ingredients differed from that in the first test. In observing 24 HAT larvae, mortality was 3, 5, 5, 10, and 17 individuals at each test dose, while the control treatment had no mortality. Observations of 48 HAT showed an increase in dead larvae at each treatment dose of 10, 9, 10, 16 and 23 individuals. Furthermore, comments on 72 HAT showed increased mortality to 16, 17, 20, 21 and 41 individuals of *S. litura* larvae. The highest mortality was established in the observation of 96 HAT, where a dose of 50 g could kill 50 larvae of *S. litura* (Figure 2).

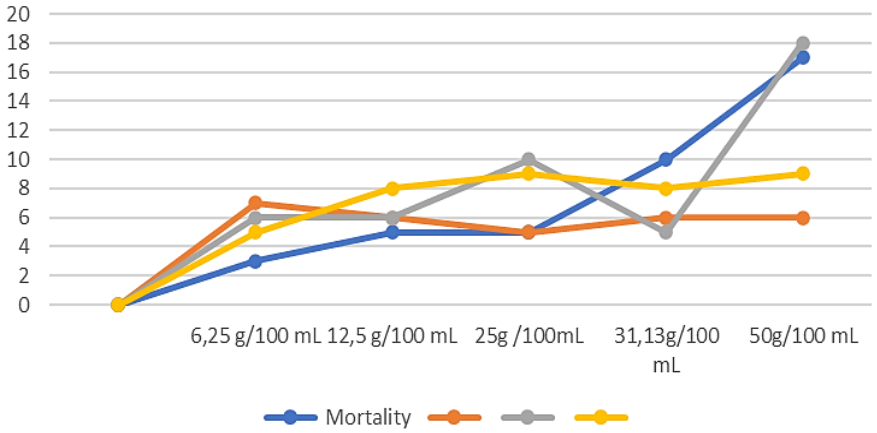

Figure 2. *S. litura* mortality due to treatment of *B. thuringiensis*

Based on the mortality data above was then analyzed to determine the relationship between dose parameters and mortality using probit analysis. Table 2 shows a dose value that is thought to be able to kill test insects with an estimator of LD50 value of 11.49 g of material and LD90 value of 90.03 g. The specificity of the insect pest host of *B. thuringiensis* can also be explained by its protein content [33].

Table 2. Estimator of toxicity parameters of *B. thuringiensis* against mortality of *S. litura* larvae in the 2nd test

Time Observation (HAT)	a ± GB	b ± GB	LD_{50} (SK 90%)(g)	LD_{90} (SK 90%)(g)
24	-2.69 ± 0.48	1.23 ± 0.33	144.30 (78.47 – 663.11)	1583.9 (421.29 – 51201)
48	-1.67 ± 0.37	0.79 ± 0.27	118.68	4785.10
72	-1.58 ± 0.34	1.14 ± 0.25	24.10	314.80
96	-1.52± 0.34	1.43 ± 0.26	11.49	90.03

Notes: a: intercept, b: slope, GB: error, LD: lethal doses, SK: confidence interval

Based on the mortality data above was then analyzed to determine the relationship between concentration parameters and mortality using probity analysis. Based on Table 2, with the estimated concentration value at each observation time, there are LC50 and LC90 values with lower concentration estimator values at 96 HAT (Table 2). This estimator value shows that to kill test insects, 50–90% within 24 HAT can use the estimator concentration range of 144.30–1583.9 g of material for up to 96 HAT. The effectiveness of microbial use is primarily determined by the toxicity properties of the microbes and the larval stadia tested or targeted. Usually, larvae with early stadia are more sensitive than those with late stadia. This is likely influenced by the size and weight of the insect's body. In small insects, the active compound is thought to reach the target site more quickly in concentrations sufficient to cause poisoning than in more giant insects [11,34].

B. thuringiensis delta endotoxin is very effective [26,35] as a stomach poison in insects that can cause larval mortality because the digestive tract absorbs it [20,36]. When sensitive insects eat spores and protein crystals, paralysis occurs, resulting in host death. *B. thuringiensis* crystals will dissolve in the digestive tract; bacteria secrete toxins that can kill insects in these tissues. Protein crystals eaten by insects will dissolve in an alkaline environment in the intestines of insects. In target insects, the protein will be activated by insect protein-digesting enzymes. The activated protein will attach to receptor proteins located on the surface of intestinal epithelial cells. This attachment results in the formation of pores or holes in the cell so that the cell undergoes lysis. Eventually, the insect will experience indigestion and die. The dead larvae turning black can be seen from observations. In addition, it can be seen that the larva's body hardens and shrinks. This condition is because the protein crystals eaten by the larvae begin in the invasion of the larval intestine and result in the formation of holes or pores in the cells so that the body of the infected larva will shrink [14,37].

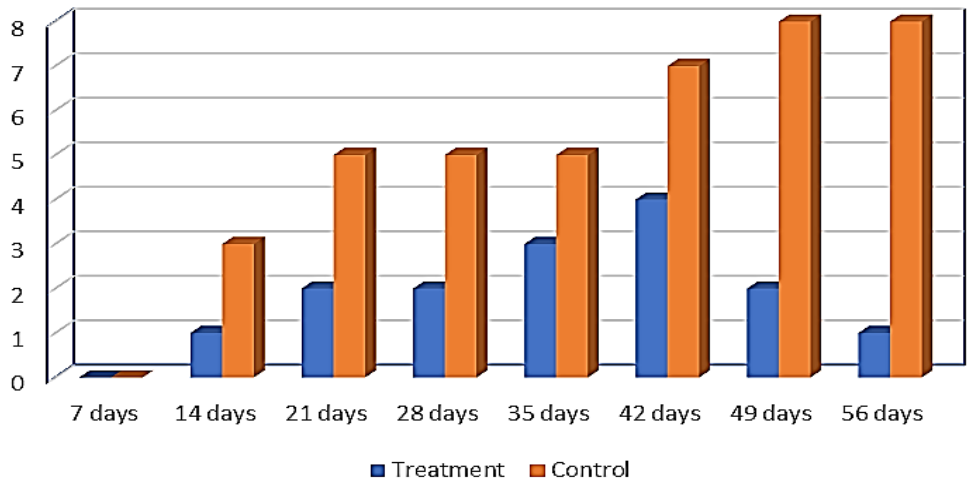

Figure 3. Insect populations of *S. litura*

Figure 3 shows that the population of *S. litura* in the spraying treatment of B. thuringiensis is lower than that of farmers. Gram-positive bacteria in *B. thuringiensis* enter the caterpillar's body, causing paralysis of the caterpillar's digestive tract.

Symptoms seen in dead caterpillars are soft bodies containing fluid, black in color, foul smelling, and their bodies are crushed and secreted black liquid. Crystal toxin is a major factor responsible for causing the death of larvae. Crystal proteins ingested by insects will dissolve in an alkaline environment in the digestive tract of insects. The dissolved prototoxin is then cut into an active toxin by the proteinase enzyme produced by the insect's digestive tract. The activated toxin then attaches to receptor proteins associated with membranes located on the surface of intestinal epithelial cells [37].

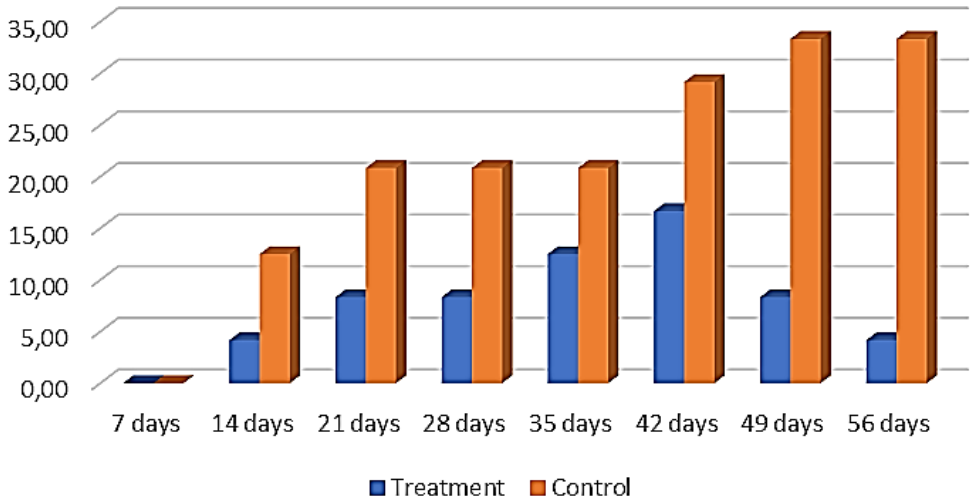

Figure 5. Intensity of attack *S. litura*

Figure 5 shows that the intensity of attacks on treatment is relatively lower than that of control (farmer land). The power of *S. litura* attacks on rice plants occurs in March or the rainy season. In spraying *B. thuringiensis* , the power of *S. litura* began to decrease at the age of 49 days. In principle, control using *B. thuringiensis* does not directly affect mortality, but after application, it will make insect pests lose appetite and cause death after a few days. This differs from using chemical insecticides because of larval mortality and being driven by nature as a stomach poison, allegedly because plants absorb the insecticide. The development of microorganisms as entomopathogens should be followed by farmers' attitudes and behaviors in reducing chemical insecticides [13,38,39]. In addition, botanical insecticides are more effective [32] than single applications when combined with microbial insecticides [28,31].

4. Conclusion

Application of *B. thuringiensis* exerts mortality influence on *S. litura* larvae in both laboratory and field testing. The highest mortality in laboratory tests was 50 larvae at a dose of 100 grams/500 ml of water. The lowest mortality was found at 20 grams of material/500 ml of water for as many as 12 larvae. In field testing, the best dose of 50 grams/14 liters of water can suppress the development of S. litura. *B. thuringiensis* is a bacterium that causes insect death and is often used to control insect pests that attack plants, and the development of microorganisms as entomopathogens must be followed by farmers' attitudes and behaviors in reducing the use of chemical insecticides.

Acknowledgements

The author would like to thank the Rector of Musamus University and LPPM Musamus University for supporting the research through DIPA Unmus 2023 funding. Thanks are also expressed to the farmer group and extension workers of Bokem Village, Merauke District, for their cooperation.

References

1. Saputro WA, Purnomo S, Salamah U. Study of food waste of farmers' households in Klaten to support food security. Anjoro Int J Agric Bus 2021;2:58–64. https://doi.org/10.31605/anjoro.v2i2.1166.
2. Sembiring J, Ofre O, Mendes J, Rante N. Respon pertumbuhan dan tingkat serangan hama penggerek batang pada berbagai varietas padi sawah (Oryza sativa L.) yang ditanam di lahan bukaan baru Kabupaten Merauke, Provinsi Papua. J Agrik 2023;34:144–53. https://doi.org/10.24198/agrikultura.v34i1.41614.
3. Damiri N, Pujiastuti Y, Mulawarman, Astuti DT, Afriani SR, Rahim SE. Short Communication: Biological control agent for Spodoptera litura on vegetable plants. Biodiversitas 2022;23:2609–13. https://doi.org/10.13057/biodiv/d230543.
4. Nelly N, Hamid H, Yunisman Y, Pratama AS, Nawir W. The diversity of insects in West Sumatera's local rice by planting refugia as an effort to conserve natural enemies. IOP Conf. Ser. Earth Environ. Sci., vol. 497, Padang: IOP Publishing Ltd.; 2020, p. 012032. https://doi.org/10.1088/1755-1315/497/1/012032.
5. Isnawan BH, Ramadhanti R. Insect population in rice ecosystems with various types of irrigation and local rice varieties. IOP Conf. Ser. Earth Environ. Sci., vol. 752, Yogyakarta: IOP Publishing Ltd.; 2021, p. 012011. https://doi.org/10.1088/1755-1315/752/1/012011.
6. Krishanti NPRA, Wikantyoso B, Zulfitri A, Zulfian D. Bakteri entomopatogen sebagai agen biokontrol terhadap larva Spodoptera litura (F.). Ber Biol 2017;16:13–21. https://doi.org/10.14203/beritabiologi.v16i1.2153.
7. Widjayanti T, Aini LQ, Kusuma RR, Istiqomah. Potential entomopathogenic bacteria isolated from extreme condition area Sidoarjo Mud, Indonesia against Spodoptera litura Fab (Lepidoptera: Noctuidae). J Buana Sains 2023;23:1–12.
8. Suwarno, Maridi, Sari DP. Uji toksisitas isolat kristal protein Bacillus thuringensis (Bt) sebagai agen pengendali hama terpadu wereng hijau (Nepotettix virescens) vektor penyakit tungro sebagai upaya peningkatan ketahanan pangan nasional. Bioedukasi 2015;8:16–9. https://doi.org/10.20961/bioedukasi-uns.v8i1.3090.
9. Vineela V, Nataraj T, Reddy G, Vimala Devi PS. Enhanced bioefficacy of Bacillus thuringiensis var. kurstaki against Spodoptera litura (Lepidoptera: Noctuidae) through particle size reduction and formulation as a suspension concentrate. Biocontrol Sci Technol 2017;27:58–69. https://doi.org/10.1080/09583157.2016.1247433.
10. Septiana L, Solikhin, Hariri AM. Pengaruh konsentrasi dan lama fermentasi ekstrak biji mahkota dewa (Phaleria macrocarpa) terhadap mortalitas hama keong emas (Pomacea sp.) di rumah kaca. J Agrotek Trop 2016;4:135–40. https://doi.org/10.23960/jat.v4i2.1862.

11. Lestari F, Suryanto E. Efikasi Bacillus thuringiensis terhadap hama ulat daun gaharu Heortia vitessoides. J Penelit Hutan Tanam 2012;9:227–32. https://doi.org/10.20886/jpht.2012.9.4.227-232.
12. Fitri, Aminah SN, Abdullah T, Widarawati R, Annisa NW. Insect pest in rice field with legumes embankment applied by Beauveria bassiana vuill. IOP Conf. Ser. Earth Environ. Sci., vol. 807, IOP Publishing Ltd.; 2021, p. 022108. https://doi.org/10.1088/1755-1315/807/2/022108.
13. Wibowo CI. Efektivitas bacillus thuringiensis dalam pengendalian larva nyamuk Anopheles sp. Biosfera 2017;34:39–46. https://doi.org/10.20884/1.mib.2017.34.1.469.
14. Salaki CL, Tarore D, Manengkey G. Prospek pemanfaatan biopestisida bakteri entomopatogenik isolat lokal sebagai agen pengendali hayati hama tanaman sayuran. Eugenia 2013;19:1–7.
15. Afifah L, Mutsla Afifah D, Surjana T, Kurniati A, Maryana R. Produksi cendawan Entomopatogen Beauveria bassiana menggunakan substrat kaya pati dan infektifitasnya terhadap Tribolium castaneum. J Ilmu Dasar 2022;23:139–48. https://doi.org/10.19184/jid.v23i2.21435.
16. Arsi A, Pujiastuti Y, Kusuma SSH, Gunawan B. Eksplorasi, isolasi dan identifikasi Jamur entomopatogen yang menginfeksi serangga hama. J Prot Tanam Trop 2020;1:70–6. https://doi.org/10.19184/jptt.v1i2.18554.
17. Amirhusin B. Penggunaan Bacillus thuringiensis sebagai Bio-Insektisida. Bul Agrobio 2014;5:21–8.
18. Gazali A, Ilhamiyah. Hama penting tanaman utama dan taktik pengendaliannya. Banjarmasin: Unversitas Islam Kalimantan Muhammad Arsyad Al-Banjary Banjarmasin; 2022.
19. Bravo A, Gill SS, Soberón M. Mode of action of Bacillus thuringiensis Cry and Cyt toxins and their potential for insect control. Toxicon 2007;49:423–35. https://doi.org/10.1016/j.toxicon.2006.11.022.
20. Rajagopal R, Sivakumar S, Agrawal N, Malhotra P, Bhatnagar RK. Silencing of midgut aminopeptidase N of Spodoptera litura by double-stranded RNA establishes its role as Bacillus thuringiensis toxin receptor. J Biol Chem 2002;277:46849–51. https://doi.org/10.1074/jbc.C200523200.
21. Belousova ME, Malovichko Y V., Shikov AE, Nizhnikov AA, Antonets KS. Dissecting the environmental consequences of bacillus thuringiensis application for natural ecosystems. Toxins (Basel) 2021;13. https://doi.org/10.3390/toxins13050355.
22. Vengateswari G, Arunthirumeni M, Shivakumar MS. Effect of food plants on Spodoptera litura (Lepidoptera: Noctuidae) larvae immune and antioxidant properties in response to Bacillus thuringiensis infection. Toxicol Reports 2020;7:1428–37. https://doi.org/10.1016/j.toxrep.2020.10.005.
23. Dadang, Prijono D. Pengembangan teknologi formulasi insektisida nabati untuk pengendalian hama sayuran dalam upaya menghasilkan produk sayuran sehat. J Ilmu Pertan Indones 2011;16:100–11.
24. Devi S, Saini HS, Kaur S. Assessing the pathogenicity of gut bacteria associated with tobacco caterpillar Spodoptera litura (Fab.). Sci Rep 2022;12:8257. https://doi.org/10.1038/s41598-022-12319-w.
25. Rahman A, Hamid H, Resti Z. Pathogenicity of endophytic bacteria as entomopathogens against Spodoptera litura Fabricius. (Lepidoptera: Noctuidae). Crop J Plant Prot 2023;6:10–8. https://doi.org/10.24198/cropsaver.v6i1.44665.

26. Mafazah A, Zulaika E. Potensi Bacillus thuringiensis dari tanah perkebunan Batu Malang sebagai Bioinsektisida terhadap Larva Spodoptera litura F. J Sains Dan Seni ITS 2017;6:E82–6. https://doi.org/10.12962/j23373520.v6i2.27447.
27. Rahayu SE, Leksono AS, Gama ZP, Tarno H. The effect of papaya leaf extract (Carica papaya L.) on the mortality rate of Spodoptera litura Fabricius Larvae and the level of damage to soybean leaves in Malang, Indonesia: A greenhouse simulation. AGRIVITA J Agric Sci 2023;45:20–30. https://doi.org/10.17503/agrivita.v45i1.3745.
28. Hermanto A, Mudjiono G, Afandhi A. Penerapan PHT berbasis rekayasa ekologi terhadap wereng batang coklat Nilaparvata lugens Stal (Homoptera: Delphacidae) dan musuh alami pada pertanaman padi. J HPT 2014;2:79–86.
29. Schünemann R, Knaak N, Fiuza LM. Mode of action and specificity of Bacillus thuringiensis toxins in the control of caterpillars and stink bugs in soybean culture. ISRN Microbiol 2014:135675. https://doi.org/10.1155/2014/135675.
30. Vakil BJ, Dalal NJ. Comparative efficacy of newer Anthelmintics. In: Jucker E, editor. Prog. Drug Res., vol. 19, Birkhäuser Basel; 1975, p. 166–175. https://doi.org/10.1007/978-3-0348-7090-0_21.
31. Afandhi A, Pratiwi VR, Hadi MS, Setiawan Y, Puspitarini RD. Suitable combination between Beauveria bassiana (Balsamo) Vuillemin and four plant leaf extracts to control Spodoptera litura (Fabricius). AGRIVITA J Agric Sci 2020;42:341–9. https://doi.org/10.17503/agrivita.v42i2.2678.
32. Lalitha C, Muralikrishna T. Laboratory evaluation of native Bacillus thuringiensis isolates against Spodoptera litura (Fabricius). Curr Biot 2012;5:428–35.
33. Pujiastuti Y, Arsi A, Sandi S. Characteristics of Bacillus thuringiensis isolates indigenous soil of South Sumatra (Indonesia) and their pathogenicity against oil palm pests Oryctes rhinoceros (Coleoptera: Scarabaeidae). Biodiversitas 2020;21:1287–94. https://doi.org/10.13057/biodiv/d210403.
34. Lestari MS, Himawan T, Abadi AL, Retnowati R. Potensi ekstrak piper methysticum (Piperaceae) sebagai insektisida botani untuk pengendalian hama Plutella xylostella. Sains Dan Mat 2014;3:26–32.
35. Legwaila MM, Munthali DC, Kwerepe BC, Obopile M. Efficacy of Bacillus Thuringiensis (var. Kurstaki) against diamondback moth (Plutella Xylostella L.) eggs and larvae on cabbage under semi-controlled greenhouse conditions. Int J Insect Sci 2015;7:39–45. https://doi.org/10.4137/ijis.s23637.
36. Abbas MST. Genetically engineered (Modified) crops (bacillus thuringiensis crops) and the world controversy on their safety. Egypt J Biol Pest Control 2018;28:1–12. https://doi.org/10.1186/s41938-018-0051-2.
37. Hanif KI, Herlinda S, Suwandi, Karenina T. Efikasi bioinsektisida Bacillus thuringiensis Barliner terhadap Gryllus bimaculatus De Geer (Orthoptera: Gryllidae) pada tanaman padi utama dan ratun. J Lahan Suboptimal J Suboptimal Lands 2017;6:95–105.
38. Wardati I, Erawati DN, Triwidiarto C, Fesdiana U. Patogenisitas bakteri, jamur dan nematoda entomopatogen terhadap hama penggerek buah kapas (Gossypium hirsutum L.). J Ilm Inov 2013;13. https://doi.org/10.25047/jii.v13i1.57.
39. Eliza T, Hasanuddin T, Situmorang S. Perilaku petani dalam penggunaan pestisida kimia(kasus petani cabai di Pekon Gisting Atas Kecamatan Gisting Kabupaten Tanggamus). J Ilmu-Ilmu Agribisnis 2013;1:334–42.