2022_BIODIVERSITAS_SPODOPTERA.pdf

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Submission date: 13-Apr-2023 09:20PM (UTC+0700)
Submission ID: 2063483049
File name: 2022_BIODIVERSITAS_SPODOPTERA.pdf (472.28K)
Word count: 4453
Character count: 23569

BIODIVERSITAS
Volume 23, Number 5, May 2022
Pages: 2609-2613

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230543

Short Communication: Biological control agent for *Spodoptera litura* on vegetable plants

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Manuscript received: 19 March 2022. Revision accepted: 27 April 2022.

Abstract. *Damiri N, Pujiastuti Y, Mulawarman, Astuti DT, Afriani SR, Rahim SE. 2022. Short Communication: Biological control agent for* Spodoptera litura *on vegetable plants. Biodiversitas 23: 2609-2613.* This study aimed to assess the population dynamics of *Bacillus thuringiensis* and its potency as a bio-agent against *Spodoptera litura*. The field study was conducted in Musi Banyuasin District, South Sumatra, Indonesia. Isolation and exploration of *B. thuringiensis* were carried out by taking the soil around the roots or rhizosphere of the fruit plants. The results showed that 13 isolates of *B. thuringiensis,* i.e., 1 isolate from *Lansium domesticum* (D_1), 3 isolates from *Artocarpus heterophyllus* ($N_{1,2,3}$), 1 isolate from *Averrhoa carambola* (B_1), 4 isolates from *Nephelium lappaceum* ($R_{1,2,3,4}$), 1 isolate from *Musa paradisiaca* (P_1), 1 isolate from *Mangifera indica* (M_1), 1 isolate from *Garcinia mangostana* (M_2) and 1 isolate from *Psidium guajava* (JB_1) were isolated from the rhizosphere of various fruit tree. The screening test results showed that isolates R_2 and R_3 had the highest toxicity, i.e., 51.14 and 50.77%, respectively, in controlling *S. litura* on vegetable plants.

Keywords: *Bacillus thuringiensis*, biological control, rhizosphere soil, *Spodoptera litura*

INTRODUCTION

Many types of pests of the Brassicaceae family that are reported to have attacked vegetable crops and caused damage in terms of quality and quantity are *Spodoptera litura*, *Plutella xylostella*, *Crocidolomia pavonana*, *Helicoverpa armigera*, *Chrysodeixis orichalcea*, *Liriomyza* sp. and *Myzus persicae* (Homoptera: Aphididae) (Supartha et al. 2014). *Spodoptera litura* is one of the important pests in vegetable crops in several countries worldwide, including Indonesia. This pest often causes a decrease in production or even crop failures as it can cause damage to leaves and fruits. This pest is polyphagous and attacks many types of vegetables. The attack by *S. litura* in Indonesia causes a decrease in the production of some crops, including soybeans and nuts, cabbage, onion, and other vegetables (Ramzan et al. 2019; Sayid et al. 2020). The crop yield loss due to attack of *S. litura* ranges between 40-70%. This pest also has a wide distribution level in various Indonesian regions, from 23 to 45 percent (Adie et al. 2012). To overcome the pest attacks, farmers generally use chemicals or insecticides. Excessive use of chemical pesticides harms the environment, disrupts the natural balance, which leads to the emergence of resistant pests, and threats to predators, parasites, fish, birds, and other animals.

One of the causes of the negative impact of pesticides on the environment is the presence of pesticide residues in the soil that can harm non-target organisms, reach to the water sources, and affect the environment. These pesticide residues reach up the food chain, which can harm consumers, both animals and humans. The negative impacts caused by the use of chemical pesticides encourage international agreements to impose restrictions on the use of chemicals in the production process, especially synthetic chemical pesticides in the control of pests in agriculture, plantation and forestry and begin to divert to the use of environmentally safe pesticides. This policy has been imposed in many countries due to the implementation of the Rio de Janeiro conference on sustainable development (Asmaliyah et al. 2010).

Bacillus thuringiensis is a gram-positive, rod-shaped, aerobic and spore-forming bacterium. One of the characteristics of *B. thuringiensis* is that it has a protein crystal that is toxic to insects (Palma et al. 2014; Peralta and Palma 2017). *Bacillus thuringiensis* has a specific target, so it does not kill insects that are not targeted and are easy to unravel and do not accumulate and pollute the environment (Hermanto et al. 2013; Kimani et al. 2019). *Bacillus thuringiensis* is highly toxic to specific host insects, thus harmless to natural enemies and non-targeted living creatures such as humans and other organisms. *Bacillus thuringiensis* may form spores during the growth cycle phase. Spores containing crystals, most of which consist of one or more cry proteins and/or Cyt (also known as delta-endotoxins) that can serve as specific insecticides and have been used as topical pesticides to protect plants (Bravo et al. 2011; Georgia et al. 2011; Schünemann et al. 2014).

Very few studies have been conducted on exploring *B. thuringiensis* dynamics in the fruit plantation land and studying its potency in controlling *S. litura*. This study aimed to assess the population dynamics of *B. thuringiensis* and its potency as a bio-agent against *S. litura*.

MATERIALS AND METHODS

The research was conducted in the fruit garden in the District of Musi Banyuasin, South Sumatra, Indonesia, and at the Phytopathology Laboratory of the Department of Plant Pests and Diseases Faculty of Agriculture Sriwijaya University Inderalaya South Sumatra.

Isolation of *Bacillus thuringiensis*

Isolation and exploration of *B. thuringiensis* were carried out by taking the soil crops around the rhizosphere of fruit plants, i.e., duku (*Lansium domesticum*), rambutan (*Nephelium lappaceum*), jackfruit (*Artocarpus heterophyllus*), banana (*Musa paradisiaca*), starfruit (*Averrhoa carambola*), mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*) and guava (*Psidium guajava*) in Musi Banyuasin District, South Sumatra. Isolation of *B. thuringiensis* from the soil was carried out according to the procedure of Rusmana and Hadioetomo (1994).

The isolation procedure was initiated by adding 1g soil in 9 mL of sterile distilled water and then mixed until homogeneous. To make a dilution of 10-1 to 10-6, take 1 ml of the sample suspension and mix it with 9 mL of distilled water (10-1) and shake until homogeneous. The procedure was repeated until 10-6 was achieved. 0.1 mL of dilution series, i.e., 10-3 and 10-6 was transferred into NA medium containing petri dishes and then spread evenly using a sterile spatula. Petri dishes were incubated at 28°C for 48 hours. Identification was made through several stages of morphological observation, gram reaction test, catalyst, gram staining, and spore staining.

Toxicity test of *Bacillus thuringiensis*

The toxicity test of *B. thuringiensis* against *S. litura* was done through the preparation stage of insect test and preparation of isolate *B. thuringiensis*. Larvae of *S. litura* were obtained from the Chinese cabbage plantation area. The larvae were maintained until they reached to the second generation. The third instar larvae produced from the second generation were used in the toxicity test. Preparation of isolate *B. thuringiensis* was done by making each isolate suspension with a density of 106/mL. Chinese cabbage leaves were washed and then put in the suspension of each isolate until wet. Furthermore, the leaves were removed and dried. The leaves of Chinese cabbage were then placed into plastic petri dishes size 15 cm × 15 cm and covered with filter paper. A total of 5 instar larvae of *S. litura* were infected into the leaves inside a plastic petri dish, triplicate was maintained for each treatment and mortality was observed.

RESULTS AND DISCUSSION

Isolation and population of *Bacillus thuringiensis*

The result showed that a total of 13 isolates of *B. thuringiensis* were isolated from 100 samples of soil around the rhizosphere of the fruit plants, i.e., 1 isolate from *Lansium domesticum* (D_1), 3 isolates from *Artocarpus heterophyllus* ($N_{1,2,3}$), 1 isolate from *Averrhoa carambola* (B_1), 4 isolates from *Nephelium lappaceum* ($R_{1,2,3,4}$), 1 isolate from *Musa paradisiaca* (P_1), 1 isolate from *Mangifera indica* (M_1), 1 isolate from *Garcinia mangostana* (M_2) and 1 isolate from *Psidium guajava* (JB_1). All bacterial isolates showed similar morphological features as *B. thuringiensis*: white and yellow rounded colonies, wavy and slippery edges, and elevated elevations (Table 1).

It was also observed that the population of *B. thuringiensis* varies on the soil around the plant rhizosphere. Most colonies were found on soil around soil rhizosphere of *M. paradisiaca*, followed by *A. heterophyllus, N. lappaceum, M. indica*, *A. carambola, L domesticum, G. mangostana* and *P. guajava* (Table 2). Such population variations may be due to the differences in existing growing media. Different soil environments can greatly determine the density of a microorganism in it.

Moisture is one of the factors that can determine the type and population of a microbe. The soil under a shade has different inorganic components in each type and soil layer so that it affects the growth of types of microorganisms that exist. The presence of large shade also results in less light intensity, lower temperatures, water content, and more nutrients, and is a possible factor in the large population of bacteria in the soil. Soil microbial communities are structured by climate, soil physical and chemical factors such as pH and soil organic matter (Thomson et al. 2015).

Table 1. Morphology and bacterial physiology of *Bacillus thuringiensis*

Isolate codes	Morphological test				Biochemical test	
	Form	Color	Edge	Elevation	Catalyst test	Gram test
D_1	Round	White	Slippery	Arise	Positive	Positive
N_1	Round	White	Wavy	Arise	Positive	Positive
N_2	Round	White	Wavy	Arise	Positive	Positive
N_3	Round	White	Wavy	Arise	Positive	Positive
B_1	Round	White	Wavy	Arise	Positive	Positive
R_1	Round	White	Wavy	Arise	Positive	Positive
R_2	Round	White	Slippery	Arise	Positive	Positive
R_3	Round	White	Slippery	Arise	Positive	Positive
R_4	Round	White	Slippery	Arise	Positive	Positive
P_1	Round	White	Slippery	Arise	Positive	Positive
M_1	Round	White	Wavy	Arise	Positive	Positive
M_2	Round	White	Wavy	Arise	Positive	Positive
JB_1	Round	White	Wavy	Arise	Positive	Positive

Note: D_1: isolate from rhizosphere *L. domesticum*; $N_{1,2,3}$: isolate from rhizosphere *A. heterophyllus*; $R_{1,2,3,4}$: *N. lappaceum;* B_1: isolate from rhizosphere *A. carambola*; P_1: isolate from rhizosphere *M. paradisiaca*; M_1: isolate from rhizosphere *M. indica*; M_2: isolate from rhizosphere *G. mangostana* and JB_1: isolate from rhizosphere *P. guajava*

Table 2. The population of *Bacillus thuringiensis* in soil around plants rhizosphere

Origin of isolate *B. thuringiensis*	Population (cfu/g soil)
Soil from rhizosphere of *M. paradisiaca*	77.33×10^7 a
Soil from rhizosphere of *A. heterophyllus*	66.00×10^7 ab
Soil from rhizosphere of *N. lappaceum*	36.77×10^7 bc
Soil from rhizosphere of *M. indica*	36.00×10^7 bc
Soil from rhizosphere of *A. carambola*	34.33×10^7 bc
Soil from rhizosphere of *L. domesticum*	33.33×10^7 bc
Soil from rhizosphere of *G. mangostana*	15.33×10^7 c
Soil from rhizosphere of *P. guajava*	6.00×10^7 c

Note: Numbers followed by the same letter mean that there is not significant difference at $p \leq 0.05$ DMRT

In the case of soil in which banana plants were grown, although the canopy was not wide, rapid plant growth in clumps allows the provision of more suitable media for microbial soil growth. The population of *B. thuringiensis* obtained from the soil around the rhizosphere of banana (*M. paradisiaca*) was more when compared with others. This could possibly be due to the environmental conditions of banana crops. The density of bacteria population from the soil around the roots also depends on the rooting type of plants where it grows. Rooting systems also appear to support habitats or growth mediums of microorganisms in terms of water availability and temperature changes (He et al. 2009; Sarr et al. 2010). However, factors such as temperature, precipitation, organic matter, soil physical and chemical properties can also affect bacterial populations in the soil (Sessitch et al. 2004). The population of bacteria is very high at the soil surface, and its amount decreases with increasing soil depth. Plants play an important role in determining microbial diversity in the rhizosphere. Plant roots cause physical and chemical changes in rhizospheric soil, affecting the diversity of microbes in and around the rhizosphere. The root exudates select to invite or fight certain microbial populations. Many plants have tolerant or resistant to microbial attacks in the rhizosphere. Plant varieties also establish the diversity of rhizosphere microbial communities (Widyati 2013).

According to Omotayo and Omotayo (2020), several factors contribute to the existence and survival of the microbes, among them, soil containing beneficial microbes is the most important one for agricultural practices. Numerous microbes have inhibited the plants rhizosphere. The ability of plants to establish symbiosis relations with soil microbes also determines the microbial community in the rhizosphere. Plant age and health level also play an important role in determining the dynamics of microbial communities in land surrounding rhizosphere. Carbon availability in the rhizosphere is sometimes a limitation. Still, generally, bacteria produce leghemoglobin compounds to activate nitrogenize enzymes and require no competition with other rhizosphere microbes in carbon utilization (Mahmud et al. 2020).

Bioassay of *Bacillus thuringiensis* against *Spodoptera litura*

A total of 13 isolates of *B. thuringiensis* from the soil of rhizosphere have been tested for toxicity against *S. litura*. Infected larvae with *B. thuringiensis* showed symptoms of slow movement and decreased eating activity. This is in line with a study by Tampubolon et al. (2013), which reported that *S. litura* infected with *B. thuringiensis* had decreased appetite and its movements became slow or even immobile. The dead larvae showed swelling, blackish-brown skin broke and discharged foul-smelling fluid. The body of the larvae became smaller and thinner (Figure 1). According to Nawrot-Esposito et al. (2020), infected larvae of *B. thuringiensis* are smaller and thinner because the digestive system is destroyed and lysed so that the larvae die.

Protein insecticides synthesized by bacteria include crystal proteins known as delta endotoxins (Cry and Cyt toxin) and vegetative insecticidal protein (Vip), such as Vip 1, Vip 2 and Vip 3. Vip Protein 1 and Vip 2 are active binary toxins against Coleoptera, while Vip 3 protein is active against Lepidoptera (Frankenhuyzen 2009; Chakroun et al. 2016).

Results of the screening test of *B. thuringiensis* isolates showed a significant effect. Further results of the test of toxicity effects of *B. thuringiensis* isolate are presented in Table 3.

A B

Figure 1. Healthy *Spodoptera litura* (A) and *S. litura* infected by *Bacillus thuringiensis* (B)

Isolates R_3 and R_2 showed the highest toxicity, i.e., 51.14 and 50.77%, respectively. Eight other isolates also showed a tendency to be toxic to *S. litura* larvae. Isolate N_3 from jackfruit, isolate R_1 from rambutan and isolate M_1 from mango showed the lowest toxicity between 17.80-22.03%, not significantly different with control and dipel (commercial trademark of *B. thuringiensis*). The high toxicity of R_3 and R_2 can be attributed to their ability to produce protein crystals during sporulation. The protein crystals produced by *B. thuringiensis* could be toxic to the digestive system of insects (Nawrot-Esposito et al. 2020). *B. thuringiensis* act as a digestive toxin where d-endotoxins (proteins) that can undergo proteolysis become actively attached to epithelial cells that cause balance of cell osmosis irritated and caused cell breaking and insect death. The protein crystals that insects eat undergo hydrolysis by the protease enzyme to become a toxin. The protein toxin attached to the receptor protein located on the surface of epithelial cells of the intestine destroys the structure and function of epithelial cells in the insect's digestive tract, resulting in lysis and decreased appetite, and insect death (Schünemann et al. 2014). *Bacillus thuringiensis* at a density of 106/mL spore density can result in high mortality on target insects because *B. thuringiensis* has high virulence (Afriani et al. 2017; Astuti 2017; Ghazwan et al. 2017; Pujiastuti et al. 2018; Sayid et al. 2020). The results of the deadline of insect mortality (LT_{50}) applied by bacterial isolates of *B. thuringiensis* showed that the ability to kill insects of each isolate tested varied between an average of 2.94 to 7.43 days. Isolate code R_1 showed the highest (7.43 days) LT_{50}, while isolate *B. thuringiensis* with code B_1, R_2 and N_2 showed the lowest LT_{50} value, which was about 2.94-2.97. Table 3 indicated that the highest mortality was shown by isolate R_3 code that was equal to 51.14%, although its LT_{50} was an average of 4.71 days. Isolate R_2 was a fairly good isolate due to its short killing time of 2.96 days and the percentage of mortality reached 50.77% (Table 4).

Table 3. Toxicity of *Bacillus thuringiensis* on the mortality of *Spodoptera litura* larva

Isolate codes	Larvae mortality percentage (%)
R_3	51.14 a
R_2	50.77 a
D_1	47.30 ab
P_1	46.92 ab
B_1	43.07 ab
R_4	39.23 ab
N_1	35.01 ab
JB_1	34.63 ab
N_2	30.09 ab
M_2	26.56 ab
N_3	22.03 ab
R_1	17.80 b
M_1	17.80 b
Control	26.56 ab
Dipel	26.56 ab

Note: Numbers followed by the same letter mean that there is not significantly difference at $p \leq 0.05$ DMRT

Table 4. Lethal time (LT50) of *Spodoptera litura* larvae treated with *Bacillus thuringiensis*

Soil codes	LT_{50} (day)	Confidence interval 95%		Regression equation
		Lowest	Highest	
D_1	3.79	2.70	5.04	Y = 0.733x - 2.782
N_1	4.47	2.01	7.27	Y = 0.733x - 3.284
N_2	2.96	2.03	3.98	Y = 0.733x - 2.173
N_3	5.78	4.90	6.74	Y = 0.733x - 4.237
B_1	2.94	2.05	3.91	Y = 0.733x - 2.160
R_1	7.43	5.99	9.10	Y = 0.733x - 5.450
R_2	2.96	2.03	3.97	Y = 0.733x - 2.173
R_3	4.71	3.65	5.92	Y = 0.733x - 3.455
R_4	4.71	3.65	5.92	Y = 0.733x - 3.455
P_1	3.79	2.70	5.04	Y = 0.733x - 2.782
M_1	4.55	3.87	5.24	Y = 0.733x - 3.342
M_2	5.78	4.91	6.74	Y = 0.733x - 4.239
JB_1	3.88	3.12	4.68	Y = 0.733x - 2.849
Dipel	4.71	3.65	5.92	Y = 0.733x - 3.455
Control	3.89	3.00	4.88	Y = 0.733x - 2.855

Note: D_1: isolate from rhizosphere *L. domesticum*; $N_{1,2,3}$: isolate from rhizosphere *A. heterophyllus*; $R_{1,2,3,4}$: *N. lappaceum;* B_1: isolate from rhizosphere *A. carambola*; P_1: isolate from rhizosphere, *M. paradisiaca*; M_1: isolate from rhizosphere *M. indica*; M_2: isolate from rhizosphere *G. mangostana*; and JB_1: isolate from rhizosphere *P. guajava*

Isolate R_2 showed the shortest time of death, which was 2.96 days, with a mortality rate of 50.77%. Allegedly this isolate, other than producing protein crystals, also produce other compounds so that the virulence level is high. *B. thuringiensis* may produce factors associated with bacterial virulence levels, such as phospholipase C, proteases and hemolysins (Infante et al. 2010; Nisnevitch et al. 2010; Martin et al. 2011). *Bacillus thuringiensis* produces a toxin that kills Lepidoptera larvae in two or three days. The toxin effects are due to the disturbance of the larval intestinal physiology and reduced protein digestion (Nawrot-Esposito et al. 2020). This is in line with the results of the study by Ghazwan et al. (2017), who found high toxicity of *B. thuringiensis* against *Tuta absoluta* larvae of instar 1, 2, and 3, pests on tomato plants.

ACKNOWLEDGEMENTS

This work was supported by "*Program Penelitian Unggulan Profesi*" University of Sriwijaya, Bandar Lampung, Indonesia.

REFERENCES

Adie MM, Krisnawati A, Mufidah ZA. 2012. The degree of resistance of soybean genotypes to *S. litura* caterpillar. In: Pratiwi H, Winarto A (eds). Increased Competitiveness and Implementation of Peanut and Bulb Commodities Development Supports the Achievement of Four Agricultural Developments. Proceedings of the National Seminar on the Results of Research on Various Nuts and Tuber. Center for Food Crops Research and Development, Malang, 5 July 2012. [Indonesia]

Afriani SR, Pujiastuti Y, Irsan C, Damiri N, Nugraha S, Sembiring ER. 2017. Isolation and toxicity test *Bacillus thuringiensis* from Sekayu

region soil, South Sumatera on *Spodoptera litura*. IOP Conf Ser: Earth Environ Sci 102: 012066. DOI: 10.1088/1755-1315/102/1/012066.

Asmaliyah E, Wati H, Utami S, Mulyadi K, Yudhistiran, Sari FW. 2010. Introduction of Vegetable Pesticide-producing Plants and Their Traditional Use. Ministry of Forestry. Agency for Forestry Research and Development, Center for Research and Development of Forest Productivity, Jakarta.

Astuti DR, Pujiastuti Y, Suparman SHK, Damiri N, Nugraha S, Sembiring ER, Mulawarman. 2017. Exploration of *Bacillus thuringiensis* Berl. from soil and screening test its toxicity on insects of lepidoptera order. IOP Conf Ser: Earth Environ Sci 102: 012063. DOI: 10.1088/1755-1315/102/1/012063.

Bravo A, Likitvivatanavong S, Gill S, Soberon M. 2011. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochem Mol Biol 41: 423-431. DOI: 10.1016/j.ibmb.2011.02.006.

Chakroun M, Banyuls N, Bel Y, Escriche B, Ferre J. 2016. Bacterial vegetative insecticidal protein (Vip) from entomopathogenic bacteria. Microbiol Mol Biol Rev 80: 329-350. DOI: 10.1128/MMBR.00060-15.

Frankenhuyzen KV. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal protein. J Invertebr Pathol 101 (1): 1-6. DOI: 10.1016/j.jip.2009.02.009.

Georgia S, Ravijay B, Richard MT, Teresa C, Paul C. 2011. *Bacillus thuringiensis*: A century of research, development and commercial applications. Plant Biotechnol J 9 (3): 283-300. DOI: 10.1111/j.1467-7652.2011.00595.x.

Ghazwan A, Ashouri A, Talaei-Hassanloui R. 2017. Evaluation of *Bacillus thuringiensis* to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory condition. Agric Sci (8): 591-599. DOI: 10.4236/as.2017.87045.

He RI, Wang GP, Liu XH, Zhang CL, Lin FC. 2009. Antagonistic bioactivity of an endophytic bacteriaum isolated from *Epimedium brevicornum* Maxim. Afr J Biotechnol 8 (2): 191-195.

Hermanto SE, Jusuf, Hero MS. 2013. Exploration of toxin protein *Bacillus thuringiensis* from land in Tangerang Regency. Valence 3 (1): 58-56. DOI: 10.15408/jkv.v3i1.329. [Indonesia]

Infante I, Morel MA, Ubalde MC, Martinez-Rosales C, Belvisi S, Castro-Sowinski S. 2010. Wool-degrading *Bacillus* isolates: Extracellular protease production for microbial processing of fabrics. World J Microb Biotechnol 26 (6): 1047-1052. DOI: 10.1007/s11274-009-0268-z.

Kimani PG, Nyambaka H, Kasina M. 2018. Screening and partial characterization of d-endotoxins from some local *Bacillus thuringiensis* isolates for insecticidal activity against the spotted stem borer. Biofarmasi J Nat Prod Biochem 17: 21-38. DOI: 10.13057/biofar/f170103.

Mahmud K, Makayu S, Ibrahim R, Missaoui S. 2020. Current progress in nitrogen-fixing plants and microbiome research. Plant 9 (97): 1-17. DOI: 10.3390/plants9010097.

Martin PAW,Gundersen-Rindal RE, Blackburn MB. 2011. Distribution of phenotypes among *Bacillus thuringiensis* strains. Syst Appl Microbiol 33 (4): 201-208. DOI: 10.1016/j.syapm.2010.04.002.

Nawrot-Esposito MP, Babin A, Pasco M, Poirie M, Gatti JL, Gallet A. 2020. *Bacillus thuringiensis* bioinsecticides induce developmental defects in non-target *Drosophila melanogaster* larvae. Insect 11: 697. DOI: 10.3390/insects13050427.

Nisnevitch M, Sigawi S, Cahan R, Nitzan Y. 2010. Isolation, characterization and biological role of camelysin from *Bacillus thuringiensis* subsp. Israelensis. Curr Microbiol 61 (3): 176-183. DOI: 10.1007/s00284-010-9593-6.

Omotayo OP, Babalola OO. 2020. Resident rhizosphere microbiome's ecological dynamic and conservation: towards achieving the envisioned sustainable development goal, a review. Intl Soil Water Conserv Res 9 (1): 127-142. DOI: 10.1016/j.iswcr.2020.08.002.

Palma L, Munoz D, Berry C, Murillo J, Caballero P. 2014. *Bacillus thuringiensis* toxins: An overview of the biocidal activity. Toxins (6): 3296-3325. DOI: 10.3390/toxins6123296.

Peralta C, Palma L. 2017. Is the insect world overcoming the efficacy of *Bacillus thuringiensis*? Toxin 9 (39): 3390-3395. DOI: 10.3390%2Ftoxins9010039.

Pujiastuti Y, Astuti DT, Afriyani SR, Suparman S, Sembirig ER, Nugraha S, Damiri N. 2018. Characterization of *Bacillus thuringiensis* Berl, indigenous from soil and its potency as biological agents of *Spodoptera litura* (Lepidoptera: Noctuidea). IOP Conf Ser: Earth Environ Sci 102: 012064. DOI: 10.1088/1755-1315/102/1/012064.

Ramzan M, Murtaza G, Javaid M, Iqbal N, Raza T, Arshad A, Awais M. 2019. Comparative efficacy of newer insecticides again *Plutella xylostella* and *Spodoptera litura* on cauliflower under laboratory condition. Ind J Pure App Biosci 7 (5): 1-7. DOI: 10.18782/2320-7051.7796.

Rusmana I, Hadioetomo RS. 1994. Isolation of *Bacillus thuringiensis* Berl. from silkworm farms and their toxicity to the larvae of Crocodolomia binotalis Zell and *Spodoptera litura* F. Hayati 1 (1): 22-23.

Sarr PS, Yamakawa T, Asatsuma S, Fujimoto S, Sakai M. 2010. Investigation of endophytic and symbiotic features of *Rasltonia* sp. TSCI isolated from cowpea nodules. Afr J Microbiol Res 4 (19): 1959-1963.

Sayid Z, Ali A, Usman M, Mujahid A, Jafar B, Kashif A, Bashir HS, Abbas Q, Tariq MU, Shakeel MD, Sultan Y, Qureshi MH, Akhtar N. 2020. Toxicity of *Bacillus thuringiensis* against second instar larvae of *Spodoptera litura* on different host plants. J Sci Agric 4: 93-95. DOI: 10.25081/jsa.2020.v4.6464.

Schünemann R, Knaak N, Fiuza LM. 2014. Mode of action and specificity of *Bacillus thuringiensis* toxins in the control of caterpillar and stink bugs soybean culture. Intl Schol Res Notices Microbiol 2014: 135675. DOI: 10.1155/2014/135675.

Sessitch A, Reiter B, Berg G. 2004. Endophytic bacteria communities of field grown potato plants and their plant growth-promoting abilities. Can J Microbiol 50 (4): 239-249. DOI: 10.1139/w03-118.

Supartha NPEY, Susila IW, Yuladhi KA. 2014. Diversity and density of parasitoids associated with *Plutella xylostella* L. (Lepidoptera: *Plutella lidae*) in cabbage plants without application and application of insecticides. J Trop Agrotechnol 3 (1): 13-21.

Tampubolon DY, Pangestiningsih Y, Zahara F, Manik F. 2013. *Bacillus thuringiensis* and *Metharhizium anisoplie* pathogens test against the mortality of *Spodoptera litura* Fabr (Lepidoptera: Noctuidae) in the laboratory. Jurnal Agroekoteknologi 1 (3): 783-793. DOI: 10.32734/jaet.v1i3.3004.

Thomson BC, Tisserant E, Plassart P, Uroz S, Griffiths RI, Hannula SE, Buee M, Mougel C, Ranjard L,Van Veen JA, Martin F, Bailey MJ, Lemanceu P. 2015. Soil condition and land use intensification on effects on soil microbial communities across a range European field site. Soil Biol Biochem (88): 403-413. DOI: 10.1016/j.soilbio.2015.06.012.

Widyati E. 2013. Rhizosphere microbes community dinamic and it contribution on plants growth. Tekno. Hutan Tanaman 6 (2): 55-64.

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