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Utilization of *Bacillus thuringiensis* in controlling armyworms (*Spodoptera litura*) on tomato (*Solanum lycopersicum*) plants

Akhmad Rizali*

Department of Agroecotechnology, Faculty of Agriculture, Lambung Mangkurat University, South Kalimantan, Indonesia

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Abstract

Tomato plants are horticultural commodities that can provide benefits to farmers, beside the market demand that continues to increase, the cultivation method is easy. Tomatoes are also one type of vegetable plant that contains many vitamins and proteins that have been known by elderly people. Numerous chemical insecticides have been used in order to control pests, which damage for agriculture. While they are too expensive in the developing countries and harmful to both human and the environment. In addition, target insect pests rapidly develop biological resistance especially at higher rates of application. The chemical insecticides are still contributing to human life enormously, but they have been distributed in ecological system of organisms including human beings because of their low specific toxicity to any organism and their low specific toxicity to any organism and their slight decomposition in nature. An alternative control is needed with microbial insecticide which is using *B. thuringiensis B. thuringiensis* used in this study is *B. thuringiensis* which is already commercial.Then carried out purification as follows *B. thuringiensis* concentration of 5g per liter of water, 10g per liter of water, 15g per liter of water, 20g per liter of water. In treatment *B. thuringiensis*10g per liter of water can stop eating at 2 hours after application, and has been able to control as much as 75 percent.

***Corresponding Author:** Akhmad Rizali ✉ arizali25@yahoo.com

Introduction

Tomato plants are horticultural commodities that can provide benefits to farmers, beside the market demand that continues to increase, the cultivation method is easy. Tomatoes are also one type of vegetable plant that contains many vitamins and proteins that have been known by elderly people.

Based on data from the service of food crops and horticulture in south Kalimantan province in 2014, productivity of tomato plants in the region are 473 tons with a harvest area of 56 ha. That means only 8.446 tons/hectare of fresh fruit is obtained, this proves that there is still a lack of productivity of tomato plants that are able to produce 12 tons/hectare of fresh fruit. This is caused by the presence of armyworm pest attacks which can reduce production (Syukur *et al*, 2015).

Numerous chemical insecticides have been used to control *S. litura*. While chemical insecticides have knock down effect, they are too expensive harmfull to both humans and the environment. In addition, target insect pests develop biological resistance rapidly especially at higher rates of application. Thus, the increase in pesticidal application to control this pest has urged to researcher to search for biological control alternatives that would be a good component of Integrated Pest Management.

Bacillus thuringiensis is a gram-positive, spore-forming bacterium that produceparasporal crystal during the sporulation stage. The crystal is made of one or more proteins toxic to some insect species. Most strains of *B. thuringiensis* produce delta-endotoxin crystal toxic to lepidopteran insect such as moth (Dulmage, 1971).

In recent year, the need for environmentally safe pesticides has encouraged the search *B.thuringiensis* from the soil as an insect pathogen and as possible agent to use in the control of the*C. Binotallis* larvae.*B. thuringiensis* is a gram-positive soil bacterium, and produce a crystalline inclusion body during sporulation (Bulla *et al*., 1980).

This parasporal body is composed of proteins termed "delta-endotoxin", and specifically toxic to insects. In addition, *B. thuringiensis* produce another toxins namely: alpha-toxin, beta-exotoxin, and gamma-exotoxin. All of the toxic substance may not present in the bacterium (Heimpel, 1967).

In another hand, Krieg (1961) has defined various toxic substance produced *B. thuringiensis* as follow: (a) thermolabileendotoxic; (b) thermostable exotoxin; (c) bacillogenic antibiotic; (d) lecithinase; (e) proteinase. Most strains of *B. thuringiensis* produce delta-endotoxin crystals toxic to lepidopteran insects such as moth (Dulmage *et al*., 1970). The objective of the studies to survey, collect imago insect in the field, determine when the insect larvae stop feeding and mortality.

Materials and method

Bacterial source

B.thuringiensis used in the research is *B. thuringiensis* (Javelin WG) which has been sold in the market, then purification is carried out.

Experimental design

The design used in this study was a completely randomized design with 5 treatments and 4 replications as follows:

A0 : without *B. thuringiensis*

A1 : *B. thuringiensis* concentration 5 g/liter of water

A2 : *B. thuringiensis* concentration 10 g/liter of water

A3 : *B. thuringiensis* concentration 15 g/liter of water

A4 : *B. thuringiensis* concentration 20 g/liter of water

Rearing of Armyworm (S. litura)

The field-collected larvae were placed in plastic trays (13.5x22x6cm). For adequate ventilation, two 4- x 2-inch holes were cut out from they tray covered over which nylon screens were fastened. The bottom of these plastic trays were lined with strips of tissue paper which serve as moisture absorbent and pupation medium for fully-grown larvae.

This technique also facilitated tray cleaning. In some cases however, a first and`second instar larvae were

placed in one tray. The some procedure was followed for the third and forth instar larvae. The larvae were provided daily with fresh cabbage leaves. Three- to four-day old larvae of the insect were transferred into separate rearing trays thickly lined with tissue paper with soil on top which served as pupation sites. The pupae were collected and kept in clean petri dishes until emergence. From this rearing, there were 200 instars 3 used as experimental material, then the larvae were fasted for 3 days and then infested with 10 larvae to tomato plants. After *B. thuringiensis* application by spraying on experimental plants.

Growing Media

Planting media transfer into small polybags 8x9cm size, then F1 varieties of tomato seeds sown into polybags that have been filled with media, after that the seeds that are 1 week old can be cultured on the bag that has been prepared as treatment material.

The cage is made of gauze with a wire frame, the size of the cage is 30x100cm, the gauze cage is used to cover polybags containing tomato plants, in order to prevent that attack of other plant pest organisms.

Results and discussion

Stop feeding

Based on the test results on stop feeding, it was shown that the application of *B. thuringensis* at a concentration of 10g per liter of water was not significantly different from the treatment of *B. thuringiensis* 15g per liter of water and 20g per liter of water but very different from the treatment of 5g per liter of water and 0g per liter of water (Table 1.). In the results of the observation shown that *B. thuringiensis* 10g perliter of water was able to stop eating all armyworm at 2, 3, 4, 5, 6, and 7 hour after application, and this procedure is followed in *B. thuringiensis* of 15 and 20g per liter of water. this is cause by gave concentration of medium doses to high doses at the treatment. It is possible that the number of spores consumed by the test insect is more rapid and developing in their body. Causing the larva to stop eating due to poisoning. Steinkraus *et al.* (2018), Spray table tests with *B. thuringiensis* (Javelin WG on wheat leaves against armyworm, *Pseudaletia unipuncta* showed that 1st and 3rd instars had LC50s of 0.09 and 0.55kg per ha, respectively, 7d after treatment, at the higher rate stop feeding and mortality at 2 days 76%

Table 1. Effect of *B.thuringiensis* concentration to armywormsstof feeding.

Treatment	Stop Feeding (tail)						
	1 HAA	2 HAA	3 HAA	4 HAA	5 HAA	6 HAA	7HSA
B. thuringiensis 0 g/liter of water	0	0	1	2	2	3	3
B. thuringiensis 5 g/liter of water	2	2	3	5	5	7	7
B. thuringiensis 10 g/liter of water	3	7	9	10	10	10	10
B. thuringiensis 15 g/liter of water	5	10	10	10	10	10	10
B. thuringiensis 20 g/liter of water	9	10	10	10	10	10	10

Description: HAA = Hour After Application.

Symtomatology

Observation on of *B. thuringiensis* from tomato plants on armyworms showed that infected insect larvae turned yellowish at the middle and hind parts of the abdomen (Fig. 1B). The integument also turned brown to black as the infection progressed and the body became sticky because of oral and anal discharges. Dead larvae become shrunken and later turned black with putrid odor (Rizali, 2017).

Mortality

Based on observations showing that *B. thuringiensis* from 1-7 days after application had a significant effect on larval mortality. On 2 days after the *B. thuringiensis* treatment, concentration of 10g per liter of water can kill 75% of the armyworm larva, followed by treatment on the the third day to the seven days after application (Table 2.). Giving *B. thuringiensis* with a concentration of 10, 15, and 20g per liter of

water has been able to kill all test insects when compared with without giving *B. thuringiensis* (control). According to Hasinu (2009) that the higher the concentration, the more spores there will be, so the number of spores consumed in the test insect will be greater. Aguskrisno (2011), stated that protein crystals consumed by insect will dissolve in alkaline environments in the intestines of insects. In target insects, these proteins will be activated by insect protein digesting enzymes, in the end the insects will be anal discharges until dead larvae.

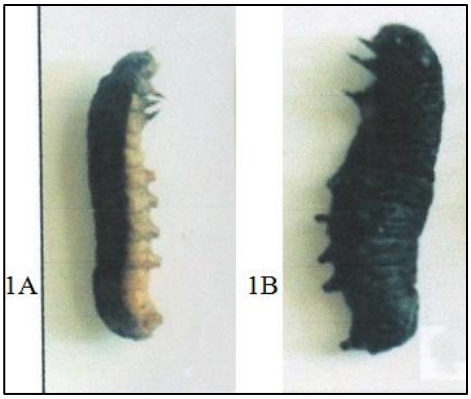

Fig. 1. Healthy insect and infected insect

Table 2. Effect of *B. thuringiensis* concentration to armyworms mortality.

Treatment	% Mortality						
	1 DAA	2 DAA	3 DAA	4 DAA	5 DAA	6 DAA	7 DAA
B. thuringiensis 0 g/liter of water	0	0	0	1	2	2	2
B. thuringiensis 5 g/liter of water	0	3	22	25	27	30	35
B. thuringiensis 10 g/liter of water	0	75	90	95	100	100	100
B. thuringiensis 15 g/liter of water	0	100	100	100	100	100	100
B. thuringiensis 20 g/liter of water	0	100	100	100	100	100	100

Description: DAA= Day After Application.

Conclusion

On 2 days after the *B. thuringiensis* treatment, concentration of 10g per liter of water *B. thuringiensis* as a microbial insecticide effective to control armyworms larvae. So itcan kill 75% of the armyworms larvae.

References

Aguskrisno. 2011. Use of *Bacillus thuringiensis* as a biopesticide. http://education. Kompasian.com. 30 Desember 2011.

Aroson A. 2002. Sorulation and delta-endotoxin synthesis by *Bacillus thuringiensis*. Cell Mol. Life Sci **59,** 417-425.

Asano S, Bando H, Iizuka T. 1993. Amplification and identification of *cryII* genes from *Baccilusthuringiensis* by PCR procedures. J. Seric. Sci. Jpn **62,** 223-227.

Asano S. 1996. Identification of cry gene from *Bacillus thuringiensis* by PCR and isolation of unique insecticidal bacteria. Mem. Fac. Agric. Hokkaido Univ **19,** 529-563.

Baba F, Asano S, Iizuka T. 1990. Purification of crystals from *Bacillus thurigiensis*by using Percoll. J. Sci. Jpn **59,** 487-489.

Balarman K, Hoti SL, Manonmani LM. 1981. An Idigenous virulent strain of *Bacillus thuringiensis*, highly pathogenic and specific to mosquitoes. Current Science **50,** 199-200.

Boonserm P, Mo, Angsuthana M, Sombat, C, Lescar J. 2006.Structure of the functional form of the mosquito larvicidal cry 4Aa toxin from Bacillus thuringiensis at 2.8-angstrom resolution. J. Bacteriol **188,** 3391-3401.

Bourquet S. 2004. Resistance to *Bacillus thuringiensis* toxin in the Eruropean corn borer: what chance for *Bacillus thuringiensis* maize?. Physiol. Entomol **29,** 251-256.

Bulla LA, Jr, Kramer KJ, Davidson LI. 1977. Characterization of the enmocidalparasporal crystal of *Bacillus thuringiensis*. J. Bacteriol **130,** 375-383.

Delucca AJII, Simonson JG, Larson AD. 1981. *Bacillus thuringiensis* distribution in soils of the United States.Canadian J. Maicrobiol **27,** 865-870.

Dulmage HT. 1992. Insecticidal activity of *Bacillus thuringiensis* and their potential for pest control in Microbial control for pests and plant diseases and plant diseases 1970-1980 (Ed.H.D Burges). Acad. Press. N.Y. PP.

Golberg LJ, Margalit J. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaeniaunguiculata, Culexunivitattus, Aedesaegypt*i and *Culexpipiens*, Mosq. New **37,** 355-358.

Hasinu JV. 2009. Isolation and *B.thuringiensis* pathogenicity test against *Crocidolomia binotalis* Zell. Agriculture cultivation journal **5(2),** 84-88.

Hastowo S, Lay BW, Ohba M. 1992. Naturally occurring *Bacillus thuringiensis* in Indonesia. J. Appl. Bacteriol **73,** 108-113.

Heimpel AM. 1967. A critical review of *Bacillus thuringiensis*Berl.And other crystalliferous bacteria. Ann. Rev. Entomol **12,** 287-322.

Held GA, Kawanishi CY, Huang YS. 1990. Characterization of the parasporal inclusion of *Bacillus thuringiensis* subsp. *Kyushuensis*. J. Bacteriol. 481-483.

Iizuka T, Ishino M, Nakajima T. 1982. Comparative morphology of Parasporal crystal and characterization of plasmid DNA from various subspecies of entomopathogenic bacteria, *Bacillus thuringiensis*. J. Fac. Agric. Hokkaido Univ **13,** 423-431.

Iizuka T, Sasaki J, Asano S, Bando H. 1995. Comparative studies on isolation and identification of *Bacillus thuringiensis*. Biotechnology and Enviro. Benefits, Vol **I,** 143-153.

Iizuka T, Yamamoto T. 1984. Serological properties of the mosquitocidal protein of *Bacillus thuringiensis* and the morphology of its parasoral crystal. J. fac. Hokkaido Univ **62,** 98-114.

Ishii, T, Ohba M. 2013. Investigation of mosquito-specific larvicidal activity of a soil isolate of *Bacillus thuringiensi* serovar *Canadensis*. Curr. Microbiol **35,** 40-43.

Kalman S, Kiehne KK, Libs JL, Yamamoto T. 1993. Cloning of novel cry IC-type gene from a strain *Bacillus thuringiensis* subs. Galleriae. Appl. Enviro. Microbio **59,** 1131-1137.

Kawalek MD, Benjamin S, Lee HL, Gill SS. 1995. Isolation and identification of novel toxin from a new mosquitocidal isolate from Malaysia, *Bacillus thuringiensis* subsp. *Jegathesan*. Apl. Enviro. Microbiol 2965-2969.

Kim K, Ohba H, aizawa K. 1984. Purification of the toxic protein from *Bacillus thuringiensis* serotype 10 isolate demonstrating a preferential larvicidal activity to mosquito. J. Invertebr. Pathol. **44,** 214-219.

Kreig A, Huger A, Langenbruch G, Schentter W. 1983. *Bacillus thurigiensis* isolate with activity againtColeoptera. In Biotechnology in invertebrate pathology and cell culture. Karl Maramorosch (ed.) p. 101-114.

Laemmli UK. 1970. Cleavage of structural proteins during tha assembly of the head of bacteriophage T4. Nature (London) **277,** 680-685).

Lee SG, Eckblad W, Bulla LA. 1985. Diversity of protein inclusion bodies and identification of mosquitocidal protein in *Bacillus thuringiensis* subsp. Israelensis. Biochem. Biophys. Res. Commun **126,** 953-960.

Lopez-Pazos SA, Martinez JM, Castilo AX, Samanca JAC. 2009. Present and significant of *Bacillus thuringiensis* Cry proteins associated with the Andean weevil *Premnotry pesvorax* (Coleotera: *Curculionidae*).

Ohba M, Aizawa K. 1986. Insect toxicity of *Bacillus thuringiensis* isolated from soils of Japan. J. Invertebr. Pathol **47,** 12-20.

Padua LE, Ohba M, Aizawa K. 1984. Isolation of a *Bacillus thuringiensis* strain (serotype 8a:8b) highly and selectively toxic against mosqouito larvae. J. Invertebr. Pathol **44,** 12-17.

Pfannenstiel MA, Ross EJ, Kramer VC, Nickerson KW. 1984. Toxicity and composition of protease-inhibited Bacillus thuringiensis var. israelensiscrystal. FEMS Microbio.Lett **21,** 39-42.

Poopathi S, Abidha S. 2010. Mosquitocidal bacterial toxin (*Bacillus spaehariicus*and *Bacillus thuringiensis* serovar *israelensis*): mode of action, cytopathological effects and mechanism of resistance.

Rizali A, Shin-ichiro Asano, Ken Sahara, Hisanori Bando, Bibiana W, Lay, Sugyo Hastowo and Toshihiko Iizuka. 1998. Novel *Bacillus thuringiensis*serovaraizawai strains isolated from mulberry leaves in Indonesia. Appl. Entomol. Zool **33(1),** 111-114.

Rizali A. 2017. Occurrence of *Bacillus thuringiensis* from different plant areas on South Kalimantan, Indonesia. JBES **11(6),** 53-58.

Steinkraus DC, Young SY. 2018. Bacillus thuringiensis for use against armyworm, Pseudaletia unipuncta (Lapidoptera: Noctuidae), On Wheat. Home Vol 82 No. **2.**

Syukur M, Helfi ES, Rudi H. 2015. Planting tomatoes in the rainy season. Jakarta.