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Toxicity of *B. thuringiensis* isolates from Indonesia and Philippines against *Crocidolomia binotalis* Zell

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Abstract

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. Ten larvae were used per isolate. Mortality was noted from 24 hours after treatment. Larvae death was determined by touching them gently with a toothpick to detect any movement. In one treatment the leaves were dipped only in distilled water before feeding them to the test inscts. The last treatment served as the control. Ten-second instar larvae were used per replication with 3 replicates per concentration and with 7 concentrations per isolate. The control larvae were fed with petchay leaves soaked in distilled water. Evaluations were conducted in two trials and each trial was conducted on separated days. The data were corrected using Abbot's formula. The initial screening of 145 *B. thurngiensis* isolates obtained from BIOTECH Philippines and one from Indonesia, showed 40 isolates with varying toxicities ranging from 40 to 100% mortality. The LC_{50} of the four isolates compared with the standard are as follow: #13 (63.34), L3 (161.17), L26 (185.12), SCSP6 (175.48) and the standard isolate (HD-1) is 51.93.

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Introduction

Cruciferous vegetables such as cabbage (*Brassica oleracea* var. *capitata* L.), petchay (*Brassica compristis* var. *chinensis*), radish (*Raphanus sativus* L.), and mustard (*Brassica junceaL.*) as horticultures are economically important crops (Sunarjono, 2005). One of the constraints in the production of cruciferous vegetables is the infestation of insect pests especially the cabbage moth (*Crocidolomia binotalis* Zell.). This insect greatly reduce both yield and quality of the produce (Pfadt, 1985). Cabbage moth, *C. binotalis* Zell. (Lepidoptera: Pyraustidae) is considered the most important limiting factor for a successful production of cruciferous vegetable not only in the Indonesia but in other country in the world. The larva feeds on foliage from seedling to harvest causing 100% yield loss if not control (Rejesus and Sayaboc, 1990).

Numerous chemical insecticides have been used to control *C. binotalis*. While chemical insecticides have knock down effect, they are too expensive harmfully 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 (Rizali. 1998). The insecticidal properties of *Bacillus thuringiensis* were recognized many years before the bacterium was identified, with some account suggesting that *B. thuringiensis* spores may have already been in used an ancient Egypt. In the modern era, the bacterium was isolated in 1901 by the Japanese biologist Shigetane Ishiwatari during an investigation into wilt disease in silk worm, and he named it *Bacillus sotto*. Ten years later, the same bacterium was isolated by Ernst Berliner from disease Mediterranean flour moth (*Ephestia kuehniella*) in the German province of Thuringia, and it was named *Bacillus thurimgiensis* (Siegel, 2001),

B. thuringiensis is a gram-positive soil bacterium, and produce a crystalline inclusion body during sporalation. This parasporal body is composed of proteins termed "delta-endotoxin", and specifically toxic to insects. In addition, *B. thuringiensis* produce another toxins namely: alpha-exotoxin, beta-exotoxin, and gamma-exotoxin. All of the toxic substances may not be present in the bacterium. In another hand, various toxic substance produced by *B. thuringiensis* as follow: (a) thermolabile endotoxin; (b) thermostable exotoxin; (c) bacillogenic antibiotic; (d) lecithinase; (e) proteinase (Iishi, *et al*, 2013).

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.The objective of the studies to determine the distribution, screening and LC_{50} of *B. thuringiensis* From Indonesia and Philippines against *Croidolomia binotalis* Zell

Materials and methods

The field-collected larvae were placed in plastic trays (13.5 x 22 x 6cm). For adequate ventilation, two 4- x 2-inch holes were cut out from the 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 instars larvae were placed in one tray. The some procedure was followed for the third and fourth 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.

Pathogenicity Test

Fifty isolates of *Bacillus* from Indonesia soil samples and 145 isolates of *B. thuringiensis* from the collection of the Microbial Insecticides Laboratory, national Institutes of Biotechnology and Applied Microbiology (BIOTECH) Philippines were screened.

A Loopful of the sporulated bacterial cultures from NA were dissolved in 10ml sterile distilled water and shaken using a magnetic stirrer until a uniform suspension was obtained. Bacterial suspension (10^{+1} spore per ml) with a corresponding optical density reading were tested on second instar larvae. Prior to treatment, larvae were starved for two hours before colonizing them on petchey dipped in desired bacterial suspension for three minute. Ten larvae were used per isolate. Mortality was noted from 24 hours after treatment. Larvae death was determined by touching them gently with a toothpick to detect any movement. In one treatment the leaves were dipped only in distilled water before feeding them to the test insects. The last treatment served as the control.

Final Testing

The isolates of *B. thuringiensis* that gave the highest mortality in the preliminary screening were further evaluated. For the production of the bacterial cells, agar slants of the selected *B. thuringiensis* isolate were used to incubate 750ml nutrient broth (NB- pH 7.6) in dextrose bottle. The bottles were incubated on rotary shaker at 100 rpm for 3 days. At the end of incubation period, the cultures were centrifuged at 5000 rpm for 20 minutes the supernatant liquid discarded. The preparation were freeze-dried and stored in the refrigerator at 4°C. seven concentration expressed in mg/l were used in determining the lethal concentration which is 50% mortality of the total population tested (LC_{50}) of the dried spore-crystal complex. Serial dilutions were made until the concentration necessary to determine LC_{50} of the isolates was found. A spreader-sticker (Tween 50-1%) was added to aid in wetting the pecthay leaves. A pecthay leaf was soaked in each bacterial preparation with known concentration prior to the introduction of the test insects starved for 3 hours. Mortality was recorded daily for 3 days.

Ten-second instar larvae were used per replication with 3 replicates per concentration and with 7 concentrations per isolate. The control larvae were fed with petchay leaves soaked in distilled water. Evaluations were conducted in two trials and each trial was conducted on separated days. The data were corrected using Abbot's formula (Reed and Muench, 1938).

$$\frac{\%\ \text{Corrected} = \%\ \text{test mortality} - \%\ \text{control mortality}}{\text{Mortality 100} - \%\ \text{control mortality}} \times 100$$

Gathering of data was started 24 hrs after treatment and daily observations were made thereafter. Larval mortalities, symptoms of infected larvae and effect on test insects that survive were recorded. The LC_{50} of the selected *B. thuriengiensis* isolates were determined using the computerized probit analysis.

Results and discussion

Pathogenicity test of different *B. thuringiensis* isolates. The initial screening of 145 *B. thuringiensis* isolates from BIOTECH Philippines and one isolate from soil samples collected in Indonesia were used against second instar larvae of *C. binotalis*. The results showed that 40 isolates have varying toxicities ranging from 40 to 100% mortality 72 hrs after treatment (Table 1).

These isolates were further screened for toxicity. Results of the second screening showed that six isolates namely #209, DD12, #13, L3, L26, SCSP 6 and HD-1 (standard isolate) were very toxic. They coused larval mortality ranging from 63.3 to 96.6% 3 days after treatment (Table. 2). The first screening had higher mortality than the second screening because in the first screening, the treatment had only one replicate with 5 ml sterile distilled water and the second one 3 replicates with 10 ml sterile distilled water. However, the second screening also revealed that majority of the other isolates were not toxic against *C. binotalis* larvae. It is probable that most of the *B. thuringiensis* isolates that were screened may be toxic to different insect species (Padua *et al.*, 1984). According to Padua some of these isolates were toxic to Diamondback moth (DBM) and slug caterpillars. Brorwnbridge (1990) reported that the screening and bioassay work on identified *B. thuringiensis* strains showed difference in their toxicities to *Chilo partellus*. There were also differences in the toxicity of isolates within the same bacterial subspecies. The other isolates took a longer period to kill susceptible host insects indicating that they are not potent enough on the insect target insect. Some isolates were unstable in which case they could pass the preliminary screening but may be eliminated in the second screening.

Final testing of potential isolates of *B. thuringiensis* .Six isolates including one from the commercial product (HD-1) were screened against second instar larvae of *C. binotalis*. Out of six, 4 isolates were selected for final testing. Two isolates discarded, because of low % mortality during first trial of LC_{50}tes. The comparative toxicities of the dried cells of *B. thuringiensis* isolates against *C. binotalis*is shown in (Table 3). The LC_{50} of the samples were analyzed from data obtained on percentage kill in each concentration tested through probit analysis within 95% confidence limits. Result revealed that isolate HD-1 was most toxic with an LC_{50} of 51.93 ug/ml. However, among the local isolates tested, #13 was the most toxic against *C. binotalis*. The results toxicities of the dried cells in decreasing order to *C. binotalis* were: HD-1 (51.93), #13 (63.34), L3 (161.17), L26 (185.12 and SCSP6 (195.48).

Table 1. Mortality of *C. binotalis* after 72 hours tereatment with different iolates of *B. thuringiensis* (First screening).

Isolate no	% Mortality after 72 hrs	Isolate no.	% Mortality after 72 hrs
# 13	100	D 1.6	80
E 5.5	100	D 1.6	80
E 5.1	100	# 209	60
D 1.7	100	D 1.9	60
E 5.3	100	D 1.3	60
DD 12	100	D 1.2	60
# 192	100	BT37	60
LED 13	100	# 1	60
# 19	100	# 6	60
EPA 18	100	# 7	60
EPA 81	100	# 22	60
T63-L4	100	# 40	60
App 26	80	# 66	60
App 22	80	# 102	60
# 4	80	# 112	40
# 8	80	#198	40
SCSP 6 (1)	80	LEP 16	40
SCSP 2 (3)	80	LEP 23	40
L26 (2)	80	LEP 25	40
L14 (3)	80	control	0
L3 (1)	80		

Toxicities of the dried HD-1 were seemingly stable compared with the local *B. thuringiensis* isolates studied. The local isolates #13 is also potential affective against *C. binotalis* as shown by the high percentage kill (Table 3).

The insecticidal activities of the remaining isolates were least effective since the required higher doses to achieve LC_{50} (Fig. 1).

Table 2. Mortality of *C. binotalis* after 72 hours treatment with different isolte of *B. thuringiensis* (Second screening).

Isolate no.	% Mortality 3 days after treatment
# 209	96.6
DD 12	96.6
# 13	93.3
L 3	86.6
L 26	76.5
SCSP 6	63.3
# 19	56.5
# 4	43.2
L 14	23.3
HD-1*	83.3
Control	0

Table 3. The LC_{50} values of the different *B. thuringiensis* isolates against *C. binotalis*.

Isolates	Lc50 ug/ ml	Fiducial limits Lower	Upper	Toxicity Ranking
HD-1	51.93	37.55	64.34	1
# 13	63.34	47.91	79.15	2
L3	161.17	142.63	179.91	3
L26	185.12	164.38	215.11	4
SCSP6	195.48	178.42	212.82	5

Likewise, the dosage mortality Thus #13 isolate could be used to complement other control approaches to control lepidopterous cabbage pests. Whitlock *et al.* (1990) reported that the standart *B. thuringiensis* kurstaki (HD-1) was most toxic against the tobacco cutworm (*Spodoptera litura*). Dulmage (1970) tested HD-1 in the laboratory and showed it to be the most toxic compared to other isolates against the pink bollworm, *Pectinophora gossypiella*, the tobacco budworm *Heliothis virescens* and the cabbage looper, *Trichoplusiani*. However, selected isolates of *B. thuringiensis* produced very effective toxins for use in insect control and the progress made from the first commercial production with the Mattes strains of Var. *thuringiensis* to the modern production with HD-1 isolate of var. *kurstaki* indicates that the research for new isolates and varieties can lead to sill more potent toxins (De Lucca., *et al.*, 1981). Toxicities of the dried were seemingly stable compared with the local *B. thuringiensis* isolates studied.

And the local isolate #13 is also potentially effective against *C. binotalis* by the high percentage kill. The insecticidal activities of the remaining isolates were least effective since they required higher doses to achieve LC_{50} (Fig. 1). Likewise, the dosage mortality `curve indicates less steep probit line (Fig. 2) which signifies that the test in fact required a higher concentration to attain 50% kill of the population.

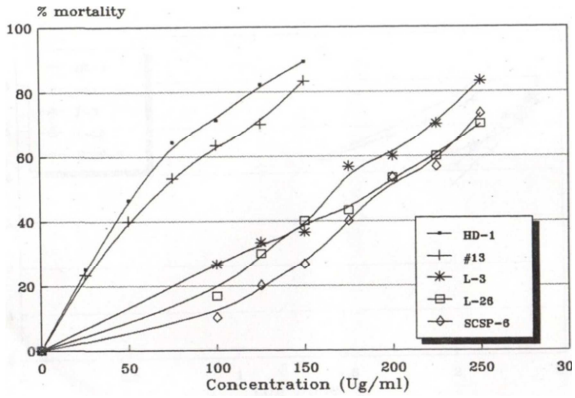

Fig. 1. Relationship between percentage of mortality and different concentrations of *B. thuringiensis* isolate against *C. Binotalis*.

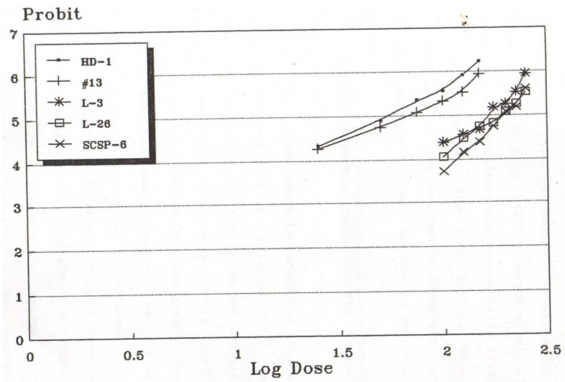

Fig. 2. Dosage-Mortality response curve of *B. thuringiensis* isolates against *C. Binotalis*.

Conclusion

145 local *B. thuringiensis* isolates from BIOTECH Philippines, The results showed that 40 isolates have varying toxicities ranging from 40 to 100% mortality 72 hrs after treatment and 1 from commercial product (HD-1) screened for toxicity against second larvae of *C. binotalis*, only 4 were selected. The dried cell local isolate # 13from Indonesia was toxic with LC50 of 63.34ug/ml followed by L3, with 161.17ug/ml, L26 with 185.12ug/ml, and SCSP 6 with 195.48ug/ml. The relative toxicities in decreasing order were: HD-1 > #13 > L3 > L26 >SCSP 6.

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