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Application of *Bacillus thuringiensis* and *Bacillus cereus* in the Field to Control the Lepidopterial Pest

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Abstract : An insect can develop evolutionary resistance to a strain of *Bacillus thuringiensis* or *Bacillus cereus* if the relationship between the two occurs continuously. The solution to this problem is to look for strains of bacteria that have never been exposed to target insects. The aim of this study was to determine the efficacy of *B. thuringiensis* in *P. xylostella, C. binotalis* and *S. litura*; and *B. cereus* in *P. xylostella*. The experimental design is a complete random factorial design with five replications. Two levels of cabbage varieties namely Grand 11 and Ishito, and three levels of agents namely *B. thuringiensis*, *B. cereus* and sterile water (control). The responses of the Grand 11 and Ishito varieties to the attack of *P. xylostella, C. binotalis* and *S. litura* were the same. *B. thuringiensis* was effective in controlling *P. xylostella, C. binotalis* and *S. litura*, while *B. cereus* was only effective in controlling *P. xylostella*. Population density of *P. xylostella* sprayed with *B. thuringiensis* on Ishito varieties was significantly different from Grand 11, while in other interactions the same.
Keywords : *Bacillus thuringiensis,Bacillus cereus,Xylostella plutella,Crocidolomia binotalis,Spodoptera litura.*

Introduction

Probably the best biopesticide and widely used comes from *B. thuringiensis*. Bacteria commonly found on this land were first discovered in Japan in 1901 by Ishawata and later in 1911 in Germany by Berliner. After that thousands of strains of *B. thuringiensis* were found. Each strain produces unique insecticidal or delta-endotoxin crystals. This *B.thuringiensis* poison can affect species of the order Coleoptera, lepidoptera and diptera. These toxins from bacteria are very specific for certain insect pests and are therefore safe for most useful insects and other animals. Additionally, toxins*B. thuringiensis* is biodegradable and not persistent in the environment[1].

The first commercial insecticide from *B. thuringiensis*, Sporine, was produced in France in 1938 and was used primarily to control flour moths. In the United States, *B. thuringensis* was first commercially fabricated in 1958[2]. An insect that is continuously exposed to *B. thuringiensis* toxin will develop a mechanism for its resistance to poisons. In Hawaii, Florida and New York, *P. xylostella* has lost its sensitivity to the toxin of *B. thuringiensis* after 30 years sprayed with a suspension of the poison[1].

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Until now thousands of strains of *B. thuringiensis* have been found and each strain may produce different insecticidal crystalline proteins. *B. thuringiensis* in the Gunung Masarang region has existed for thousands of years and has never been isolated so that it is *B. thuringiensis* strains that have never been contacted with lepidopteran pests in cabbage plants. So these pests are probably still vulnerable to the *B. thuringiensis* protein insecticides. The workings of toxins from these bacteria are generally as follows: when toxins are swallowed by insects, proteins are processed in a proteolytic manner, cross the peritrophic membrane, and bind to high affinity receptors in the middle intestinal epithelium. Furthermore, the integrity of the damaged membrane forms a pore, resulting in electrolyte imbalance which ultimately kills insects[3].

B. cereus in the Mount Masarang region has never been explored and exploited in lepidopteran pests in cabbage plants. It is likely that *B. cereus* strains from these locations synthesize secondary metabolites that are toxic to these pests because these pests have not yet developed a resistance mechanism.

The study aimed to determine the efficacy of *B. thuringiensis* against *P. xylostella*, *C. binotalis* and *S. litura*; and *B. cereus* against *P. xylostella*.

Material and Methods

Preparation of suspension concentrations of *B. thuringiensis* and *B. cereus* isolates was carried out in the Laboratory of Microbiology and Plant Disease, Faculty of Agriculture, The University of Sam Ratulangi, Manado. Field tests were carried out in fields in the administrative area of Kolongan Village, Central Tomohon District, Tomohon City.

The experimental design of *B. thuringiensis* and *B. cereus* applications was based on a complete random factorial design with five replications. Factors of hybrid cabbage varieties have two levels, namely Grand 11 (V1) and Ishito (V2) varieties, and the agents that are sprayed have three levels: *B. thuringiensis* (*Bt*), *B. cereus* (*Bc*), and Control (Cont.). Plots for combinationseach treatment was planted with 10 plants, so that the number of plants from six treatment combinations (six plots) x five replications x 10 plants per plot was 300 plants. The plot distance from one another is one meter. Cultivation of cabbage plants is carried out according to the cabbage cultivation method that is commonly practiced by farmers in Tomohon, but the dosage for *B. thuringiensis* and *B. cereus*, each 3 ml / l. Cabbage cultivation by farmers in Tomohon is as follows:

1. Nursery

The most commonly used varieties of farmers in Tomohon (Rurukan Village and its surroundings) are the varieties of Grand 11 and Ishito 3 (80-90 days). The beds are made in locations that have enough sun exposure. Cabbage seeds are planted directly on the beds of planting cabbage that has been prepared in advance. The plot size is made of 2.50 x 1 m by 30 plots. The area of land prepared is 2 x 15m x 15 m. In addition to the cabbage planted onions, *S. litura* visits this area.

2. Land preparation

The land area needed is 600 m2 with a size of 30 x 15 m. The garden used for planting cabbage was hoeed as deep as 20-30 cm. The remains of plants and grass are buried in beds. The size of the beds used is 2.50 x 1 m and 30 cm high. The distance between beds with one other bed is 1 m.

3. Thinning

At the age of 25 days after the seeds are planted in experimental plots, one plant is left in each planting hole (thinning is done in the afternoon). Cabbage seeds were planted in beds with a spacing of 50 x 50 cm in rows and between rows of 60 cm with a triangle pattern. Each plot was planted with 10 plants.

4. Fertilization

Basic fertilizer per plant is given at planting, ie 1 kg of chicken manure, 2 g of urea and 10 g of petroorganic fertilizer. Supplementary fertilizers were given when 4 weeks old plants are 2 g urea and gandasil fertilizer was sprayed at the age of 7 HST, 21 HST, 35 HST and 49 HST (every 2 weeks).

5. Stitching

Plants that die or stunt growth are removed and embroidered with healthy plants.

6. Weeding

Weeding is carried out if there are already disturbing plants that can interfere with cabbage growth and at the same time with soil scrubbing.

7. Sprinkling

Watering was carried out especially at times of initial growth as needed. The soil must be moist enough but not wet. In this study, when one-month-old plants were watered because they did not rain (dry season).

8. Application of *B. thuringiensis* and *B. cereus*

B. thuringiensis and *B. cereus* isolates to be applied in the field were cultured on four NA plates to calculate bacterial cell suspension concentrations. The calculation of bacterial spores / cells uses haemacytometer. Preparation of suspension of these bacterial isolates was carried out in the Laboratory of Microbiology and Plant Disease, Faculty of Agriculture, The University of Sam Ratulangi. The method of application of this bacterial suspension is the spray method. Specifically, *B. cereus* was only applied at the beginning of planting (from 2 weeks to 30 days old), after that it was applied by spraying *B. thuringiensis* every three days until the plant was 75 days old. Note that *B. cereus* was only applied to control *P. xylostella* because the toxicity test did not cause pain in *C. binotalis* and *S. litura*.

9. Harvest

Harvesting is done after the cabbage plant has formed the crop perfectly with an average weight of 2-2.5 kg at the age of 95 days.

6. Observation and Data Analysis

Observation of pain symptoms and behavior of the three pests, and specifically larval mortality were carried out at 12th, 24th, 48th, 72th and 96th hours after treatment. Observation of symptoms of damage to cabbage plants (heavy or light) was carried out until the end of the observation.

The population density of *P. xylostella*, *C. binotalis* and *S. litura* in Grand 11 and Ishito varieties after being applied with *B.thuringiensis* and water (control) on each plot until the 6th week after treatment was analyzed by Two-way Factorial ANOVA in the program. SPSS20.

Results and Discussion

1. *B. thuringiensis* Controls *P. xylostella*

The number of *P. xylostella* larvae in Grand 11 and Ishito varieties sprayed with *B.thuringiensis* suspension and water (control) on each plot until the 6th week after treatment was presented in Table 1. Data in Table 1 were then analyzed by Two-Way factorial ANOVA in the SPSS20 program. Figure 1 points to the interaction between the controlling agents and the number of *P. xylostella* larvae in the Grand 11 and Ishito varieties.

Table 1. Population Density of *P. xylostella*Larvae in a Combination of Treatments between two Cabbage Varieties and Controlling Agents.

Cabbage Varieties	Treatments	Replication to:					Amount	Average
		1	2	3	4	5		
		……..Individual……..						
Grand II (V1)	Control	17	14	35	33	29	128	25.6
	Bt	5	4	2	2	0	13	2.6
Ishito (V2)	Control	24	45	40	74	43	226	45.2
	Bt	0	2	0	0	0	2	0.4

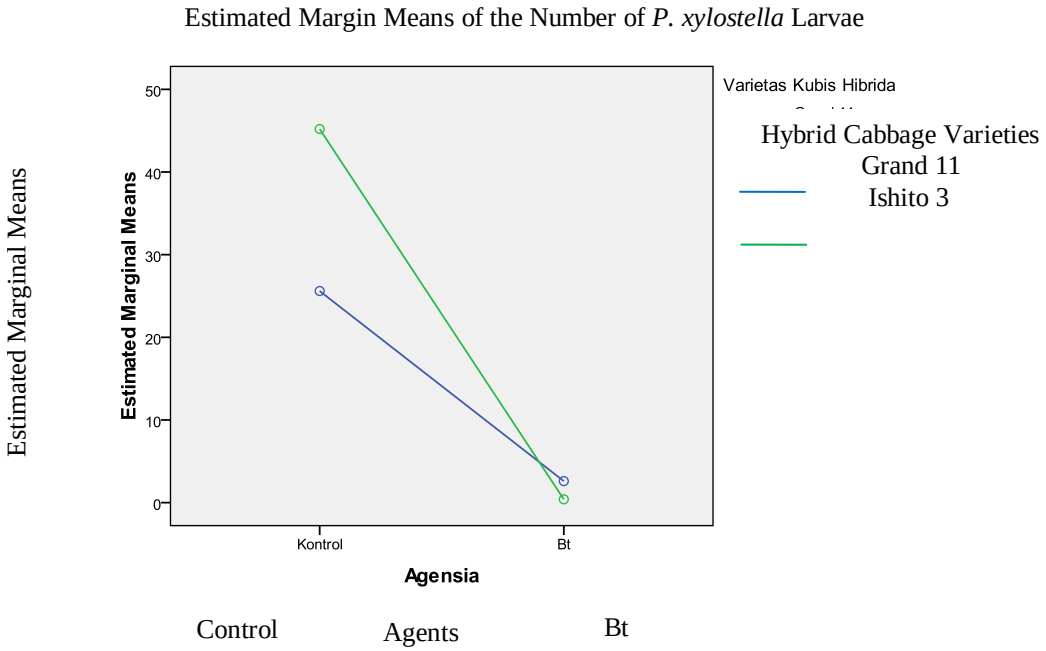

Figure 1. Interaction between Agents Control in Grand 11 and Ishito Varieties.

The p value for the main effect of the variety is 0.077, and because this value is> 0.05, it was concluded that the effect of cabbage varieties is not significant, meaning that the resistance response to *P. xylostella* is the same. From B, the p value for the agency is 0.00 (<0.05); therefore it can be concluded that the main effect of the agent on densely populated *P. xylostella* population was significant. The p value for interaction is 0.031 (<0.05), meaning the interaction is significant and it can be concluded that the effect of controlling agents on *P. xylostella* populations in varieties Grand 11 and Ishito are not the same.

In Table 1 it can be seen that the average number of *P. xylostella* larvae in the Grand 11 varieties after treatment was 25.6 individuals and those sprayed with *B. thuringiensis* 2.6 individuals; on the Ishito variety, controls 45.2 individuals and with *B. thuringiensis* 0.4 individuals. This information shows that the dense differences in the population of *P. xylostella* were significant in both cabbage varieties sprayed with water (control) and *B. thuringiensis*.

The success of *B. thuringiensis* isolates from the Gunung Masarang region in controlling *P. xylostella* in Grand 11 and Ishito varieties of cabbage is likely because *B.thuringiensis* isolates were new strains so that the first interaction with *P. xylostella* occurred, so the response - Resilience response from this pest is not yet active. According to[4] that insects which are continuously exposed to *B. thuringiensis* may activate innate cellular resistance so that they can be resistant to the insecticidal action of these bacteria. According to[5] stated that *P. xylostella* which is susceptible to *B. thuringiensis* has at least three different ICP receptors on the middle intestine brush-border membrane. The resistant *P. xylostella* strain does not have the capacity to bind Cry IA (b).

The results of research[6] that of the 39 *B. thuringiensis* isolates collected from Chikamagalur, mortality ranged from 20.30 to 83% after 72 hours of application. Among the indigenous isolates with the highest mortality have spherical crystals. Among 28 indigenous isolates from Goa, mortality was more than 70%. These isolates have crystals in the form of pyramids and balls.

Insects that are sensitive to endotoxin delta endotoxins have protease enzymes in alkaline conditions in the middle intestine. The activated endotoxin delta endotoxin-delta binds to the receptor in the vesicle membrane brush that is present in the middle intestinal epithelium and punctures the cell membrane resulting in ionic imbalances and dead insects[7].

2. *B. thuringiensis* Control *C. binotalis*

The number of *C. binotalis* larvae in Grand 11 and Ishito varieties sprayed with *B. thuingiensis* suspension and water (control) on each plot until the sixth week after treatment are shown in Table 2. The data in Table 2 are then analyzed by Two-Way Factorial ANOVA in the SPSS 20 program. Figure 2 points to the

interaction between the controlling agents and the number of *C. binotalis* larvae in the Grand 11 and Ishito varieties.

Table 2. Amount of *C. binotalis* Larvae in Combination Treatment between Two Cabbage Varieties and Control Agents.

Cabbage Varieties	Treatments	Replication to :					Amount	Average
		1	2	3	4	5		
		.....I n d i v i d u a l						
Grand II (V1)	Control	18	48	120	135	43	363	72.6
	B.thuringiensis	0	0	2	2	0	4	0.8
Ishito (V2)	Control	148	115	160	136	67	626	125
	Bt	0	0	0	0	0	0	0

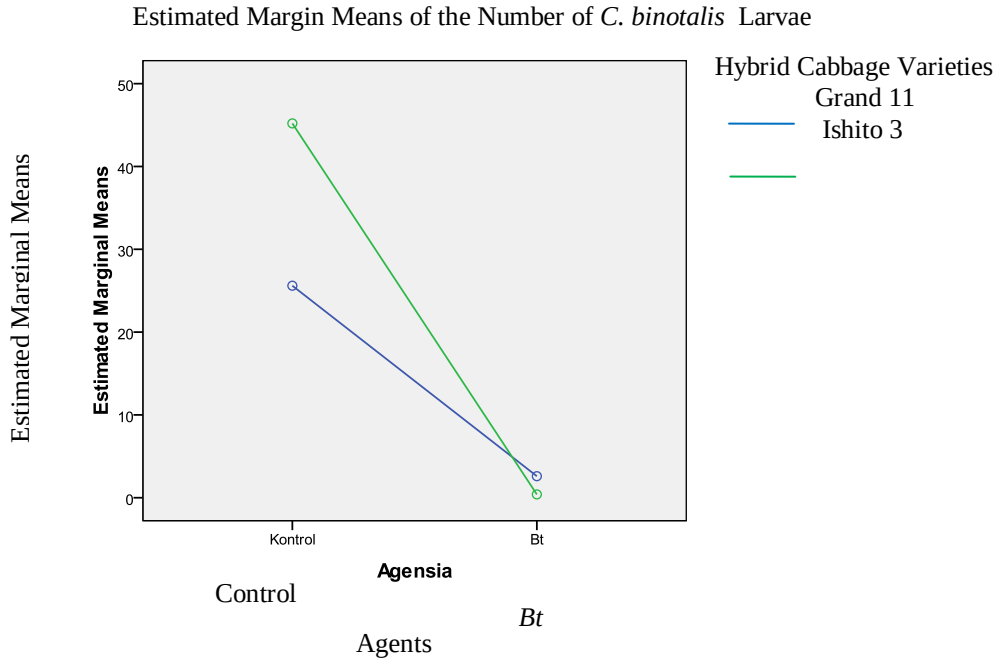

Figure 2. Interaction between Control agents and Amount of *C.binotalis* Larvae in Grand 11 and Ishito Varieties.

The p value for the main effect of the variety is 0.086, the value of this p> 0.05 means that the effect of the cabbage varieties is not significant. In conclusion, the resistance response of Grand II and Ishito varieties to *C. binotalis* is the same. The p value for the agent 0.00 (<0.05) means the main effect of the controlling agent on the number of *C. binotalis* larvae is significant. The p value for interaction is 0.07 (> 0.05), meaning that the interaction is not significant, so it can be concluded that the effects of controlling agents on the number of *C. binotalis* larvae in the Grand II and Ishito varieties are the same.

Information from Table 2 shows that the average number of *C. binotalis* larvae in the Grand II variety until the end of the observation (6th week after application), namely control 72.6 individuals and sprayed with *B. thuringiensis* 0.8 individuals; in Ishito varieties, control of 125 individuals and with *B. thuringiensis* 0 individuals. It is clear that the number of *C. binotalis* larvae in the Grand II and Ishito varieties treated with *Bt* is significantly different from the control.

Strains of *B. thuringiensis* from the Masarang Mountain region have a high ability to control *C. binotalis* in cabbage probably because new contact with these pests has occurred, so the expression of resistance genes is not yet active. According to[1] that 30 years after *B. thuringiensis* toxin was used in Hawaii, Florida and New York to control *P. xylostella*, this strain appeared which had a high resistance to *B. thuringiensis* toxin. This phenomenon also occurs in several countries including Japan, China, the Philippines, Thailand and Malaysia.

In general, insects are susceptible to *B. thuringiensis*. has two types of middle intestine receptors. The first type consists of the family N aminopeptidase which binds to CryIAc toxins. The second type is that which has the ability to bind CryIAb toxin[8].

3. *B. thuringiensis* Controls *S. litura*

The population density of *S. litura* in Grand II and Ishito varieties after application with *B.thuringiensis* and water (control) on each plot until the 6th week after treatment is shown in Table 3. The data in Table 3 were analyzed by Two-way Factorial ANOVA in the SPSS20 program. Figure 3 points to the interaction between the controlling agents and the number of *S. litura* larvae in the Grand 11 and Ishito varieties.

Table 3. Population of *S. litura* Larvae in a Combination of Treatments between Two Cabbage Varieties and Control Agents.

Cabbage Varieties	Treatments	Replicationto :					Amount	Average
		1	2	3	4	5		
		.....I n d i v i d u a l.....						
Grand II (V1)	Control	186	20	74	49	39	368	73.6
	Bt	0	0	0	2	2	4	0.8
Ishito (V2)	Control	8	4	16	41	23	92	18.4
	Bt	0	9	9	3	2	14	2.8

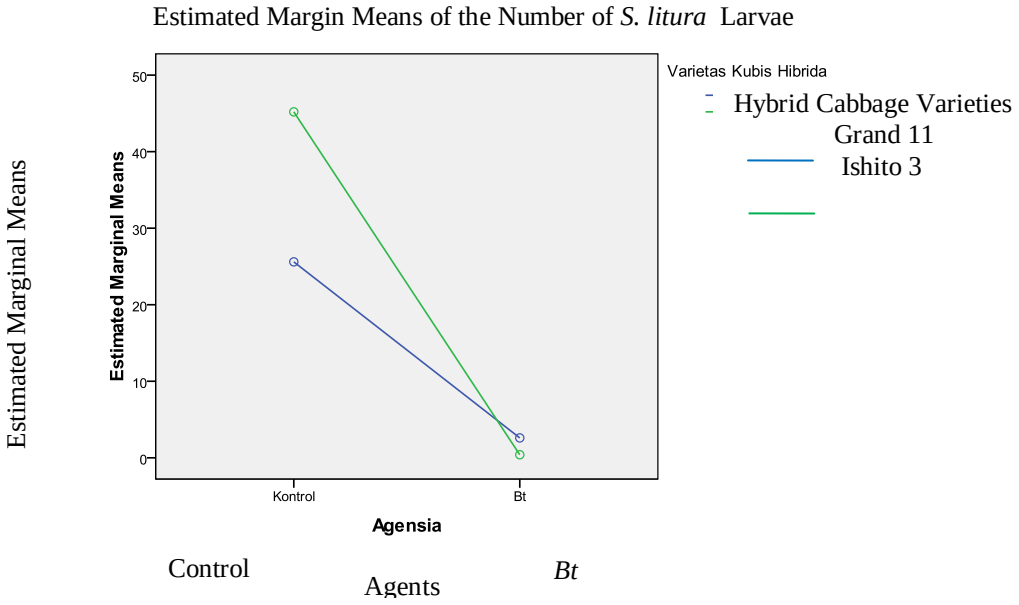

Figure 3. Interaction between Control Agens and Amount of *S.litura* Larvae in Grand 11 and Ishito Varieties.

The p value for the main effect of cabbage varieties is 0.97 (> 0.05), meaning that the effect of cabbage varieties is not significant. The conclusion of this p value is the resistance response to *S. litura* is the same. The p value for the agent is 0.01 (<0.05) so it can be concluded that the main effect of the agent on the density of the *S. litura* population is significantly different. The p value for interaction is 0.76 (> 0.05), meaning that the interaction is not significant, so it can be concluded that the number of *S. litura* larvae in the Grand 11 and Ishito varieties is the same.

In Table 3 it appears that the average number of *S. litura* larvae in the Grand 11 varieties after application is 73.6 individual controls and *Bt* sprayed 0.8 individuals; in Ishito varieties, controls 18.4 individuals and with *Bt* 2.8 individuals. This information shows that indeed controlling *S. litura* causes the population density to decrease dramatically compared to controls.

In Thailand, from 91 soil samples found 121 isolates *Bt*[9]. All isolates were tested for insecticidal activity in *S. litura* and *S. exigua*. Seven isolates included toxin activity against these pests, mortality was more than 90%. The detection of cry genes with the polymerase chain reaction shows that on the DNA chromosomes there are the CryIAb, CryIAc, CryIC, CryID, Cry II, CryGA, CryGb and Cry2A genes, whereas in plasmid DNA there are CryIAa, CryIAb, CryIAc, CryIC, CryID, CryII and Cry2A genes .

Bt isolates from the Gunung Masarang region have a large capacity to control *S. litura* probably because these pests have only been exposed to new strains from the region. Neppl (2000) states that insect resistance to *Bt* appears after approximately 30 years this bacterium has been continuously contacted.

4. *B. cereus* Controls *P. xylostella*

The population density of *P. xylostella* in Grand II and Ishito varieties sprayed with *Bc* and water (control) in each plot until the last observation (six weeks after treatment) is presented in Table 4. The data in this table is then analyzed by Two-way Factorial ANOVA SPSS20 program. Figure 4 points to the interaction between the controlling agents and the number of *S. litura* larvae in the Grand 11 and Ishito varieties.

Table 4. Population Density of *P. xylostella* in a Combination of Treatments between Two Cabbage Varieties and Controlling Agents.

Cabbage Varieties	Treatments	Replicationto :					Amount	Average
		1	2	3	4	5		
		.....I n d i v i d u a l.....						
Grand II (V1)	Control	186	20	74	49	39	386	73.6
	Bc	0	0	0	3	4	7	1.4
Ishito (V2)	Control	148	115	160	136	67	626	125.2
	Bc	3	1	0	0	0	4	0.8

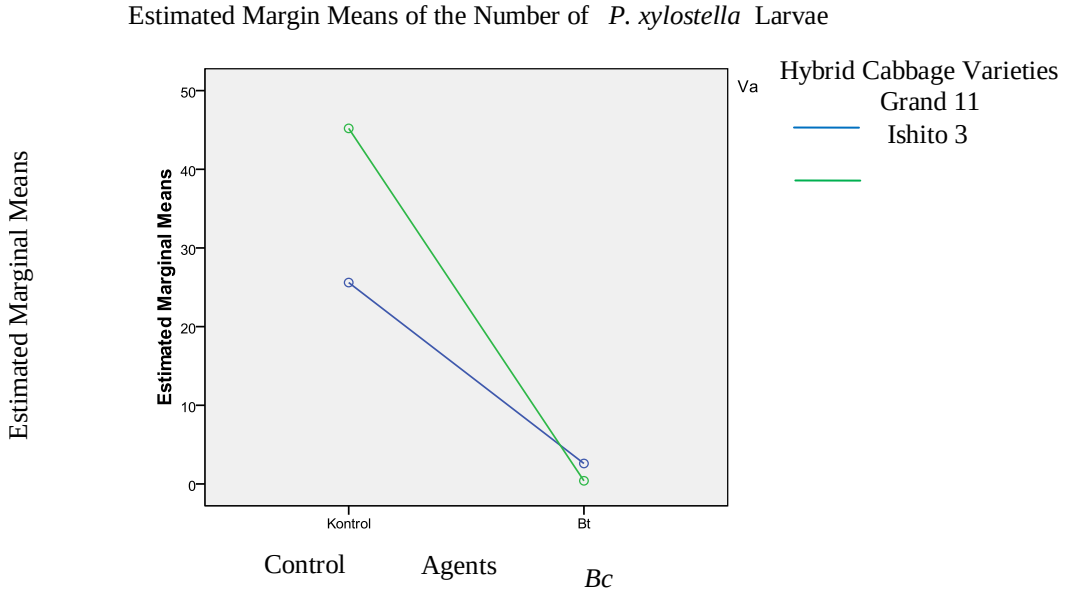

Figure 4. Interaction between Control Agents (*Bc* and Water) on the Number of *P. xylostella* Larvae in Grand 11 and Ishito Varieties.

The p value for the main effect of the variety is 0.149 (> 0.05), meaning that the effect of cabbage varieties is not significantly different. In conclusion, the resistance response of Grand II and Ishito varieties to P. *xylostella* is the same. The p value for the controlling agent is 0.00 (<0.05), so it can be concluded that the main effect of controlling agents on the densely populated *P. xylostella* population is significantly different. The

p value for interaction is not significantly different so it can be concluded that the effect of the controlling agent on the densely populated *P. xylostella* in the Grand 11 and Ishito varieties is relatively the same.

In Table 4 shows that the average number of *P. xylostella* larvae in the Grand II variety until the last observation (six weeks after treatment) is 73.6 individual controls and sprayed with *Bc* 1.4 individuals; in Ishito varieties, controls 125.2 individuals and with *Bc* 0.8 individuals. These data indicate that the effect of the controlling agent (*Bc*) on both cabbage varieties is significant.

The results of research[10] that four strains of *B. cereus* that were toxic to *Anthonomusgrandis*, *Spodoptera littoralis* and *Aphis fabae*. The toxin produced by this bacterium is some nonprotein exotoxins. According to[11] that that *B. cereus* produces cellular degradative enzymes such as phospholipases, enterotoxins and haemolysis. These compounds play a role as a cause of insect death due to degradation of the epithelium.

The *B. cereus* isolates from the Gunung Masarang region have a high ability as a controlling agent in *P. xylostella*. Applications in other lepidoptera pests that attack Cruciferae are not effective. This phenomenon may occur because each insect pest has a different response to resistance to *B. cereus*.

D. Conclusion

1. The responses of the Grand 11 and Ishito varieties to the attack of *P. xylostella*, *C. binotalis* and *S. litura*, and *B. cereus* were relatively the same.
2. *B. thuringiensis* effectively controls *P. xylostella*, *C. binotalis* and *S. litura*, in the field; whereas *B. cereus* is only effective in controlling *P. xylostella*.
3. The densely populated *P. xylostella* sprayed with *B. thuringiensis* and water surged in the Ishito variety compared to Grand 11, while in other interactions the same.

References

1. Neppl CC. Management of Resistance to *Bacillus thuringiensis* Toxins. camillapede.tripod.com/bapaper.html., 2000.
2. Ibrahim MAN, Junker GM, Bulla LA.*Bacillusthuringiensis*: A Genomics and Proteomics Perspective. www.ncbi.nih.gov/pmc/articles/PMC3035146/., 2010.
3. MacIntosh S, Stone CTB, Jokerst RS, FuchsRL. Binding of *Bacillus thuringiensis* Proteins to a Laboratory-Selected Line of *Heliothis virescens*. www.pnas.org/content/8930.full.pdf, 1991.
4. Candas M, Loseva O, Oppert B, Kosaraju P, Bulla JrLA. Insect Resistance to *Bacillus thuringiensis* Alterations in the Indianmeal Moth Larval Crut Proteome. www.mcponline.org/content/2/1/19. full. 2002.
5. Terre J, Real MD, Rie JV, Jansen S, and Peferoen M. Resistence of *Bacillus thuringiensis* Bioinsectcide in a Field Population of *Plutellaxylostella* is due to a Change in a Midgut membrane Receptor. www.ncbi.nlm.nih.gov/pnas01062-0062.pdf. 1991.
6. Maruthesh SA. 2007. Molecular Characterization and Efficacy of Native Isolates of *Bacillus thuringiensis* (Berliner) Against Cruciferous Pests with Special Reference to Diamondback. etd. uasd.edu/ft/th9618.2007.
7. Eswarapriya B, GopalsamyB, Kameswari B, Meera R, Devi P. Insecticidal Activity of *Bacillus thuringiensis* IBT-15 Strain against *Plutella xylostella*. International Journal of Pham Tech Research 2 (3): 2048-2053 pp. sphinxsal.com/pt.61%20%20(2048-2053).pdf. 2010.
8. Franklin SE, Young L, Watson D, Cigan A, Meyer T, Bulla LA. Southern Analysis of BT-R1, the *Manducasexta* Gene Encoding the Receptor for the CryIAb Toxin of *Bacillus thuringiensis*. research.biology.arizona.edu/BtReceptor.html. 1997.
9. Thaphan P, Keawsompong S, Chanpaisaeng J. Isolation, Toxicity andDetectionof Cry Gene in *Bacillus thuringiensis* Isolates in KrabiProvince, Thailand. rdo.psu.ac.th/0125-3395-30-5-597-601.pdf. 2008.
10. Perchat S, Buisson C, Chaufaus J, Sanchis V, Lereclus D, Gohar M. *Bacillus cereus* Produces Several Non Proteinaceous Insectisidal Exotoxins. www.ncbi.nlm.gov/pubmed/16214163. 2005.

11. Salamitou S, Ramisse F, Brehelin M, Bourguet D, Gilois N, Gominet M, Hernandez E, Lereclus D. The plc Regulon is Involved in the Opportunistic Properties of *Bacillus thuringiensis* and *Bacillus cereus* in Mice and Insects. http://mic.sgmjournals.org/content/146/11/2825.full. 2000.

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