B. thuringiensis is a gram-positive soil bacterium, and produce a crystalline inclusion body during sporulation. 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. Most strains of B. thuringiensis produce delta-endotoxin crystals toxic to lepidopteran insects such as moth. Recently, however several researches have shown that B. thuringiensis is also widely distributed in natural soils of various area. that B. thuringiensis made up less than 0.5% of more than 46,000 bacterial isolates recovered from various soils in the United States. Out of the 50 Bacillus sp. isolates from the soil of South Kalimantan. Indonesia, only one (2%) was identified as B. thuringiensis based on phase contrast microscope examination for the presence of parasporal inclusion bodies.

Akhmad Rizali
Yusriadi Marsuni

Occurrence of Bacillus Thuringiensis from South Kalimantan, Indonesia

Isolation of Bacillus Thuringiensis

The author was born on February 26, 1959 in Kotabaru, South Kalimantan, Indonesia. He graduated Bachelor degree at Faculty of Agriculture, Lambung Mangkurat University. Then he completed his Master degree in Entomology Department, Faculty of Agriculture, (UPLB) at Los Banos and Doctor Course program was completed at Hokkaido University.

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Occurrence of *Bacillus Thuringiensis* in South Kalimantan. Indonesia

[1]Akhmad Rizali and [2]Yusriadi Marsuni

[1]Department of Agrotechnology, Faculty of Agriculture, Lambung Mangkurat University. Indonesia

[2]Department of Plant Protection, Faculty of Agriculture, Lambung Mangkurat University. Indonesia

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INTRODUCTION

Cruciferous vegetable such as cabbage (Brassica oleracea var. capitata L.), petchay (Brassica compristis var. chinensis, radish (*Raphanus sativus* L.), and mustard (*Brassica juncea* L.) are economically important crops grown in Indonesia. The area devoted for these vegetables was 10,522 ha with a production of 137,381 metric tons (Bureau of agricultural Statistics, 1990).

One of the constrains in the production of cruciferous vegetables is the infestation of insect pests especially the cabbage moth (*Crocidolomia binotalis*). This insect greatly reduced both yield and quality of the produce (Pfadt, 1985).

Cabbage moth, *Crocidolomia binotalis* Zell. (Lepidoptera: Pyraustidae) is considered the most important limiting factor for a successful production of cruciferous vegetables not only in the Indonesia but in other countries of the word. The larva feeds on foliage from seddling to harvest causing 100% yield loss if not controlled (Rejesus and Sayaboc, 1990). To control this dreaded pest, farmers rely on conventional insecticide. Crucifer production in Indonesia is higly dependent on chemical insecticides for pest control. The frequent and regular use of chemical insecticides however has brought about a number of problems, namly: development of insect resistance, toxic residues and destructive affects on non-target but beneficial organisms. This situation has necessitated the search for other non-chemical material for pest management with the hope of an effective and safe method of insect pest control (Cadapan and Gabriel.,1972).

Numerous chemical insecticides have been used to control *C. binotalis*. While chemical insecticides have knock down effect, they are too expensive and harmful to both humans and the environment. In addition, target insect pests develop biological resistance rapidly especially at higher rates of application to control this pest has urged researchers to search for biological control alternatives that would be agood component of integrated Pest management.

Currently, researches on the use of pathogenic microorganisms to control insect pests are increasing. Microbial pest control is practiced in different parts of the world through utilization of pathogens like fungi, bacteria, viruses and nematodes (Burges and hussy., 1971); Burges., 1981). Te most promising microbial control agent is the crystalliferous bacterium, *Bacillus thuringiensis* Berliner. It has been mass produced s microbial insecticides in seeral countries. Since its discovery around the turn of this century, *B. thuringiensis* has been considered primarily as a pathogen of Lepidopterous larvae. Now 137 insect species belonging to orders Lepidoptera, Hymenoptera, Diptera and Coleoptera were effectively infected by *B. thuringiensis* (Heimpel., 1967).

Padua el al., 1982 started the isolation of this bacteriumfrom soils collected from various regions of the Phillipines, However, it is nt yet known if *B. thuringiensis* also occurs indigenously in different crops growing areas and if it does, it is consequently important to

determine its potency against *C. binotalis*. This study therefore was conducted with the following objectives :

1. To survey, collect and determine the distribution of *B. thuringeinsis* in selected diverse crop-growing areas.
2. To screen isolates collected for pathogenicity to cabbage worm.
3. To determine the LC_{50} of the selected isolates.

REVIEW OF LITERATURE

A. Taxonomy and Description of *B. thuringiensis* Berliner

Bacteria causing diseases in insects began in the late nineteenth century with Pasteur's (1870) study of flacherie of silkworm, and with Cheshire and Cheyne's (1885) description of bacillus alvey and its role in Eropean foulbrood disease of the honey-bee (Falcon., 1971).

Although the discovery of *B. thuringiensis* took place in the early part of the 20th century, its development for biological control ofinsect pests was slow, partly due to a long-lasting confusion between the different varieties. The first important attempt to differentiate and classify the early isolated *B. thuringiensis* (Heimpel and Angus., 1958) used morphological and biochemical characters. Thsese isolates were isolates were named *B. fintimus, B. entomocidus* var *entomocidus* and var. *subtoxicus*, and*B. thuringiensis*var. Berliner, var. *sotto* and var. *alesti*.

B.thurngiensis had earlier been discovered by Ishiwata in 1901 from a severe flacherie of the silkworm, *Bombyx mori*. Ishiwata named it "soto" (fainting or sudden collapse) bacillus although his taxonomical description of the causative bacillus of the "sotto" disease was incomplete. In 1991, Berliner proposed the name of *B. thuringiensis* for the *bacillu*s which he isolated from the diseased larvae the Mediterranean flour moth, Anagasta (Ephestia) *kuhniell*a Zell. He noted infection of the larvae after the ingestion of the *bacillus* or its spores. He described the bacterium in 1915 and named it *Bacillus thuringiensis* Berliner. Mattes (1927) isolated the same bacillus from the same insect host which Berliner had found earlier. This strain is now maintained as *B. thuring*iensis subsp. *thuringiensis* (serotype 1).

Berliner (1915) and Mattes (1927) noticed that the vegetative remains of the sporulating cells assumed a rhomboid shape. Hannay in 1953, described this crystalline inclusion in the sporangium of the organism and made further interpretation of the data being accumulated on the bacillus at that time. Neither Berliner nor Mattes attributed those parasporal bodies any role in the diseases process caused by the ingestion of sporulating *B. thuringiensis*. Hannay (1953). On the other hand, speculated that the inclusions were connected with the pathogenicity of the bacillus. The results of Angus's work (1954) confirmed the findings of early workers and of hannay's suggestion. He reported that the paralysis-inducing principle of the bacterium is present only in sporulating cultures. Furthermore, paralysis was notcaused

by growth of the microorganisms in the host tissue but associatd with crystalline inclusions soluble in insect and juice or dilute alkali.

The *B. thurngiensis* varieties produce a lecithinase identical with thet of *B. cereus* in insects with appropriate pH conditions the enzyme is undoubtedly active. Burcher (1960) pointed out that some facultative pathogens are proteolytic, and these include *B. cereus* and *B. thuringiensis* varieties.

B. thuringiensis is a gram-positive isect pathogenic bacterium in which the protein body is strongly toxic to Lepidopteran or Dipteran larvae and called delta-endotoxin, and closely related to *B. cereus*. The important characteristic of *B. thuringiensis* is the production of a toxic crystalline inclusion (parasporal body, toxic crystal, and endotoxin)while*B. cereus* does not produce any such inclusion (Aizawa., 1982).

The classification of *B. thuringeinsis* has been done by the flagellar agglutination which demonstrates the existence of flagellar antigen (H-antigen). Presviously,the classification was done by the combination of H-antigen (serotypes) and biotypes, particularly the seterase types. Recently, however, new subspecies of *B. thuringiensis* have been proposed based on the antigen. This is because serotyping by flagellar agglutination proved to be the most sensitive, specific, reliable and rapid method of identification (Barjac., 1981). The classification of *B. thuringiensis* is shown in Table 1.

A. Toxins of *B. thuringiensis* and their Mode of Action

Crystal formation begins when growth and nucleic acid synthesis stop and cells start to sporulate. Heimpel and Angus 1959) stated that the crystal is synthesized from small molecular weight compounds into a final form of protein. The crystal is alkaline soluble, proteinaceous substance toxic to certain species of Lepidoptera. Besides this crystal, delta endotoxin, bacillus thuringiensis subspecies possess other toxins namely: alpha-exotoxin, beta-exotoxin and gamma-exotoxin. All of the toxic substances may not be present in the bacterium (Heimpel., 1967).

Krieg (1961) has defined the ariuos toxic substances produced by *B. thuringiensis* as follows: (a) thermolabile endotoxin; (b) thermo stable exo-toxin; (c) bacillogenic antibiotic; (d) lecithinase; (e) proteinase. In Krieg's system the thermolabile endotoxin refers to the parasporal crystal.

Table 1. Classifiction of *Bacillusthuringiensis*

SEROTYPE (H-ANTIGEN)	ESTERASE TYPE REFERENCE	SUBSPECIES	PRODN. OF B-EXOTOXIN IN
1	*thuringiensis*	*thuringeinsis*	+
Subsp.			
2	*finitimus*	*finitimus*	-
3a 3b	*alesti*	*alesti*	-
4a 4b	*kurstaki*	*kurstaki*	-
4a 4c	*sotto*	*sotto*	-
5a 5b	*kenyae*	*kenyae*	-
5a 5c	*galleriae*	*galleriae*	-
6	*canadiensis*	*canadiensis*	-
7	*entomocidus*	*entomocidus*	-
8a 8b	*morrisoni*	*morrisoni*	+
8a 8c	*ostriniae*	*ostriniae*	-
8b 8d	*nigeriensis*	*nigeriensis*	-
9	*tolworthi*	*tolworthi*	+
10	*darmstadiensis*	*darmstadiensis*	-
11a 11b	*toumanoffi*	*toumanoffi*	+
11a 12c	*kyushuensis*	*kyushuensis*	-
12	*thompsoni*	*thompsoni*	-

Table 1.continued…….

SEROTYPE (H-ANTIGEN)		ESTERASE TYPE REFERENCE	SUBSPECIES	PRODN. OF B-EXOTOXIN IN
	13	*pakistani*	*Pakistani-*	
	14	*israelensis*	*israelensis-*	
	15	*dakota*	*Dakota*	-
	16	*indiana*	*Indiana-*	
17		*tohokuensis*	*tohokuensis*	-
18		*kumamotoensis*	*kumamotoensis*	+
19		*tochigiensis*	*tochigiensis*	-
20a	20b	*yunnanensis*	*yunnanensis*	-
20a	20c	pondicheriensis	pondicheriensis	-
	21	*colmeri*	*colmeri*	-
	22	*shadongiensis*	*shadongiensis*	-
	23	*japonensis*	*japonensis*	-
	24	*neoleonensis*	*neoleonensis*	-
	25	*coreanensis*	*coreanensis*	-
	26	*silo*	*silo*	-
	27	*mexicanensis*	*mexcanensis*	-

Source : Barjac, D.H. and E. Frachon., 1990.

The crystal-protein produced during sporulation acts as a toxin on a number of insects. *B.thuringiensis* has been used in the control of defoliating lepidopterous larvae as a biocide (Percy and Fast., 1983). The proteinaceous crystal, termed delta-endotoxin is dissolved and activated by the enzymes of the gut of the host insect in the lumen (Murphy et al., 1978; Nishiitsufsuji-Uwo and Endo., 1980). The organ most affected in the digestive tract has been found to be the midgut. The crystal toxin acts on the midgut epithelium causing it to sweel and to eventually disintegrate (Mathavan et al., 1989). The important change produced in the cell are reported to be hypertrophy of cells. Swelling of the plasma membrane at the free surface and the disruption of microvilli, vacuolation of cytoplasma, change in the cisternae of the endolasmic reticulum, loss of ribosomes, and changes in the configuration of mitochondria (Mathavan et al., 1989).

Angus and Heimpel (1959) and Heimpel and Angus (1959) suggested that the midgut of silkworm and other lepidopterans was affected by the crystal protein as a result of which the gut wall bcome leaky; the highly alkaline midgut contents, with their high concentration of K^+, leak into the hemolymph and increase the pH of blood from 6.8 to 8.1. The increase in K^+ concentration in thehemolyph is reported to cause the paralysis of the larva and its ultimate mortality.

The alpha-exotoxin (phospholipase C) causes breakdown of essencial phospholipids in the insect tissue (Falcon., 1971).Another toxin that has been extensively studied is the heat stable beta-exotoxin. This toxin has been found in *B. thuringiensis* var. *thuringiensis* and *B. cereus* (McConnell and Richards., 1959), but not in *B. thuringiensis* var. sotto, or var. *dendrolimus, B. thuringiensisentomomocidus* var. *entomocidus*, and *B. thuringiensis* var s*ubtoxicus*.

The thermostable exotoxin is of low moleculer weight water soluble substance of unknown composition which affect several orders of insects, but only when injected into body cavity. Production of the exotoxin begins toward the end of the logarithmic phase of growth and attains its maximum at the beginning of sporulation. The toxin has the ability to prevent pupation in certain Diptera and also produces teratological effects in developing Diptera, Coleoptera and Lepidoptera (Wolfenbarger et al., 1972). Although the toxin had been purified, its use as purified toxin is prohibited.

The Gamma-exotoxin (labile exotoxin) is composed of free amino acids and one or more peptides of low molecular weight (200-2000) (Lysenko and Kucera., 1971). The importance of these toxic fraction aside from the parasporal bodies is that they extend the host range of *B. thuringiensis* to a wider range of insect (Angus., 1965).

Generally, infected insects become sluggish, exhibiting oral discharge. The integument turns brown or black (Poinar and Thomas., 1978) and the whole body becomes sticky, later drying up into a scale-like structure.

B. Development of *B. thuringiensis* as Microbial Insecticide

Steinhaus (1951) reported on some aspects of the biological control potentialities of certain sporeforming bacteria, together with some general consideration of *B. thuringiensis* Berliner as an insect pathogen and as a possible agent to use in the control of the alfalfa caterpillar.

The first commercial material containing *B. thuringiensis* var. *thuringiensis* were made available for testing in the field in the United States in 1958. The initial field test of the various preparations were applied by hand sprayer to cauliflower plants for the control of cabbage looper. In addition, one material, in which the bacillus ingredient was blended with a Celite carrier to a concentration of approximately 3 billion viable spores per gram, was applied as a dust, gave 100 percent of the dead larvae. There are four major brands of microbial insecticides containing *B. thuringiensis* var.thuringiensis in the USA :

(1) Bankthane L-69
(2) Biotrol BTB
(3) Parasporin
(4) Thuricide

At least one other company in the United States has made considerable progress with the manufacture of *B. thuringiensis* var, *thuringeinsis* preparations. Therefore, it is possible that more products will make their appearance if the agriculture and home markets continue to develop.

The *bacillus* materials have been tested in many parts of the United States, primarily against susceptible insect pest on vegetable and field crops. At present, all the American product have received an exemption from the requirement of a residue tolerance from Food and Drug Administration of the Federal Government on the following raw agricultural commodities : alfalfa, apples, artichokes, beans, broccoli, cabbage, cauliflower, celery, cottonseed, lettuce, melon, potatoes, spinach and tomatoes (Hall., 1963).

The data obtained by McEwen and Harvey (1959) in field trials with sprays of several *bacillus* preparations on broccoli and cauliflower at Geneva, New York, in 1958, gave the indication that the imported cabbage worm could be controlled easily with relatively low dosages of infective material where as control of the cabbage looper requires a much higher rate of application. Subsquent tests conducted in 1959 with a different series of wet table powder preparations showed that sprays of *B. thuringeinsis* var.*thuringiensis* can provide good control of these two inscts. Because of the great difference in susceptibility, control of the imported cabbage worm required about one-eighth the amount of bacillus preparation necessary to suppress the cabbage looper (McEwen et al., 1960).

This difference in susceptibility is in general agreement with the earlier findings of Tanada (1956), Hall and Dunn (1958), and Hall and Andres (1959). Entirely different results

were obtained by Semel (1961), who repots thet commercial preparations of *B. thuringiensis* var. *thuringiensis* used as sprays or a transplant-dip on cauliflower at long island, New York, were unable to give control of the cabbage looper. The latter findings are difficult to understand in the light of the much better results attained by McEwen and his associates with spray applications and the excellent control with the use of dust formulations reported by Shorey and Hall (1962). It would appear that the poor control obtained by Semel resulted more from the inherent coverage difficulties encountered with the use of sprays and the drastic effect of the heavy rainfall that followed the dip treatment than the ineffectiveness of the microbial materials. Beyond a doubt, no chemical insecticide that required more than a few hours for control would have fared better under similar circumstances.

Other tests of *bacillus* materials have been reported from Canada and Europe. Jaques and Fox (1960) stated that *B. entomocidus* var. *entomocidus* Heimpel and Angus generally were more effective as sprays than as against the imported cabbage worm in insects on cabbage in Nova Scotia in 1959. Both pathogens, although not equal in effectiveness to certain chemical insecticides, gave adequate protection to to the cabbage plants. Performance was improved through the use of skim-milk powder and Geon latex as sticking agents. The successful use of sprays and dusts containing *B. thuringiensis* for control of the cabbage worm, *P. brassicae* (Linnaeus), on cabbage has been reported from France (Lemoigne et al., 1956); Martouret., 1959). It was found that for good results sprays containing 1 pound of a powder with a toxicity level of 900 biological gallons should be applied at the rate of 126 gallons per acre and dusts containing 5 percent of the powder at 27 pounds per acre.

Sprays and dust applications of experimental commercial preparations of *B. thuringiensis* var.*thuringiensis* have been found to be equally effective in limiting infestations of the artichoke plume moth, *Platyptilia carduidactyla* (Riley),on young sprouts and developing artichokes in field tests conducted in California by Tanada and Reiner (1960). The effectiveness of the bacterium is dependent on its high virulence for the first-instar larvae which are killed within 1 or 2 days after infection and before they can mine into the plant tissues. This study has demonstrated low economic thresholds. It has been followed by the registration of the commercial bacillus preparations for use on artichokes. However, additional field evaluations and comparisons with accepted chemical control programs will be needed before the microbial products can be recommended and adapted for use against artichokes plume moth infestations.

An attempt was made to determine the effectiveness of sprays of *B. thuringiensis* var. *thuringiensis* against the corn earworms, *Heliothis zea* (Boddie) on sweet corn in Nova Scotia (Jaques and Fox., 1960). There was no evidence that the treatments caused any reduction in the number of larvae in the ears. This lack of control reflects not only the mode of activity of the corn earworm, which, like the larvae of the artichoke plume moth, feeds very little on the surface and quickly enters into the protected inner parts of the plant, but also the relatively low degrre of susceptibility of the insect to the *bacillus*.

Fox and Jaques (1961) reported interesting results of field tests conducted in Nova Scotia during 1960 involving seven sprays schedules using a commercial preparation of *B. thuringiensis* var.*thuringiensis*, DDT, and rotenone for control of the imported cabbage worm and the diamondback moth. Up to three sprays treatments were applied at 2-weeks intervals. It was found that none of the spray schedules tested eliminated either insect. However, the pasts were adequately controlled by two sprays schedule consisting of three applications of a microbial insecticide, or one application of DDT followed by two of the bacillus. Although mortality of the cabbage worm was high immediately following one or two applications of themicrobial material, the plants were not protected for a sufficientlylong period. Likewise , a single application of DDT followed by one application of *bacillus* did not protect the plants up to the time of harvest. Although the effectiveness of the microbial material against the insects was not outstanding, the control was acceptable and demonstrated the potential usefulness of *B. thuringiensis* var. *thuringiensis*an an alternative to DDT or rotenone.

There has been considerable interest shown by entomologists in the United States in the use of microbial preparations for the control of insect pests on tobacco. These investigations have been motivated by need to develop new materials or control methods that will result in reduced insecticidal residues for protection of the smoking public. However, Rabb et al. (1957) reported on the results of a series of small-plot field tests conducted in North Carolina with sprays of a noncommercial preparation of *B. thuringiensis* var. *thuringiensis*. It was found that low dosage of this material, which at concentrations of 40 to 50 billion spores per gram was within the range of the present commercial products, gave good control of the tomato hornworm, *P. sexta* (Johannson). At a dosage of approximately 0.3 pound in 50 to 60 gallons of water per acre, the *bacillus* suspension gave a good control for 72 hours after treatment as TDE, a recommended hornworm toxicant. It was observed that the infected larvae ceased to feed almost immediately after treatment, thereby minimizing the more rapid larval kill by the chemical material. One noted drawback to the use of the pathogen against the complex of tobacco insects was the lack of effect on budworms, *Heliothis sp*. primarily *H. virescens* (Fabricius) by the low dosages that controlled the hornworms. It would appear that the tobacco budworm, like its close relative the corn earworm, may have a low degree of susceptibility to the bacillus and other control strategies will have to be developed to place concentrated amounts of infective material into contact with the larvae. Test by Jaques and Fox (1960) in Nova Scotia showed that an experimental commercial wettable powder preparation of *B. thuringiensis* var. *thuringiensis* could be used for controlling the winter moth, *Operophtera brumata* (Linnaeus) and the fall cankerworm, *Alsoptrila pom*etaria (Harris), on apple.

Later studies on the same crop with several commercial *bacillus* materials indicated that spray applications provided good control of not only the above species, but also the eastern tent caterpillar, *Malacosoma americanum* (Febricius). The microbial materials were less effective against larvae of the gray-banded leaf roller, *Argyrotaenia mariana* (Fernald), and the eye-spotted bud moth, *Spilonota ocelana* (Denis and Schiffermuler). Good control of the red-banded leaf roller, *A. velutinana* (Walker), with the use of *bacillus* products has been reported by McEwen et al., (1960), although a relatively high dosage was required to provide

suppression equal to that of normally recommended chemical insecticides. Additional tests have been conducted against the codling moth, C. pomonella, on apple with commercial preparations of *B. thuringiensis* var. t*huringiensis* (Mcewen et al., 1960) and *B. cereus* Frankland and Frankland (Stephens., 1957). In these experiments, the microbial materials gave some kill, but in general, very inadequate control of the pest larvae.

Commercial bacillus materials have been tested against defoliating insects of forest and shade trees in the United States and Canada. Cartwell et al., (1961) reported the results of aerial tests made in Woodlands in Connecticut and Vermont in which sprays of *B. thuringiensis* var. *thuringiensis* control of heavy infestations of the gypsy moth, *Porthetria dispar* (Linnaeus), that compared favorably with chemical treatments.

Many more tests of entomogenous bacteria have been made in Europe for the control of forest insect pests during the few years. A manufactured powder consisting mainly of toxic crystals of the E-58 strain of *B. thuringiensis* was found to be fairly effective against infestations of the tent caterpillar, *M. Neustria*, when applied in a water spray on elm trees in Holland (Van damme and van der Laan., 1959).

Investigations on the use of nontoxic bacterial pathogens to suppress lepidopterous pests of stored agricultural products have been renewed following the advent of the manufactured bacillus materials in the United States. Kantack (1959) repots on laboratory studies with commercial preperations of *B. thuringiensis* var. *thuringiensis* against the Indian-meal moth, *Plodia interpunctella* (Hubner), and suggests that further studies may show that field control of this insect is possible. Additional tests should be expected as new and better microbial materials make their appearance.

There is great interest in the United states in the possibility of utilizing the commercial *B. thuringiensis* var. *thuringiensis* materials as feed additives to prevent the emergence of adults of the housefly, *M. domestica*, from the droppings of livestock. Larvae of the housefly were first reported to be susceptible to a toxin in cultured bacillus material by Hall and Arakawa (1959), Briggs (1960) found that a filterable substance interfered with the development of the larvae and reduced the number of adults issuing from a treated substrate. Application of laboratory and commercially produced *bacillus* preperations as an additive to chicken feed resulted in 99 percent reduction of adult emergence when 3 grams per day were consumed by laying hens. No deleterious effects on the hens, or on the quantity or quality of their eggs, were noted after continued consumption of the microbial insecticide materials.

Comparable studies conducted by Dunn (1960) showed that cattle given *B. thuringiensis* var. *thuringiensis* as an additive in their feed produced droppings thet prohibited normal development of 92 percent of the 48-hours-old housefly larvae placed in the feces. At the concentrations of the toxic materials used in the tests, it was found that most of the mortality occurred in the prepupal rather than the larval stage. Other studies with a commercial bacillus material involving feeding to livestock and adding to manure to suppress housefly development have been reported (Harvey and Brethour., 1960). The microbial

insecticide when mixed directly with cattle feces at rates of 0, 50, 100, 200, and 300miligrams perkilogram and inoculated with 100 housefly eggs resulted in n average of 43, 41, 9, 5 and 0 adult flies completing development, respectively. Similar findings have come from tests with layer hens (Burns et al., 1961)

The development of biological pesticide is being encouraged in many countries, and various isolates of *B. thuringiensis* are in commercial use against a number of Lepidoptera pests. Commercial formulation of *B. thuringeinsis* such as Dipel, Thuricide HP, Bachre, Bactospeine 6000 and Tarsal have been used in large scale for the control of insect pests of crops in the United States and in the German Federal Republic (Burges and Hussey., 1971). Such material is only effective against Lepidoptera and is harmless to honeybees and entomophagous insects. Field application of commercial preparation of *B. thuringiensis* increase the level of spores occurring naturally on the treated plants but environmental conditions can greatly influence the results (Kreig., 1978).

The result of Bullock and dulmage (1969) were successful in controlling a population of pink bollworm, *Pectinoophora gossypiella*, in field cages with a commercial formulation of *B. thuringiensis*. Padua et al., (1982) found that an isolate designated PG-14 (subsp. Morrisoni, serotype H 8a: 8b) exhibited rapid and high toxicity to mosquito larvae but showed no toxicity to the larvae of silkworm, *Bombyx mori*, or to adults of a daphnid.

Resistance to *B. thuringiensis* Berliner was induced in houseflies (*Musca domestica* Linn.) by selecting survivor from treated medium during 50 generations of rearing. The degree of resistance based on the ratios of LD_{50} values, varied from 8 to 14 fold between the 27^{th} and 50^{th} generation inclusive. Resistance developed during 30 generations of selections, appeared to decline slightly during 20 generations without election pressure (Harvey et al., 1965).

Commercially produced products from the HD-1 isolate of *B. thuringiensis* var. *alesti* indicated that the delta-endotoxin produced by biological agents can provide adequate control of the cabbage caterpillar complex when applied on a regular weekly basis throughout the growing season (Libby and Chapman., 1971).

In the laboratory tes, Alcantara (1981) tested 13 subspecies and two commercial preparation of *B. thuringiensis* Berliner for pathogenicity on the fourth and fifth larvae of *C. binotalis* Zell. *B. thuringiensis* subspecies *israelensis*, *kyushuensis* and sotto gave the highest toxicity among the serotypes.

At present, some strains of *B. thuringiensis* have been isolated shown toxic activity against Lepidoptera and dipteral, and several economically important pests in the order Coleoptera (beetles). An isolate with activity against some species of Coleoptera has been reported by Krieg et al., 1984).

However,two new isolates of *B. thuringiensis* (K-2074) and 2170) from Taiwan have been identified trough an active screening program to be highly pathogenic against the tobacco cutworm (Whitlock et al., 1990).

C. Cabbage Worm, *Crocidolomia binotalis* Zell.

1. Origin and distribution.

Zeller described *C. binotalis* in 19852 from a spciemen Collected in South Africa. Early synonyms of C. binotalis were *C. comalis* (Guenee) 1854 from java. The native range of the moth probably embraced both Africa and Asia. Hompson (1896) listed *Pjonea comalis* Guen. Delt.And Pyr.And P.incomalis Guen.Delt.And Pyr.As synonyms. The pyralid is known commonly as cabbage worm (Knott and Deanon., 1967), cabbage moth (Sanchez et al.,1969; deang., 1969; Delos Reyes., 1960), cluster caterpillar (Smith., 1943), and cabbage caterpillar (Sison., 1927).

On cabbage, the larvae bore into the heads and feed inside until they are ready to pupate. Extensive larval feeding may result in stunting, non-formation of heads or even death of plants (Sanchez et al., 1969). *C. binotalis* occurs in extensive areas of west, south and east of Africa, throughout the warmer parts of Asia, and in north-eastern Australia, Caroline Is., Cook Is., Fiji, Indonesia, Marianas, New Caledonia, Nive, Norfolk Is., Papua New Guinea, the Philippines, American and Western Samoa, Solomon Is., Tonga (Anon., 1979). It is not recorded from Kiribati, Marquesas, Tokelau, Tuvalu or Willis and Futuna Is. (Waterhouse., 1985) or from Hawaii or the Americas (Anon., 1979).

In the Philippines, infestation by *C. binotalis* is severe from November to January (Delos Reyes., 1960) while Esguerra and Gabriel (1969) reported that this pest is abundant from June to November. Furthermore, this pest is scarce in the months of March, april and may (Delos Reyes., 1960; Gabriel and Esguerra., 1969). Cham (1962) considered this pyralid a major pest from October to January in Australia.

2. Life Cycle

The moths are strong fliers and seek out *Brassicacaeae* over considerable distance. The eggs are laid in a cluster, overlapping each other on the lower surfaces of the leaves, the egg mass having a brown (Fig 1.), furry appearance due to scales detached from the abdomen of the female adhering to them. The eggs hatch in 5 to 25 days, depending upon the temperature. Newly hatched larvae cluster near the egg mass and chew the leaf surface. As the grow, the larvae disperse and destroy the young leaves and the growing point of the plant. In cabbage they are found in the heart, which is riddled, to produce a mass of webbing and frass. There are five larval instars, and feeding occupies 19 to 42 days. Rainfall of 15 mm in 1 hour caused about 15% mortality of early larval instars. Pupation occurs in a silken, soil-covered coccon up to 10 cm underground, and the pupal stage occupies 10 to 42 days (Waterhouse and Norris., 1987).

Delos Reyes (1960) reported that the species pass through five larval intars but Sison Claimed that the larvae undergo only four molts. Variation in the number of larval instars in some insect species had been reported also. C. binotalis pupate in the soil for 6 to 8 (Sison., 1927) up to 9 days (Delos Reyes., 1960). The total developmental period (from egg laying to adult emergence) is usually 24 days (Delos Reyes., 1960).

3. Host Range

C. binitalis Zell.,is common on cultivated and wild crucifers. It attacks many types of Brassica and other crucifers including sesawi, petsai, lobak, radish, wild nasturtium, and Capparidaceae (weeds such as Polanisia and Gyandropsis) (Kalshoven., 1981).

4. Natural enemies.

The parasitoid attacking *C. binotalis* are listed in table 2.

Table 2.Natural Enimies of *Crocidolomia binotalis* Zell.

SPECIES	FROM	REFERENCE
Hymenoptera		
Ichneumonidae		
Trathala flavoorbitalis	hilipines	Cushman, 1933 and Esguerra and Gabriel,1969
Enytus spp.	Indonesia	Kalshoven, 1981
Atrometus spp.	Indonesia	Kalshoven, 1981
Mesochorus spp.	Indonesia	Kalshoven, 1981
Diadegma sp.	Papua New guinea	Grave and Ismay, 1983
Exochus sp.	Papua New Guenea	Grave and Ismay, 1983
Braconidae		
Apanteles crocidolomiae	India	Ahmad, 1964
Baracon leffroyi	India	Ayyar, 1928, Ayyar And Margabandu, 1934
Braconhebetor	India	Singh, 1978
Bracon melleus	India	Ayyar, 1928, Ayyar and Margabandu, 1934
Bracon sp.	India	Ayyar, 1928, Ayyar and Margabandu, 1934
Disophrys sp.	Malawi	Smee, 1942
Chelonus	Indonesia	Kalshoven, 1981

Table 2. Continued…

SPECIES	FROM	REFERENCE
Diptera		
Sarcophagidae		
Boettcherisca peregrine	Sri Lanka	Baranov, 1934
Techinidae		
Pales blepharipus	South Africa	Gunn, 1925
Palexorista inconspicuoidea	Papua New Guinea	Baranov, 1934
Palexorista solennis	India	Singh, 1978
Unidentfied techinid	Pakistan	Anon., 1981
Winthemia neowinthemoides	Pakistan	Baranov, 1934
Two of spp.	Indonesia	Kalshoven, 1981

SOURCE : Kalshoven, 1981 and Waterhouse et al., 1987.

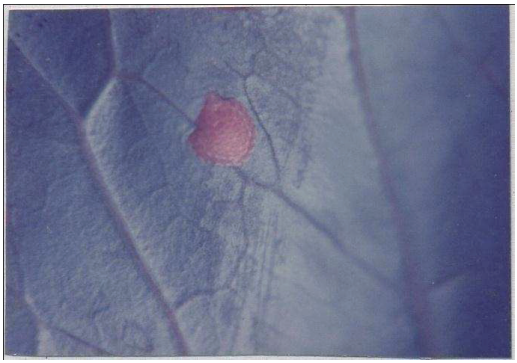

Figure 1. The egg mass of *Crocidolomia binotalis*

Figure 2.*Crocidolomia binotalis* larvae damaging a cabbage leaf

Figure 3.*Crocidolomia binotalis* damaged cabbage hearts in the field.

Figure 4.*Crocidolomia binotalis*pupa (A) and fourth instar larva (B)

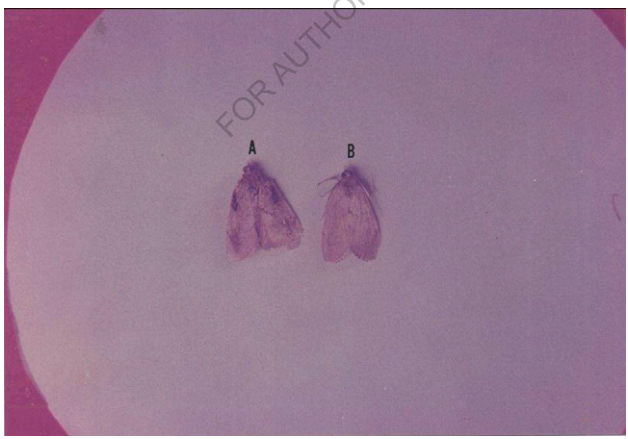

Figure 5. Adults of *Crocidolomia binotalis* (A) male and (B) female

MATERIALS AND METHODS

The study was undertaken at the Insect Pathology Laboratory, Department of Entomolgy and National Institutes of Biotechnology and Applied Microbiology (BIOTECH), University

A. Collection and Mass Rearing of *C. binotalis*

Eggs, larvae, and adults of *C. bin*otalis were collected from the cabbage plots at the central Experiment Station, U.P. College og Agriculture, Los Banos,Masapang, Caluan and cabuyao, Laguna.

The field-collected larvae were placed in plastic trays (13.5 x 22 x 6 cm). 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. In some cases however, a first and second instar larvae were placed in one tray. The same procedure was followed for the third and fourth intar 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.

Newly-emerged adults were kept in 30 x 32 x 49 cm screen cages. The cages have wooden frames and the floor with a sliding glass door. Two wads of absorbent cotton saturated with 30% solution of honey were hang inside the cages for the adults to feed on honey. The floors of the cages were lined fully with polyethylene sheet trough which whole cabbage leaves were changed and inspected daily for newly-laid eggs. Portions of cabbage leaves and polyethylene sheet with egg masses were cut out and transferred into petri dishes. At the black head stage, the egg masses were placed over fresh cabbage leaves kept inside the above-described rearing plastic trays.

The first two larval instars at times may share one rearing tray. However, the succeeding larval instars were separated individually to minimize cannibalism. Canibalism were observed in crowded trays. The larvae were transferred into clean rearing trays 2 to 3 times a week. Fresh cabbage leaves were provided daily.

B. Isolation of *B. thuringiensis*

Soil samples were collected in areas planted to vegetables, rice, citrus, peanut and corn in South Kalimantan (Indonesia) following multistage random sampling. Soil samples were collected at random in a 1- hectare area for a total of 5 kg. The soil sample were taken from the top 1 cm of the soil layer. The 5-kg soil samples were mixed thoroughly and composite sample of 1 kg was taken from which isolation were made for as long as one month. The samples were labeled denoting date, place of collection and crops planted.

Five 1-g soil samples were separately suspended to 9 ml of distilled water. After allowing the suspension to stand for 5 minute, 3-4 ml of the the suspension were taken. One half of the suspension was transferred to a test tube and heated in a waterbath of 80^{o}C for 15 minutes, so that all microorganisms were killed except Bacillus and other sporeforming

bacteria, then allowed to cool at room temperature. Ten-fold serial dilutions of the heated suspension in sterile distilled water were placed on nutrient agar (NA-pH 7.5). After two days of incubation at 28°C, Bacillus colonies were recorded. After 2 to 3 days incubation, crystalliferous sporeforming bacteria were determined in phase contrast microscope.

C. Pathogenicity Test

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

A Loopful of the sporulated bacterial cultures from NA were dissolved in 10 ml 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 inscts. The last treatment served as the control.

D. 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 750 ml 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 (LC50) of the dried spore-crystal complex. Serial dilutions were made until the concentration necessary to determine LC50 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 inscts 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 concentration 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 corerected using Abbot's formula (Reed and Muench, 1938).

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

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 LC50 of the selected *B. thuriengiensis* isolates were determined using the computerized probit analysis.

RESULTS AND DISCUSSION

Isolation and distribution of *B. thuringiensis* in different crop growing areas.

Fifty *Bacillus sp*. were isolated from 6 soil samples collected from diverse crop growing areas in the South Kalimantan (Indonesia). The different isolates were obtained from the same area planted with diverse crop. Two soil samples great were found in this area namely: typic calciborrols with pH 6.5 was planted to rice and typic tropudults pH ranging from 4.8 – 5.7 planted to vegetable, corn, citrus, peanut and cabbage. Out of the 50 *Bacillus sp*. isolates, only one (2%) was identified as *B. thuringiensis* based on phase contrast microscope examination for the presence of parasporal inclusion bodies. Only the soil sample from citrus yielded *B. thuringiensis*. This result suggest that *B. thuringiensis* is rare and not widespread in the places where sampling was done.

The possible reason for the low incidence of B. thuringiensis isolated from the samples taken in the areas surveyed are the small number of samples size from which the isolation were made, the area where sampling was don and also the difference in the physic-chemical characteristics of the soil where samples were taken. The low incidence of *B. thuringiensis* was also reported in Japan by Ohba and Aizawa (1986) in soil sample from non-sericultural areas. Out of 6910 isolates only 189 (2.7%) isolates were identified as *B. thuringiensis*, which produce parasporal inclusion in contrast to 11.4% obtained in sericultural farms. Obviously, the low incidence could be attributed to the limited area and occurrenceof drought.

Recently, Delucca et al., (1982) reported that in the United States, of the 46,373 soil samples collected only 250 (0.5%) contained *B. thuringiensis*. The difference in the distribution of B. thuringiensis between soils of Japan and USA may be due to the difference in soil types examined or to geographical difference (Ohba and Aizawa et al., 1982) on soil samples collected from areas treated with B. thuringiensis var. kurstaki and var. galleriae showed that the variety recovered were the same varieties applied to the area indicating that the bacterium is stable in the soil.

Saleh et al., (1970) recovered *B. thuringiensis*, ranging from 7,800 to 170,000 propaguules, ssentially spores, per gram of soil from a silty clay loam, and two silt loams, but not from a muck soil to which either of the commercial products known as Thuricide and Biotrol had been applied for the control of insect pest on cabbage and lettuce. Furthermore, soil samples with a pH 7.3, silty clay loam texture and pH 5.2 silt loam soils were known to contain *B. thuringiensis*.

According to Ignoffo and Graham., (1967) spore viability is maintained in soil amended with high levels, (billion) of B. thuringiensis spores. B. thuringiensis spores can

remain ungerminated and viable for long periods of time in the field of diverse soil texture and pH (Saleh et al. 1970). B. *Thuringiensis* can persist for a considerable length of time in treated soils (Saleh et al., 1970), and it is common in soils adjacent to sericulture farms in Japan (Aizawa et al.,1961; Ohba et al., 1979). It will persist in the dust of abandoned mills where grains had been stored, even many years after the mills have been closed (Vankova and Purini., 1979). It can also very quikly appear in mass insect rearing facilities (Burges and Hurst., 1977). No *B. thuringiensis* was isolated from cabbage worm larvae manifesting sign and symptoms of bacterial infection (Pacumbaba., 1987). Individual insects could have been infected with *B. thu*ringiensis under field condition but epizootics due to *B. thuringiensis* do not normally occur in nature (De lucca et al., 1982). *B thuringiensis* HD-1 was isolated from a mass reared colony of pink bollworm, *Pectinophora gossypiella* (Dulmage., 1970). This showed that epizootics of B. thuringiensis could occur in or around areas where Lepidoptera insects have been mass reared in a confined environment.

According to Sneh and Schuster (1981), the number of other bacteria present per mg of healthy larva increased with larval weight. These being predominantly Streptoccoccus and *Erwinia spp*. While in dead larvae, the increase of *Erwinia sp*. was higher than that of *Corynebacterium sp., Micrococcus sp., Serratia marcascens* and *Bacillus sp*.

De Lucca et al., (1982) reported that the precence of *B. thuringiensis* in the soil does not indicate any enhanced value in insect control. However, the search for new isolates and varieties can lead to more poent toxin, with activity against new nd unexpected insect species.

Pathogenicity test of diferrent *B. thuringiensis* isolates

The initial screening of 145 *B. thuringiensis* isolates from BIOTECH 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. 3). 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 caused larval mortality ranging from 63.3 to 96.6% 3 days after treatment (Table. 4).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., 1984, some of these isolates were toxic to Diamondback moth (DBM) and slug caterpillars.

Brownbridge (1990) reported the the screening and bioassay work on identified *B. thuringiensis* strains showed differences in their toxicities to *Chilo partellus*. There were also difference 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 target insect. Some isolates were unstable in which case they could pass the preliminary screening but may be eliminated in the second screening.

Table 3.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		

Table 4.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

*Standard isolate in the bioassay.

The comparative toxicities of the dried cells of *B. thuringiensis* isolates against *C. binotalis*is shown in Table 5. 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 the 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 same isolate is also effective against DBM (*Plutella xylostella*) and coconut slug caterpillar indicating its potential to other lepidopterous pests (Padua et al., 1984). Thus #13 isolate could be used to complement other control approaches to control lepidopterous cabbage pests.

Whitlock et al., (1990) reported that the standard *B. thuringiensis kurstaki* (HD-1) was the most toxic against the tobacco cutworm (*Spodoptera li*tura). 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, Trichoplusia ni. However, selected isolates of b. thuringiensis produced very effective toxin for use in insect control and the progress made from the first commercial production with the Mattes strain of var. *thuringiensis* to the modern production with the HD-1 isolate of var. *kurstaki* indicates that a search for new isolates and varieties can lead to still more potent toxins (De lucca et al., 1981).

The relative toxicities of the dried cells in decreasing order to *C. binotalis*were : HD-1 (51.93), #13 (63.34) L3 (161.17), L26 (165.12) and SCSP6 (195.48), respectively. The different toxicity of isolates are not statistically significant (Appendix Table 6).

The potential dried cells of local isolates were #13 and L3 (Appendix table 1 and 4). Toxicities of the dried HD-1 was seemingly stable compared with the local *B. thuringiensis* isolates studied. The local isolate #13 is also potentially effective against *C. binotalis* as shown by the high percentage kill (Appendix Table 4). The insecticidal activity of the remaining isolates were least effective since they required higher doses to achieve LC_{50}(Fig. 6). Likewise, the dosage mortality curve indicates less steep probit line (Fig. 7). Which signifies that the test in fact required a higher concentration to attain 50% kill of the population.

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 LC50 tes. The comparative toxicities of the dried cells ob *B. thuringiensis* isolates against *C. binotalis*is shown in Table 5. The LC50 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 LC50 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).

Toxicities of the dried HD-1 was 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 5). The insecticidal activity of the remaining isolates were least effective since the required higher doses to achieve LC50 (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.

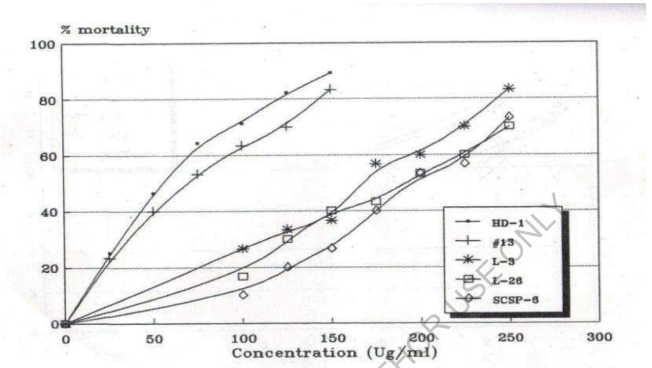

Figure 6. Relationship between percentage of morality and different concentrations of *B. thuringiensis* isolate against *C. binotalis*.

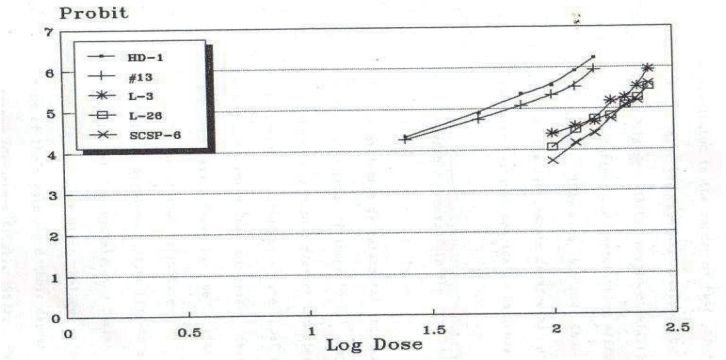

Figure 7. Dosage-mortality response curve of *B. thuringiensis* isolates against *C. binotalis.*

Results indicate that the variation of toxicity of the other bacterial isolates from the commercial produc could be attributed to the manner of mass production and formulation. This was commonly observed by Angus and Noris (1968), Dulmage (1970), Beegle et al., (1986). They noted that fermentations and formulations effect potencies of the bacterium. They also contend that there are differences in the response to commercial products and difference between insect species in response to the same product.

Another possible reason in the variation of the insecticidal activity of the different B. thuringiensis against the target insect is parasporal inclusion or toxic crystals of the isolates. Dulmage (1971) reported thet the crystal structures from different isolates used are affected according to bacterial strain, host species and even the medium in which they are grown. Even if these factors are the same. There may be variation in the parasporal bodies or crystal structures.

A significant difference exist between sizes of the protein molecules of the crystals of different serotypes. Thus, there is a correlation between the moleculer organization of the crystals and teir degree of toxicity with proteases (Grigovora et al., 1967). Burgerjon and Martouret (1971) attributed the variability in the efficacy of the crystals of different srains to various host to the structure of the crystal as a substrate and the specificity of the host enzyme.

Toxicity differences of the different isolates of B. thuringiensis against C. binotalis indicate that systematic screening is important in utilizing this bacterium for microbial control.

Symptomatology

Observations on the toxicitiy and pathogenicity of *B. thuringiensis* on *C. binotalis* showed that infected larvae turned yellowish at the middle and hind part of the abdomen (Fig.8). The integument also turned brown to black as the infection progreesed and the body became sticky because of oral and anal discharges. Dead larvae become shrunken and later turned black with putrid odor. The mechanism of toxicity is due to the digestion of the crystal in the insect gut and release of the soluble active toxin (Pendleton., 1968) which later on cause the midgut to swell and eventually disintegrate. Heimpel and Angus (1959) suggested that the histopathological effects observed might be due to the breakdown of cell-cementing substance under the influence of the crystal.

Table 5. The LC_{50} values of the diff*erent B. thuringiensis* isolates against *C. binotalis*

ISOLATES	LC50 UG/ ML	FIDUCIAL LIMITS		TOXICITY RANKING
		Lower	Upper	
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

Figure 8. Dead larvae of *C. binotalis* showing the progress of symptom of *B. thuringiesis*

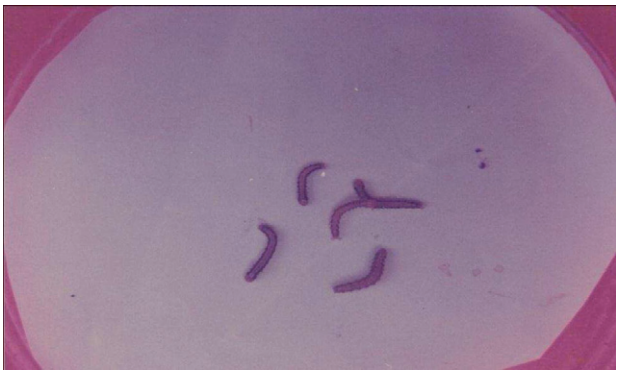

Figure 9. Healthy larvae of *C. binotalis*

SUMMARY AND CONCLUSION

The study was conducted at the insect Pathology Laboratory, Deparment of Entomology and BIOTECH, University of the Philippines at Los banos, Laguna. Primarily to isolate and screen *B. thuringiensis* against *C. binotalis*.

Six soil samples were collected from differen crop growing areas in South Kalimantan (Indonesia). Isolation from six soil samples yielded about 50 isolates, one was indentified as *B. thuringiensis*.

Of the 145 local *B. thuringiensis* isolates from BIOTECH and 1 from commercial product (HD-1) screened for toxicity against second instar larvae of C. binotalis, only 4 were selected. The dried cell local isolate #13 was most toxic with LC_{50} of 63.34 ug/ml and SCSP6 with 195.48 ug/ml. The relative toxicities in decreasing order were: HD-1 > #13 > L3 > L26 > SCSP6.

The result indicates that *B. thuringiensis* could be used in suppressing *C. binotalis* as a complement to other control measure to conclusively demonstrate its utility in pest suppression.

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Appendix Table 1. Effect of dried cells of *B. thuringiensis* isolate L3 on *C. Binotalis*.

CONCENTRATION Ug/ml	NO. OF INSECT TESTED	NO. OF DEAD INSECT	% MORTALITY
250	30	25	83.3
225	30	21	70.0
200	30	18	60.0
175	30	17	56.6
100	30	11	36.7
125	30	10	33.3
100	30	8	26.7
C	30	0	0

Appendix Table2. Effect of dried cells of *B. thuringiensis* isolate L26 on *C. Binotalis*

CONCENTRATION Ug/ml	NO. OF INSECT TESTED	NO. OF DEAD INSECT	% MORTALITY
250	30	21	70.0
225	30	18	60.0
200	30	16	83.3
175	30	13	43.3
100	30	12	40.0
125	30	9	30.0
100	30	5	16.0
C	30	0	0

Appendix Table3. Effect of dried cells of *B. thuringiensis* isolateHD-1 on *C. Binotalis*

CONCENTRATION Ug/ml	NO. OF INSECT TESTED	NO. OF DEAD INSECT	% MORTALITY
150	30	27	90.0
125	30	25	83.3
100	30	22	75.3
75	30	20	66.6
50	30	15	50.0
25	30	9	30.0
C	30	2	6.67

Appendix Table4. Effect of dried cells of *B. thuringiensis* isolate #13 on *C. Binotalis*

CONCENTRATION Ug/ml	NO. OF INSECT TESTED	NO. OF DEAD INSECT	% MORTALITY
150	30	25	83.3
125	30	21	70.0
100	30	19	63.3
75	30	16	53.3
50	30	16	40.0
25	30	7	23.3
C	30	0	0

Appendix Table5. Effect of dried cells of *B. thuringiensis* isolateSCSP on *C. Binotalis*

CONCENTRATION Ug/ml	NO. OF INSECT TESTED	NO. OF DEAD INSECT	% MORTALITY
150	30	27	90.0
225	30	25	83.3
200	30	22	75.3
175	30	20	66.6
150	30	15	50.0
125	30	9	30.0
100	30	2	6.67
C			

Appendix Table6. Probit regression equation and estimated LC50s of the selected *B. thuringiensis* isolate tested against *C. Binotalis*

B. THURINGIENSIS ISOLATES	REGRESSION EQUATION	$x^2 - TEST^a$	LC50 X = LOG (DOSE)	DOSE LC50
#13	Y = 1.3609 + 2.0196	$X\ 0.9785^{ns}$	1.80	63.38
L3	$Y = -3.4331 + 3.8206$	$X\ 2.3684^{ns}$	2.21	161.17
L26	$Y = -2.8186 + 3.4482$	$X\ 0.4444^{ns}$	2.27	185.12
SCSP6	$Y = -5.5515 + 4.6054$	$X\ 0.7912^{ns}$	2.29	195.48
HD-1	$Y = 0.9320 + 2.3714$	$X\ 0.5256^{ns}$	1.72	51.93

ns = not significant
a x^2 = Tabular value with 5 dif. At 5% = 11.07
a x^2 = Tabular value with 4 dif. At 5% = 9.48

Appendix Table7. Indonesian isolates with soil sources.

ISOLATE	SOIL SOURCES		
	Crop Planted	Great Soil	pH
AA	Rice	Typic Calciborolls	6.5
AA 1.1			
AA 1.2			
AA 2.1 (1)			
AA 2.2			
AA 2.3			
AA 4.1			
AA 2.5			
AA 2.5 (1)			
AA 2.5 (2)			
BB	Vegetable	Typic tropudults	4.8
BB 1.1			
BB 1.2			
BB 2.3			
BB 1.4			
BB 3.1			
CC	Corn	"	5.2
CC 1.2			
CC 2.1			
CC 2.3			
CC 2.4			
CC 3.2			
CC 5.1			
CC 5.1 (1)			
DD	Citrus	"	5.7
DD 1.2 (1)			
DD 1.3			
DD 1.5			
DD 2.2			
DD 3.1			
DD 4.1			
DD 4.2			

Appendix Table7 continued …

ISOLATE	SOIL SOURCES		
	Crop Planted	Great Soil	pH
EE	Peanut	"	4.7
EE 1			
EE 2			
EE 1.3			
EE 1.4			
EE 3.1			
EE 4.3			
D	Cabbage	"	5.1
D 1.2			
D 2.1			
D 2.2 (1)			
D 2.3			
D 2.4			
D 4.1			
D 3.1			
D 3.2			
D 4.2			
D 5.2			

Appendix Table8. *B. thuringiensis* isolate from BIOTECh.

ISOLATE NO.			
PSB 9103	# 31	60 LAC	HD – 1
PSB 9125	# 32	PG -02	SCSP6 (4)
PSB 1939	# 33	LEP 13	SCSP6 (1)
PSB 1957	# 34	LEP 22	SCSP2 (3)
PSB 9108	# 35	LEP 23	SCSP2 (6)
PSB 9143	# 36	LEP 25	L16 (4)
PSB 9134	# 37	LEP 37	L26 (2)
PSB 9112	# 38	LEP 27	L14 (3)
PSB 9132	# 39	LEP 28	L3 (1)
PSB 9133	# 40	LEP 30	L14 (2)
PSB 9102	# 41	# 102	D 1.8
# 1	# 42	# 112	D 1.6
# 2	# 43	# 139	D 1.1
# 3	# 44	# 143	D 1.2
# 4	# 45	# 156	D 1.9
# 5	# 46	# 160	E 5.3
# 6	# 47	# 167	D 1.3
# 7	# 48	# 183	E 5.4
# 8	# 49	# 191	E 2.8
# 9	# 50	# 192	E 5.2
# 10	# 51	# 198	D 1.2
# 11	# 52	# 200	SCSP6 (2)
# 12	# 53	# 209	E 5.2
# 13	# 54	APP 26	E 5.5
# 14	# 55	APP 1	
# 15	# 56	SCSP 2 (11)	
# 16	# 57	SCSP 2 (8)	
# 17	# 58	SCSP 2 (7)	
# 18	# 59	M 242	
# 19	# 60	EPA 18	
# 20	# 61	SCSP 2 (19)	
# 21	# 62	SCPS 2 (10)	
# 22	# 63	APP 27	
# 23	# 64	PSB 1991	
# 24	# 65	T 63-L4	
# 25	# 66	APP 11	
# 26	# 67	SCSP 2 (8)	
# 27	# 68	SPB 9132	
# 28	# 69	SPB 9122	
# 29	# 70	SPB 9123	
# 30			