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# Monitoring Insecticide Resistance Development in Beet Armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

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## ABSTRACT

Field collected larvae of beet armyworm, *Spodoptera exigua* (Hübner) were reared and evaluated for resistance against cypermethrin 10 EC, neem extract (Azadirachtin 0.1%) and *Bacillus thuringiensis* Berliner var. *kurstaki* (53,000 SU per mg) for 12 generations under laboratory condition by using leaf dip bioassay. Cypermethrin showed $LC_{50}$ value of 251 ppm at $F_1$ generation and decreased gradually to onward generations resulting $LC_{50}$ value of 90 ppm at $F_{12}$ generation for non-selection, whereas $LC_{50}$ increased rapidly with a value of 8625 ppm at $F_{12}$ generation under selection pressure. Neem extract exhibited $LC_{50}$ value ranging from 3.98 ppm to 9.99 ppm for non-selection, whereas under selection pressure $LC_{50}$ ranged from 4.38 ppm to 13.04 ppm throughout the generations. *Bacillus thuringiensis* var. *kurstaki* showed $LC_{50}$ value which ranged from $7.3 \times 10^6$ SU/L to $15.9 \times 10^6$ SU/L for non-selection, and selection for resistance monitoring gave $LC_{50}$ value ranged from $7.6 \times 10^6$ SU/L to $73.9 \times 10^6$ SU/L . Compared with non-selection strain, selection strain exhibited 95.83-fold, 1.44-fold and 5.6-fold increase in the $LC_{50}$ for 12 generations against cypermethrin, neem extract and *Bacillus thuringiensis* var. *kurstaki*, respectively.


**Key words:** *Spodoptera exigua*, cypermethrin, neem extract, *Bacillus thuringiensis* var. *kurstaki*, resistance monitoring, leaf dip

## INTRODUCTION

Beet armyworm, *Spodoptera exigua* (Hübner) is a polyphagous and widely distributed pest on many crops and vegetables including ornamental plants. The history of insecticide resistance development has earned the beet armyworm as one of the most costly insect pest, in terms of control, of vegetables grown in Thailand. Overtime, excessive use of synthetic insecticides has resulted in serious problem , for instance in the development of insect resistance to insecticides, insecticide-induced resurgence of insect pests, adverse effect on non-target organisms namely parasitoids, predators, pollinators, fishes, birds, cattle, and human beings. In addition, phytotoxicity, environmental pollution, and an alarming increase in the cost of pesticides have dictated the need of effective and biodegradable pest control materials with greater selectivity. For this reason natural

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pesticides are often preferred over synthetic ones

Among several options, the extracts from neem has evoked a great deal of interest because of its bio-efficacy and bio-degradability. The main substance, azadirachtin influences the hormone system of insects, exerting a pesticidal effect. Feeding activity, reproduction, and flying ability of insects are also affected. Botanical insecticide has been taking attention to control a variety of insect pest in crops, especially vegetables. It has no harmful effect on natural enemies, keeping the ecosystem safe and sound. The use of *Bt* is increasing dramatically because of recent development in genetic engineering, including insertion and expression of *Bt* toxin in several major crop plants such as cotton, tobacco and tomato ( Tabashnik 1994). However, like any kind of chemical insecticide, the value of *Bt* could be diminished seriously by widespread development of resistance in insect population to *Bt* toxin. Recently, several common species of insect pest have been selected for resistance to *Bt* in the laboratory, indicating that biological pesticides can suffer the same fate as chemical pesticides (Tabashnik *et al*., 1992; Chaufaux *et al*., 1997). Improved monitoring of resistance would decrease the number of ineffective pesticide applications that are made when a resistance problem exists but has not been diagnosed. Resistance often lead to replacement of one pesticide with another that are more expensive and less compatible with alternative control. Therefore, the study was designed to observe the potential of cypermethrin, neem extract and *Bacillus thuringiensis* var. *kurstaki* to induce resistance in field collected strain of *Spodoptera exigua* under laboratory condition.

## MATERIALS AND METHODS

### Collection of field strain and rearing

Larvae of beet armyworm were collected from the field of soybean, chilli, onion and mungbean at Kamphaeng Saen, Nakhon Pathom, Thailand in the month of December, 1998. The collected larvae were reared on artificial diet without exposure to any insecticides in Laboratory of Entomology Department, Kamphaeng Saen Campus of Kasetsart University, Thailand under 27 ± 2°C and 50 - 80% R.H. The diet (modified from the diet described by Shorey and Hale 1965) was composed of soaked mungbean (150 g), baking yeast (10 g), methyl paraben (2.5 g), sorbic acid (1.5 g), ascorbic acid (3 g), casein (3 g), choline chloride (0.5 g), agar (14 g), vitamin mixture (10 ml), formalin 40% (2 ml ) and distilled water (750 ml). Larvae were reared on artificial diet in both petridish and plastic cup, each with three larvae until they pupated. After the color of pupae turned brown, soaked them in 10% formalin for 10 minutes. Then they were dried in a good aeration condition. The pupae were then placed in box for adult emergence. The adults were then introduced in new boxes, each with 10 males and 10 females. Diluted honey was used in cup with cotton wool for adult feeding. A piece of wax paper was inserted inside the box for egg laying and covered with cheese cloth. The moth's diet was changed everyday. The eggs were collected from waxy paper, cheese cloth and cup. Then the eggs were soaked in 10% formalin for 10 minutes. After that they were passed through the running water twice and dried up in a good aeration condition. The clusters of eggs were then placed in diet box (upside down) and sealed with sticky tape. After the larvae hatched for a week, the wax paper, cheese cloth and cup were removed. The larvae were then placed in petridish and plastic cup on artificial diet. This rearing process was followed for every generation.

### Insecticides

Commercial formulation of cypermethrin (10%EC), neem extract (Azadirachtin 0.1%) and

*Bacillus thuringiensis* Berliner var. *kurstaki* (53,000 SU per mg) (Delfin) was used as for the bioassay.

**Toxicity test**

Newly molted 3rd instar larvae from the $F_1$ laboratory generations were treated with different insecticides by using leaf dip technique. Soybean leaves were collected from the plants grown in pots throughout the study. The leaves of similar size were selected and dipped into the test solutions for 10 seconds with gentle agitation. Surfaces were allowed for air dry, and then placed into a 8 cm diameter transparent plastic cup with 20 larvae per cup. One leaf was placed in a cup. In total, 80 larvae (4 replications of 20 larvae per cup) were used for each concentration. Seven serial concentrations including one control (distilled water as the diluent) were used for each test insecticide. Untreated controls were dipped in distilled water. To avoid desiccation of leaves in the test containers, moistened cotton wool were attached with leaf petiole. Serial dilutions of the test insecticides were expressed in ppm (part per million) of active ingredient for cypermethrin and neem extract, and SU/L (spodoptera unit per liter) for *B. thuringiensis* var. *kurstaki*.

Larval mortality was assessed after 48 hours for cypermethrin and 72 hours for neem extract and *B. thuringiensis* var. *kurstaki*. Larvae were considered dead if they gave no coordinated response to stimulation by touch with a blunt needle. Results were expressed as percentage of mortality, correcting for untreated (control) mortality using Abbott's (1925) formula. Data were analyzed by probit analysis (Finney, 1971).

Selection pressure procedure

At first $F_1$ laboratory generation of beet armyworm were divided into two groups. The first group was reared without any insecticide pressure (non-selection) and the other group was divided into three sub-groups subjected to selection pressure with cypermethrin, neem extract and *B. thuringiensis* var. *kurstaki*. After selection progeny for each insecticide, each sub-group was divided into two groups: one for next selection (allowed for feeding insecticide treated leaves for inducing resistance) and other for toxicity test with different concentrations. In every generation, larvae under selection pressure were assessed for $LC_{50}$ for each insecticides. On the other hand, the untreated stock was assessed for $LC_{50}$ for each insecticide throughout the generation. This process was continued up to 12 generations for monitoring resistance development in beet armyworm . To determine the resistance ratio (RR), the $LC_{50}$ of each insecticide for the selected strain was divided by the corresponding $LC_{50}$ for the non-selected strain.

## RESULTS AND DISCUSSION

**Selection for cypermethrin**

The $F_1$ generation (non-selection) of beet armyworm was tested for resistance to cypermethrin exhibited 251 ppm for $LC_{50}$ whereas $F_1$ generation (selection pressure) showed 383 ppm for $LC_{50}$ which indicated the induced cypermethrin in the later (Table 1). It was observed from the study that cypermethrin provided a decrease values of $LC_{50}$ to onward generations in non-selection stock which reached to 90 ppm at 12th generation. Mishra (1989) found $LC_{50}$ value of 95 ppm for cypermethrin against 6 days old larvae of *Heliothis armigera* which was similar to $LC_{50}$ of non-selection stock (90 ppm) at 12th generation. After 11 generations of continuous selection pressure, beet armyworm showed a rapid increase value of $LC_{50}$ to proceeding generations which reached 8625 ppm at 12th generation. This result indicated that cypermethrin was induced in beet armyworm for resistance by insecticide selection pressure in the laboratory, whereas continuous rearing without selection

**Table 1** Selection and progression of resistance to cypermethrin of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

| Generations | Non-selection strain $LC_{50}$ (ppm) | Selection strain $LC_{50}$ (ppm) | Resistance ratio (RR)[1/] |
|---|---|---|---|
| $F_1$ | 251 | 383 | 1.53 |
| $F_2$ | 200 | 805 | 4.03 |
| $F_3$ | 176 | 1237 | 7.03 |
| $F_4$ | 172 | 1677 | 9.75 |
| $F_5$ | 166 | 2897 | 17.45 |
| $F_6$ | 155 | 3188 | 20.57 |
| $F_7$ | 151 | 4867 | 32.23 |
| $F_8$ | 144 | 6734 | 46.76 |
| $F_9$ | 135 | 7049 | 52.21 |
| $F_{10}$ | 108 | 8046 | 74.50 |
| $F_{11}$ | 100 | 8368 | 83.68 |
| $F_{12}$ | 90 | 8625 | 95.83 |

[1/] $LC_{50}$ of selection strain / $LC_{50}$ of non-selection strain.

pressure caused reduced resistance level. The resistance ratio compared with non-selection at $LC_{50}$ was 95.83. Chau (1995) reported that beet armyworm had become resistance to pyrethroids such as cypermethrin, fenvalerate and deltamethrin. Aldosari *et al.* (1996) found that selection of the Marana strain for resistance to cyfluthrin for 9 generations resulted in a 70.7-fold increase in the $LD_{50}$. These results support the resistance development in beet armyworm against cypermethrin by selection under laboratory condition.

**Selection for neem extract**

Neem extract showed less efficacy to develop resistance in beet armyworm. It might be due to the disrupted metamorphosis which resulted in the larval mortality. Even at low concentration larvae faced abnormal molting. The investigation revealed 3.98 ppm for $LC_{50}$ in non-selection $F_1$, whereas selection $F_1$ showed 4.38 ppm for $LC_{50}$. Non-selection $F_2$ generation exhibited 6.13 ppm for $LC_{50}$ and the same generation of neem selection pressure yielded 6.32 ppm for $LC_{50}$. The ranges of $LC_{50}$ values from $F_2$ to $F_{12}$ (non-selection) varied from 6.13 ppm to 9.99 ppm, whereas under selection pressure it varied from 6.32 ppm to 13.04 ppm (Table2). The result showed the highest 9.99 ppm for $LC_{50}$ within 12 generations in non-selection, whereas selection stock provided its highest 13.04 ppm for $LC_{50}$. Resistance ratio varied from 0.98 to 1.44 within 12 generations . The investigation revealed that neem could not induce in beet armyworm for resistance development up to 12 generations under laboratory condition. Moar and Trumble (1987) reported the toxicity of neem to beet armyworm, *Spodoptera exigua* by using neonate larvae in a diet-incorporation bioassay. Neem showed 0.116 µl/ml for $LC_{50}$. Lee *et al.* (1991) reported that one dose (1 µg) being sufficient

**Table 2** Selection and progression of resistance to neem extract of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

| Generations | Non-selection strain $LC_{50}$ (ppm) | Selection strain $LC_{50}$ (ppm) | Resistance ratio (RR)[1/] |
|---|---|---|---|
| $F_1$ | 3.98 | 4.38 | 1.10 |
| $F_2$ | 6.13 | 6.32 | 1.03 |
| $F_3$ | 6.56 | 7.14 | 1.09 |
| $F_4$ | 8.35 | 8.20 | 0.98 |
| $F_5$ | 8.97 | 10.36 | 1.15 |
| $F_6$ | 8.46 | 9.81 | 1.16 |
| $F_7$ | 8.76 | 9.53 | 1.09 |
| $F_8$ | 9.14 | 11.09 | 1.21 |
| $F_9$ | 9.08 | 11.78 | 1.30 |
| $F_{10}$ | 8.36 | 11.74 | 1.40 |
| $F_{11}$ | 9.99 | 13.04 | 1.31 |
| $F_{12}$ | 8.23 | 11.87 | 1.44 |

[1/] $LC_{50}$ of selection strain / $LC_{50}$ of non-selection strain.

to disrupt subsequent development and /or reproduction stages. Azadirachtin at 20 and 30 ppm caused higher mortality than at 10 ppm and also retarded larval development (Adhikary 1981). Adel and Sehnal (1999) reported that larvae of *Spodoptera littoralis* feeding with 10-1000 ppm sunneem oil (containing about 0.1-10 ppm azadirachtin) causes a cessation or reduction of feeding, delay of molts, death of larvae and pupae, and sterility of emerged adults. No references on the development of resistance against neem products has been found except Vollinger (1986) who reported the development of resistance against neem extract in comparison with deltamethrin in case of *Pleutella xylostella*. He reported that NSKE (neem seed kernel extract) treated line showed no sign of resistance up to 42 generations whereas deltamethrin treated lines developed 20 and 35 times resistance.

**Selection for *B. thuringiensis* var. *kurstaki***

The toxicity test for *Bt. kurstaki* resulted 7.3 $\times 10^6$ SU/L for $LC_{50}$ in non-selection stock ($F_1$), whereas selection stock ($F_1$) exhibited $7.6 \times 10^6$ SU/L for $LC_{50}$. From $F_2$ to $F_{12}$ generations, *Bt* (non-selection) showed $LC_{50}$ values from $11.2 \times 10^6$ - $15.9 \times 10^6$ SU/L, whereas selection stock exhibited $12.3 \times 10^6$ - 73.9 x $10^6$ SU/L for $LC_{50}$ (Table 3). The resistance ratios from $F_1$ - $F_5$ generations were more or less similar showing no resistance development occurred within the periods. After $F_6$ generation, resistance ratios became double compared to $F_1$ generation. At 12th generation resistance ratio raised to 5.6 indicating beet army worm acquired resistance against *Bt* var. *kurstaki* under laboratory condition . The toxicity of *Bt* var. *kurstaki* was evaluated in laboratory and field against *Spodoptera exigua* by Tamez-Guerra *et al*. (1998). Results of laboratory tests showed that some *Bt* strains isolated from Mexico (GM-7 and

**Table 3** Selection and progression of resistance to *Bacillus thuringiensis* var. *kurstaki* of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

| Generations | Non-selection strain $LC_{50}$ ($\times 10^6$SU/L) | Selection strain $LC_{50}$ ($\times 10^6$SU/L) | Resistance ratio (RR)[1/] |
|---|---|---|---|
| $F_1$ | 7.3 | 7.6 | 1.04 |
| $F_2$ | 11.2 | 12.3 | 1.10 |
| $F_3$ | 12.5 | 16.2 | 1.30 |
| $F_4$ | 14.2 | 16.8 | 1.18 |
| $F_5$ | 13.7 | 18.2 | 1.33 |
| $F_6$ | 13.7 | 24.3 | 1.77 |
| $F_7$ | 14.1 | 25.6 | 1.82 |
| $F_8$ | 15.1 | 30.4 | 2.01 |
| $F_9$ | 14.7 | 38.4 | 2.61 |
| $F_{10}$ | 15.9 | 43.1 | 2.71 |
| $F_{11}$ | 15.9 | 63.1 | 3.97 |
| $F_{12}$ | 13.2 | 73.9 | 5.60 |

[1/] $LC_{50}$ of selection strain / $LC_{50}$ of non-selection strain.

GM-10), were sufficiently toxic to warrant further investigation as potential bio-insecticides for lepidopteran control. The toxicities of different components of the spore-parasporal body complex of the NRD-12 and HD-1 strains of *Bt* var. *kurstaki* Berliner to neonate *Spodoptera exigua* were determined using diet incorporation bioassays . Spore preparations from HD-1($LC_{50}$=117 µg/ml diet) were slightly more toxic than those from NRD-12($LC_{50}$=166 µg/ml). Belda *et al*. (1994) reported that mortality against 2nd instar larvae of beet armyworm by the application of *Bacillus thuringiensis* subsp. *aizawai* : *kurstaki* @ $6 \times 10^6$ IU/L (international unit/liter) reached up to 50% after 168 hours which supported the results of bioassay. Many field populations of diamondback moth, *Plutella xylostela* have evolved resistance to *Bacillus thuringiensis* var. *kurstaki* (Tabashnik, 1994). Moar *et al*. (1995) reported resistance of *Spodoptera exigua* to CryIC, a major toxin in *Bt* a*izawai*. All of these documents support the present study.

## CONCLUSION

Larvae of beet armyworm, *Spodoptera exigua* (Hubner) were collected from different vegetable fields and their progeny were evaluated for resistance to cypermethrin, neem extract and *Bacillus thuringiensis* var. *kurstaki* under laboratory conditions by selection pressure. The study revealed that beet armyworm larvae developed resistance to cypermethrin which caused the rapid increase of $LC_{50}$ values with 95.83-fold within 12 generations. In case of neem, larvae faced molting problem even at low concentration resulting larval mortality. It is assumed that beet armyworm could not develop resistance sharply against neem extact by selection pressure for generations studied. Beet armyworm had been gaining resistance against *Bacillus*

*thuringiensis* var. *kurstaki* within 12 generations. Resistance ratio increased to 5.6-fold indicating biological pesticides can suffer the same fate as chemical pesticides.

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