Developing a neem-based pest management product: Laboratory evaluations of neem extracts on insect pests resistance to synthetic pesticides

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Abstract

Laboratory studies has been conducted as part of a project aimed at development of a neem-based insecticide for pest management purposes. Data are presented for permethrin, a pyrethroid insecticide, and neem (*Azadirachta indica*) products tested against larvae of Diamondback Moth *Plutella xylostella* and *Helicoverpa armigera* which were collected from several locations in West Java, Indonesia. The results of bioassay showed that the average LC_{50} values of *Plutella xylostella* strain of Pangalengan, Garut and Lembang had been 60-100 fold higher to permethrin as compared with that of the normal dosage recommended. Similarly, the LC_{50} values obtained from *Helicoverpa armigera* collected from Lembang, Pangalengan, and Jatinangor strains showed the same trend with LC_{50} values which had been 46-73 fold as compared with that recommended dosage. The fact that the LC_{50} values of both *Plutella xylostella* and *Helicoverpa armigera* were much higher than the recommended rates for control of either insect, suggest that both insects have developed resistance to permethrin. The results of bioassay with neem products tested against *Plutella xylostella* and *Helicoverpa armigera* larvae showed that, in general, neem products could provide adequate control of insects which had developed resistance to permethrin. Interestingly, the findings with neem products indicated that the susceptibility of each strain on insect in question showed the same trend. Viz., statistically LC_{50} values for each strain of either *Plutella xylostella* or *Helicoverpa armigera* to neem products was not significantly different one to another. However, our findings showed that neem treated insects, even though they were not killed directly by the insecticide, the insects were not able to molt to the next instar or pupae, very low percentage of adults emerged. Therefore, we suggest that the susceptibility of neem products tested could not be easily determined by only measuring the LC_{50} values from the larval stage, but other aspects should be considered as well, i.e., the disruption of the growth and development of the insect in question. In short, our findings suggest that neem products could be used effectively to control insects which have developed resistance to conventional insecticide

Key words : Insect, Plutella xylostella, Helicoverpa armigera, permethrin, insecticide resistance, neem

1 Introduction

The overuse and misuse of insecticides in agriculture practices have caused target pests become resistant for a wide range of insecticides (Georghio 1990, Ahmad 1995). Owing to this matters, it has become increasingly difficult to find insecticide(s) that is (are) still able to control target pest effectively. This situation has led many scientists to look for new methods (without pesticides) of controlling agricultural pests. However, it is difficult to foresee how insect pests can be controlled effectively without insecticides. In fact, during the past two decades the concept of the judicious use of insecticides has been formalized in Integrated Pest Management (IPM), making insecticides still play a central role in current and future crop protection (Metcalf, 1982).

In Indonesia two of the major vegetables grown are cabbage and tomato. Cabbage, as many other brassicas, are very susceptible to some insect pests. Feeding damage by the diamond-back moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is a primary factor limiting the production of cabbage in many highlands' areas of Indonesia, whereas tomato plant is widely known to have been attacked by *Helicoverpa armigera* (Lepidoptera: Noctuidae), a polyphagous insects that feeds not only on tomato but also on other agricultural crops

Due to the severity of this problem, substantial efforts have been devoted, including the development of IPM program. Unfortunately, insecticides are still the principal means of control by farmers. Even worse, there is mounting evidence that the growing use of insecticides to control diamond-back moth has resulted in the development of resistance to

almost all major insecticide groups, including pyrethroid (Tabashnik, 1994; Soekarna, *et.al.* 1982, Sastrodihardjo, 1986, Adiputra, 1984; Sastrosiswojo, 1990), as well as undesirable ecological side effects to our environment.

The situation to *Helicoverpa armigera* is no different than that of *Plutella xylostella*. Insecticide resistance in *H. armigera*, especially to pyrethroids, is now widespread in many countries, including Indonesia (McCaffery and Walker 1991, Ahmad et.al. 1995)

Owing to this problem, we believe that it is becoming increasingly important to find an alternative for conventional insecticides by searching to the natural products; one of the candidates is neem-based insecticide (Schmutterer, 1990, Quarles 1994). The use of neem-based insecticides as a source of biologically active substances for pest control is increasing worldwide, and have recently gained popularity as components of integrated pest management (Banken and Stark, 1997). Fortunately, in Indonesia, neem trees are grown in many parts of Java and Bali and the seeds are not difficult to obtain to be extracted as insecticide (Ahmad *et.al.*, 1992)

Neem products are unique in that they are medium to broad spectrum insecticides, yet appear to have little or no toxicity to warm-blooded animals (National Research Council, 1992). Interestingly, to the farmers advantages, the scientists believe that due to its mixed mode of action, neem products could be used to control insects that are resistant to, or inherently difficult to control with conventional insecticides.

To the best of our knowledge, in Indonesia, no one has ever reported or tried to see whether neem products have the potential to control pyrethroid resistant diamond-back moth *Plutella xylostella* or *Helicoverpa armigera*. Therefore, the experiments reported here were designed to measure the susceptibility of *Plutella xylostella*, and *Helicoverpa armigera* collected from four localities to permethrin and neem products.

2 Materials and methods

2.1 Insects Collections and Rearing

The *Plutella xylostella* and *Helicoverpa armigera* strains for the bioassay were obtained by sampling strains from several predominantly cabbage and tomato agroecosystem in areas where difficulties in controlling these insects had been reported, *i.e.*, Pangalengan, Jatinangor, Lembang and Garut areas, all in West Java: larvae and pupae were collected from each site.

For *Plutella xylostella*, the emerging adults were placed in cages and fed on a 30 % honey solution; seedling of cabbages are used as rearing materials, three days after emergence, adults would lay eggs on the head of cabbage seedlings and the eggs would hatch after 3-4 days. The newly hatched larvae would feed on the seedling. after reaching instar III, then they were ready for the experiments. In general, method of rearing developed by Liu and Sun (1984) were followed with slight modification as needed. In brief they were kept in 12:12 h photoperiod, RH about 80 % and at room temperature.

Rearing of *Helicoverpa armigera* was based on the method developed for *Heliothis zea*, developed by Waldbauer *et.al.*, (1984). In brief a laboratory culture of *Helicoverpa armigera* was started with larvae collected from Lembang, Jatinangor, and Garut. The culture was maintained on an artificial diet, wheat germ-based diet as described by Waldbauer et al., (1984), they were kept in 12:12 h photoperiod, and room temperature. All experiments were started with newly molted unfed 4th instar larvae.

2.2 Insecticides Preparation

The commercial preparations of pyrethorid permethrin (Ambush 2 EC of ICI) was used: as the neem products, we used neem extract of our laboratory (Inter University Center for Life Sciences ITB) and the commercial preparation of neem, Margosan-O (W.R. Grace & Company, Columbia, Md, USA)

2.3 Bioassay of Plutella xylostella Larvae

Bioassays and rearing were conducted at the same laboratory and environment. Each bioassay was performed on a separate date; the bioassays were carried out by methods similar to the leaf residue methods described by Tabashnik *et. al.*, (1990). In brief, leaf disks (6 cm diameter) were cut from 2 months old cabbage plants. Depending on the insecticide being tried, each disk was individually dipped for 5 seconds in one of either neem solutions or permethrin, or controls. As the suspension on the leaf surface dried, the disk was then placed in a 9-cm diameter petri disk, lined with Whatman filter paper on the bottom. Eight different concentrations of permethrin were used, i.e. 100, 200, 300,

400, 500, 700, 900 and 1000 ppm. Whereas, for the neem extract, one of either 10, 20, 40, 60, or 80 %(v/v) for the neem extract made in our laboratory and 6 concentrations for Margosan-O, *i.e.* 1, 15, 30, 60, 120 and 240 ppm was used.

Third instar larvae were separated from the colony (normally 7 days after eggs were placed on cabbage leaves). The larvae were then placed on treated cabbage leaf disk and left in the laboratory. Observations were made every 24 hours.

2.3 Bioassay of Helicoverpa armigera larvae

Permethrin insecticide and neem extracts were used against 4th instar larvae of *Helicoverpa armigera*. The method used was based on the Entomological Society of America (Anonymous, 1970). In brief, 4th instar newly molted larvae were given a 5 µl permethrin solution dorsally. The solution containing either 10, 100, 200, 300, 400, or 500 ppm of permethrin. Neem treatment was based on neem extract product of our laboratory (IUC Life Sciences ITB), six concentrations were given, *i.e.* 1, 10, 20, 50 or 80 % (v/v). Observation were made every 24 hours.

2.4 Data Analysis

Concentration-mortality regression for the larvae from each bioassays were evaluated statistically using probit analysis (Polo-PC Probit and Logit analysis; LeOra Software 1994). Differences in toxicity were considered significant when 95 % Fiducial Limit (FL) did not overlap (Adams *et. al.*, 1990).

3 Results and discussion

3.1 Plutella xylostella

Table 1 shows the response of each strain to permethrin. Lembang strain apparently was the most susceptible strain (LC_{50} = 369 ppm) as compared to the other two (LC_{50} Garut Strain = 483 ppm, and LC_{50} Lembang strain = 538 ppm).Based on these findings, we decided to consider Lembang strain as our base for comparing the resistance ratio. Resistance ratio in this experiment, as will be mentioned elsewhere, is calculated as follows: Resistance ratio = LC_{50} of resistant strain : LC_{50} of the most susceptible strain. The steepest slope was 21.33 (Lembang strain) and the flattest was 1.48 (Pangalengan Strain). Even though statistically not significant (based on the Fiducial limit values), the low LC_{50} and the steep slope at Lembang strain suggest that *Plutella xylostella* in this locality shows a relatively more susceptible to permethrin as compared to the other two strains. However, if we compared the dosages that would normally used by the farmers to control this insects, we found that all strains have shown some degree of resistance to pyrethroid. For example, the normal dosage for controlling *Plutella xylostella* is 3-5 ppm. Interestingly, our findings shown that at least 300 ppm are needed to have an LC_{50}. The very high LC_{50} values (approximately 60-100 higher as compared to the recommended dosage), suggests that resistance has already reached a serious level. It is also interesting to note that based on the information that was gathered from the farmers, it turned out that the farmers had been normally used carbamates and organophosphate insecticides and had never used any pyrethroid insecticides to control this *Plutella xylostella*. Therefore, we reasoned that some cross resistance to pyrethroid might has been occurred due to the previous insecticide application, including organochlorine, or the population of the insects in question always got new infestation from different place (s) which might have been sprayed by pyrethroid insecticides. In short, this finding and the regression line suggest, as we expected before, that all three strains were already resistant to pyrethroid as indicated by Solang (1995).

Table 1 Response of several strains of *Plutella xylostella* to permethrin

Strain	n	LC_{50}	Slope	RR
Pangalengan	30	369 [a] (195-577) ppm	1.48 ± 0.41	
Garut	30	483 [a] (217-748) ppm	2.75 ± 0.30	1.31
Lembang	30	538 [a] (480-568) ppm	21.33 ± 6.76	1.46

Means within columns followed by the same superscripts are not significantly different (Calculated by Fiducial Limit on 95 % Level of Confidence) (Adams, *et. al.* ,1990)

Having known that our *Plutella xylostella* has been resistanced to permethrin (See Table 1), our tests with neem preparation as can be seen in Table 2 indicate that neem could provide adequate control of *Plutella xylostella*. Neem extract which was made in our laboratory showed a little variation in the average LC_{50} values which ranged from 6.47 % to 11.25 % (range of application is 10 % - 100 %).

Tests with commercial neem preparation with Margosan-O yields similar findings with LC_{50} in 53.79 ppm and 57.58 ppm respectively, for *Plutella xylostella* strains from Lembang and Garut. Even though without the results from Pangalengan for *Plutella xylostella* tested against Margosan-O, our findings suggest that both neem extract preparation (Product of IUC, our Lab.) and commercial preparation (Margosan-O) have shown to have the potential to be developed as alternatives to conventional insecticides for controlling the pyrethroid resistant *Plutella xylostella*.

Table 2 Responses of several strains of *Plutella xylostella* to neem insecticides

Strain	n	Insecticide	AVERAGE LC_{50}	Slope ± SE
Pangalengan	30	Neem extract IUC	11.25 [a] %	1.50 ± 0.37
Garut	30	Neem extract-IUC	9.55 [a] %	1.62 ± 0.40
Lembang	30	Neem extract -IUC	6.47 [a] %	1.35 ± 0.38
Pangalengan	30	Neem Margosan-O	103 [b] ppm	3.61 ± 0.53
Garut	30	Neem -Margosan -O	249 [b] ppm	0.96 ± 0.21
Lembang	30	Neem Margosan-O	146 [b] ppm	2.46 ± 0.40

Means within columns followed by the same superscripts are not significantly different (Calculated by Fiducial Limit on 95 % Level of Confidence) (Adams, *et al.* 1990)

Table 3 and 4 show the effect of different concentrations of neem extracts on the development of *Plutella xylostella* larvae. It shows that pupation was significantly reduced started at 1 ppm in the insects collected from Lembang (63.3 % pupation) and Garut (50 % pupation). No pupation occurred in the 120 ppm and 60 ppm in Lembang strain, only 6.6 % in 60 ppm (Pangalengan) and 3.3 % (Garut). Table 4 shows similar trend that the formation of normal adults was significantly affected by neem treatment. Only 13.3-30 % normal adults were recorded in the 30 ppm, and in the 60 ppm no emergence of normal adults from Garut and Lembang strain, but 3.3 % of normal adults were still emerged from Pangelengan strain. No normal adults occurred in the 120 ppm.

Table 3 Neem treatment and the formation of normal pupae of *P. xylostella*

Strain	Normal pupae (%)					
	Control	1 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Pangalengan	100.0	96.6	76.6	43.3	6.6	0.0
Garut	90.0	50.0	63.3	16.6	3.3	0.0
Lembang	96.6	63.3	60.0	3.3	0.0	0.0

Table 4 Neem treatment and the formation of normal adults of *Plutella xylostella*

Strain	Normal adults (%)					
	Control	1 ppm	15 ppm	30 ppm	60 ppm	120 ppm
Pangalengan	100.0	90.0	30.0	13.3	3.3	0.0
Garut	86.6	76.6	70.0	30.0	0.0	0.0
Lembang	100.0	96.6	70.0	13.3	0.0	0.0

3.2 *Helicoverpa armigera*

Table 5 shows that *Helicoverpa armigera* from Jatinangor had the lowest LC_{50} value (73 ppm) as compared to the other two strains, viz., Pangalengan (104 ppm) and Lembang (189 ppm). This finding suggests that among the three strains, *H. armigera* from Jatinangor, relatively, was the most susceptible to permethrin than those from Lembang and Pangelengan. Based on this, we put Jatinangor strain as our standard to measure the resistance ratio. Therefore, Pangalengan and Lembang strains have resistance ratio of 1.99 and 2.59, respectively. However, if we take into account the recommended dosage for the application of permethrin to control this insect, we found that our lowest LC_{50} value (73 ppm) obtained from the experiments is exceedingly higher than the recommended dosage which only used 1-2 ppm. Therefore, we have reason to believe that all strains have developed some degree of resistance to permethrin.

Table 5 Responses of several strains of *Helicoverpa armigera* to permethrin

Strain	n	LC_{50}	Slope	RR
Jatinangor	30	73 (45 - 104)^{a}	1.43 ± 0.21	
Pangalengan	30	104 (52 - 168)^{ab}	1.35 ± 0.21	1.99
Lembang	30	189 (157 - 230)^{b}	3.18 ± 0.45	2.59

Means within columns followed by the same superscripts are not significantly different
Calculated by Fiducial Limit on 95 % Level of Confidence) (Adams, *et.al.* 1990)

Table 6 shows the dose-response regression estimates for neem against *Helicoverpa armigera* larvae. LC_{50} values ranged from 2.74 to 12.11%. The steepest slope was 1.60 (Lembang strain) and the flattest was 1.16 (Pangalengan strain). However, statistically there was no significant difference among them as the fiducial limit values were not overlap (Adams *et.al.* 1990). This finding was in agreement with our expectation that all strains has never been exposed to neem insecticide, which suggest the insect in question are still very susceptible to neem.

Table 6 Responses of several strains of *Helicoverpa armigera* to neem extract

Strain	n	LC_{50}	Slope	RR
Pangalengan	30	2.74 (0.694 - 5.706)*	1.16 ± 0.19	
Jatinangor	30	3.58 (1.883 - 5.681)*	1.32 ± 0.20	1.31
Lembang	30	12.11(5.498 - 22.447)*	1.60 ± 0.23	4.42

Means within columns followed by the same superscripts are not significantly different
(Calculated by Fiducial Limit on 95 % Level of Confidence) (Adams, *et.al.* 1990)

As we did with *Plutella xylostella*, we also evaluated the effect of neem treatment on the pupal and adult development of *Helicoverpa armigera*. The results as can be seen in Tables 7, 8 and, 9, in general, show that pupal development was severely affected as the concentration of neem was increased. For example, 50 % neem extract could reduce the pupation down to only 6.7 % in Lembang strain, 3.3.% in Pangalengan strain, and no pupation occurred in Jatinangor strain.

Similarly, the emergence of adults was severely affected by neem treatment (Table. 8) no emergence was recorded in 50 % and 80 % neem. Table 9 shows that neem treatment would still allowed the pupae to eclose to adult form but in abnormal shaped. In 20 % concentration, neem was able to produce 71.4 - 100.0 % adults with some degrees of abnormalities.

Table 7 Pupal development and neem treatment (%)

Strain	Normal pupae, %					
	Concentration of neem extracts					
H. armigera	0 %	1 %	10 %	20 %	50 %	80 %
	v/v	v/v	v/v	v/v	v/v	v/v
Lembang	100.0	70.0	60.0	53.3	6.7	0
Pangalengan	100.0	66.7	30.0	16.7	3.3	0
Jatinangor	100.0	56.7	36.7	26.7	0	0

Table 8 Adults emergence and neem treatment (%)

Strain	Formation of normal adults (%) Neem concentration					
H. armigera	0 % v/v	1 % v/v	10 % v/v	20 % v/v	50 % v/v	80 % v/v
Lembang	100.0	70.0	40.0	23.3	0	0
Pangalengan	100.0	46.7	16.7	6.7	0	0
Jatinangor	96.0	33.3	26.7	13.3	0	0

Table 9 The emergence of abnormal adults and neem treatment (%)

Strain	Abnormal adults (%) Neem concentration					
H. armigera	0 % v/v	1 % v/v	10 % v/v	20 % v/v	50 % v/v	80 % v/v
Lembang	0	9.5	41.8	71.4	-	-
Pangalengan	0	21.4	60.0	100.0	-	-
Jatinangor	0	30.0	50.0	75.0	-	-

Table 10 shows the overall effects of neem treatment which as calculated are total mortality of larvae, pupae and adults. We thought that this kind of measurement is important for neem as the effect of neem is ready seen in the mortality of larval stage only. Therefore, Table 10 shows that even in 1 % of neem concentration, it was enough to disturb the growth and development of the insect in question. The effect, apparently, is dosage dependent.

Table 10 Total mortality (%) of *H. armigera* larval, pupal, and adults as a result of neem treatment

Strain	Total mortality: larvae, pupae and adults (%) Neem concentration					
H. armigera	0 % v/v	1 % v/v	10 % v/v	20 % v/v	50 % v/v	80 % v/v
Lembang	0	30.0	60.0	76.6	100.0	100.0
Pangalengan	0	53.3	83.3	93.3	100.0	100.0
Jatinangor	3.3	66.7	73.3	86.7	100.0	100.0

Our experiments show that both neem extract product of our laboratory and Margosan-O, the commercial preparation, both gave satisfactory results. However, we have to mention here that Margosan-O and neem extracts are not exactly the same in terms of active ingredients. Margosan-O contains 0.3 % azadirachtin (the main agent for battling insect) and 14 % neem oil, whereas neem extract, as the name implies, contains not only azadirachtin and neem oil but also other active ingredients such as salannin, meliatriol, and nimbin. Each of these has an insecticidal activity. Nonetheless, from our experiment it was difficult to notice if there was any difference in insecticidal activity against *Plutella xylostella* or *Helicoverpa armigera* between neem extract and Margosan-O since both gave good results as we expected. Both neem products were not only able to kill the treated larvae, but also, which is unique to neem treatment, cause several symptoms to happen such as extended larval stage, no pupation at higher neem concentration, loss of appetite, formation of pupal-adult intermediates, inability to complete pupal ecdysis, and deformation of adult characters. All of the observed morphogenetic abnormalities expressed by the treated larvae were similar to those reported by Ladd *et.al.* 1984 and Banken and Stark 1997.

Permethrin's mode of action, as pyrethroid insecticide, is interfering neural transmission in insect nervous system, and it's entirely different from that of neem's. Neem extracts, on the other hand, as we mentioned before, contains several active ingredients, and they act in different ways ranging from antifeeding to disrupt the growth and development of

the insect through hormone regulation. Fortunately, neem's active ingredients bear no resemblance to the active ingredients in many marketed conventional insecticides. Therefore with its mixed mode of actions, and with the fact that there is no reported case on insect resistance to neem, neem products would be a good candidate to control any insects that have developed resistance to conventional insecticides. This notion become more important considering the fact that *Plutella xylostella* and *Helicoverpa armigera* are well known problem insects world wide and easily developed high level resistance in the field to any modern insecticides (Ahmad and McCaffery, 1988; Ascher, 1993).

In summary our, laboratory finding and also our previous experiments (Ahmad *et.al* 1992) clearly suggest that neem products both of IUC ITB and the commercial one (Margosan O) are still effective to be used as natural insecticide against the apparent resistance to pyrethroid *Plutella xylostella* and *Helicoverpa armigera*. Therefore it is fair to say that to combat the problem with the ever increasing insect resistance to insecticides, neem is one of the answers, and deserves more thorough study before it could be used just like other commercial insecticides available in the market. However, one has to be careful in measuring the effectiveness of neem insecticides as symptoms of exposure to neem were generally not evident until the onset of molting or pupation in the control animals. Mortality of the affected insects occurred not only during the larval treatment but also during pupal stages, or upon adults emergence.

In conclusion, our data indicate that neem insecticide shows hopes as effective alternative insecticide to control insects that were normally difficult to control by using conventional insecticides.

4 Acknowledgments

Thanks and appreciation due to the Asahi Glass Foundation for funding this project. Thanks also due to Mashuri and Siane for collecting some of the *Plutella xylostella* and *Helicoverpa armigera* larvae, and laboratory assistance.

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