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Dosage Mortality Studies with *Bacillus thuringiensis* and Neem Extract on Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera:Plutellidae)

Kajian Dosis Mortalitas *Bacillus thuringiensis* dan Ekstrak Nimba pada Ulat Daun Kubis *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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ABSTRACT

The objective of the study was to evaluate the responses of the diamondback moth, *Plutella xylostella* (L.) larvae to B*acillus thuringiensis* and Neem extract. Therefore, dosage mortality studies by bioassay method were conducted on populations of *Plutella xylostella* from Garut, Pangalengan, and Lembang. Tests with *Bacillus thuringiensis*, resulted in no significant differences in susceptibility between Garut and Pangalengan populations. However, those two populations were differed significantly in susceptibility to Lembang Population which had the highest value of LC50 with Resistance Factor of 2.63, suggesting that a significant level of resistance against *Bacillus thuringiensis* already occurred. In response to neem extract treatment, the results, as expected showed that there were no significant differences in susceptibility among the three populations. This indicates that neem extract could be used to control *Plutella xylostella* that has developed resistance to *Bacillus thuringiensis.*

Key Words: *Plutella xylostella, Bacillus thuringiensis*, Neem extract, resistance

INTISARI

Penelitian ini bertujuan untuk melihat respons ulat daun kubis *Plutella xylostella* (L.) terhadap *Bacillus thuringiensis* dan ekstrak nimba. Untuk itu, kajian dosis mortalitas dengan cara bioasai telah digunakan terhadap populasi *Plutella xylostella* (L.) yang diambil dari Garut, Pangelengan, dan Lembang. Perlakuan dengan *Bacillus thuringiensis* tidak menunjukkan perbedaan yang nyata dalam hal kerentanan diantara populasi Garut dan Pangelengan. Tetapi, populasi Lembang menunjukkan kerentanan yang berbeda nyata jika dibandingkan dengan populasi Garut dan Pangalengan yang ditunjukkan dengan nilai LC50 dan Faktor Resisten tertinggi dengan nilai 2,63. Penemuan ini mengindikasikan bahwa populasi dari Lembang telah resisten terhadap *Bacillus thuringiensis*. Perlakuan dengan ekstrak nimba, hasilnya seperti telah diduga menujukkan bahwa tidak ada perbedaan secara nyata dalam hal kerentanan diantara ketiga populasi tersebut. Hasil ini,mengindikasikan bahwa ekstrak nimba dapat dipergunakan untuk mengendalikan *Plutella xylostella* yang sudah resisten terhadap *Bacillus thuringiensis.*

Kata Kunci: *Plutella xylostella, Bacillus thuringiensis,* Ekstrak nimba, resisten.

INTRODUCTION:

The Diamondback Moth *Plutella xylostella* (L.) was cosmopolitan pest and considered as the most destructive pest on crucifers worldwide (Vandenberg *et al.*, 1998). In Indonesia, this pest has been reported as a primary factor in limiting the production of cabbage in many areas (Ahmad *et al.*, 1998).

Insecticides are used regularly to control this pest. Unfortunately, in many part of Indonesia, over the years, there are mounting evidences that the growing use of conventional insecticides to control this insect has resulted in the development of resistance to several major group of insecticides (Soekarna *et al.*, 1982; Adiputra, 1983; Sastrodihardjo, 1986; Sastrosiswojo, 1990).

Previous study has indicated that *Plutella xylostella* collected from Lembang, Pangalengan and Garut, developed resistance to permethrin (Ahmad *et al.*, 1998). Therefore, in the study reported here the responses of *Plutella xylostella* larvae to the most recent insecticides used against this insect i.e., *Bacillus thuringiensis* and to neem product were evaluated. Neem product was tested because it is the fact that although the specific mode of action of neem products on a given insect is not completely understood, interestingly, resistance of insect species toward neem metabolites has not been substantiated to date.

MATERIAL AND MENTODS

Insects Collections and Rearing.

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

The insects were reared on their natural diet, methods used to rear larvae and adults were essentially as described by Liu and Sun (1984). Briefly they were kept in 12:12 h photoperiod, RH about 80 % and at room temperature.

Dosage - Mortality Bioassay: *Bacillus thuringiensis* and Neem Extract

Bioassay were conducted to determine the effect of Turex WP *(water soluble powder; Bacillus thuringiensis* var. *aizawai-kurstaki*, 3.8% [AI]/wt:wt (Ciba-Geigy), and neem extract; 20 % seed extract [azadirachtin, neem oil, salannin, meliantriol, and nimbinen] (Inter University Center for Life Sciences ITB) against *Plutella xylostella* larvae.

Bioassays and rearing were conducted at the same laboratory and environment. The bioassay 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. Each disk was individually dipped for 5 seconds in one of either *Bacillus thuringiensis* solutions or neem extract, or controls. As the suspension on the leaf surface dried, the disk then was placed in a 9-cm diameter petri disk, lined with Whatman filter paper on the bottom.

Eight different concentrations of *Bacillus thuringiensis* were used, *i.e.*, 0; 1; 10; 100; 1000; and 10,000 ppm. Whereas, for the neem extract, one of either 0; 10; 20; 40; 60; or 80 % (v/v) were used.

Third instar larvae were separated from the colony (normally 7 days after eggs were placed on cabbage leaves. Three-four replicates of 10 larvae were treated at each of five insecticide concentrations. The larvae were then placed on treated cabbage leaf disk and left in the laboratory. Mortalities were recorded after 72 h; larvae that were unable to move after being prodded with a blunt probe were considered dead.

Data Analysis

Concentration-mortality regression for the larvae from each bioassay was 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). Unavailability of known susceptible strain of *Plutella xylostella* has led comparison of LD50 between a laboratory (susceptible) strain and field strain could not be made.

RESULTS AND DISCUSSION

Table 1 shows the LC50 values for *Bacillus thuringiensis*, among larvae from three locations. LC50 for Lembang population was significantly 2-fold higher than that of Garut and Pangalengan. Having known that this insecticide is not widely used on cabbage, this finding is somewhat surprising, which suggest that the *Plutella xylostella* from Lembang has developed resistance to *Bacillus thuringiensis*. Nevertheless, while the regression-line slope for *Bacillus thuringiensis* is not significantly different among the three populations, these data are still cause concern. One has to concern with this finding because as has been reported earlier in similar study with pyrethroids by Ahmad *et al.* (1998), they showed that it was *Plutella xylostella* from Lembang which had the highest resistance against permethrin with LD50 500 fold higher than that of recommended dosage. Therefore, theoretically, if *Plutella xylostella* in Lembang has also developed resistance to *Bacillus thuringiensis (*resistance factor 2.63*)* , this condition will probably leave the farmers in Lembang with no other conventional insecticides for effectively controlling damage populations of *Plutella xylostella.* Besides, this finding is somewhat expected due to the fact that some *Bacillus thuringiensis* products in South East

Asia had suffered resistance development to *Plutella xylostella* (Tabashnik *et al.*, 1990).

Because the mode of action for *Bacillus thuringiensis* differs from that for pyrethroid or other conventional insecticides (Sarnthoy *et al.*, 1997), it seem unlikely that resistance to *Bacillus thuringiensis* found in *Plutella xylostella* from Lembang resulted from cross-resistance to other insecticides. Moreover, available data suggest that some cases of resistance to *Bacillus thuringiensis* has been shown to be associated with loss of the toxin binding to the cell receptor in the midgut (Lee *et al.*, 1995)

Table 1. Responses of several population of *Plutella xylostella* against *Bacillus thuringiensis*

Population	**n**	**AVERAGE LC_{50}**	**Slope ± SE**	**Resistance Factor**
Garut	30	31.62 [a] ppm	1.99 ± 0.34	1
Pangalengan	30	36.35 [a] ppm	1.31 ± 0.19	1.15
Lembang	30	83.16 [b] ppm	1.43 ± 0.21	2.63

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)
Resistance Factor: the highest LC50:the lowest LC50

Results obtained for neem extract against populations of *Plutella xylostella* larvae from three locations are shown in Table 2. It shows that there was a little variation in the average LC50 values which ranged from 7.51 % - 11.01 %. . With no significant differences among the LC50 values and the slopes, this finding suggests that *Plutella xylostella* was still very susceptible to neem product. It is not surprising though to observe that the LC*50* values found here are similar to those reported earlier by Ahmad *et al.*, 1998, this could be the case since the experimental larvae were taken from the same colony reared in the Laboratory of Inter University Center for Life Sciences Institut Teknologi Bandung.

Table 2. Responses of several population of *Plutella xylostella* against neem extract

Population	**n**	**AVERAGE LC_{50}**	**Slope ± SE**	**Resistance Factor**
Garut	40	11.01 % [a]	1.55 ± 0.43	1.19
Pangalengan	40	10.99 % [a]	1.49 ± 0.39	1.14
Lembang	40	7.51 % [a]	1.30 ± 0.40	1

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)
Resistance Factor: the highest LC50:the lowest LC50

Findings in this experiments and those reported earlier by Ahmad *et al.* (1992 and 1998) showed that neem-based insecticides showed hope as effective alternative insecticide to control insects that is normally difficult to control by using conventional insecticides as well as *Bacillus thuringiensis.* This finding is actually not very surprising considering the fact that neem's active ingredients bear no resemblance to the active ingredients found in many marketed insecticides, including to *Bacillus thuringiensis.* Therefore with its mode of actions, which ranging from antifeedant to disrupt the growth and development of the insect through hormone regulation [Mordue (Luntz) and Blackwell, 1993], resistance to neem products can not be developed rapidly. In fact, studies with *Plutella xylostella* found there was no sign of resistance in feeding response or reproductive success after 35 generations (Schmutterer, 1990). However, interestingly, Budianto (1999), working with a predatory mite *Amblyseius deleoni* showed a 2-fold increase in LC50 values after only 8 application of neem extract in 8 generations in the laboratory. His finding alarmingly suggests that resistance to neem extracts, despite its mode of actions, is possible to be developed.

In conclusion, although results obtained in this study suggest that *Plutella xylostella* larvae which collected from Lembang has developed some degree of resistance to *Bacillus thuringiensis*, but this insecticide still provide excellent control to *Plutella xylostella* in other locations. Although neem extract seems to be promising in the future as a non conventional insecticide for control of resistant insects, it is wiser to adopt resistance management in the field, therefore development of further resistance can be delayed significantly (Tabashnik, 1994).

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