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SUSCEPTIBILITY OF THE DIAMONDBACK MOTH, PLUTELLA XYLOSTELLA (L.), FROM WEST JAVA, INDONESIA TO FIVENON-CLASSICAL INSECTICIDES

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ABSTRACT

This research was conducted to evaluate the susceptibility of Plutella xylostellalarvae from three locations in West Java to five non-classical insecticides, i.e. abamectin, chlorfenapyr, emamectin benzoate, metaflumizone, and spinetoram. The insecticides were tested against second-instar larvae P. xylostella with a leaf-dip feeding method with a 96-h feeding treatment. The number of dead larvae was counted at 96 h after treatmentand larval mortality data was analyzed with the probit method. Based on insecticide LC95,P. xylostella from Cipanas, Cianjur was the least susceptible to all five insecticides followed by that from Pacet, Cianjur and Cisarua, Bogor. P. xylostella from Cipanas, Cianjur was not susceptible to abamectin and chlorfenapyr but that from the other two locations was still susceptible. LC95 of emamectin benzoate against P. xylostella from the three locations was 0.08 to 2.94 times and thatof spinetoram was 0.02 to 0.12 times as high as their respective recommended field rates whereas that of metaflumizone was 5.2- to 22.2-fold higher than its field rate.Thus, P. xylostella from the three locations were still susceptible to emamectin benzoate and spinetoram but were not susceptible to metaflumizone. The insecticides that are still effective against P. xylostella, when needed,should be used in the context of integrated pest management.

Keywords: Cabbage pest, field rate, non-classical insecticides, susceptibility

1. INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.), is an important pest of Brassicaceae vegetable crops. Heavy infestation by this pest can cause a substantial yield loss (Grzywacz et al., 2010).Integrated pest management (IPM) has been adopted as the basis of cabbage pest control in Indonesia since 1992 (Sastrosiswojo et al., 2005). Major tactics of the cabbage IPM include empowerment of the DBM parasitoid,*Diadegma semiclausum* Hellen, and cultural control techniques.In practice, however, cabbage growers often use broad-spectrum insecticides (Rauf et al., 2005) that may interfere with the function of natural enemies of cabbage pests. In addition, regular use of insecticides can result in the development of resistant pest populations (Georghiou, 1986).

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Insecticides used for insect pest control can be categorized as classical neurotoxic compounds, which include organochlorines, organophosphates, carbamates, and pyrethroids, and non-classical compounds developed after the 1980s (Sparks, 2013). Among the non-classical insecticides, five insecticides registered for vegetable pest control in Indonesia are abamectin, chlorfenapyr, emamectin benzoate, metaflumizone, and spinetoram (Direktorat Pupuk dan Pestisida, 2018). The effectiveness of these insecticides may decrease over time as they are frequently used. Information on resistance of *P. xylostella* to those five insecticides in Indonesia is still limited.

Changes in susceptibility of *P. xylostella* to commonly-used insecticides should be monitored periodically as a part of insecticide resistance management program (Brent, 1986).This study was carried out to assess the susceptibility of *P. xylostella* larvae from three locations in West Java to five non-classical insecticides, i.e. abamectin, chlorfenapyr, emamectin benzoate, metaflumizone, and spinetoram.

2. MATERIALS AND METHODS

2.1. Location and Dates of Testing

This research was done at the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Bogor Agricultural University from September 2015 to May 2016. The sites and dates of collection of *P. xylostella* parental larval colonies and the dates of insecticide testing are shown in Table 1.

Table 1. The sites and dates of collection of *P. xylostella* and the dates of insecticide testing

Site of insect collection	Date of insect collection	Insecticide	Date of insecticide testing
Pacet District, Cianjur Regency, West Java 6°45’13”S, 107°2’25”E	20/12/2015	Abamectin	12/2/2016
		Chlorfenapyr	17/2/2016
	24/1/2016	Spinetoram	2/3/2016
		Emamectin benzoate	20/3/2016
	15/2/2016	Metaflumizone	22/4/2016
Cipanas District, Cianjur Regency, West Java	20/12/2015	Chlorfenapyr	11/2/2016
	24/1/2016	Abamectin	1/3/2016
		Spinetoram	9/3/2016

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6°43'8"S, 107°1'16"E	11/2/2016	Emamectin benzoate	8/4/2016
	4/3/2016	Metaflumizone	30/4/2016
Cisarua District, Bogor Regency, West Java 6°42'0.2"S, 106°56'0.3"E	27/12/2015	Chlorfenapyr	23/2/2016
	29/1/2016	Abamectin	24/3/2016
	11/2/2016	Spinetoram	6/4/2016
		Emamectin benzoate	15/4/2016
	4/3/2016	Metaflumizone	27/4/2016

2.2. Rearing of Test Insects

P. xylostella larvae collected from farmer's fields in locations as shown in Table 1 were reared in the laboratory. The larvae were placed in a plastic box (35 cm x 26 cm x 6 cm) and fed with pesticide-free broccoli leaves obtained from Bina Sarana Bhakti organic farm at Cisarua, Bogor, West Java. Four days after pupation, the pupae were transferred to a steel-framed gauze cage (60 cm x 30 cm x 30 cm)and the emerging adults were fed 10% honey solution soaked a cotton swab suspended inside the cage. A pot of 10-14 days chinese cabbage seedlings were placed in the adult cage for oviposition. The eggs were collected daily and placed in a plastic box and the emerging larvae were fed with pesticide-free broccoli leaves as above. Second-instar *P. xylostella* larvae from the second laboratory generations were used for bioassays.

2.3. Test Insecticides

Insecticides tested were Agrimec 18 EC (active ingredient [a.i.] abamectin 18 g/L), Alverde 240 SC (a.i. metaflumizone 240 g/L), Endure 120 SC (a.i. spinetoram 120 g/L), Proclaim 19 SG (a.i. emamectin benzoate 5%), and Rampage 100 EC (a.i. chlorfenapyr 100 g/L), which were obtained from pesticide kiosks in Bogor and Cianjur, West Java.

2.4. Insecticides Bioassays

Insecticide toxicity tests against second-instar larvae *P. xylostella* were done by using a leaf-dip feeding method adapted from IRAC method No. 18 (http://www.irac–online.org/content/uploads/method_018_v3.4_Mar10.pdf).Each insecticide was tested at five concentrations that were expected to give 15% to 95% larval mortality. The concentration ranges tested for each insecticide was determined in preliminary tests. Control was included in each insecticide test. Each insecticide formulation was diluted to a desired concentration with distilled water containing an insecticide sticker alkylarylpolyglycol ether 80 mg/L (Agristick 400 L, 0.2 mL/L). Distilled water containing Agristick 0.2. mL/L served as a control solution.

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In the treatment with each insecticide, portions of broccoli leaves (4 cm x 4 cm) were dipped singly in particular insecticide dilutions and then air-dried on absorbent paper at ambient temperature. Leaves for control larvae were dipped in the control solution. Two portions of treated or control broccoli leaves were placed in an upside-down petri dish lined with moistened absorbent paper and then 10 second-instar larvae *P. xylostella* were put into the dish. Each treatment was replicated five times. After 48h, the remaining treated or control leaves was replenished with other freshly treated or control leaves and test larvae were allowed to feed for an additional 48 h. The number of dead larvae was counted at 96 h after treatment and then larval mortality data was analyzed with the probit method using PoloPlus for Windows (Robertson et al., 2002-2003). $LC_{95}$of each insecticide was compared with its recommended field rate to determine the status of susceptibility of *P. xylostella* from three locations in West Java, Indonesia to that insecticide.

3. RESULTS

LC_{50} of all five insecticides to *P. xylostella* from Pacet, Cianjur and Cisarua, Bogor were still lower than their respective recommended field rates, but LC_{50} of three insecticides, i.e. abamectin, chlorfenapyr and metaflumizone, to *P. xylostella* from Cipanas, Cianjur were higher than their respective field rates (Table 2). Furthermore, LC_{95} of all five insecticides to *P. xylostella* from Cipanas, Cianjur were the highest compared with those to *P. xylostella* from Pacet, Cianjur and Cisarua, Bogor. Thus, based on comparison of $LC_{95}$of the test insecticides, *P. xylostella* from Cipanas, Cianjur was the least susceptible to all five insecticidesfollowed by that from Pacet, Cianjur and Cisarua, Bogor.

LC_{95} of abamectin and chlorfenapyr to *P. xylostella* from Cipanas, Cianjur were 55.7- and 5.3-fold higher than their respective field rates, those to *P. xylostella* from Pacet, Cianjur were 2.7- and 1.3-fold higher, whereas those to *P. xylostella* from Cisarua, Bogor were only 1.6 and 0.9 times as high as their respective field rates (Table 2). Field population of a pest species is considered resistance to an insecticide if LC_{95} of the insecticide against the pest field population is at least 4-fold higher than that against the standard susceptible strain (Winteringham, 1969). By analogy, based on comparison between LC_{95} and the recommended field rate of the test insecticides, it can be stated that *P. xylostella* from Cipanas, Cianjur was not susceptible to abamectin and chlorfenapyr but that from the other two locations was still susceptible.

Table 2. Toxicity of five insecticides to *P. xylostella* larvae from three locations in West Java

Site of insect collection	Insecticide	$b \pm SE^{a}$	LC_{50} (mg a.i./L)^{b}	LC_{95} (mg a.i./L)^{b}	Field rate (mg a.i./L)
Pacet, Cianjur	Abamectin	1.073 ± 0.216	0.14	4.77	1.8

	Chlorfenapyr	1.429 ± 0.226	8.84	125.13	100
	Emamectin benzoate	1.835 ± 0.334	0.24	1.89	1.9
	Metaflumizone	2.246 ± 0.300	23.30	125.83	24
	Spinetoram	1.816 ± 0.296	0.05	0.36	12
Cipanas, Cianjur	Abamectin	2.232 ± 0.310	18.36	100.20	1.8
	Chlorfenapyr	2.693 ± 0.321	129.50	528.47	100
	Emamectin benzoate	2.426 ± 0.316	1.17	5.58	1.9
	Metaflumizone	2.657 ± 0.309	127.92	532.09	24
	Spinetoram	3.812±0.455	0.54	1.47	12
Cisarua, Bogor	Abamectin	2.084 ± 0.247	0.47	2.87	1.8
	Chlorfenapyr	1.758 ± 0.227	10.81	93.19	100
	Emamectin benzoate	2.424 ± 0.306	0.03	0.15	1.9
	Metaflumizone	1.247 ± 0.260	10.97	228.73	24
	Spinetoram	2.648 ± 0.408	0.07	0.29	12

[a]*b*: slope of probit line. SE: standard error.

LC_{95} of emamectin benzoate against *P. xylostella* from the three locations were 0.08 to 2.94 times and those of spinetoram were 0.02 to 0.12 times as high as their respective field rates whereas those of metaflumizone were 5.2- to 22.2-fold higher than its field rate (Table 2). Thus, it can be stated that *P. xylostella* from the three locations were still susceptible to emamectin benzoate and spinetoram but were not susceptible to metaflumizone.

4. DISCUSSION

P. xylostella from Cipanas, Cianjur, West Java, Indonesia was the least susceptible to five non-classical insecticides, i.e. abamectin, chlorfenapyr, emamectin benzoate, metaflumizone, and spinetoram, compared with that from Pacet, Cianjur and Cisarua, Bogor. The highest susceptibility of *P. xylostella* from Cipanas, Cianjur might be due the most intensive insecticide application in Cipanas, Cianjur.The more frequent and the higher dose of insecticide application may accelerate the development of insect resistance to insecticides or the decrease in pest susceptibility to insecticides (Georghiou & Taylor, 1986).

P. xylostella from Cipanas, Cianjur was not susceptible to abamectin and chlorfenapyr but that from the other two locations was still susceptible. At the time of *P. xylostella* larvae collection in the field, cabbage farmers at Cipanas, Cianjur still used abamectin and chlorfenapyr in alternation with some other insecticides for controlling*P. xylostella*. On the other hand, cabbage farmers at Pacet, Cianjur and Cisarua, Bogor often used abamectin for controlling *P. xylostella* in the past but at present they use that insecticide only sparingly. Meanwhile, cabbage farmers in the latter two locations have not been familiar with chlorfenapyr so that this insecticide has not been used intensively for controlling *P. xylostella.*

P. xylostella from the three locations were still susceptible to emamectin benzoate and spinetoram but were not susceptible to metaflumizone. These three insecticides belong to the relatively new classes of insecticidal compounds.Emamectin benzoate is a semi-synthetic analogue of abamectin and the latter is a mixture of avermectin B1a and B1b which were isolated from a fermentation product of a soil bacteria *Streptomyces avermitilis* (Pitterna, 2012). This insecticide is a nerve and muscle poison which acts by allosterically opening chloride channels in postsynaptic nerve membranes which eventually causes paralysis and death of the poisoned insects (Casida &Durkin, 2013).

Spinetoram is a mixture of semi-synthetic derivatives of spinosyn J and spinosyn L which were isolated from a fermentation product of a soil bacteria *Saccharopolyspora spinosa* (Crouse et al., 2012). Spinosyn is a nerve poison as an allosteric modulator of nicotinic acetylcholine receptors (nAchR). This insecticide allosterically activates nAchR at postsynaptic nerve membranes resulting in the opening of sodium channels and continuous nerve transmission which subsequently producesthe symptoms of hyperexcitation, convulsion, paralysis, and death (Salgado & Sparks, 2010; IRAC, 2018).

The aforementioned susceptibility of *P. xylostella* to emamectin benzoate and spinetoram suggests that these two non-classical insecticides do not show cross-resistance to classical neurotoxic insecticides belonging to organophosphate and pyrethroid classes to which resistance in *P. xylostella* has been reported (Moekasan et al., 2004; Udiarto et al., 2007). At nerve level, the first two insecticides have different mode of actions from the latter two. Organophosphates act as inhibitors of acetylcholinesterase enzymes at the nerve synapses and pyrethroids as axonic excitotoxins which prevent the closure of the voltage-gated sodium channels in the axonal membranes allowing sodium ion influxes and nerve impulse transmission to proceedand these subsequently produce the symptoms ofhyperexcitation, convulsion, paralysis, and death (Yu, 2008).

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Metaflumizone is a semicarbazone insecticide and acts as a nerve poison (Kuhn et al., 2012). It blocks the sodium channels in the axonal membranes so as to interfere with sodium ion influxes and nerve impulse transmissions which eventually result in paralysis and death (Salgado & Hayashi, 2007).Metaflumizone is a relatively new insecticide and has not long been used in the study areas. It seems that cross-resistance occurred between this insecticide and previously used insecticides that interferes with the function of the axonal sodium ion channels like pyrethroids so that *P. xylostella* from the study areas was not susceptible to metaflumizone.

Previously, it was reported that*P. xylostella* populations from some cabbage growing regions in Java, Indonesia were no longer susceptible to abamectin (Moekasan et al., 2004; Udiarto et al., 2007). Resistance of *P. xylostella* to abamectin has been reported from some other countries including Brazil, China, Malaysia, Pakistan and Taiwan (Furlong et al., 2012).Resistance of *P. xylostella* to chlorfenapyr and metaflumizone has never previously been reported in Indonesia. In China, Jiang et al. (2015) reported that out of 31 populations of *P. xylostella*, 32% showed low level resistance, 36% exhibited moderate resistance, and one population showed very high resistance to chlorfenapyr. Wang et al. (2016) reported that the BY12 strain of *P. xylostella* collected from Baiyun, Guangdong Province, China in 2012 showed a medium level of resistance to metaflumizone.

Like the results of this study, *P. xylostella* from some cabbage producing regions in Java, Indonesia was reported still susceptible to emamectin benzoate (Chenta & Prijono, 2014; Tarwotjo et al., 2014; Rakhman & Prijono, 2015).In other countries, there is no report yet on resistance of *P. xylostella* to emamectin benzoate. Previously, spinetoram was also reported still effective against *P. xylostella* from West and Central Java, Indonesia (Chenta & Prijono, 2014; Rakhman & Prijono, 2015). In Brazil, Neto et al. (2016) reported that *P. xylostella* populations from the Agreste region of Pernambuco showed a large variation in their susceptibility to spinetoram and there was cross-resistance between spinosad (the parent compound of spinetoram) and spinetoram.

Emamectin benzoate and spinetoram have been registered with the Environmental Protection Agency of the United States as an organophosphate alternative and reduced-risk insecticide, respectively (EPA, 2018). These relatively safe insecticides can serve as a precious tool in cabbage IPM. Therefore, their effectiveness should be preserved through appropriate insecticide resistance management strategies. Those insecticides can be used at appropriate time and dosages in the context of IPM, e.g. by considering the control threshold of *P. xylostella* and the population ofparasitoids of the pest(Sastrosiswojo et al., 2005). In addition, alternation or rotation with other effective and safe insecticides can be undertaken to reduced selection pressureof insecticide resistance (Leeper et al., 1986).

5. CONCLUSION

Based on insecticide LC_{95}, *P. xylostella* from Cipanas, Cianjur, West Java, Indonesia was the least susceptible to five non-classical insecticides, i.e. abamectin, chlorfenapyr, emamectin benzoate, metaflumizone, and spinetoram, followed by that from Pacet, Cianjur and Cisarua, Bogor. *P. xylostella* from Cipanas, Cianjur was not susceptible to abamectin and chlorfenapyr but that from the other two locations was still susceptible., *P. xylostella* from the three locations were still susceptible to emamectin benzoate and spinetoram but were not susceptible to metaflumizone. The insecticides that are still effective against *P. xylostella*should be used, when needed,in the context of cabbage IPM.

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