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Research Article

Resistance Level of *Plutella xylostella* L. (Lepidoptera: Plutellidae) on Cypermethrin in the Regency of Kupang

Tingkat Kepekaan Plutella xylostella *L.* (*Lepidoptera: Plutellidae) terhadap Insektisida Sipermetrin di Kabupaten Kupang*

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ABSTRACT

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the major pests of the Brassicaceae family. Presently, farmers mostly use insecticide to control this pest. The improper use of insecticide, however, may lead to target pest resistance. Resistance to pyrethroid (cypermethrin) insecticide cases have been widely reported. This research aimed to know whether the farmers use cypermethrin based on the recommended practices and to determine the resistance level of *P. xylostella* collected from Tarus, Noelbaki, Oesao and Pukdale Villages collected from the Kupang Regency, East Nusa Tenggara, which were then compared to the population of Cangkringan (Sleman, Yogyakarta) as a susceptible population. The survey showed that these four locations in Kupang, used insecticides intensively and did the mixing of two or three insecticides. Insecticide application in these four locations was mostly not scheduled as recommended on the label of insecticides. The susceptibility test showed that the RR (resistance ratio) value from Kupang (Tarus = 9.2, Noelbaki = 7.2, Oesao 7.3, and Pukdale = 3.8, respecively) was higher than susceptible population (Cangkringan=1.0). Therefore, *P. xylostella* larvae collected from Kupang has been resistant to cypermethrin.

Keywords: cypermethrin, *Plutella xylostella*, resistance

INTISARI

Plutella xylostella *L. merupakan salah satu hama utama pada tanaman Famili Brassicaceae (Cruciferae). Pengendalian dengan insektisida masih menjadi andalan petani di lapangan. Dampak negatif dari insektisida seperti resistensi, resurgensi dan munculnya hama sekunder tidak membuat efek jera karena minimnya pengetahuan tentang dampak negatif tersebut. Resistensi terhadap golongan piretroid sudah banyak dilaporkan. Salah satunya sipermetrin. Tujuan dari penelitian ini untuk melihat cara penggunaan insektisida oleh petani dan tingkat kepekaan dari populasi lapang asal Tarus, Noelbaki, Oesao, dan Pukdale (Kupang, NTT) dibandingkan dengan populasi Cangkringan (Sleman, DIY) terhadap insektisida sipermetrin. Data survei tentang cara penggunaan insektisida oleh petani diketahui bahwa penggunaan insektisida di keempat lokasi tersebut cukup intensif dan terjadi pencampuran insektisida yang tidak kompatibel. Penyemprotan dilakukan secara rutin di Desa Tarus; di Desa Noelbaki dan Oesao ada yang secara rutin dan ada yang kondisional; sedangkan penyemprotan secara kondisional di Desa Pukdale. Data uji kepekaan menunjukkan bahwa* P. xylostella *asal Kupang telah resisten terhadap sipermetrin dengan nilai RR (rasio resistensi) tertinggi dari populasi Tarus (9,2), Noelbaki (7,2), Oesao (7,3), Pukdale (3,8) dibandingkan dengan populasi peka asal Cangkringan.*

Kata kunci: Plutella xylostella*, resistensi, sipermetrin*

INTRODUCTION

The cabbage (*Brassica oleracea* L.) is a horticultural crop having high demand and economic value, and a good source of vitamines, minerals, carbohydrates, protein, and fat (Pracaya, 1993). In Indonesia, *Plutella xylostella* is a major pest in Brassicaceae Family including mustard greens and cabbage in Java, Bali, Sumatra, and other areas. In the tropical regions, the population of *P. xylostella* might reach 12 generations per year. The cabbage planted every year caused the host of this pest is available all the time (Setiawati, 2000; Listyaningrum *et al*., 2003b). *P. xylostella* is an oligophagous pest only attacking plants of Cruciferae/Brassicaceae family (Talekar & Shelton, 1993). In the dry season, the population of *P. xylostella* increases, but decreases in the rainy season due to the higher mortality of the first and second instar larvae than the third and fourth instars due to rainfall (Sudarwohadi, 1975; Elzinga, 2004). Seven larvae of *P. xylostella* per plant may cause damage up to 28% and if the insecticide was not applied, yield losses can reach 100% (Setiawati, 2000; Winasa & Herlinda 2003; Winarto & Nazir, 2004).

Insecticide is to be the first resort to control *P. xylostella*. Pyrethroid insecticide, especially cypermethrin, is a synthetic insecticide commonly used by farmer, because it is fast and effective to kill the target pest, a side from its inexpensiveness (Furlong *et al*., 2008). As other pyrethroid insecticides, cypermethrin is a synthectic insecticide with a chemical structure derived from pyrethrum, a compound extracted from chrysanthemum (Ware & Withacre, 2004). Cox (2002), reported that cypermethrin used to control Lepidopteran pests in cotton, fruit and vegetables. It mode of action is by disrupting the nerve system of the pest. Cypermethrin delayed the closing of the "entry gate" of sodium through the nerve, causing some nerve impulses to bundle into a single impulse. Therefore, nerves then release neurotransmitter acetylcholine and stimulate other nerves (Ells, 1992).

The improper use of insecticides causes negative impact, such as resistance, resurgence, outbreak of secondary pest and the death of natural enemies (Untung, 2006). The resistance of *P. xylostella* has developed very fast in the field (Shelton *et al*., 1993). *P. xylostella* has been known to be resistant to deltamethrin, methamidophos, chlorpyrifos, and spinosad (Branco & Gatehouse, 1997; Agboyi *et al*., 2016). Yu (1993), reported that *P. xylostella* has been resistant to some insecticides. One of those insecticides is cypermethrin. The resistant population of *P. xylostella* to pyrethroid (such as permethrin, cypermethrin, fenvalerate, cyhalothrin, deltamethrin, and fluvalinate) can reach the range of 298−82400 times compared to the susceptible population. Nuryanti & Trisyono (2002), also reported the populations of *P. xylostella* collected from Cepogo, Boyolali and Kopeng Salatiga, both in Central Java, showed that population of Kopeng has resistance level 469 times compared with Cepogo population. (Listyaningrum *et al*., 2003a) reported that *P. xylostella* resistant to deltamethrin was inherited homogeneously, as a recessive gene, and enhanced by a maternal effect.

The observation conducted in central growing areas of cabbage in Kupang Regency showed that cypermethrin was one of the most insecticides used by that local farmers. Spraying and the mixing different insecticides were not applied based on the recommendation on the label insecticide. Therefore, this study aimed to determine the resistance level of *P. xylostella* collected from the Tarus, Noelbaki, Oesao and Pukdale villages, Kupang, East Nusa Tenggara (NTT) to cypermethrin.

MATERIALS AND METHODS

Observation of the Use of Insecticides

The observation of the use of insecticides by farmers from, in the Tarus, Noelbaki Village, Oesao, and Pukdale Villages, Kupang Regency, East Nusa Tenggara Province, Indonesia, began in July−December 2016. The research location was determined based on the consideration that those areas are the central growing areas of cabbage in East Nusa Tenggara. Nine farmers as respondent were selected from four locations. The data was collected using interview method. Parameters used to justify the use of pesticide in those villages were the kind of insecticides used, spraying schedule, and the number of insecticides mixed.

The Initial Population of Plutella xylostella

Stadia collected from each location were larvae, pupae and imago stored in different containers. The population from Cangkringan (Sleman, DIY) collected from organic cabbage field was designated as a susceptible population.

Mass Rearing of Plutella xylostella

The larvae were placed in plastic jars with diameter of 20 cm and height of 20 cm and fed using organic caisim until became pupae. After the adults emerged, those moved into the net cage with a white cloth on the top length of 40 cm, width of 28 cm and height of 30 cm then the caisim plants aged of 2 weeks were put in for laying egg, and the adults were fed using honey 10% dropped on cotton. After 2 days, the plants contained of eggs masses were moved into a new plactic jar with diameter of 20 cm and height of 20 cm until the eggs hatched. This method was repeated until reached 280 of the second-generation larvae (F2) for testings.

Susceptibility Test of **Plutella xylostella** *Larvae to Cypermethrin*

Each population were tested twice. The first was preliminary test to determine the right concentrations used for second test. Six of concentrations of 400, 200, 100, 50, 25, and 12.5 ppm of cypermethrin (trade name Rizotinand) and 0 ppm as a control using distilled water. 200 ppm equivalent to 2 ml/L which is the recommended dose. Organic caisim plants aged of two weeks were used to feed 10 larvae of second instar larvae (F2) for 72 hours. The treatments were done as follows organic caisim plants aged of two weeks were dipped into each concentration of cypermethrin until 10 seconds, started from the lowest concentration until the higher concentrations. Then those plants were air-dried, and 10 larvae of second instar (F2) were put in a plastic jar (diameter of 8 cm and height of 20 cm) removed one side (length of 10cm and width of 5cm) and replaced it with white cloth, and labeled based on the series of concentration. Observations were conducted at 24, 48, and 72 hours after treatment (hat) by calculated the percentage of larval mortality. Each treatment was replicated four times. The second test was similar with the first test, the only difference was the concentrations used (1600, 800, 400, 200, 100, 50, 25, and 12,5 ppm) that was determined based on the first test.

Data Analysis

Data of the use of insecticides observation were analyzed descriptively and made into table and graphic using Microsoft Excel 2007. The level of resistance was analyzed using probit analysis to obtain the LC_{50} value (Finney, 1971). Probit analysis was conducted using SPSS Version 24. The resistance ratio was determined by comparing the LC_{50} value of the tested-populations with the most susceptible population. The LC_{50} value of those populations is different significantly if the confidence value does not overlap (Trisyono & Chippendale, 2002).

RESULTS AND DISCUSSION

The Use of Insecticides by Farmers

Farmers of Tarus village used six insecticides which were applied regularly (4 weeks after planting) by mixing three insecticides, and started spraying intensively until approximately the harvesting season (Table 1 and Table 2). The spraying of insecticide in Oesao and Noelbaki villages used four insecticides, which were applied regularly based on the damage. Where as in Pukdale Village the application used three insecticides, that was based on damage. If the damage was light, the spraying was done with an interval of 3 days and when the damage was heavy, the spraying was conducted every day. Here, as this survey found that the damage was very heavy, the spraying was shorter than the recommended spraying i.e 4−5 days.

Table 1. Consideration of insecticide spraying by farmers where the population of *Plutella xylostella* were collected, Kupang, East Nusa Tenggara

Insecticide Application	Number of Farmers (%)			
	Tarus	Noelbaki	Oesao	Pukdale
Decision of spraying:				
Scheduled	22	11.1	11.1	
Based on damage*		11.1	11.1	33.3
Mixtures:				
Two insecticides		11.1	11.1	33.3
Three insecticides	22	11.1	11.1	

Remark: *Number of farmers interviewed was nine
*If the damage was light, the spraying was done with an interval of 3 days; when the damage was heavy, the spraying was conducted every day.

Table 2. Various insecticides used by farmers in locations where *Plutella xylostella* were collected, Kupang, East Nusa Tenggara

Trade name (active ingredient)	Number of Farmers (%)			
	Tarus	Noelbaki	Oesao	Pukdale
Sidamethrin 50 EC (Cypermethrin)	22.2	22.2	22.2	33.3
Demolish 18 EC (Abamectin)	22.2	11.1	11.1	
Curacron 500 EC (Profenofos)	22.2		11.1	
Furadan 3 G (Carbofuran)	22.2	22.2	22.2	33.3
Toxafine 400 EC (Dimethoate)	22.2	22.2	22.2	11.1
Manthene 75 SP (Acephate)	22.2	22.2	22.2	

Remark: Number of farmers interviewed was nine

Farmers mostly mixed several insecticides with different active ingredient in purpose to make the mixture which was thought to be more effective to control the target pests. Two or three insecticides were mixed in four villages surveyed (Tarus, Noelbaki, Oesao, and Pukdale). The mixing of insecticides would be less effective when the target pest has been resistant to one or both of those insecticide (IRAC, 2012). If that mixing was done without knowing the right composition, it would increase the cost for controlling the pest. If the dosage used was over the recommended dosage, it would lead to the resistance of the target pest, polluting the environment, and outbreak of secondary pest.

There were six insecticides used with different active ingredient by farmers in Kupang, i.e. Sidamethrin 50 EC (cypermethrin), Demolish 18 EC (abamectin), Curacron 500 EC (profenofos), Furadan 3 G (carbofuran), Toxafine 400 EC (dimethoate), and Manthene 75 SP (asefat), respectively (Table 2). Farmers from Tarus village used those six insecticide and applied them regularly to their crops with different mixture for every application.

Cloyd (2011) stated that if the application of two or more different insecticides which increase the effectiveness of pest control, the insecticides were called synergistic, however if that the resulting mixture was lower in its effectiveness, the were categorized as antagonists or incompatible. Therefore, the agricultural extension is in a need to increase the knowledge of the farmers in mixing insecticides to be more compatible.

Toxicity of Cypermethrin to **Plutella xylostella**

The mortality of *P. xylostella* larvae for each treatment increased along the duration of observation (Figure 1). The population of *P. xylostella* collected from Kupang District was indicated as resistant populations and the population collected from Cangkringan as a susceptible population. The result of preliminary test showed that mortality at the highest concentration of 400 ppm at 72 hat from Cangkringan reached 87.18%, whereas the mortality of Kupang was lower than that, i.e 55.56, 63.16, 67.57, and 78.95% for Tarus, Noelbaki, Oesao, and Pukdale respectively). Therefore, the tested concentrations were tested for Kupang populations were made higher up to 1600 ppm. Cypermethrin disrupts the nervous system of target insect by effecting the Na^+ membrane (sodium channel). Cypermethrin delays the closing of the "gate" of the flow of sodium (Na^+) causing nerve impulses to be single impulse thus the insect had a seizure. Then that insect will be dead or paralyzed. The knockdown effect by the pyrethroid to target insect occurred within 1−2 minutes (Kazachkova, 2007).

Bioassay was done by using second instar of F2. Probit analysis was conducted at 48 and 72 hat, because of the mortality of *P. xylostella* at 24 hat was less than 50%. The four populations from Kupang was indicated resistant to cypermethrin by comparing the resistance ratio (RR) of those with Cangkringan population (Table 3). The highest RR value was 9.2 times (from Tarus) and the lowest was 3.8 times (from Pukdale). The slope of the populations of *P. xylostella* collected from Kupang was higher than that from Cangkringan (Table 3). The higher of the slope value from a certain population, the more similar the resistance level of each insect from that population. Because the higher of the slope value, the higher of the concentrations (Kerns *et al.,* 1998).

The increase of resistance level was affected by enzymes activity. Generally, esterase enzymes were found involved to breakdown the insecticide molecules of organophosphate, carbamate, and pyrethroid insecticides (Srigiriraju *et al.,* 2009). Previous study showed that resistance to organophosphate and indoxacarb had correlation with the increase of the esterase activity (Sayyed & Wright, 2006). Qian *et al*., (2008) also reported that *P. xylostella* resistant to abamectin was due to the increase of the mixed function oxidase (MFO) activity.

Tarus is a village has a wide area growing areas of horticulture and it is nearby to rice fields. Therefore, the application of insecticide and the resistance level in this village were high. The resistance level of *P. xylostella* larvae collected was different geographically, showed the natural variation among populations due to selection when those were exposured to insecticides (Siegfried *et al.,* 2005). Gong *et al.* (2013), stated that differences of ecological and environmental contributed to variation in enzyme activity among populations. For example, the increase of esterase activity was related to latitude. Pesticide resistance may also be caused by other mechanisms and factors, such as regional, genetic, and environmental differences among different populations (Maa *et al*., 2001). Other researcher also stated that the resistance level of *P. xylostella* collected in autumn was higher than that in spring or early summer (Jiang *et al*., 2015). Therefore, the locations of the *P. xylostella* collected affected the resistance level or RR value and the causal factor might climate.

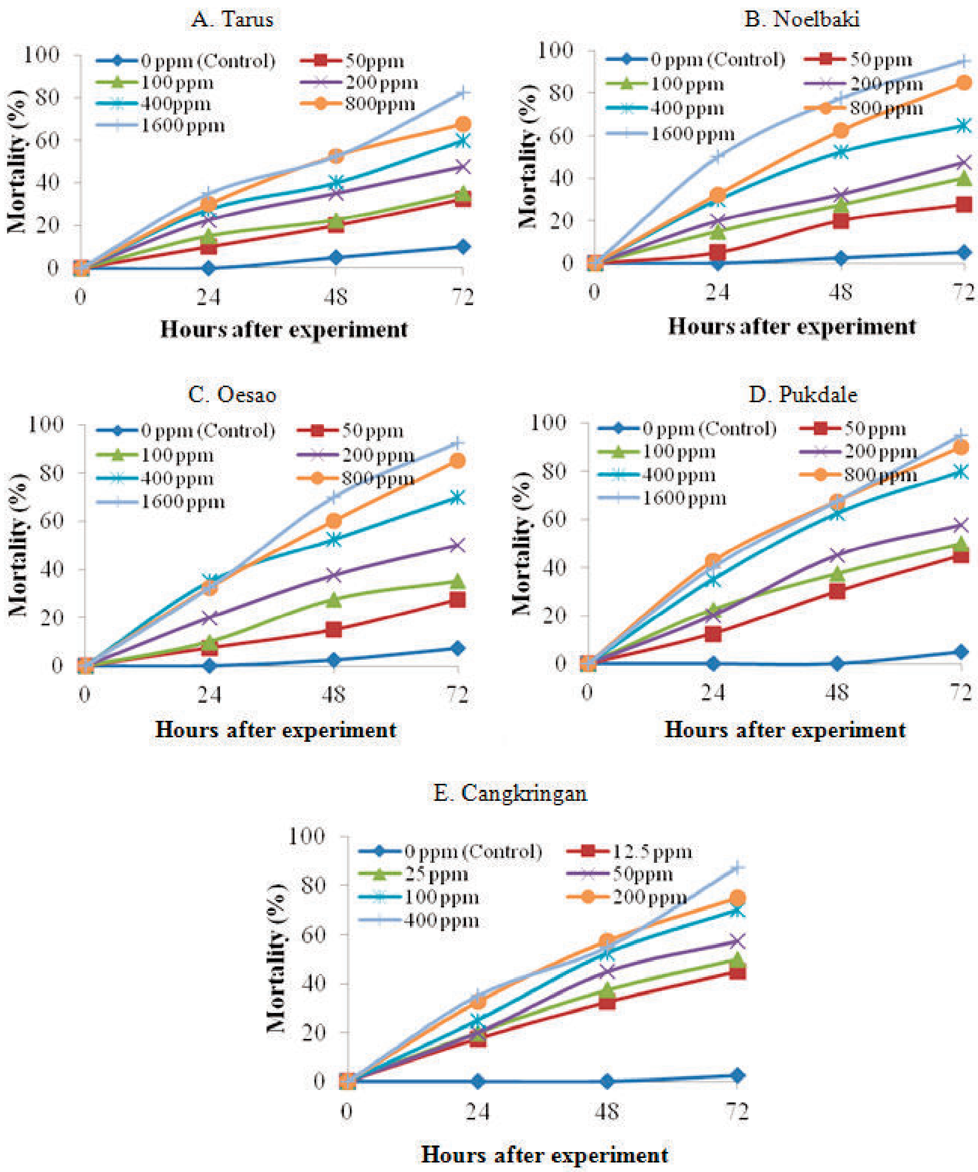

Figure 1. Comulative larval mortality (%) of *Plutella xylostella* after feeding with caisim leave treated with cypermethrin; the tested larvae were originally collected from Tarus (A), Noelbaki (B), Oesao (C), Pukdale (D) (Kupang, East Nusa Tenggara) and Cangkringan (E), (Sleman, Yogyakarta)

Table 3. Resistance level of *Plutella xylostella* collected from Kupang to cypermethrin

Province	District	No. Larvae	Time (hour)	Slope ± SEM	LC_{50} (95% CI), ppm	χ^2	Resistance Ratio
Kupang, East Nusa Tenggara	Tarus	280	48	0.60 ±0.167	9386.324 (5081.133−34380.005)	0.846	
			72	0.75 ±0.169	2103.596 (1285.468−3200.439)	1.009	9.2
	Noelbaki	280	48	1.00 ±0.173	3746.835 (2607.330−5669.021)	0.986	
			72	1.50 ±0.191	1653.482 (1177.078−2206.943)	2.536	7.2
	Oesao	280	48	0.90 ±0.173	4267.268 (2913.082−6793.296)	0.487	
			72	1.25 ±0.19	1663.161 (1185.805−2217.779)	1.024	7.3
	Pukdale	280	48	0.60 ±0.164	2462.307 (1339.795−4249.075)	1.409	
			72	1.25 ±0.194	880.852 (508.400−1273.903)	2.76	3.8
Special Province of Yogyakarta	Sleman	280	48	0.35 ±0.16	1406.259 (535.518−36239.086)	0.276	
			72	0.70 ±0.172	229.128 (90.501−383.835)	0.885	1.0

Remark: Resistance ratio= LC_{50} of Kupang population : LC_{50} of Sleman population at 72 hours after treatment.

CONCLUSION

This research showed that farmers from Tarus, Noelbaki, Oesao and Pukdale Villages applied the insecticide intensively and mixed several insecticides with different active ingredients without having the knowledge for the right combination to be synergistic. The susceptibility test showed that Tarus population has the highest level of resistance (9.2) compared with other populations. The resistance ratio of Noelbaki and Oesao population was similar (7.2 and 7.3, respectively). The lowest of the resistance ratio was Pukdale population of 3.8, all compared with the Cangkringan population. Therefore, *P. xylostella* larvae collected from Kupang was resistant to cypermethrin with the highest resistance level was from tarus, that due to the improper use of insecticide by farmers.

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