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Resistance Inheritance of *Plutella xylostella* Population to Residual of Emamectin Benzoat

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Abstract

Excessive use of insecticides drives the increasing ability of pests to become resistant. The objectives of this research were to study the susceptibility and the resistance inheritance of the eleven population of *P. xylostella* to emamectin benzoate. The leaf-dip bioassay was applied to determine the sensitivity of *P. xylostella* to emamectin benzoate. The offspring of backcrossed F2 were tested whether the resistance was controlled by monogenic. The results showed that the LC_{50} of the Selo population was 53.42 ppb, and the Puasan population was 212.13 ppb. The genetic analysis showed that the backcrosseddegree of dominance (D) was less than 1. It was indicated that the *P. xylostella* resistance to emamectin benzoate was recessive. The value of LC_{50} of the backcrossed F1♀ x ♂S (177.99 ppb) and its reciprocals x ♀R (F1) (201.69 ppb) were not significantly different with the value of LC_{50} resistance population. This suggests that the nature of *P. xylostella* resistance to emamectin benzoate was controlled by monogenic.The result of the study would be beneficial for developing strategy to maintain susceptible population using refugee plant during lack of their host.

How to Cite

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INTRODUCTION

Larvae of *Plutella xylostella* (L) and *Crocidolomia binotalis* (Zell) (Lepidoptera: Pyrallidae) are two types of highly destructive pests of cabbage plants, especially during the dry season (Winarto, & Nazir, 2004). Cabbage yield loss in Indonesia by *P. xylostella* together with *C. binotalis* (Zell) in dry season reached 100% when did not apply insecticides. Meanwhile, the loss in the cabbage harvest in Malaysia because of the *P. xylostella* reached 87.5% without insecticide application. The average loss of crop harvest in Segunung was 58% (Bynum & Archer, 2000; Setiawaty, 2000)

Cabbage pest control in Indonesia is still heavily dependent on the use of insecticides. The use of insecticides in cabbage crop especially in highland area is very intensive, whether using high-dose and spraying a very short interval (Abate et al., 2000; Nuryanti, 2001). This situation cause many serious problems, including developing insecticides resistance, reduced toxicity values, changes in toxicological properties, and changes in population genetics characters (Shelton et al., 2000).

Excessive use of insecticides could increase the ability of pests to become resistant to a particular chemical insecticide.It is because individuals of pests which are susceptible will be eliminated by insecticides (Matsumura & Boethel, 2000). Insect resistance to insecticides is the development of the ability of a population of insects to tolerate doses of toxicant which previously has been shown to turn off most of the individuals from the normal population of the same species (Zhao et al., 2000; Marcon et al., 2000). Naturally, individual insects do not grow and develop into resistant individuals, but the offspring become susceptible individuals. Individual susceptible allele frequency in nature is greater than individual resistant allele frequencies. Individuals resistant allele frequency (RR) range from 10^{-2} until 10^{-13} (Georghiou & Taylor, 1986; Groeters & Tabashnik et al, 1992)

Attique et al. (2006) stated that the use of insecticide lambda-cyhalothrin in New Zealand affected resistance of *P. xylostella* population significantly, up to 885 fold, while the spinosad and indoxacarb affected light resistance or even no resistance at all. Moreover, Attique et al. 2006 also showed that resistance properties was reduced to methamidophos. Bailey (2001) showed that the differences of susceptibility within populations of *Heliothis virescens* against Dipel and endotoxin were 3.6 and 8 fold, respectively. The level of resistance of *Pseudoplusia includends* population in the United States against emamectin benzoate vary between 1 to 6.21 fold (Moulton et al., 2002). Susceptibility of *P. xylostella* populations in California against emamectin benzoate were 1-13 fold (Shelton et al., 2000). Wearing et. al. (2000) showed that the resistance of *Planotortri xocto* against tebufenozida increased 269 fold compared to the susceptible strain. According Moulton et al. (2002), the LC_{50} of metoksifenozida on field strain of *Spodoptera exigua* was higher than the laboratory strain (Harwanto, 2014). Resistance occur not only to conventional insecticides, but also to 3rd generation insecticides i.e. Insect Growth Regulators (IGRs) such as ecdison agonists, juvenile hormone (JH). Juvenile hormone that already applied to control insects in the United States are metropin, kinoprin, and hidroprin, while chitin inhibitors insecticides is diflubenzuron (Ware, 2004).

The pattern of inheritance of insect resistance to insecticides can be determined by doing a cross between individuals that are resistant and susceptible individuals. If the resistance trait is controlled by two alleles at one locus, the R allele for resistance properties and the S allele to the natural susceptible, then in the population there are three possible genotypes of insects, namely RR, RS and SS. A cross between a homozygous resistant (RR) is recessive with homozygous susceptible (SS) which will predominantly produce offspring (F_1) heterozigot (RS) that is susceptible. When F_1 is allowed to do cross each other there will be segregation in the F_2 phenotype ratio of 3: 1 (Cummings & Klugs, 1997). Characteristic patterns of inheritance include the influence of the female parent (maternal genetic effect) linkage resistance properties of a specific gender (sex linkage), the dominance of inheritance, and the number of genes controlling (Tabashnik, 1992).

Inheritance of resistance can be controlled by one or a few genes. The estimation of the number of loci involved in inheritance of resistance needs is important to determine whether the resistance is controlled by a single gene (monogenic) or by many genes (polygenic). Estimation of the involvement of a single gene or multiple genes in the inheritance of resistance is important to know how heredity may influence the rate of change in the nature of the population (Sato et al., 2004.)

Inheritance of insect resistance to insecticides can be either dominant or recessive and monogenic or polygenic controlled. In general, allele controlling insect resistance properties are recessive, inherited in an autosomal, and controlled monogenic. It is important to know the cha-

racteristics of resistance properties as the base of resistance management strategies which are developed to anticipate and to slow the development of resistance (Moekasan, 2004.).

Tabashnik et al. (2002) showed that the resistance of *Leptinotarsa decemlineata* against *B. thuringiensis* was dominant, while Listyaningrum et al. (2003) reported that the resistance of *P. xylostella* on deltametrin was recessive. Hence, the resistance characteristic on particular insect may vary different depend on their insecticide. The objectives of this research were to study the susceptibility and the resistance inheritance of the eleven population of *P. xylostella* to emamectin benzoate. The resistance inheritance comprehension is important as basic strategy for resistance management. The hypothesis of single gen or multiple genes involvement in resistance inheritance was important due to their role in affecting the rate of resistance characteristic on population.

METHODS

The collection of *P. xylostella* populations was conducted in ten districts where these locations were main production areas of cabbage. The collected *P. xylostella* were cultured in the laboratory to test the susceptibility and the resistance of *P. xylostella* against emamectin benzoate. Sex identification was determined by rearing 20 pupae of resistant insects at a jar, and 20 pupae in another jar of susceptible insects. The top of the jar was covered using strimin cloth. Cotton that has been oiled using 10% solution of honey was put on the cloth surface. Imago which emerged from either resistant or susceptible insects were observed to determine their gender. Males imago have abdominal smaller than the female imago (Pérez et al., 2000; Carriere et al., 2001).

Resistance characteristics properties test was reciprocally performed by mating the male population of susceptible (S) *P. xylostella* to the female population of resistant (R). The purpose of crossing is to know the effect of reciprocal maternal and domination resistance properties. Relationship with the concentration of F_1 hybrid larval mortality is used to determine the presence of maternal effects. Bioassay was conducted using leaf immersion method (Nuryanti & Trisyono, 2002; Listyaningrum et al., 2003). Concentration of emamectin benzoate which were used to test the F_1 hybrid females of susceptible offspring (S♀) and male resistant (R♂) were used for testing the concentration of resistant populations. The same concentration was also used to test the F_1 hybrid females marriages resistant (R♀) and males are vulnerable (S♂). To determine dominance (D) resistance to emamectin benzoate in F_1 used the formula of Listyaningrum, et. al. 2003; Huang & McGaughey (1999), as followed:

$$D = \frac{2Xb - Xa - Xc}{Xa - Xc}$$

D = the level of dominance. Xa = $\log_{10}$ [LC_{50}] resistant population. Xb = log10 [LC_{50}] heterozygote population, and Xc = $\log_{10}$ [LC_{50}] vulnerable populations. If the value of D = -1 resistance is recessive, the value of D = 0 resistance is intermediate and D = +1 resistance is dominant.LC_{50} value is calculated using probit analysis (Busvine, 2002). Abbott formulation (1925) was used to correct mortality control. Two LC_{50} values are not significantly different if the 95% confidence interval was overlap (Trisyono, 2002).

To determine whether the resistance is controlled by single or multiple genes, it was applied backcross between F_1 hybrid with one of the parents (Tabashnik et al., 1992; Tabashnik et al., 2002). The determination of elders was conducted after inheriting resistance properties of F_1 hybrid was knew. Resistance properties was controlled by monogenic when the value χ^2calculation < χ^2tables. χ^2value was calculated using the following formula:

$$\chi^2 = \frac{(o - c)^2}{o}$$

Note: o is the number of dead insects on backcross observations on the concentration x, and cis the expected number of dead insects.

The calculation of expected F_2 mortality (backcrossed offspring) was F_2 = (percentage of F_1 larval expected mortality on concentration + percentage of expected vulnerable mortality on the concentration c) x 0.5 (Tabashnik et al., 2002; Siegfried et al., 2000). If the value χ^2 calculated<value χ^2 table then the nature of resistance is controlled by a single gene (monogenic) (Tabashnik et al., 2002; Brewer & Trumble, 1991).

RESULTS AND DISCUSSION

The susceptibility test of *P.xylostella* to emamectin benzoate showed that the LC_{50} values of the eleven tested population ranged from 53.42 up to 212.13 ppb.

Selo (Cepogo) Population had the lowest LC_{50}values and showed significant difference to the Kejajar, Plalar (Getasan), Babrik (Ngablak), Kaponan (Ferns), Kertek (Kertek), Keteb (Sawangan), and Puasan (Ngablak) population, where

their lower and upper limit value of 95% confidence interval were not overlap. The susceptibility level of Selo population wasnot significantly different to Gondosuli (Tawangmangu), Kenteng (Sumowono), and Gedongsongo (Bandungan) population (Table 1),

Figure 1. Adult Diamondback moth, *Plutella xylostella.*

The results showed that Selopopulation has the lowest LC_{50} value, which is equal to 53.42 ppb, and is the most susceptible population among the population being tested. The Selo homozygote population can be obtained from the testing of the seventh generation ofSelo population (F_7) against emamectin benzoate with a concentration of 3.97 to 1000 ppb, to obtain the LC_{50} values of 46.63 ppb. Remaining F_7 larvae was used as a parent (P) of the susceptible population. Based on the resistance test,Puasan population had the highest of LC_{50}values , which was amounted to 212.13 ppb. To get the homozygote Puasan population, the selection was applied using emamectin benzoate in the second to fifth generation (F2-F5) with a concentration of 7.81 to 2000 ppb, which was obtained the LC_{50} values of 218.91 ppb. Parent of Puasan population was chosen from the rest of fifth generation of larva selection (Table 2). Since the LC_{50} value of F_1 reciprocal crosses were not significantly different compare to the former LC_{50}, the F_1 offspring was combined (pooled) for the next test.

The results of genetic analysis showed that the degree of dominance (D) from crosses between ♂R x ♀S (F_1) was 0.77 and ♂S x ♀R (F_1) was 0.93. When the degree of dominance (D) = -1, it indicates that the nature of *P. xylostella* resistance against emamectin benzoate was recessive. Due to the resistance is recessive, then F1 hybrid was backcrossed with sensitive elders, otherwise when F_1hybridisbackcrossed with resistant elders, then the resistance is dominant (Tabashnik et al. 2002). Because of the nature of resistance was recessive, then crossing between F1♀ x ♂S was applied with LC_{50} values was 177.99 ppb and crossing between F_1♂ x ♀S with LC_{50} values was 158.09 ppb.LC_{50} value of the backcrossed between F_1♀ x ♂S (177.99 ppb) and its reciprocal ♂S x ♀R (F_1) (201.69 ppb) was not significantly different compared to the value of crossed LC_{50} between ♂R x ♀R (218.9 ppb). This suggests that the nature of resistance of *P. xylostella* against emamectin benzoate was controlled by monogenic.

Larval mortality caused by emamectin benzoate from the crossing of ♂S x ♀R (F_1) was not significantly different compare to crossing♂R x ♀S (F_1). This showed that there was no effect of maternal on inheritance of *P. xylostella* resistance against emamectin benzoate. The absence of ma-

Table 1. Suceptibility of *P.xylostella* populations originating from eleven Central Java province population against emamectin benzoate

Population	Sub Distric	N	Control Mortality	Slope (SE}	LC_{50} (SK 95%) ppb)	RF	$\chi 2$
Puasan	Ngablak 1	270	2.27	0.893 ± 0.125	212.13(128.15-351.16) b	3.97	16.61
Keteb	Sawangan	270	2.27	0.791 ± 0.122	153.44 (87.87-267.89) b	2.87	22.19
Babrik	Ngablak 2	270	2.27	0.741 ± 0.135	152.13 (96.30-242.83) b	2.85	13.29
Kaponan	Pakis	270	2.27	0.794 ± 0.121	149.77 (86.74-258.58) b	2.80	14.18
Plalar	Getasan	270	2.27	1.127 ± 0.135	144.44 (88.96-199.71) b	2.70	1.12
Kertek	Kertek	270	4.65	0.734 ± 0.124	137.85 (75.11-252.99) b	2.58	19.65
Kejajar	Kejajar	270	9.09	0.834 ± 0.114	82.81 (70.70 – 96.99) c	1.55	6.94
Kenteng	Sumowono	270	0.00	0.798 ± 0.118	78.32 (45.86-123.75) a	1.47	14.04
Gondosuli	Tawangmangu	270	4.65	0.846 ± 0.124	78.16 (45.71-121.83) a	1.46	1.94
Gdongsongo	Bandungan	270	2.27	0.844 ± 0.122	75.46 (63.45 – 89.14) a	1.41	6.01
Selo	Cepogo	270	2.27	0.667 ± 0.111	53.42 (44.95 – 63.84) a	1.00	5.85

Note: The larvae used were the third instar larvae; LC_{50} followed by the same letter are not significantly different; RF = resistance factor; Value $\bar{\kappa}^2$ table (α = 0. 05) = 7.81.

Table 2. LC_{50} value of crossbred third instar larvae of *P. xylostella* three-FI hybrid result of reciprocal cross breeding and backcross result.

Crossbreeding	Larva number (n)	Slope (SE)	LC_{50} (95% confidence interval) (ppb)
♂S x ♀S	45	0.68 (0.11)	46.63 (25.70 - 84.61) [a]
♂R x ♀R	45	0.89 (0.13)	218.91 (131.39 – 364.72) [b]
♂R x ♀S(F_1)	45	0.72 (0.11)	180.51 (96.10 – 339.07) [b]
♂S x ♀R(F_1)	45	0.73 (0.12)	201.69 (108.84 – 373.79) [b]
F_1 pooled	45	0.77 (0.11)	196.52 (113.38 – 340.59) [b]
F_1♀ x ♂S	45	1.28 (012)	177.99 (120.23 – 263.03) [b]
F_1♂ x ♀S	45	1.13 (0.13)	158.09 (107.55 – 232.38) [b]

Note: LC_{50} values followed by the same letter are not significantly different because of the overlapping value of the lower and upper 95% confidence interval.

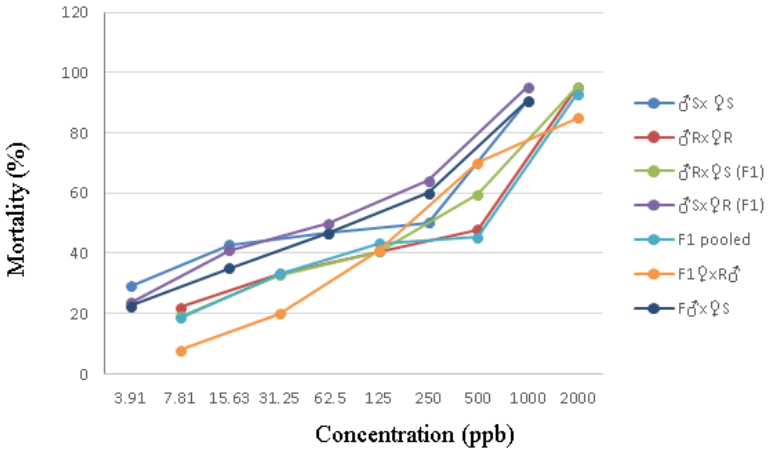

Figure 2. Response of *P. xylostella* population of crossbred F_1 hybrid, reciprocally, and backcrossing against emamectin benzoate

Table 3. Chi-square analysis of crossbacked *P.xylostella* mortality of F_1♀x♂S against emamectin benzoate of monogenic model.

Concentration (ppb)	Mortality (%)		χ^2 calculated	χ^2 table
	Expected	Observed		
2000	94.10	92/85	0.02	3.8
500	43.39	44.18	0.01	3.8
125	39.26	41.86	0.17	3.8
31.25	29.04	31.78	0.26	38
7.81	15.53	16.71	0.09	3.8

ternal effects could be seen in Figure 2.

Figure 2 indicates, that the larval mortality arising from the cross ♂S x ♀R (F_1) (LC_{50} = 201.69 ppb) with the results of a cross ♂R x ♀S (F_1) (LC_{50} = 180.51 ppb) did not significantly different and equally high chart position.LC_{50} value emamectin benzoate in backcross and its reciprocal test were not significantly different for the population F_1pooled LC_{50} and LC_{50} cross ♂R x ♀R. This means that resistance is inherited monogenic trait. According to Tabashnik (2002) when the LC_{50} value of the backcrossing test were not significantly different to LC_{50} of the resistant population, then it is controlled by monogenic resistance, but when there is a significant difference, it is controlled by polygenic resistance. These results can be tested using chi-square analysis (Table 3).

Chi-square analysis showed that the mortality of larvae was greater compared with each tested concentration. Chi-square analysis of monogenic models show that the 2 count each con-

centration smaller than $\bar{\chi}^2$tables at P = 0.05 and df = 1 ($\bar{\chi}^2$tables = 3.84). This proves that the inheritance of resistance of*P. xylostella* was controlled by a single gene (monogenic). According to Sato et al. (2004), inherited monogenic resistance was likely to be spread in the population than polygenic.

The information about resistance inheritance is important as fundamental strategy for resistance management strategy. The hypothesis about the involvement of single gen or multiple genes in resistance inheritance is important because it could affect the rate of resistance change within population. The resistance inheritance comprehension is important as basic strategy for resistance management. The hypothesis of single gen or multiple genes involvement in resistance heritance was important due to their role in affecting the rate of resistance characteristic on population. Due to the resistance of *P. xylostella* to emamectin benzoat was recessive and being controlled by single gene, it is recommended that the strategy for its management could be using refugee plant as media for susceptible insects was developed. Plant refugee should be chosen from the same family with cabbage crop which were preferred by insects. The availability of refugee was expected to be useful for susceptible population to develop and occur mating with resistance population, thus at the end it will produce susceptible population.

CONCLUSION

The Selo population was the most sensitive population compared to other populations. While the nature of *P. xylostella* resistance against emamectin benzoate was recessive and controlled by a single gene (monogenic).

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