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RESISTANCE STATUS OF *Crocidolomia pavonana* F. (LEPIDOPTERA : CRAMBIDAE) FROM PASIRWANGI GARUT, WEST JAVA TO THE INSECTICIDE PROFENOFOS AND ITS SUSCEPTIBILITY TO THE METHANOLIC LEAF EXTRACT OF *Nicotiana tabacum* L. (SOLANACEAE)

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ABSTRACT

This research was conducted to determine the resistance status of *Crocidolomia pavonana* to the synthetic insecticide profenofos and to know the toxicity of leaf extract of tobacco (*Nicotiana tabacum*) against *C. pavonana*. The study used two bioassay methods, dry film and feeding assay. *C. pavonana* larvae was obtained from Sirnajaya, Karyamekar, and Padasuka village, Pasirwangi district, Garut, West Java, Indonesia. A standard susceptible strain of C. *pavonana* larvae (more than 20 generation in the laboratory) was used as comparison. The results of this study showed that *C. pavonana* from all villages (Karyamekar, Sirnajaya, Padasuka) indicating slight resistance ($1 < RR \leq 5$) to profenofos with Resistance Ratio (RR) values of 1.04, 1.65, and 1.20, respectively (dry film method) and 1.23, 1.86, and 1.42 (feeding assay). These results suggest that the resistance mechanism of *C. pavonana* in these populations occur mainly in the insect alimentary canal. On the other hand, the field population of *C. pavonana* had a RR to tobacco leaf extract of less than one (0.92 in Sirnajaya; 0.89 in Karyamekar; 0.90 in Padasuka) suggesting that the larvae of *C. pavonana* tested were not resistant to the tobacco leaf extract.

Key words : Resistance ratio, resistance mechanism, botanical insecticide.

INTRODUCTION

Pest resistance cases to inorganic insecticide have been known since 1910, but the number of cases have increased significantly since the discovery of the synthetic organic insecticide of DDT (dichloro diphenyl trichloroethane) in 1945. In 1986, it was reported that 447 insect species became resistant to most groups of insecticide (organochlorine, oganophosphate, carbamate, synthetic pyrethroid, fumigant), including *Bacillus thuringiensis* (Georghiou and Mellon, 1983).

Uhan and Sulastrini (1993) reported in 1993 that *C. pavonana* populations from Lembang (West Java, Indonesia) were not resistant to a number of synthetic insecticides tested. Resistance status of *C. pavonana* to profenofos synthetic insecticide was reported previously by Santoso (1997). Suharti (2000) stated that *C. pavonan*a of Lembang (Cibogo and Cikidang) had a Resistance Ratio (RR) value more than 4 times, which were 6.81 and 7.88. In Pangalengan (Pulosari and Warnasari) and Cisurupan (Sukawargi), *C. pavonana* also became resistant to profenofos with RR of 3.19, 1.59,

and 1.50, respectively. Due to resistance cases of *C. pavonana* to profenofos synthetic insecticide, it is necessary to find new insecticides which are more effective and safer to the environment. Regular monitoring of resistance status is also required.

One alternative to manage insect resistance to insecticides is through the use of botanical insecticides such as leaf extract of tobacco (*Nicotiana tabacum*) which has long been used for insect control. Tobacco leaves contain nicotine as the main active component, which was isolated in 1828, and the potency of nicotine is already well recognized (Gilbert, 2011). Sohail et al. (2012) report that tobacco water leaf extract at a concentration of 2% was effective against aphid, *Toxoptera aurantii* and Khapra, *Trogoderma granarium* (Sarmamy et al. 2011).

According to our survey, farmers from Pasirwangi applied insecticides with profenofos as active ingredient since 1990, without replacement with other insecticides. Therefore, this research aimed to study the resistance status of *C. pavonana* from Pasir Wangi district Garut to profenofos insecticide and tobacco (*N. tabacum*) leaf extract as an insecticide alternative for the management of *C. pavonana* resistance.

MATERIALS AND METHODS

The experiments were conducted from August to December 2011 at the Pesticide Laboratory, Department Plant Pest and Diseases, Agriculture Faculty, Universitas Padjadjaran, Jatinangor-West Java, Indonesia. The experiments consisted of three stages: determination of standard insect susceptibility, determination of resistance level, and determination of resistant insect susceptibility to botanical insecticide.

Rearing of Test Insect

A susceptible population (standard) of *C. pavonana* was obtained from the Physiology and Toxicology Laboratory, Department of Plant Pests and Diseases, Bogor Agricultural University. The insect field populations were obtained from three locations in Pasirwangi district, Garut, which were Karyamekar village, Sirnajaya, and Padasuka. The colony was reared as described by Prijono and Hassan (1992). The larvae were fed with pesticide-free broccoli leaves and the adults were fed with 10% honey solution in a cotton swab.

Preparation of *N. tabacum* Leaf Extract

Tobacco leaves were obtained from Cijambu village, district of Tanjungsari, Sumedang. Newly harvested tobacco leaves were sliced and air dried for one week and ground with a blender into powder. The powder was soaked with methanol (technical grade) for 72 hours in a glass jar. The solution was filtered with Whatman filter paper No. 41. Filtrates were evaporated using a rotary evaporator under reduced pressure to yield crude extracts. The crude extracts were kept under low temperature (-2^{o}C) in a freezer until use.

Preparation of Insecticide Solution

The leaf extract of *N. tabacum* and a synthetic insecticide with active ingradient profenofos were dissolved in distilled water, containing alkyl aryl polyglycol ether (0.5 ml L^{-1}) as spreader and sticker agent, and applied using dry film and feeding assay methods.

Dry Film Method

The profenofos solution tested were 0.0004%, 0.0008%, 0.0016%, 0.0032%, 0.0048y% (v/v) and control (distilled water containing alkyl aryl polyglycol ether 0.5 ml L^{-1}). The profenofos solution (4 ml) was pipetted into a Petri dish and spread on both inner surfaces of the Petri dish. The Petri dish was air dried for 30 minutes. Ten larvae of *C. pavonana* were placed in the Petri dish using a smooth paintbrush. After two hours of exposure, the larvae were transferred to another Petri dish and fed with fresh mustard leaf. Each treatment was replicated four times.

Feeding Assay Method

The profenofos concentration tested and the dilution procedure was similar with the dry film method. Fresh mustard leaf (4 x 4 cm) was dipped in a specific concentration of profenofos for one minute, air dried and placed inside a Petri dish. After which ten second instar *C. pavonana* larvae were placed in the Petri dish. Two pieces of mustard leaves were provided during a 48 hour period. All treatments and control were replicated four times.

Determination of Susceptibility Level of Standard Insect

Test on susceptibility of standard *C. pavonana* was done at five concentration levels of profenofos insecticide (0.0004%, 0.0008%, 0.0016%, 0.0032%, 0.0048% (v/v) and control. The concentrations of synthetic insecticide applied was expected to cause mortality $0 < x < 100$ % based on preliminary tests. Treatments were replicated four times. Each replication used ten second instar *C. pavonana* larvae. *C. pavonana* larvae were exposed to the insecticide using dry film (contact effect) and feeding methods (Busvine, 1970). Larval mortality was observed at 24 and 48 hours after treatment and mortality was calculated using the following formula:

$$M = \frac{a}{b} \times 100\%$$

M = Mortality (%)
a = Number of dead *C. pavonana* larvae
b = Number of *C. pavonana* tested

Mortality data would be corrected with Abbott formula, if the control resulted in larval mortality of less than 20% (Busvine, 1971):

$$\text{Pt}(\%) = \frac{\text{Po} - \text{Pc}}{100 - \text{Pc}} x100\%$$

Po = Insect mortality due to treatment (%)
Pt = Corrected mortality (%)
Pc = Insect mortality of control (%)

Mortality data obtained at 24 and 48 hours after treatment was used to determine LC_{50} value of profenofos synthetic insecticide to *C. pavonana* standard by using program POLO-PC Le Ora software 1987.

Determination of Resistance Level

The determination of resistance level was done at five concentrations of profenofos insecticide (0.0004%, 0.0008%, 0.0016%, 0.0032%, 0.0048%) and one control against *C. pavonana*

larvae from cabbage plantation. The concentrations of synthetic insecticide applied was expected to cause mortality $0 < x < 100$ % that determined based on preliminary test. The method used to evaluate resistance level was similar to that used to determine the susceptibility of standard insect. Based on mortality of larvae, probit analysis was used to determine LC_{50} of profenofos insecticide to field population of *C. pavonana*.

Observations were done at 48 hours after treatment by counting the number of dead *C. pavonana* larvae. The resistance level was determined by calculating the Resistance Ratio (RR) using the following formula:

$$RR = \frac{LC_{50} \text{ field population}}{LC_{50} \text{ standard population}}$$

The resistance of *C. pavonana* larvae to an insecticide can be categorized into three levels: slightly resistant ($1 < RR \leq 5$), moderately resistant ($5 < RR \leq 10$) and highly resistant ($RR > 10$) (Rodríguez et al. 2007).

Susceptibility of resistant insect to *N. tabacum* leaf extract

After determination of resistance level, standard and field population of *C. pavonana* were tested for their susceptibility to tobacco (*Nicotiana tabacum*) leaf extract. The evaluation used five concentration levels of extract solutions (0.1%, 0.2%, 0.4%, 0.5%, 0.6%) that were determined using the same testing of resistance level and one control (without tobacco leaf extract). The experiments were conducted using completely randomized design with four replications. Each treatment used ten second instar *C. pavonana* larvae. The extract was applied on the leaves using the dipping method and then the treated leaves were exposed to *C. pavonana* larvae as food (feeding assay). Data on the mortality of both populations of *C. pavonana* obtained at five days after application were used to determine LC_{50} of *N. tabacum* leaf extract.

Insect mortality was observed every 24 hours, until test larvae reached fourth instar.

The susceptibility level of field population of insect test to tobacco leaf extract was determined by calculating the Resistance Ratio (RR) using the following formula:

$$RR \text{ (tobacco)} = \frac{LC_{50} \text{ field population}}{LC_{50} \text{ standard population}}$$

A computed value for RR (tobacco) < 1, indicates that the insect field population, which was determined to be resistant to profenofos synthetic insecticide, was observed to be sensitive to tobacco leaf extract.

RESULTS AND DISCUSSION

Determination of Susceptibility level of Test Insect Standard

The lethal concentration 99% (LC_{99}) value of profenofos against *C. pavonana* larvae standard was 0.0442% in dry film method and 0.0179 in feeding assay method (Table1). The LC_{99} value in feeding assay method was lower than that in dry film method. This indicates that synthetic insecticide with profenofos as active ingredient had higher toxicity through oral application. Profenofos was active as a stomach and contact poison and has a wide spectrum against insect pests (Worthing,1991).

Table 1. Toxicity of synthetic insecticide profenofos against second instar larvae of *C. pavonana* standard at 48 haa (hour after application).

Test method	Regression parameter		LC_{50} (%) CI (95%)	LC_{99} (%) Cl (95%)
	a ± sd	b ± sd		
Dry film	4.353 ± 0.717	1.496 ± 0.250	0.00123 (0.00089- 0.00164)	0.0442 (0.01833 - 0.2431)
Feeding assay	5.588 ± 0.777	1.867 ± 0.268	0.00102 (0.00077- 0.00129)	0.0179 (0.00973 - 0.0515)

a = intercept sd = standard deviation
b = slope Cl = Confidential limits 95%

Determination of Resistance Level

The results of the study showed that *C. pavonana* larvae from three locations (Karyamekar, Sirnajaya, and Padasuka village) in Garut district had RR values of more than one but less than or equal to five (Table 2 and Table 3). This indicates that the field populations of *C, pavonana* were slightly resistant to the synthetic insecticide profenofos.

Table 2. Resistance ratio (RR) of *C. pavonana* to profenofos in dry film method at 48 HAA (hour after application).

Population	Regression parameter		LC_{50} (%) Cl (95%)	RR
	a ± sd	b ± sd		
Laboratory (standard)	4.353 ± 0.717	1.496 ± 0.250	0.00123 (0.00089 – 0.00164)	-
Karyamekar	4.064 ± 0.709	1.405± 0.247	0.00128 (0.00091 – 0.00174)	1.04
Sirnajaya	5.276 ± 0.768	1.960± 0.275	0.00204 (0.00162 – 0.00263)	1.66
Padasuka	4.051 ± 0.706	1.632 ± 0.248	0.00148 (0.00108 – 0.00202)	1.20

Table 3. Resistance ratio of *C. pavonana* synthetic insecticide profenofos in feeding method (48 HAA).

Population	Regression parameter		LC_{50} (%) Cl (95%)	RR
	a ± sd	b ± sd		
Laboratory (standard)	5.588 ± 0.777	1.867 ± 0.268	0.00102 (0.00077 - 0.00129)	-
Karyamekar	4.792 ± 0.734	1.651 ± 0.256	0.00125 (0.00069 - 0.00203)	1.23
Sirnajaya	4.963 ± 0.747	1.824 ± 0.266	0.00190 (0.00149 - 0.00248)	1.86
Padasuka	4.393 ± 0.717	1.548 ± 0.252	0.00145 (0.00108 - 0.00193)	1.42

The Resistance Ratio values (RR) of the three populations of test insect using the dry film method (contact effect) were lower than those in feeding method (oral effect) (Tables 2 and Table 3). This may indicate that the test insects have developed resistance through certain mechanisms mainly occurring in the insect alimentary canal. Rapid excretion of toxicant from alimentary canal is one of

the resistance mechanisms in insects. Generally, insect resistance occurs through the following mechanisms : (i) reduced penetration, (ii) increased sequestration or excretion or both, (iii) behavioral resistance, (iv) metabolic resistance, and (v) target site insensitivity (FAO, 2012; Pittendrigh *et al.,* 2008; Kranthi, 2005).

The level of resistance of *C. pavonana* to profenofos synthetic insecticide in Garut district was considered low if compared to that reported by other researches in other locations. A study by Suharti (2000) indicates that resistance ratio value of *C. pavonana* in Sukawargi village, Cisurupan district is 1.50. Sajali (2011) reported that resistance ratio value of *C. pavonana* in Mekarjaya, Cikandang, and Simpang village of Cikajang district ranged from 1.2 – 2.5. In another study, Santoso (1997) reported that resistance ratio value in Cipeusing and Barukai village, Pangalengan district reached 15.4 and 13.3. The resistance ratio value indicates resistance status. The higher the resistance ratio, the more resistant the test insects.

The emergence of resistance insect can be caused by several factors, such as frequency of application, the use of a single insecticide for a long time, and improper dose or concentration of application. According to Sajali (2011), the use of synthetic pesticides and fertilizers in Garut are relatively high in the early part of the planting season. Local farmers reported that profenofos synthetic insecticide has been applied since 20 years ago, with an application interval of 7 - 10 days.

The application of pesticide by farmers in Garut is less intensive compared to that in Pangalengan. According to Santoso (1997), spraying interval by farmers in Pangalengan is 2 - 3 days and have been conducted since 1980 (Soeriatmadja and Sastrosiswoyo 1988). The difference in application interval of the insecticide could be the reason why resistance ratio value detected in Garut and Pangalengan district was not the same.

Santoso (1997) and Suharti (2000) reported that the control of *C. pavonana* in Pangalengan always use insecticide with profenofos as active ingredient for a long time and the inappropriate application has triggered a quicker development of resistant insects. This was indicated with hight level of resistance ratio value detected in Pangalengan if compared to resistance ratio value detected in Garut. RR status in Garut possibly would be increased if application of the pesticide was imprecise.

Target insects could become resistant because of selection of population of insect having effective mechanism of detoxyfication of toxicant so that the resistant population in the next generation will grow uncontrollable with initially effective insecticide. Detoxyfication of toxic compound by insects may occur by various enzymes available in the insect body such as oxidation enzyme of polysubstrate mono oxigenase (PsMO), reductase, hydrolase, glutathione S-transferase, and conjugation reactions (Matsumura, 1985; Pittendrigh et al. 2008; Hollingworth and Dong, 2008; Dono et al. 2010). Gunning and Moores (2001) reported that insect resistance to organophosphate compounds may occur through insensitivity of the common molecular target site acetylcholinesterase which breaks down the neurotransmitter acetylcholine.

According to Suharti (2000), RR value was often detemined by the behavior of farmers in using synthetic insecticides such as spraying frequency and application pattern. Less frequent application of insecticides can reduce selection pressure so that the development of resistant insect population can be minimized. Therefore, the development of resistant *C. pavonana* larvae in Pasirwangi, Garut may also occur as result of inappropriate use of insecticide and continuous use of insecticides, without rotation by chemical family.

The high RR value of *C. pavonana* from Sirnajaya village could be caused by the large area of monocultural planting system of Brassicaceae (such as cauliflower, green mustard, and white

mustard) and intensive use of profenofos insecticide for about five to seven years. As a result, the development of resistant insect population was quicker.

In applying profenofos synthetic insecticide, farmers often did not use the recommended concentration as written on the label (1.5 ml L^{-1}). Farmers usually apply insecticides at concentrations they predict would kill the insect pests. Based on the result of an interview with farmers, the concentration applied by farmers was 2 - 3 ml L^{-1} in order for a quicker death of the pest. The interval of spraying was 7 - 10 days depending on the rate of insect infestation in the field.

According to Moekasan (1998),the irrational use of insecticides, such as spraying frequency and high doses, will accelerate the development of resistant insects.

Profenofos is active as a contact and stomach poison. A reduction in insect susceptibility to contact insecticide is often caused by a changing in cuticle characteristic such as thickness, hardness, and lipid content. Insect larvae are usually more susceptible to contact insecticide during molting prosses and become less susceptible with the increase of age. Penetration rate of an insecticide into insect body influenced by cuticle thickness (Matsumura, 1985; Prijono, 1999; Hollingworth and Dong, 2008). An increase in the thickness of the cuticular layer usually results in lower lipid content, causing a decrease in the number of insecticides dissolved in the cuticle (Bushvine, 1971). Profenofos is non polar and is lipid soluble. Low content of lipid can cause slow penetration rate and this results in an increase in the tolerance of the larvae to insecticide.

A stomach poison insecticide is active if it enters through insect digestion organ or alimentary canal. Therefore, the insecticide contaminated crop must be eaten in enough amount to kill the insect (Matsumura, 1985; Djojosumarto, 2008). The reduction in the susceptibility of larvae to stomach poison insecticide could be because of an increase in the resistance of gut wall or rapid elimination of toxicant from the insect alimentary canal (Onstad, 2008; Kranthi, 2005).

In the cellular level, an improvement in enzymatic activity which detoxify insecticides is among the mechanisms of resistance of insects (Bushvine, 1971). Yu et al. (2003) reported that *Spodoptera frugiperda* which is resistant to carbaryl insecticide and methyl parathion showed an increase in detoxyfication enzymatic activity of MFO (microsomal mixed function oxidases), hydrolase and glutathione S-transferase enzyme. Therefore, analysis of these detoxification enzyme should be done in future studies.

Susceptibility of resistant insects to *N. tabacum* leaf extract

Results of the susceptibility test of *C. pavonana* larvae against leaf extract of *N. tabacum* showed that the test insect indicated resistant to profenofos was susceptible to *N. tabacum* extract. Susceptibility of test insect larvae was shown by Resistance Ratio (RR) value that was less than one (Table 4).

Profenofos insecticide and tobacco extract have different modes of action. Therefore, tobacco extract could become an alternative method to manage *C. pavonana* larvae that have developed resistance to organophosphate insecticides. Organophosphate insecticides act on the synapse of neuron. Organophosphates interfere with acetylcholinesterase, the enzyme that breaks down of neurotransmitter acetylcholine in the synapse (Matsumura, 1985; Stenersen, 2004). On the other hand, the active compound of tobacco extract, nicotine's mode of action is interfering with acetylcholine receptor at nervous system (Stenersen, 2004; Yu, 2008). Since insect resistance mechanism to profenofos insecticide occurs due to be lower sensitivity of acetylcholinesterase to the active site of the insecticide (Fournier et al. 1992; Hemingway et al. 2004), a different mode of action

(molecule or organ target) between organophosphate and tobacco leaf extract makes this botanical insecticide effective against *C. pavonana* which is resistant to profenofos.

Table 4. Resistance ratio (RR) of *C. pavonana* to tobacco leaf extract (5 DAT).

Population	Regression Parameter		LC_{50} (%) Cl (90%)	RR
	a ± sd	b ± sd		
Laboratory (standar)	1.034 ± 0.199	2.207 ± 0.357	0.34022 (0.2156 - 0.57631)	-
Karyamekar	1.309 ± 0.208	2.259 ± 0.369	0.30364 (0.20530 - 0.43276)	0.89
Sirnajaya	0.805 ± 0.193	1.604 ± 0.332	0.31500 (0.20053 - 0.50486)	0.92
Padasuka	1.261± 0.204	2.458 ± 0.361	0.30690 (0.22735 - 0.40790)	0.90

DAT = days after treatment; CI= confidential limits

CONCLUSION

The population of *C. pavonana* larvae from Pasirwangi district, Garut, West Java, was observed to be slightly resistant to profenofos because its resistance ratio (RR) value was more than one but less than five. Resistance ratio in feeding assay method was higher than dry film method indicating that the resistance mechanism involved may primarily occur in the alimentary canal of the insect. Field populations of *C. pavonana* were susceptible to tobacco leaf extract. Therefore, tobacco leaf extract could be used as an alternative insecticide for the management of *C. pavonana* resistance to profenofos insecticide.

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