Proceeding of The International Seminar on Chemistry 2008 (pp. 589-594)
Jatinangor, 30-31 October 2008

Bioactivity methanolic seed extract of *Barringtonia asiatica* (Lecythidaceae) against *Crocidolomia pavonana* (Lepidoptera:pyralidae)

Danar Dono*

Plant Protection Department, Agriculture Faculty, Padjadjaran University,
km 21 Bandung-Sumedang Main Road, Jatinangor, 45300
*e-mail: danardono21@yahoo.com

Abstract

Methanolic seed extract of *Barringtonia asiatica* has insecticidal activity, however its effect on *Crocidolomia pavonana* oviposition, fecundity, and food utilization efficiency were not observed yet. *B. asiatica* seed extract was tested with residual method application on mustard leaf at concentration of 0,02%; 0,05%; 0,1%; 0,2%; 0,3% and control to get LC_{50} value. The effect of *B. asiatica* seed extract on *C. pavonana* fecundity was conducted by residual method application of *B. asiatica* seed extract at concentration of 0.09%, 0.15%, 0.22% and control on mustard leaf. The fecundity of adult insect develops from the larvae that feeding with treated food was observed. The effect of *B. asiatica* seed extract on oviposition of *C. pavonana* female was conducted by spraying seed extract at concentration of 0,66%, 0,77% and 0,96%. The effect of extract on food utilization efficiency of *C. pavonana* larvae was done based on gravimetric method at concentration of 0,08%; 0,14%; and 0,21%. The result of the research showed that *B. asiatica* seed extract were toxic on *C. pavonana* larvae with LC_{50} value of 0.15% and had antifeedant effect. *B. asiatica* seed extract at range concentration of 0.09%-0.22% delayed and inhibited egg production of *C. pavonana*. Seed extract at concentration of 0,96% (equal with LC_{90} value) totally inhibited *C. pavonana* oviposition. seed extract of *B. asiatica* caused decreasing of consumption rate (CR), relative consumption rate (RCR), growth rate (GR), and relative growth rate (RGR), so the ability of the larvae to damage crop become decreasing.

Keywords: *Barringtonia asiatica, Crocidolomia pavonana*, extract, toxicity, antioviposition, fecundity, food utilization efficiency

Introduction

Indonesia has high diversity of plant. Various plants are reported to have insecticidal activity. One of potential plant can be developed as source of insecticide is *Barringtonia asiatica* (L.) Kurz. (Lecythidaceae) which general name is Sea Poison Tree (Indonesian name is Bitung) (Ecology & Evolutionary Biology Greenhouses [EEBG], 2006).

In some places, *B. asiatica* is used as traditional medicine and fish poison (The Cook Islands Natural Heritage Trust, 2005). In Philippine, *B. asiatica* leaf is used for stomachache drug and rheumatic (EEBG, 2006). In Pacific Archipelago, seed of *B. asiatica* applied to poisoning fish by the flowing fraction of seed in water (Cannon *et al.*, 2004). Methanol seed extract of *B. asiatica* has strongest insecticide activity compared to bar skin and leaf extract. According to *C. pavonana* larval response after the application of extract indicated that the extract has antifedant activity to larva (Dono & Sujana, 2007).

Active compound in *B. asitica* extract, which cause poisoned at fish is group of saponin compound, this compound detectable at all part of crop (Tan, 2002; EEBG, 2006). In general, saponin has poison character to poikilothermal animals (Cannon *et al.,* 2004). Ranuncoside VIII is one of saponin compound group from *B. asiatica* extract has poison activity against fish (Burton *et al.,* 2003). Two other main saponins isolated from methanolic seed extract of *B. asiatica* have antifeedant effect to larvae of *Epilachna* (Herlt *et al.,* 2002). Besides, leaf methanol extract, fruit, seed, and bar skin of *B asiatica* shows activity antibacterial broad spectrum. A number of its fraction showing anti mushroom activity (Khan & Omoloso, 2002).

Literature studies indicated that, there are only a few information reporting compounds insecticide activity from *B. asiatica* extract. Therefore, effort for exploitings *B. asiatica* as botanicals insecticide still need to be developed. At this research, insecticide activity and anti oviposition and reproduction from seed *B. asiatica* extract was investigated.

Materials and Methods

Test Insect

C. pavonana field colony was reared in mass rearing laboratory of Plant Protection Department, Faculty of Agriculture, Padjadjaran University. Rearing technique of insect was adapted from Prijono & Hassan (1992) procedure. Adult of *C. pavonana*

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would be maintenance in mica-plastic cage (40 x 40 x 40 cm) and feeding with honey at concentration of 10%. A number of sheath mustard leaves (*Brassica juncea*) dipping into plastic cup as oviposition site of insect. Egg obtained every day was put into plastic box (10 x 5 x 6 cm). After the egg hatch larval keeping in plastic box until pupal formed. *B. juncea* leaves was used as feeding of *C. pavonana* larvae. Egg parasitoid *Trichogramma* obtained from PT Jati Tujuh, Majalengka.

Extraction

Seed of *B. asiatica* obtained from Jatinangor, Sumedang which is located ± 700 metre above sea level. Methanolic seed extract of *B. asiatica* was prepared by Dadang & Nugroho (1999) methods.

Fraction of seed (more or less 2 cm in diameter) was air-drying in one week. Seed was refined by using blender become seed flour with water content 15%. The seed flour then soaked in methanol with comparison 1:10 (w/v) during 3 x 24 hours. The mixture was filtered by using glass funnel. Result of screening is evaporated with rotary evaporator at temperature of 50°C and pressure 580-600 mmHg. Crude extract obtained kept in freezer at temperature of 4°C.

Experiment1. Toxicity of B. Asiatica Seed Extract Against C. Pavonana Larvae

Examination of seed methanol extract insecticide activity *B. asiatica* to larva *C. pavonana* is done by using leaf residual method (Prijono, 2003). Methanolic seed extract of *B. asiatica*. was diluted in methanol to obtain concentration needed for examination. The concentration was determined based on preliminary test. The mixture was added with alkylaril polyglycol ether (400 g l^{-1}) as sticker-spreader and Tween 80 as emulsifier at concentration 1 ml l^{-1}. As control was applied with mixture of 2%methanol, alkylaril polyglycol ether 400 g l^{-1} and Tween 80 (1 ml l^{-1}).

Two mustard leaves (4 x 4 cm) were dipped into each mixture in few second. Air-drying mustard leaves were put into petri disc (9 cm diameter), then 10 *C. pavonana* larvae second instars (2 hour after molting) was entered in its. After 48 hours the *C.* larvae feed on leaf mustard treated with extract, the larvae to be feed on mustard leaf without treatment until reaching fort instar.

Larval *C. pavonana* mortality was observed every day after treatment until reaches fort instar larvae by counting dead larvae. If at control happened death of larva C. pavonana less than 20%, mortality every treatment is corrected by using Abbott formula (Finney, 1971).

The correlation of extract concentration and larval *C. pavonana* mortality was determined with probit analysis (Finney, 1971).

Experiment 2. Effect of B. asiatica Seed Extract to Oviposition of C. pavonana Female

The experiment was done to know influence of *B. asiatica* seed extract to oviposition activity of *C. pavonana* on mustard crop with spraying method. Extract was tested at concentration equivalent with LC_{50}, LC_{70}, and LC_{90}. The extract diluted with water in hand sprayer 100 ml by adding 0,1 ml Tween and Agostick as sticker and emulsifier 100 ml. *B. juncea* leaf was sprayed with extract until run off, then the crop was put in screen-plastic cage (diameter 25 cm and height 30 cm). A couple of two days emerge *C. pavonana* adult inoculated into the screen-plastic cage by using small film plastic tube. Amount of eggs oviposite by *C. pavonana* on treated mustard leaf and control collected dayly then calculated by using microscope.

Number of egg cluster and amount of egg analysed with varians analysis, then continued with doubled distance test of Duncan by using SAS program.

Experiment3. Effect of B. asiatica Seed Extract to C. pavonana Fekundity

Influence of *B. asiatica* seed extract to fecundity of adult female of *C. pavonana* was conducted by leaf dipping method of larval feeding leaf mustard into dilution of extract. The extract tested at concentration of 0.09% (LC_{30}), 0.15% (LC_{50}), 0.22% (LC_{70}). and control. *C. pavonana* second instar was used in this experiment. Two mustard leaves (4 x 4 cm) were dipped in mixture of extract at concentration determined previously. After solvent evaporated, two treatment leaf cuts put down in plastic box, then 10 larvae of *C. pavonana* second instar put in the plastic box by using paintbrush. Control larvae feed on leaf which only be plunged in solvent. The larvae feed on lef treatment as long as 72 hours, then the larvae was given leaf without treatment until reaching adult.
Nine couple of *C. pavonana* adult were keept in screen-plastic cage separately (6.5 cm in diameter, height 30 cm). The insect was given 10% mixture of honey which was absorbed at cotton. Mustard plan as site of egg laying put down in screen plastic cage. Amount of *C pavonana* egg observed every day until the insect death.

Data obtained analysed with analisys of varians continued with Duncan multiple rang test at significance level 5% applies program SAS version 6.12.

Experiment4. The Effect of B. asiatica Seed Extract to Food Utilization Efficiency of C Pavonana Larvae

As correction factor of larval and leaf weight, five larvae and five leaves samples were deliberated separately and dried in oven at temperature of 100°C during 48 hours. Wet and dry weight of larval and

leaf was used to know dry weight percentage of Larvae and leaf. Correction factor of percentage of dray weight larvae and leaf was calculated by using formula: material dry weight = dry weight (after draining) / fresh weight (before draining) x 100%.

Three pieces of mustard leaf (4 x 4 cm) were plunged into each mixture at concentration of LC_{30}, LC_{50}, and LC_{70} and control during one minute. Three treatment leaf cuts which has been air-drought put in plastic box. Every larvae and leaf cutting before examination was deliberated its weight, then every five *C. pavonana* larvae put into one plastic box.

Result of multiplication correction factor with fresh weight of larvae and leaf before examination showed initial dry weight of larva (BKAWL) and initial dry weight of leaf (BKAWD).

After larvae consume treatment leaf during 24 hours, every larva, rest of feed leaf and faces of larvae were wrapped tissue paper separately and dried by using oven at temperature 100^{o}C during 48 hours separately. After drought, larvae and rest of feed leaf and faces of larvae were deliberated again to get final dry weight of larvae (BKAKL), final dry weight of leaf (BKAKD), and final dry weight of faces (BKK).

Data BKAWL, BKAWD, BKAKL, BKAKD and BKK was used to calculate consumption rate (CR), growth rate (GR), digestibility (D), efficiency conversion of consumption (ECC), and food efficiency conversion of digested (ECD) from larva, based on gravimetry method (Waldbauer, 1968) :

1. CR = (BKAWD - BKAKD) / period eats

Wight average of larva = (BKAWL + BKAKL) / 2

Relative consumption rate (LKR) = LK / Wight average of larvae

2. GR = (BKAKL-BKAWL) / period eats

Relative growth rate (LPR) = LP / Wight average of larvae

3. D = (BKAWD - BKAKD) - BKK / (BKAWD - BKAKD) x 100 %

4. ECC = (BKAKL-BKAWL) / (BKAWD - BKAKD) x 100 %

5. ECD = (BKAKL-BKAWL) / (BKAwD - BKAkD) - BKK x 100 %

Obtained data was analysed with analisys of varians continued with doubled distance test of Duncan applies program SAS.

Results and Discussion

Toxicity OF B. Asiatica Seed Extract to Larva C. Pavonana

Mortality of larvae generally happened at two days after treatment and increase till fifth day after treatment. The mortality of larvae in line with concentration of extract applied. Based on visual observation, body death of *C. pavonana* larvae treated with *B. asiatica* seed extract showed the smaller size body, having color black, larva body gradually runs dry. Active compound responsible to mortality of larvae that were saponin and alkaloid. According to Harborne (1987) and Robinson (1995) saponin is triterpene glycoside and sterol which has surface active compound and character like soap which determine its toxicity to various insects. saponin also can pursue exhalation of insect (Vincent, 1995).

Result of probit analysis done to mortality data by one up to five days after application (daa). Result of probit analysis to mortality of *C. pavonana* larvae shows impairment LC50 to two hsa to five hsa, value LC50 equal to 0,15% at day to five after application (Tables 1). This thing because of increase of big larva mortality at two to five hsa causing influences value LC50 obtained. By paying attention to value LC50 by five hsa, explainable that seed extract B. asiatica has strong insecticide activity to larva C. pavonana.

Tabel 1 Parameter probit regression correlation of concentration of *B. asiatica* seed extract and mortality of *C. pavonana* larvae

Waktu analisis (hari)	a ± SE	b ± SE	LC_{50}	Confidencial limits (%)
1	-	-	-	-
2	-3.34 ± 0.88	6.35 ± 3.42	0.53	-
3	-1.66 ± 0.21	5.01 ± 1.09	0.33	0.27 – 0.48
4	-1.36 ± 0.18	6.66 ± 1.03	0.20	0.14 – 0.34
5	-1.21 ± 0.17	7.84 ± 1.09	0.15	0.09 – 0.27

Keterangan : a = Intercep
b = Slope
SE = Standard Error

The Effect of Seed B. asiatica Extract to Oviposition C. pavonana Female

Number of eggs oviposite by *C. pavonana* at leaf crop treated with extract at concentration of 0.66%, 0.77% and 0,96% significantly different compared with control treatment. On crop treated with extract at concentration of 0.96% was not found a number of eggs at 1 - 6 day after application (Tables 2). In the nature, adult female of *C. pavonana* could yielding 180 - 320 eggs during its life span (Thayib, 1983).

Inhibition of oviposition activity of *C. pavonana* on mustard crop treated with extract at concentration of 0,96% was caused by specific repellent compound (anti-oviposition) from seed extract of *B. asiatica*. This would be different with the result of the research by Syahputra (2007), that extract of *Calophyllum soulattri* at concentration of 0,075% didn't have antioviposition activity to adult female of *C. pavonana*. Oviposition preferensi of *C. pavonana* closely related with intensity of special stimulus from crop (Pelealu, 2004). Spraying of extract *B. asiatica* on mustard leaf could inhibit special stimulus from plant to excite the insect to laid eggs. In general, egg laid by *C. pavonana* female determined by crop secondary metabolite (Honda, 1995).

Effect of B. asiatica Seed Extract to Fecundity of Female C. pavonana

Result of experiment indicated that treatment of seed extract of *B. asiatica* could be delay egg formation of *C. pavonana* female. Time formation of egg at all the extract treatment was longer than control. *B. asiatica* seed extract treatment at concentration of 0.09%, 0.15, and 0.22% could delayed time formation of *C. pavonana* egg as long as 1-3 days compared with control. At control, top production of eggs happened at sixth day with total egg amount of 570, while at extract treatment at concentration of 0.09%, 0.15%, and 0.22% happened at 7^{th} , 8^{th}, and 9^{th} with egg total 163, 154, and 106 (Figure 1). The result of this experiment equal with experiment conducted by Syahputra (2007), dichloromethane active fraction of *C. soulattri* bar skin extract at equivalent concentration of LC_{50}-LC_{99} delayed and reduced eggs production of *C. pavonana* female. Sudarmo (2005) said that botanical insecticide could inhibit reproduction of insect. Reproduction inhibition of *C. pavonana* female possibility caused by active compound from seed extract of *B. asiatica* directly affected formation insect reproductive organ or effected by hormonal disruption that controlling egg formation. Egg formation controlled at least by juvenile hormon (Chapman, 1998). In addition treatment of *B. asiatica* seed extract at all concentration caused oviposition period of *C. pavonana* female becomes shortly compared with control.

The Effect of B. Asiatica Seed Extract to Food Utilization Efficiency of C. pavonana Larvae

Treatment of *B. asiatica* seed extract at concentration of 0.08% tends to reducing consumption rate (CR), relative consumption rate (RCR), growth rate (GR) and relative growth rate (RGR) of *C. pavonana* larvae. Treatment of extract at concentration of 0,14% caused the biggest decreasing of CR, RCR and RGR of the larvae. There is improvement tendency of digestibility (D), efficiency conversion consumption (ECC), and efficiency conversion digestibility (ECD) of *C. pavonana* larvae at concentration of 0,08% and 0,14% if compared to control (Table 3).

Consumption of food containing allelochemical caused abnormal growth ofinsect, Allelochemical had negative effect to insect in three mode of action mechanisms. First, lessens food entering by inhibit feeding activity of insect. Second, after digested allelochemistry can reduce efficiency utilization of insect food. Third, allelochemicals poisoning insect through various physiology processes. At the certain of time, allelochemical affected insect by using third of the mechanism (Schoonhoven *et al*., 1998).

Allelochemicals, included saponin, found in food consumed by insect could reduce digestive enzymatic activity and absorption of insect food (Berenbaum *et al.,* 1986; Chapman, 1998). Digestive enzyme take role in breaking down of nutrition so important in absorption and utilization of food consumed by insect for growth (Bell & Carde, 1984).

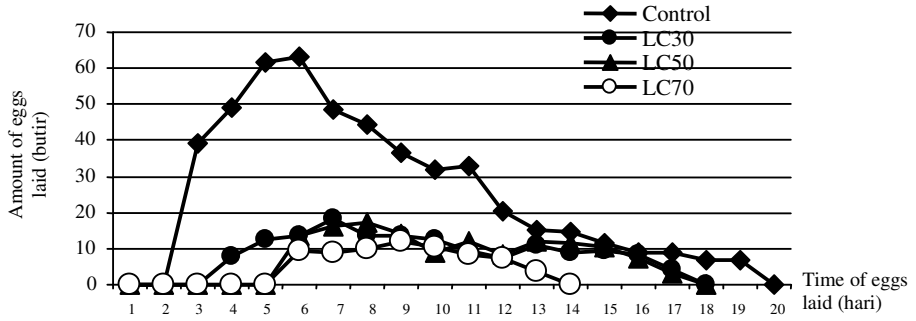

Figure 1 The effect of *B. asiatica* seed extract treated to *C. pavonana* larvae on amount and formation time of eggs of *C. pavonana* adult

Table 2 Amount of eggs and egg cluster oviposite by *C. pavonana* on mustard leaf treated with *B. Asiatica* seed extract

extract Concentration (%)	Amount of eggs and egg cluster oviposite by *C. pavonana* on mustard leaf (day after aplication)											
	1		2		3		4		5		6	
	Amount of eggs	Egg cluster	Amount of eggs	Egg cluster	Amount of eggs	Egg cluster	Amount of eggs	Egg cluster	Amount of eggs	Egg cluster	Amount of eggs	Egg cluster
0,96%	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
0,77%	0a	0a	0a	0a	8.5a	0.25 a	17.5a	0.25a	0a	0a	0a	0a
0,66%	0a	0a	0a	0a	19.5a	1a	29.5a	0.5a	38.5b	0.75 b	0a	0a
Kontrol	19.5a	0.25 a	28.6b	0.5a	55.9b	1a	184.9 b	2b	127.8 c	1.75 b	61b	1a

The value followed by the same letter in the same collum did not significantly different according Duncan multiple range test at level of 5%

Table 3 The effect of *B. Asiatica* seed extract on consumption rate (CR), relative consumption rate (RCR), growth rate (GR), relative growth rate (RGR), digestibility (D), efficiency conversion consumption (ECC), and efficiency convertion digestibility (ECD) of *C. pavonana* larvae

Treatment	CR (g/day)	RCR (g/g/day)	GR (g/day)	RGR (g/g/day)	D (%)	ECC (%)	ECD (%)
Control	0.0088 ± 0.004 b	3.62 ± 1.91 b	0.0002 ± 0.0004 b	0.005± 0.144 b	78.53± 2.84	2.40± 4.98	1.95± 6.98
$LC_{30\ (0.08\%)}$	0.0041 ± 0.005 ab	2.62 ± 3.48 ab	-0.0004± 0.0006 a	-0.35± 0.406 ab	95.62± 18.75	25.32± 40.32	19.42± 38.74
$LC_{50\ (0.14\%)}$	0.0004 ± 0.004 a	-0.76 ± 3.46 a	-0.0007± 0.0002 a	-0.57± 0.373 a	113.3± 32.99	54.15± 109.37	29.74± 63.49
$LC_{70\ (0.21\%)}$	0.0014 ± 0.004 a	1.17 ± 2.88 ab	-0.0009± 0.0004 a	-0.56 ± 0.19 a	63.95± 76.66	-6.67± 22.19	-12.5± 20.07

The value followed by the same letter were not significant difference according Duncan multiple range test at 5% level.

Conclusions

Methanolic seed extract of *B. asiatica* had strong insecticidal activity against *C. pavonana* larvae with LC_{50} value of 0.15% and had antifeedant effect. *B. asiatica* seed extract at range concentration of 0.09% - 0.22% delayed and inhibited egg production of *C. pavonana*. Seed extract at concentration of 0,96% totally inhibited *C. pavonana* oviposition. seed extract of *B. asiatica* caused decreasing of consumption rate (CR), relative consumption rate (RCR), growth rate (GR), and relative growth rate (RGR), so the ability of the larvae to damage crop become decreasing.

Acknowledgements

The research is a part of the project carried out through a grant awarded under Research Program Hibah Kompetisi A3, Plant Protection Department, Agriculture Faculty, Padjadjaran University. To Emelda anggraeni, Puguh Hari Susetyo, and Ifan Wirahadian, thanks for their assist in this research.

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