Synergistic action of mixed extracts of *Brucea javanica* (Simaroubaceae), *Piper aduncum* (Piperaceae), and *Tephrosia vogelii* (Leguminosae) against cabbage head caterpillar, *Crocidolomia pavonana*

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ABSTRACT

Extracts seeds, fruits, and leaves of *Brucea javanica, Piper aduncum*, and *Tephrosia vogelii* were tested separately and also in mixture (3:2.5:1) in the laboratory for their insecticidal activity against the cabbage head caterpillar, *Crocidolomia pavonana*. *B. javanica, P. aduncum*, and *T. vogelii* plant materials were extracted with ethyl acetate-methanol (9:1), ethyl acetate, and ethyl acetate respectively by using maceration method. Insecticidal bioassays were done by a leaf-residue feeding method. Second-instar larvae *C. pavonana* were fed extract-treated broccoli leaves for 48 hrs and then were presented untreated leaves until the surviving larvae reached the fourth-instar stage. Larval mortality was assessed at 5 days after treatment, and the data analyzed by the probit method. The results showed that larval mortality started at first day treatment and increased at second day's treatment. After changing with untreated leaves the mortality decreased significantly. Based on LC_{50} and LC_{95} values, *T. vogelii* leaf extract was more toxic (LC_{50}= 0.06%, LC_{95}= 0.12%) than *P. aduncum* fruit extract (LC_{50} = 0.24%, LC_{95}=0.32%) and *B. javanica* seed extract (LC_{50}=0.17%, LC_{95} =0.41%). Based on value of LC_{50} (0.03%) and LC_{95} (0.05%), the toxicity of a mixture of *B. javanica, P. aduncum,* and *T. vogelii* extract (3:2.5:1) against *C. pavonana* larvae was very toxic. Based on the combination index according to the independent joint action model, the extract mixture had a strongly synergistic joint action against *C. pavonana* larvae, at level LC_{50} and LC_{95}, with a combination index of 0.225 and 0.190. The mixture extract worked better than each single extract against *C. pavonana*

Keywords: Botanical insecticides, cabbage pest, extract mixture, joint action.

INTRODUCTION

Crocidolomia pavonana (F.) (Lepidoptera: Crambidae) is a main pest on Cruciferae plant family. This pest attacks on cabbage crop, causing significant yield losses without control effort (Sastrosiswojo and Setiawati, 1993). A pest control using synthetic insecticides left many negative impacts, including the target pest resistance and resurgence, killing natural enemies and untargeted organisms, environmental pollution and residues (Metcalf, 1982).

Botanical insecticides relatively have lower negative impact than synthetic insecticides, because they are more easily degraded in the environment and are compatible with other integrated pest management techniques as well. Raw material shortage is a problem faced in botanical insecticide field applications. Dependence on one type of raw material would affect pest control as well as interfere in ecosystems balance. To minimize the problems, a combination of extracts from several plant species can be tried. The use of a mixture of insecticides is recommented as it would delay the onset of pest resistance in certain insecticides. It would control some types of pests, improve the efficiency of the application because mixture often used at lower doses than the doses of each component separately, and also reduce the side effects of non target organism and environment (Prijono, 1992). More than 2400 species of plants belonging to 235 families reported pesticide-containing materials (Grainge and Ahmed, 1988). Among them are *Brucea javanica, Piper aduncum,* and *Tephrosia vogelii*.

Brucea javanica is a member of Simaroubaceae Family known to possess active compound quassinoid and derivatives of quasinoid. The quasinoid compound including isobrucein A and B, brucein B and C, glaucarubinon, and quasin have feeding inhibitors effect on *Myzus persicae* (Polonsky *et al*., 1982). Fractionation of methanol extract of *B. javanica* seeds yielded an active fraction which was eluted with ethyl acetate-

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methanol 9:1. Emulsifiable concentrate (EC) and wettable powder (WP) formulations of melur fruits were active against *C. pavonana* and *P. xylostella* larvae (Lina *et al*., 2010)

Many researches utilized various parts of *P. aduncum*. Almeida *et al.* (2009) and Jantan *et al.* (1994) mention that dilapiol is a main component from of *P. aduncum* oil (64-90%). Essential oil from *P. aduncum* showed bioactive potential to be used as a repellent against mosquito *Aedes albopictus* (Misni *et al.,* 2009). Fazolin *et al.* (2005) also report that essential oil from *P. aduncum* leaves caused mortality in *Cerotoma tingomarianus* beetle by contact method.

Leaves of *T. vogelii* contain insecticidal compound known as rotenone and rotenoid such as deguelin, tefrosin, and elipton (Delfel *et al.,* 1970; Lambert *et al.* 1993). Leaves and twigs of *T. vogelii* are common plants used for poison fishing in entire tropical Africa (Neuwinger, 2004). A powder of *T. vogelii* leaves caused mortality, reduce the longevity, and oviposition of *Callosobruchus maculatus* (Boeke *et al.,* 2004).

Several studies have shown that the leaf extract of *T. vogelii* provides a synergistic effect with other plant extracts. Abizar and Prijono (2010) reported ethyl acetate leaf extracts of *T. vogelii* toxic to *C. pavonana* larvae and a mixture of leaf extract of purple-flowered *T. vogelii* and fruit extract of *P cubeba* (5:9 w/w) was more toxic to *C. pavonana* larvae than each extract tested separately. The mixture extract is expected to increase the mortality of target pests. It becomes necessary to study the combination of a mixture of extracts of *B. javanica, P. aduncum*, and *T. vogelii* to get the correct dosage, so that the use of three types of material in controlling *C. pavonana* can be more effective and efficient. Phytotoxicity tests of mixed formulation was also conducted at the same time. The purpose of this study was to determine the insecticidal activity of extracts from *B. javanica, T. vogelii*, and *P. aduncum* against *C. pavonana* and to know the activity of the mixed extract combination from three types of plants.

MATERIALS AND METHODS

The research was conducted at the Laboratory of Insect Physiology and Toxicology, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural Institute (IPB). Broccoli Plant Propagation for Insect Rearing and Test (*Brassica oleracea* L. var. Brand Sakata, Green Magic Broccoli F1-Hybrid) were grown on black plastic bag (polybag). Broccoli seeds sown on seedling trays filled with 50 holes contained organic growing media. Along with seeding fertilization was done with slow release compound fertilizer "Dekastar" (NPK 22-8-4). After 4 weeks, broccoli seedlings were transferred to polybag (5 L) containing growing media soil. Maintenances were done daily, including watering, weeding, and mechanical pest control if pests were found in plants. Broccoli leaves aged approximately 2 months old were used as feed larvae *C. pavonana* maintenance and treatment.

Insect Rearing

C. pavonana from laboratory breeding was carried out following the procedure used by Basana and Prijono (1994). The larvae was fed with broccoli leaves free of pesticides as mention above, the imago was fed with honey solution (10%) using cotton ball.

Plant Materials

Plant materials used in this research are *T. vogelii* leaves, *B. javanica* seed, and *P. aduncum* fruits. All materials were collected from West Sumatera (*B. Javanica*) and West Java (*T. vogelii* and *P. aduncum*), Indonesia. Each plant material was cut (± 3 cm) and then placed on plastic box and allowed to air dry without direct sunlight. After drying each plant part was milled using a grinder, then sieved into powder. Powder (50 g) of each plant part was inserted into Erlenmeyer flask and immersed on a suitable solvent based on previous research. *Tephrosia vogelii* and *P. aduncum* was immersed in 500 mL of ethyl acetate, while *B. javanica* was soaked in 500 mL ethyl acetate: methanol-9: 1 at least for 24 hrs. The liquid extract was filtered using glass funnel (diameter 9 cm) repose with filter paper. Distillate was collected in flask evaporator, then evaporated with a rotary evaporator at 45°C and a pressure of 337 mbar. The extract was stored in the refrigerator at ± 4°C until used for testing.

Test of Toxicity Extracts

The treatment using leaf-residue feeding method where each extract tested at five concentration level and six replication based on preliminary test. Each extracts mixed with the methanol solvent and Tween as an emulsifier, then diluted with water. Final concentration of methanol and emulsifier in the test mixture were 1% and 0.2% respectively. A piece of broccoli leaves (4 cm x 4 cm) dipped one by one in certain extract suspension, then air-dried. Control leaves dipped in a control solution. One piece of treatment leaves or control leaves separately placed in a petri dish (diameter 9 cm) cover with a tissue on the bottom of Petri dish.

Fifteen second instars larvae of *C. Pavonana* were put in to petri dish containing treated or control leaves, and were allowed the larvae to feed for 48 hrs. After 48 hrs the treated leaves were replaced with untreated leaves. Each treatment and control was repeated 5 times. The number of dead larvae and developmental period were recorded. Larval mortality data were analyzed using POLO-PC program (Le Ora Software, 1987). Larval developmental period data were expressed as mean plus minus standard deviation.

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Analysis of joint action of extract mixture

The analysis was based on the independent joint action model. The combination index (CI) at LC_{50} and LC_{95} level was calculated as follows (Chou and Talalay, 1984):

$$\frac{LC_x{}^{1(cm)}}{LC_x{}^{1}} + \frac{LC_x{}^{2(cm)}}{LC_x{}^{2}} + \frac{LC_x{}^{3(cm)}}{LC_x{}^{3}} + (XY) + (XZ) + (YZ) + (XYZ)$$

X Y Z

LCx1, LCx2, and LCx3 are LC_x of components 1, 2, and 3 in separate tests. LCx1 (cm), LCx2 (cm), LCx3 (cm) of each LC component 1, 2, and 3 in the mixture that resulted in mortality of x (50% and 95%). LC value is obtained by multiplying the LCX mixture proportions of components 1, 2, and 3 concentrations in the mixture.

The interaction categories were adapted from Kosman and Cohen (1996) and Gisi (1996) based on the inverse co-toxicity ratio values: (1) CI < 0.5, the mixture components were strongly synergistic, (2) CI 0.5 to 0.77, the mixture components were less synergistic, (3) CI > 0.77 to 1.43, the mixture components were additive and, 4) CI > 1.43, the mixture components were antagonistic.

RESULTS AND DISCUSSION

Plant Extraction

Tephrosia vogelii leaves have the highest activity at 0.1% and at 0.5% caused 100% mortality of insects test. The activity of *B. javanica* fruit extract was lower than *T. vogelii* leaf extract at the same concentration. At 0.1% and at 0.5% concentrations of *B. javanica* caused 55.5% and 100% insects test mortality respectively. *P. aduncum* fruits extract at concentrations 0.1% and 0.5% caused 26.6% and 100% mortality respectively.

Test of Extract Toxicity

The results showed that *T. vogelii* has a positive relationship with the increasing of concentration and the number of mortality of *C. pavonana*. The mortality of *C. pavonana* treated with *T. vogelii* at the lowest concentration and the highest concentration are 8.89% and 88.89% respectively. Developmental period of the surviving larvae from second instar to third and second instar to fourth instar between 1.45 to 2.39 days and 1.5 to 2.85 days respectively were compared to control (Table 1). As Neuwinger (2004) reported, rotenoid occur mainly on leaves and twigs of *T. vogelii* and contain insecticidal compound known as rotenone and rotenoid such as deguelin, tefrosin, and elipton (Delfel *et al.,* 1970; Lambert *et al.,* 1993). Rotenone as an active compound work as respiration poison by block electron transfer in NADH from transport electron system in mitochondria (Hollingworth, 2001). Then ATP production will reduce and cell activity decrease, causing paralysis and mortality (Perry *et al.,* 1998). Larval *C. pavonana* poisoned of *T. vogelii* appeared to be blackened due to the death of cell and tissue (Abizar and Prijono, 2010).

The activity of *B. javanica* seed extract has a similar pattern with *T. vogelii* leaf extract, larval mortality increase as increasing

Table 1. Mortality and developmental period of *C. pavonana* larvae in the treatment with *T. vogelii* leaf extract

Extract Concentration (%)	Mortality (%)	Mean duration ± SE (days)	
		Instar 2-3	Instar 2-4
0 (control)	0.00	2.01 ± 0.11	3.55 ± 0.52
0.019	8.89	3.46 ± 0.53	5.05 ± 0.22
0.029	20.22	3.72 ± 0.54	5.39 ± 0.49
0.044	29.21	4.05 ± 0.33	5.60 ± 0.49
0.066	60.00	4.36 ± 0.64	6.39 ± 0.49
0.1	88.89	4.4 ± 0.52	6.4 ± 0.52

SE: standard error

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Table 2. Mortality and developmental period of *C. pavonana* larvae in the treatment with *B. javanica* seed extract

Extract Concentration (%)	Mortality (%)	Mean duration ± SE (days)	
		Instar 2-3	Instar 2-4
0 (control)	0.00	2.03 ± 0.8	4.80 ± 0.48
0.05	2.22	2.97 ± 0.18	3.95 ± 0.21
0.087	13.33	3.58 ± 0.57	5.37 ± 0.56
0.15	34.83	3.62 ± 0.59	5.62 ± 0.62
0.26	78.89	4.05 ± 0.23	6.00 ± 0.00
0.45	98.89	4.00 ± 0.00	7.00 ± 0.00

of extract concentrations. The highest concentration caused 98.89% mortality of *C. pavonana*. Developmental period of the surviving larvae was shortened when compared with controls far both instars 2- 3 as well as instars 2 - 4 (Table 2.). More than 150 quasinoids have been isolated from many species of Simaroubaceae. Guo *et al.* (2005) identified several quasinoid from *B. javanica* such as bruceocide C, D, E, and F, but the activity on insect is unreported yet. Lina *et al.* (2010) investigated the activity of fruit extract of *B.javanica* against *C. pavonana* and *P. xylostella*. The result showed that extract ethyl acetate: methanol-9:1 has strong insecticidal activity on both insects. Dayan *et al.* (1999) indicate that the mode of action of quassinoids is associated with inhibition of the plasma membrane NADH oxidase. The activity of quassinoid caused mortality and developmental period inhibitors.

Piper aduncum fruit extract showed 100% mortality at highest concentration against *C. pavonana* larvae (Table 3.). Dilapiol (fenilpropanoid) is known as the main compound of *P. aduncum* and has insecticidal and synergism activity (Bernard *et al.,* 1995; Fazolin *et al.,* 2005). Dilapiol worked by blocking the activity of cytochrome P450 enzyme in *Ostrinia nubilalis* midgut (Bernard *et al.,* 1990). Dilapiol has metilendioksifenil group in its structure which is characteristic of various synergies component block cytokrom P450 enzyme activity (Perry *et al.,* 1998; Scott *et al.,* 2008).

Table 3. Mortality and developmental period of *C. pavonana* larvae in the treatment with *P. aduncum* fruit extract

Extract Concentration (%)	Mortality (%)	Mean Duration ± SE (days)	
		Instar 2-3	Instar 2-4
0 (control)	0.00	2.03 ± 0.18	4.00 ± 0.26
0.15	3.33	2.21 ± 0.41	4.17 ± 0.38
0.19	5.56	2.35 ± 0.48	4.32 ± 0.47
0.24	48.31	2.57 ± 0.50	5.04 ± 0.42
0.30	93.33	3.00 ± 0.00	5.83 ± 0.41
0.375	100.0	-	-

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Table 4. Mortality and developmental period of *C. pavonana* larvae in the treatment with a mixture of *T. vogelii, B. javanica,* and *P. aduncum* extracts (1:3:2.5)

Extract Concentration (%)	Mortality (%)	Mean Duration ± SE (days)	
		Instar 2-3	Instar 2-4
0 (control)	0.00	2.00 ± 0.00	3.83 ± 0.38
0.015	6.45	3.94 ± 0.44	5.78 ± 0.47
0.022	24.44	4.04 ± 0.70	5.72 ± 0.64
0.033	80.89	4.11 ± 0.58	6.83 ± 0.38
0.05	93.33	5.00 ± 1.00	7.00 ± 1.00
0.075	100.0	-	-

Toxicity tests of the extracts mixture of *T. vogelii*: *B. javanica*: *P. aduncum* (1: 3: 2.5) against *C. pavonana* are presented on Table 4. The concentrations used on mixture were lower than the concentration of each extract in a single test. The pattern of *C. pavonana* mortality was increased with increasing of extract concentration. The lowest concentration (0.015%) and the highest concentration caused mortality of *C. pavonana* 6.45% and 100% respectively. The developmental period of *C. pavonana* was longer than that of controls, which ranged from 1.94 to 3 days for instars 2-3, and 3 to 3.17 days for instars 2-4.

The pattern of *C. pavonana* mortality treated with the mixture extracts shown in Figure 1. The mortality started at first day observation for each concentration and increase at second day observation. The mortality was constant after three days observation because the larvae fed with untreated leaf. The pattern suggests that characters of the extract mixture are more toxic than inhibiting growth and development of *C. pavonana*. Probit analysis performed to determine the concentration-mortality relationships between each single extracts and mixture and *C. pavonana*. The results appear as shown in Table 5. The value of slope of *P. aduncum* extract is the highest among *T. vogelii* and *B. javanica*. This suggests that the addition of concentration of *P. aduncum* extract will increase the larvae mortality higher than other extract at the same concentration.

Figure 1. Time-course mortality of *Crocidolomia pavonana* larvae caused by mixture of *T. vogelii, B. javanica,* and *P. aduncum* extracts (1:3:2.5)

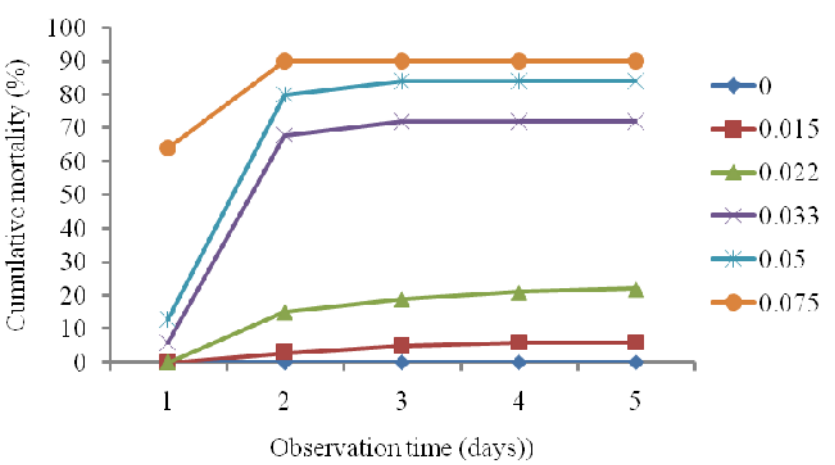

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Table 5. Parameters of probit regression between concentrations and mortality *C. pavonana* larvae treated with *T. vogelii, B. javanica. a*nd *P. aduncum,* and their mixture

Extract source	b± SE[a)]	LC_{50} (CI 95%)	LC_{95} (CI 95%)
B. javanica	4,28± 0,33	0,17 (0,14-0,20)	0,41 (0,31-0,63)
P. aduncum	13,03 ± 1,04	0,24 (0,20-0,28)	0,32 (0,27-0,58)
T. vogelii	3,48± 0,30	0,05(0,04-0,07)	0,16 (0,10-0,42)
Mixture extracts	6,03± 0,33	0.06 (0.05-0.08)	0.12 (0.09-0.24)

[a)] b = slope, SE: standard error, CI: confidence interval.

Analysis of extract mixture

Analysis of the mixture extracts of *B. javanica*, *P. aduncum*, and *T. vogelii* against *C. pavonana* showed that the index combination value was smaller than 0.5 both on level LC_{50} and LC_{95}. These results indicate strong synergism interaction properties, these properties are most likely dominated by *P. aduncum*. Dilapiol compound from *P. aduncum* works as synergistic properties caused activity of cytokrom P450 enzyme block (Bernard *et al.,* 1990, 1995; Fazolin *et al.,* 2005). Inhibitions of cytochrome P450 enzyme will cause derive from *B. javanica* and *T. vogelii* toward the target site and work maximum.

CONCLUSION

Tephrosia vogelii leaf, *Brucea javanica* seed, and *Piper aduncum* fruit extracts, tested separately, had good insecticidal activity against *Crocidolomia pavonana* larvae (LC_{95} < 0.5%). At LC_{50} and LC_{95} level, a mixture of *T. vogelii*, *B. javanica* and *P. aduncum* extracts (1:3:2.5) were strongly synergistic against *C. pavonana* larvae.

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Received: 10.4.2013 Revised: 27.5.2013 Accepted: 12.6.2013

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