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EVALUATION OF INSECTICIDAL ACTIVITY OF MELIACEOUS PLANT EXTRACTS AGAINST *Crocidolomia binotalis* ZELLER (LEPIDOPTERA: PYRALIDAE)

Eka Candra Lina[1)] and Djoko Prijono[1, 2)]

[1)] Department of Plant Pests and Diseases, Faculty of Agriculture, Bogor Agricultural University
[2)] Corresponding author

RINGKASAN

Pengujian Aktivitas Insektisida Ekstrak Tumbuhan Meliaceae terhadap *Crocidoomia binotalis* Zeller (Lepidoptera: Pyralidae)

Aktivitas insektisida ekstrak daun, ranting, kulit batang dan/atau biji tujuh spesies tumbuhan Meliaceae diuji terhadap larva instar-2 Crocidolomia binotalis *dengan metode residu pada daun. Bahan uji yang digunakan adalah fraksi etil asetat dari ekstrak metanol setiap bahan tumbuhan. Perlakuan kontaminasi pakan selama 2 hari dengan ekstrak biji* Aglaia elliptica, A. harmsiana, A. odoratissima *dan* Trichilia trijuga *0,25% mengakibatkan kematian larva uji berturut-turut 98,3, 100, 100 dan 91,5%. Ekstrak ranting* A. harmsiana *agak aktif (kematian larva 72,9%), ekstrak kulit batang* Dysoxylum mollissimum *memiliki aktivitas sedang (kematian 50%), ekstrak daun dan ranting* T. trijuga *tidak aktif (kematian 0%), sementara ekstrak lainnya (kulit batang* A. aspera, *daun* A. harmsiana, *serta daun dan ranting* A. elliptica, A. odorata *dan* A. odoratissima*) kurang aktif (kematian 1,7-28%). Berdasarkan persentase kematian instar-2 dan instar-3, LC_{50} ekstrak biji* A. elliptica, A. harmsiana, A. odoratissima *terhadap larva* C. binotalis *berturut-turut 0,11, 0,03 dan 0,04%. Selain mengakibatkan kematian larva, ekstrak yang aktif juga memperpanjang lama perkembangan larva dari instar-2 ke instar-4. Untuk spesies yang sama, lamanya penundaan perkembangan bersesuaian dengan tingkat pengaruh letal ekstrak bagian tumbuhan yang bersangkutan.*

Kata kunci: *Aglaia, Crocidolomia binotalis, Dysoxylum,* insektisida botani, Meliaceae, *Trichilia.*

ABSTRACT

Evaluation of Insecticidal Activity of Meliaceous Plant Extracts against *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae)

Insecticidal activity of leaf, twig, stem bark and/or seed extracts of seven species of Meliaceae was evaluated against second-instar larvae of Crocidolomia binotalis *using leaf residual method. Ethyl acetate soluble fraction of methanolic extract of each plant material was used in this study. The feeding treatment for 2 days with seed extracts of* Aglaia elliptica, A. harmsiana, A. odoratissima *and* Trichilia trijuga *at 0.25% caused 98.3, 100, 100 and 91.5% larval mortality, respectively.* A. harmsiana *twig extract was somewhat active (72.9% larval mortality),* Dysoxylum mollissimum *bark extract was moderately active (50% mortality),* T. trijuga *leaf and twig extracts were inactive (0% mortality), while the other extracts (*A. aspera *barks,* A. harmsiana *leaves, and leaves and twigs of* A. elliptica, A. odorata *and* A. odoratissima*) were only weakly active (1.7-28% mortality). Based on mortality of the second and third instar, LC_{50} of* A. elliptica, A. harmsiana *and* A. odoratissima *seed extracts against* C. binotalis *larvae were 0.11, 0.03 and 0.04%, respectively. In addition to lethal effect, the active extracts also prolonged the developmental time of the surviving larvae from the second to fourth instar. For the same plant species, the length of developmental delay caused by extracts of different parts corresponded with the degree of their lethal effect.*

Key words: *Aglaia*, botanical insecticides, *Crocidolomia binotalis, Dysoxylum,* Meliaceae, *Trichilia.*

INTRODUCTION

Crocidolomia binotalis Zeller (Lepidoptera: Pyralidae) is a notorious pest of brassicaceous crops (Sastrosiswojo & Setiawati 1993). The problem of this pest in some highland vegetable producing regions is increasingly becoming severer, not only in the dry season but also in the rainy season. Most registered insecticides for this pest are currently no longer effective when applied at recommended doses, and as a consequence, many farmers apply insecticides more frequently at higher dosages, but with little or no avail (D Prijono, personal observation in the field). This problem is compounded by the lack of effective natural enemies and modest soundness of other nonchemical control measures. Thus, effective and safe alternatives to synthetic insecticides need to be searched for and developed. One alternative that meets these criteria and is worth to be studied is natural insecticides from plants (botanical insecticides).

It has been well established worldwide that the plant family Meliaceae represents one of the potential sources of botanical insecticides (Mikolajczak *et al.* 1989; Xie *et al.* 1994; Isman *et al.* 1995; Nugroho *et al.* 1997a, 1997b; Prijono 1998). Among the meliaceous plants that receive much attention in recent years are those in the genera *Aglaia*, *Dysoxylum* and *Trichilia*.

There are some 70 species of *Aglaia* in Indonesia (Pannell 1992), but not more than 35% of them have been evaluated for their insect control property. Extracts of some species such as *A. elliptica*, *A. harmsiana* and *A. odorata* were reported to possess strong insecticidal activity against some lepidopteran pests including *C. binotalis* and *Spodoptera littoralis* (Boisd.) (Ishibashi *et al.* 1993; Nugroho *et al.* 1997b; Gussregen *et al.* 1997; Prijono 1998; Prijono *et al.* 1999). Rocaglamide derivatives have been established as the main insecticidal principles in most *Aglaia* species hitherto studied. Recently, Prijono *et al.* (1999) reported that stem bark extract of *A. angustifolia* showed strong insect growth regulating (IGR) activity against *C. binotalis*, in which the symptom of poisoning was distinctly different from that caused by extracts of other *Aglaia* species. Active compounds in the *A. angustifolia* extract, however, have not been identified

Extracts of some species of *Dysoxylum* and *Trichilia* were also reported active against some insects (Mikolajczak and Reed 1987; Mikolajczak *et al.* 1989; Prijono 1997, 1998; Charnelis *et al.* 1998). Limonoid compounds have been known as the main insecticidal principles in some species of *Trichilia* (Nakatani *et al.* 1981; Xie *et al.* 1994). Russell *et al.* (1994) has isolated a terpenoid compound from *D. spectabile* fruits as an ant repellent. Insecticidal compounds in other species of *Dysoxylum* studied by those authors have not been identified.

Given the number of meliaceous species growing in Indonesia (Pannell 1992; Mabberley *et al.* 1995), there is still a vast opportunity to find further new sources of botanical insecticides among the species of Meliaceae. In view of this potential, this study was conducted to evaluate insecticidal properties of extracts of seven species of Meliaceae in the genera *Aglaia*, *Dysoxylum* and *Trichilia* against *C. binotalis* larvae.

MATERIALS AND METHODS

Test Insect

C. binotalis larvae were taken from the laboratory colony maintained at the Laboratory of Insect Physiology and Toxicology (LIPT), Bogor Agricultural University (BAU). The insect colony has been maintained in the laboratory since September 1992 under ambient conditions (2531.5°C, 65-85% RH, and ca. 12 L:12 D regime). The larvae were fed pesticide-free broccoli leaves and the adults were fed 10% honey solution in cotton swab as described by Basana and Prijono (1994).

Plant Materials for Extraction

Leaves, twigs and seeds of *Aglaia elliptica*, *A. harmsiana*, *A. odoratissima* and *Trichilia trijuga*; leaves and twigs of *A. odorata*; and stem barks of *Dysoxylum mollissimum* were obtained from Bogor Botanic Garden. Stem barks of *A. aspera* were collected from Bukit Raya, West Kalimantan. This species was identified by a botanist at the National Herbarium in Bogor. The seeds were received in cool-dried condition, while leaves, twigs and stem barks were extracted in fresh condition.

Extraction

Plant materials were ground separately with a blender, then a known amount of particular ground materials was extracted with methanol by stirring in an erlenmeyer flask for 24 hours. The extract was filtered and the marc was washed repeatedly with methanol until the filtrate was colorless. The filtrates were pooled, then the solvent was evaporated in a rotary evaporator (rotavapor) at 50°C under reduced pressure. The extract obtained was partitioned between ethyl acetate and water. The water phase was discarded and the ethyl acetate phase was collected and evaporated in a rotavapor as above. The ethyl acetate fraction obtained was weighed and then kept in refrigerator (≤4°C) until used in the bioassay. To estimate the dry weight of plant materials extracted, a sample of particular plant materials of about 2 g was baked in an oven at 105°C for 2 days and then weighed again. The yield of extract (Y) on a dry-weight basis was calculated as follows:

$$Y\ (\%) = \frac{WE}{FP \times DS/FS} \times 100\%$$

where WE = weight of extract obtained, FP = fresh weight of ground plant materials extracted, FS = fresh weight of a sample dried in the oven, DS = dry weight of the sample.

Extract Bioassay

The bioassay was conducted at the LIPT-BAU under room conditions as above. Ethyl acetate soluble fraction of each extract was tested against second-instar larvae of *C. binotalis* using leaf residual method. In the initial screening, each extract was tested at a concentration of 0.25% (w/v). Each extract was dissolved in a mixture of acetone-methanol (3:1) to the desired concentration, then 25 µl of a particular extract solution was applied uniformly on each side of broccoli leaf disks (3 cm in diameter) using a microsyringe. Control leaf disks were treated with solvent only. After the solvent had evaporated, two treated or control leaf disks were placed in a glass petri dish (9 cm in diameter) lined with towel paper, then 15 second-instar larvae were introduced into the dish. Each extract treatment and control were replicated four times. The larvae were allowed to feed on treated or control leaves for 48 hours, then were provided untreated leaves until they reached the fourth-instar stage. The number of dead or moulting larvae was recorded daily from the second to fourth instar. Insect mortality in the treatment was corrected with control mortality using Abbott's formula (Abbott 1925).

Extracts that gave more than 95% mortality were tested further at seven concentration levels to bracket a range of concentrations which were expected to cause 0-100% mortality as determined in preliminary tests. Treatment procedures were the same as above, but in these tests each treatment was replicated six times. Larval mortality was recorded daily until the larvae reached the fourth instar and the developmental time of the surviving larvae was also recorded. Larval mortality data were analyzed by the probit method (Finney 1971) via PROC PROBIT of the SAS Package (SAS Institute 1990).

RESULTS

Yield of Extracts

As the available data indicate, the leaves gave the highest extract yield compared to other plant parts studied. The yield of leaf extracts ranged from about 9.8% to 15.8% (Table 1). Other plant parts (twigs, stem barks and seeds), excluding *T. trijuga* seeds, gave about 1.7% to 3.3% of extract. The high yield of *T. trijuga* seed extract was due to the high oil content in the extract as can be seen upon evaporation of the extract.

Insecticidal Property

Results of the bioassay showed that insecticidal effect of the test extracts on *C. binotalis* larvae varied widely depending on species and plant parts extracted. The seed extracts exhibited the strongest insecticidal effect and the leaf extracts generally had the lowest activity (Table 1). The feeding treatment with seed extracts of *A. harmsiana* and *A. odoratissima* at a concentration of 0.25% yielded 100% larval mortality. At the same concentration, seed extracts of *A. elliptica* and *T. trijuga* were less active than the two aforementioned extracts, twig extract of *A. harmsiana* was somewhat active, stem bark extract of *D. mollissimum* was moderately active, leaf and twig extracts of *T. trijuga* were inactive, whereas the other test extracts, including *A. odorata* leaf and twig extracts, were only weakly active (Table 1).

The treatment with extracts of particular plant parts also caused a delay in larval development to the same degree as their lethal effect, i.e. the higher the lethal effect of an extract the longer the larval development caused by the extract. For example, the mean developmental time of *C. binotalis* larvae from the second to fourth instar in the treatment with *A. elliptica* seed extract was 5.8 days longer compared to control, whereas in that with *A. elliptica* leaf extract was only 0.2 days longer. The same tendency is true for extracts from different parts of the other kinds of test plants (Table 1).

Results of further tests with *A. elliptica*, *A. harmsiana* and *A. odoratissima* seed extracts showed that, in particular test extracts, the slope of probit regression for mortality of instar-2 was not significantly different from that of instar-2 and 3 (b $\pm$ SE overlapped, Table 2). The slope of probit regression of *A. elliptica* seed extract was not significantly different from that of *A. harmsiana* seed extract suggesting that the two extracts had similar action. *A. odoratissima* seed extract had a steeper probit regression slope than did the two aforementioned extracts. This suggests that *A. odoratissima* extract has a higher lethal effect at higher concentrations as reflected by the lower LC_{95} of this extract compared to that of *A. harmsiana* (Table 2).

There was no significant difference between LC_{50} against instar-2 and that against instar-2 and 3 for the seed extracts of *A. elliptica*, *A. harmsiana* and *A. odoratissima* (95% CI overlapped, Table 2). This indicates that there was no marked mortality increase during the third-instar stadium (after the feeding treatment was removed). Based on LC_{50} values, *A. odoratissima* seed extract had about the same level of activity as *A. harmsiana* seed extract, and *A. elliptica* seed extract was about 3.6-4 times less active than *A. harmsiana* seed extract.

Consistent with the above tendency that the higher the lethal effect the longer the larval development, in the treatment with the three most active extracts, larval developmental time was generally longer with the increase in extract concentration (Table 3).

Table 1 Effects of extracts of seven species of Meliaceae on mortality and developmental time of *C. binotalis* larvae

Extract[a]	Extract yield[b] (%)	Mortality[c]	Mean developmental time $\pm$ SD (days) (n)[d]	
			Treatment	Control
A. aspera				
Stem bark	1.66	5.2	4.9 $\pm$ 0.7 (55)	4.1 $\pm$ 0.4 (58)
A. elliptica				
Leaf	15.81	1.7	4.2 $\pm$ 0.4 (58)	4.0 $\pm$ 0.1 (60)
Twig	1.89	8.9	3.8 $\pm$ 0.6 (50)	4.1 $\pm$ 0.4 (58)
Seed	3.29	98.3	9.0 (1)	3.2 $\pm$ 0.4 (59)
A. harmsiana				
Leaf	11.00	28.0	6.3 $\pm$ 0.5 (39)	3.0 $\pm$ 0.1 (60)
Twig	1.99	72.9	7.6 $\pm$ 0.8 (16)	4.1 $\pm$ 0.4 (59)
Seed	2.97	100.0	-[e]	3.9 $\pm$ 0.5 (60)
A. odorata				
Leaf	10.57	16.6	4.2 $\pm$ 0.5 (49)	3.5 $\pm$ 0.5 (60)
Twig	2.31	23.3	6.3 $\pm$ 0.5 (46)	3.2 $\pm$ 0.4 (59)
A. odoratissima				
Leaf	9.76	6.7	4.5 $\pm$ 1.0 (56)	3.5 $\pm$ 0.6 (60)
Twig	2.56	1.9	4.4 $\pm$ 0.6 (55)	4.1 $\pm$ 0.4 (58)
Seed	1.96	100.0	-[e]	4.0 $\pm$ 0.9 (58)
D. mollissimum				
Stem bark	2.63	50.0	6.8 $\pm$ 1.0 (30)	3.5 $\pm$ 0.5 (60)
T. trijuga				
Leaf	10.32	0	4.3 $\pm$ 0.4 (60)	4.0 $\pm$ 0.0 (59)
Twig	1.71	0	4.0 $\pm$ 0.2 (59)	4.0 $\pm$ 0.2 (58)
Seed	9.95	91.5	6.0 $\pm$ 0.7 (55)	4.0 $\pm$ 0.2 (58)

[a] Test extract: ethyl acetate soluble fraction of methanolic extract at 0.25%; [b] On a dry-weight basis; [c] Mortality from the second to fourth instar, corrected with control mortality using Abbott's formula (1925); [d] Development from the second to fourth instar, SD = standard deviation, n = number of surviving larvae; [e] All larvae died before reaching the fourth instar.

Table 2 Results of probit analysis of mortality of *C. binotalis* larvae from the second to fourth instar as affected by the treatment with extracts of three species of *Aglaia*

Extract	Mortality assessed	b ± SE[a]	LC_{50} (95% CI)[b] (%)	LC_{95} (95% CI) (%)
A. elliptica	Instar-2	3.17 ± 0.50	0.12 (0.11 - 0.13)	0.36 (0.29 - 0.48)
	Instar-2 + 3	2.61 ± 0.50	0.11 (0.09 - 0.16)	0.47 (0.26 - 2.82)
A. harmsiana	Instar-2	3.12 ± 0.54	0.03 (0.02 - 0.04)	0.09 (0.06 - 0.26)
	Instar-2 + 3	3.17 ± 0.57	0.03 (0.02 - 0.04)	0.09 (0.06 - 0.26)
A. odoratissima	Instar-2	4.88 ± 0.67	0.04 (0.03 - 0.05)	0.08 (0.07 - 0.13)
	Instar-2 + 3	4.99 ± 0.72	0.04 (0.03 - 0.05)	0.08 (0.07 - 0.13)

[a] b = slope of probit regression, SE = standard error; [b] CI = confidence interval.

Table 3 Effects of extracts of three species of *Aglaia* on developmental time of *C. binotalis* larvae from the second to fourth instar

Extract	Concentration (%, w/v)	Mean developmental time ± SD (days) (n)[a]
A. elliptica	0	3.4 ± 0.5 (90)
	0.025	4.7 ± 0.8 (85)
	0.055	5.9 ± 1.1 (57)
	0.085	6.3 ± 0.8 (52)
	0.115	6.7 ± 0.7 (45)
	0.145	6.8 ± 0.8 (42)
	0.175	7.1 ± 0.7 (17)
	0.205	7.2 ± 0.5 (26)
A. harmsiana	0	3.9 ± 0.4 (87)
	0.010	5.1 ± 0.7 (78)
	0.020	6.1 ± 0.9 (57)
	0.030	6.7 ± 0.7 (40)
	0.040	7.1 ± 0.4 (38)
	0.050	6.9 ± 0.4 (17)
	0.060	6.7 ± 0.6 (3)
	0.075	8.0 ± 0.0 (1)
A. odoratissima	0	4.0 ± 0.3 (89)
	0.020	5.1 ± 0.8 (82)
	0.030	6.5 ± 1.0 (62)
	0.040	6.8 ± 0.9 (43)
	0.050	7.2 ± 0.9 (25)
	0.060	5.0 ± 1.7 (7)
	0.070	7.1 ± 1.0 (15)
	0.080	7.0 ± 0.7 (5)

[a] SD = standard deviation, n = number of surviving larvae.

The development of *C. binotalis* larvae from the second to fourth instar in the treatment with *A. elliptica*, *A. harmsiana* and *A. odoratissima* seed extracts at concentrations of 0.025-0.205%, 0.01-0.075% and 0.02-0.08%, respectively, was delayed by 1.3-3.8, 1.2-4.1 and 1.1-3.2 days, compared to their respective controls (Table 3).

DISCUSSION

Variation in insecticidal activity of different species of Meliaceae and of different parts of the same species has been well recognized (Mikolajczak *et al.* 1989; Satasook *et al.* 1994; Xie *et al.* 1994; Isman *et al.* 1995; Prijono 1998). Insecticidal activity of a particular species may even vary with its geographical origin. For example, Satasook *et al.* (1994) reported that methanolic extract of *A. odorata* leaves from northern Thailand had significantly different activity from that of materials from southern Thailand. Thus, it is not surprising that *A. odorata* extracts used in this study were only weakly active against *C. binotalis* larvae, although this species has been known containing rocaglamide that has comparable activity to azadirachtin (Ishibashi *et al.* 1993; Nugroho *et al.* 1997a), a natural insecticide from the widely known neem tree (*Azadirachta indica*).

Variation in insecticidal activity of different species and plant parts might be due to the difference in the type, composition or content of active compounds, or combination of these factors. Rocaglamide derivatives have been established as the main insecticidal principles in *Aglaia* spp. hitherto studied (Ishibashi *et al.* 1993; Nugroho *et al.* 1997a, 1997b), triterpenoids were reported as the primary insecticidal compounds in some species of *Trichilia* (Nakatani *et al.* 1981; Xie *et al.* 1994), while insecticidal principles in *Dysoxylum* are largely unknown. Available literature indicated that different parts of *Aglaia* spp. had different composition and content of rocaglamide derivatives (Ishibashi *et al.* 1993; Janprasert *et al.* 1993; Dumontet *et al.* 1996; Gussregen *et al.* 1997).

Nugroho *et al.* (1997b) has isolated and identified six insecticidal benzofuran compounds, including rocaglamide and didesmethylrocaglamide which have comparable activity to azadirachtin, from the seeds of *A. elliptica* and a rocaglamide derivative from the leaves of *A. harmsiana*. Active compounds in *A. harmsiana* and *A. odoratissima* seeds, however, have never been reported. Considering the similarity of effects of seed extracts of the latter two species to that of *A. elliptica* seed extract, it seems that the seeds of *A. harmsiana* and *A. odoratissima* also contain rocaglamide derivatives. The content of rocaglamide derivatives in *A. harmsiana* and *A. odoratissima* seeds, however, may be higher than that in *A. elliptica* seeds since seed extracts of the first two species were about 3-4 times more active than seed extract of the latter (Table 2). Further work is needed to prove this supposition.

The potent insecticidal rocaglamide derivatives in *Aglaia* spp. belong to the chemical class of benzofuran which is different in chemical nature from all existing groups of synthetic insecticides. These compounds may serve as alternatives to synthetic insecticides in overcoming resistant problem in *C. binotalis* (Ratna & Prijono 1999) since these compounds may still attack target biochemical sites which have apparently been saturated by all existing synthetic insecticides.

In conclusion, some Meliaceae plants particularly *A. harmsiana* and *A. odoratissima* are potential sources of botanical insecticides which could be used as alternatives to synthetic insecticides in coping with *C. binotalis* pest problem. Isolation and identification of active compounds in those two species are worthwhile to be pursued.

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