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

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RINGKASAN

Aktivitas Insektisida Ekstrak Biji Meliaceae terhadap Larva *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae)

Ekstrak aseton biji 21 jenis tanaman Meliaceae diuji aktivitas insektisidanya terhadap larva instar II Crocidolomia binotalis *Zeller dengan metode residu pada daun brokoli. Larva diberi makan daun perlakuan selama 2 hari, kemudian diberi makan daun tanpa perlakuan hingga mencapai instar IV. Data yang dicatat adalah luas daun yang dimakan selama 2 hari perlakuan, jumlah larva yang mati serta lama perkembangan larva yang bertahan hidup dari instar II ke instar IV. Ekstrak biji* Aglaia harmsiana, Azadirachta indica *(mimba) dan* Dysoxylum mollissimum *pada konsentrasi 0,25% menunjukkan aktivitas insektisida yang kuat terhadap* C. binotalis *(kematian larva 95-100%), ekstrak* Aglaia odoratissima *dan* Trichilia trijuga *menunjukkan aktivitas cukup kuat (kematian larva 78% dan 87%), sedangkan ekstrak lainnya memiliki aktivitas sedang sampai tidak aktif (kematian larva 0 - 73%). Ekstrak yang aktif umumnya memiliki ketiga sifat yang dianalisis, yaitu sifat penghambat makan, insektisida dan penghambat perkembangan, dalam proporsi yang sebanding. Ekstrak yang aktif (pada konsentrasi 0,25%) menghambat aktivitas makan sebesar 78,4 - 96,6% dan memperpanjang lama perkembangan dari instar II ke instar IV selama 2,2 - 4,2 hari. Kajian lanjutan perlu dilakukan untuk mengidentifikasi senyawa insektisida dalam ekstrak yang aktif tersebut, kecuali ekstrak mimba yang telah banyak diteliti.*

Kata kunci: Ekstrak biji, Meliaceae, insektisida botani, *Crocidolomia binotalis*.

ABSTRACT

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

Acetone seed extracts of 21 species of Meliaceae were evaluated for their insecticidal activity against second-instar larvae of Crocidolomia binotalis *Zeller by a leaf-residue feeding method. The larvae were fed extract-treated broccoli leaves for 2 days, then were maintained on untreated leaves until the fourth-instar stage. Records were kept in regard to the area of leaves eaten during the 2-day feeding treatment, daily larval mortality, and developmental time of surviving larvae from instar II to instar IV. The seed extracts of* Aglaia harmsiana, Azadirachta indica *(neem) and* Dysoxylum mollissimum *at a concentration of 0.25% exhibited a strong insecticidal activity against C. binotalis larvae (95-100% mortality), those of* Aglaia odoratissima *and* Trichilia trijuga *showed a fairly strong activity (78% and 87% mortality), whereas the activity of other extracts varied from inactive to moderately active (0 - 73% mortality). The active extracts generally exhibited the three kinds of effects, i.e. antifeedant, insecticidal and developmental derangement, at similar levels. The active extracts at 0.25% inhibited feeding by* C. binotalis *larvae on treated leaves by 78.4 - 96.6% and prolonged the developmental time from instar II to instar IV by 2.2 - 4.2 days as compared with controls. Further studies are needed to identify insecticidal compounds in the said active extracts, except neem which has been exhaustively studied.*

Key words: Seed extracts, Meliaceae, botanical insecticides, *Crocidolomia binotalis*.

INTRODUCTION

In the past three decades there has been a high level of interest in the study of meliaceous plants as possible sources of insect control agents. To date, at least 14 genera of Meliaceae, i.e. *Aglaia, Azadirachta, Cedrela, Chickrassia, Chisocheton, Dysoxylum, Khaya, Lansium, Melia, Sandoricum, Swietenia, Toona, Trichilia* dan *Turraea*, have been reported to contain one or more species possessing

insect control properties (Mikolajczak & Reed 1987; Jacobson 1989; Mikolajczak *et al.* 1989; Satasook *et al.* 1994; Xie *et al.* 1994; Isman *et al.* 1995; Prijono 1997). Among thos plants, neem (*Azadirachta indica*) is the most frequently studied species (Schmutterer 1995), and in some countries such as India and the USA, certain neem products have been developed commercially (Knauss & Walter 1995; Parmar 1995). Development of a commercial product from another species of Meliaceae, i.e. *Aglaia odorata*, has also been attempted in Thailand (Isman 1995).

Insecticidal preparations from Meliaceae generally act as slow-acting toxicants which may also exert feeding and growth inhibition effects (Isman *et al.* 1995). In most species that have been studied, meliacins (or limonoids) are responsible for such activities (Jacobson 1989; Champagne *et al.* 1992; Isman *et al.* 1995), whereas in *Aglaia* spp., some insecticidal benzofurans have been identified as the main active principles (Ishibashi *et al.* 1993; Nugroho *et al.* 1997a, 1997b).

Despite intensive studies on insect control properties of meliaceous plants abroad, information on botanical insecticides from this group of plants in Indonesia is still limited. At most only one-third of the total number of meliaceous species in Indonesia have been studied for their insecticidal bioactivity. Moreover, the level of activity of a given species may vary with geogaphical origin of the source plants, test insects, methods of preparation and methods of application. Therefore, it is necessary to evaluate the insecticidal property of locallyavailable meliaceous plants against appropriate target insects.

This study was conducted to evaluate the insecticidal activity of acetone seed extracts of 21 species of Meliaceae against the cabbage head caterpillar, *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae), a notorious pest of crucifers in Indonesia and some other tropical regions of Asia, Africa, Australia and the Pacific Islands (Brown & Hargreaves 1979; Kalshoven 1981). This pest is difficult to control nonchemically due to the absence of effective natural enemies. Thus, availability of environmentally nondisruptive botanical insecticides should be of great help for brassica growers in controlling the pest.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Insect Physiology and Toxicology, Bogor Agricultural University (LIPT-BAU) under ambient conditions (24,5-32°C, 65-85% RH and ca. 12 L : 12 D regime).

Source Plant Materials

In this study, seeds of 21 species of Meliaceae were used as the source of extracts. The seeds of *Sandoricum koetjape* were taken from the fruits bought at a local market (Bogor Market), neem seeds were collected in Probolinggo, East Java, and other meliaceous seeds were obtained from Bogor Botanic Garden. The seeds were kept in refrigerator ($\leq 4^{\circ}$C) until extracted.

Test Insect

C. binotalis larvae were obtained from a stock colony at the LIPT-BAU. The colony has been reared in the laboratory since September 1992. The larvae were maintained on pesticide-free broccoli leaves and the adults were fed 10% honey solution as previously described (Basana & Prijono 1994).

Seed Extraction

The seeds were extracted with acetone under ambient temperature as previously described (Prijono & Manuwoto 1997). In brief, the ground seeds of particular species were extracted with acetone (1:10, w/v) by stirring for 24 hours. The extract was filtered, the marc was washed with half of the initial volume of the solvent, and the filtrates were combined. Then, the solvent in the filtrates was evaporated, and the extract was kept in refrigerator ($\leq$4°C) until used.

Extract Bioassay

The extracts were tested against second-instar larvae of *C. binotalis* using a leaf-residue feeding method similar to that described by Prijono *et al.* (1997). Initially, each extract was tested at a concentration of 0.25% (w/v). A certain amount of each extract was mixed with an emulsifier alkyl glycerol phthalate (Latron 77 L, 77% a.i.), a mixture of acetone-methanol (1:1) was added, then the mixture was diluted with water to a desired volume. The final concentrations of emulsifier and acetone-methanol were 0.154% and 10%, respectively. Water containing the emulsifier and acetone-methanol at the same concentrations served as a control solution.

Portions of broccoli leaves (ca. 4 cm x 4 cm) were dipped in particular extract emulsions for ca. 10 seconds and air-dried. Control leaves were dipped in the control solution. Treated or control

leaf portions were placed singly in glass petri dishes (9 cm diameter) lined with moist towel paper, and 15 third-instar *C. binotalis* larvae (within 6 hours after moulting) were placed into each dish. A total of 75 larvae (15 larvae/ dish) were used in the treatment with each extract or control. The test larvae were fed treated leaves for 48 hours, and then fed untreated leaves until they reached the fourth-instar stage.

The area of treated leaves eaten during the 48-hour feeding treatment was estimated using a mm^2 grid paper and compared with that of controls. The number of dead larvae was recorded daily during the period from the second to fourth instar. In addition, the developmental time from the second to fourth instar was also recorded.

Larval mortality in the treatment was corrected with control mortality using Abbott's formula (Abbott 1925). Extracts which caused ≥ 50% mortality were tested further at five times dilution.

Based on the larval mortality, the activity of each extract was arbitrarily classified into the following categories: (i) strong activity: mortality (m) ≥ 95%; (ii) fairly strong: 75% ≤ m < 95%; (iii) sufficiently strong: 60% ≤ m < 75%; (iv) moderate: 40% ≤ m < 60%; (v) somewhat weak: 25% ≤ m < 40%; (vi) weak: 5% ≤ m < 25%; (vii) practically inactive: m< 5%.

RESULTS

Aglaia harmsiana seed extract exhibited comparable activity to neem extract which has been widely known for its control properties (Schmutterer 1995). Both extracts at 0.25% exerted 100% larval mortality, all of which occurring in the second instar, and at 0.05% yielded about 50% mortality. In this system, *A. harmsiana* extract was more active as antifeedant as neem extract (Table 1). Moreover, *A. harmsiana* extract caused a longer delay in the development of *C. binotalis* larvae than did the neem extract (Table 2).

Extracts of three other species, i.e. *A. odoratissima*, *Dysoxylum mollissimum* and *Trichilia trijuga*, at 0.25% possessed a fairly strong activity, exerting a total of 78 - 95% larval mortality, 78.4 - 90.9% feeding inhibition and 2.2 - 4.2 days developmental delay (Table 1 and 2). *A. elliptica* extract was sufficiently active in regard to its lethal effect and fairly strong in respect to its antifeedant and developmental derangement effects. Extracts of five other species, i.e. *A. rufa*, *Aphanamixis grandifolia* and three species of *Swietenia*, showed a moderate to fairly strong antifeedant effect, but did only a weak to moderate lethal effect (Table 1). The other test extracts exhibited a weak overall activity or inactive. Some extracts, such as *A. grandis*, *Dysoxylum caulostachyum* and *D. parasiticum*, even seem stimulating feeding.

In the treatment with *A. odoratissima*, *D. mollissimum* and *Swietenia* spp. extracts, there were considerable increases in larval mortality during the third instar stadium or after the treatment has been removed (Table 1) suggesting insect growth regulating activity. In other treatments, larval mortality generally did not increase markedly after the surviving larvae moulted to the third instar.

In the treatment with active extracts, the delays in developmental time from instar II to instar IV were more pronounced than those in the duration of instar II. For example, the treatment with *Aglaia* spp. extracts except *A. grandis* prolonged the duration of instar II by 0 - 3.0 days when compared with controls, whereas the delays in the development from instar II to instar IV ranged from 0.4 - 3.9 days (Table 2). Such difference was more pronounced in the treatment with *D. mollissimum* extract at 0.25%, i.e. 0.4 days vs. 4.2 days. A probable explanation for the longer delay in the total developmental time is that more active substances reach the target site(s) after the larvae moult to the third instar.

DISCUSSION

This study reveals some new promising sources of botanical insecticides, at least in terms of their activity against *C. binotalis*. These include, in decreasing order of activity, the seeds of *A. harmsiana*, *Dysoxylum mollissimum*, *Trichilia trijuga* and *A. odoratissima*. Active insecticidal compounds in these seeds have never been isolated and identified.

A. harmsiana seed extract showed comparable activity to neem extract and more active than *A. elliptica* extract, whereas the activity of *D. mollissimum*, *T. trijuga* and *A. odoratissima* extracts were comparable to that of *A. elliptica* extract. The neem with azadirachtin as its main active principle has been widely known for its antifeedant and insect growth regulating activities. Six insecticidal benzofurans, including rocaglamide and didesmethylrocaglamide which possess comparable insecticidal activity to azadirachtin, have been isolated from *A. elliptica* fruits (Nugroho *et al.* 1997a). These

Table 1. Effects of acetone seed extracts of the Meliaceae on feeding and mortality of *C. binotalis* larvae

Extract	Concentration (%)	Initial no. of test larvae[1)]	Ave. no. of survivors in the first 2 days	Feeding activity[2)] (% relative to control)	Mortality[3)] (%)	
					Instar II	Instar II + III
Aglaia elliptica	0.25	73	54.5	14.0	73.3	73.1
	0.05	75	75.0	45.6	0	0
A. grandis	0.25	75	75.0	103.4	0	0
A. harmsiana	0.25	73	39.5	3.4	100.0	100.0
	0.05	74	68.5	16.1	41.3	49.3
A. odoratissima	0.25	68	60.5	21.6	50.0	78.1
	0.05	75	75.0	69.5	0	0
A. rufa	0.25	75	73.0	17.9	22.7	26.7
Aphanamixis grandifolia	0.25	74	63.0	8.0	41.9	54.1
	0.05	75	75.0	43.4	0	0
Azadirachta indica	0.25	66	58.0	11.5	100.0	100.0
	0.05	75	74.0	32.4	40.5	51.4
Carapa guianensis	0.25	75	75.0	91.7	0	0
Cipadessa baccifera	0.25	75	75.0	96.8	0	0
Dysoxylum alliaceum	0.25	75	75.0	99.7	0	0
D. cauliflorum	0.25	74	74.0	95.4	0	0
D. caulostachyum	0.25	74	74.0	103.2	0	0
D. mollissimum	0.25	74	67.0	9.1	52.0	94.7
	0.05	75	75.0	75.8	0	0
D. parasiticum	0.25	75	75.0	102.0	0	0
Khaya ivorensis	0.25	74	74.0	57.6	1.3	1.3
K. senegalensis	0.25	75	75.0	90.1	0	0
Sandoricum koetjape	0.25	73	72.5	71.8	1.4	5.2
Swietenia candollei	0.25	65	64.5	48.9	6.2	33.9
S. macrophylla	0.25	71	71.0	23.8	5.6	43.9
S. mahagoni	0.25	67	66.0	32.6	10.4	43.7
Trichilia trijuga	0.25	74	53.0	19.7	78.4	86.6
	0.05	75	75.0	94.3	0	0

[1)] One or more larvae were missing if the initial number of larvae was less than 75.
[2)] Based on the area of leaves eaten divided by the average number of surviving larvae during the first two days.
[3)] Corrected with control mortality using Abbott's formula (Abbott 1925).

authors also isolated a rocaglamide glycoside derivative from *A. harmsiana* leaves.

Previously, Prijono (1997) reported that extracts of four species of *Dysoxylum*, i.e. *D. alliaceum, D. cauliflorum, D. caulostachyum* and *D. parasiticum* showed a good contact effect against the mungbean beetle, *Callosobruchus maculatus* (F.), whereas *D. mollissimum* was inactive. Under the present system, the reverse is true, i.e. only *D. mollissimum* extract was active against *C. binotalis* larvae. Extracts of the first four species of *Dysoxylum* were probably inactivated in the gut of *C. binotalis* larvae.

In a study by Mikolajczak & Reed (1987) it was found that ethanolic seed extracts of *D. binectariferum, D. malabaricum, D. reticulatum* and *D. spectabile* at 1% showed a strong insecticidal activity against the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and at 0.5% inhibited feeding by the striped cucumber beetle, *Acalymma vittatum* (F.). In a further study, Mikolajczak *et al.* (1989) reported that the treatment with ethanolic extracts of *D. malabaricum* and *D. spectabile* at 0.2% in artificial diet caused a complete kill in *S. frugiperda*. Russell *et al.* (1994) isolated an ant repellent (2S,3R)-2,3-dimethyl-3-(4-methyl-3-pen-

Table 2. Effects of acetone seed extracts of the Meliaceae on developmental time of *C. binotalis* larvae

Extract	Concentration (%)	Mean developmental time ± SD (days) (N)[1]			
		Instar II to instar III		Instar II to instar IV	
		Treatment	Control	Treatment	Control
Aglaia elliptica	0.25	4.5 ± 1.5 (20)	1.5 ± 0.5 (66)	7.6 ± 1.3 (19)	3.9 ± 1.1 (62)
	0.05	2.3 ± 0.5 (75)	2.0 ± 0.2 (74)	5.2 ± 0.8 (75)	4.1 ± 0.4 (74)
A. grandis	0.25	2.0 ± 0.0 (75)	2.0 ± 0.2 (75)	4.1 ± 0.5 (75)	4.2 ± 0.8 (74)
A. harmsiana	0.25	-[2]	1.1 ± 0.3 (75)	-[2]	3.1 ± 0.4 (75)
	0.05	4.1 ± 1.5 (44)	2.1 ± 0.4 (74)	7.2 ± 1.7 (38)	3.3 ± 0.6 (74)
A. odoratissima	0.25	2.3 ± 1.2 (34)	1.5 ± 0.5 (66)	7.0 ± 1.8 (14)	3.9 ± 1.1 (62)
	0.05	2.0 ± 0.3 (75)	2.0 ± 0.2 (74)	4.5 ± 0.6 (75)	4.1 ± 0.4 (74)
A. rufa	0.25	2.1 ± 0.6 (75)	1.1 ± 0.3 (75)	4.9 ± 0.5 (55)	3.1 ± 0.4 (75)
Aphanamixis grandifolia	0.25	4.8 ± 0.6 (43)	1.1 ± 0.3 (75)	6.9 ± 0.8 (34)	3.1 ± 0.4 (75)
	0.05	1.4 ± 0.7 (75)	1.1 ± 0.3 (75)	4.6 ± 0.9 (75)	3.1 ± 0.4 (75)
Azadirachta indica	0.25	-[2]	1.1 ± 0.3 (75)	-[2]	3.1 ± 0.4 (75)
	0.05	1.2 ± 0.3 (44)	1.1 ± 0.3 (75)	5.1 ± 1.0 (36)	3.1 ± 0.4 (75)
Carapa guianensis	0.25	2.0 ± 0.2 (75)	2.0 ± 0.2 (74)	4.1 ± 0.4 (75)	4.1 ± 0.4 (74)
Cipadessa baccifera	0.25	1.0 ± 0.2 (75)	1.1 ± 0.3 (75)	2.8 ± 0.2 (75)	3.1 ± 0.4 (75)
Dysoxylum alliaceum	0.25	1.0 ± 0.1 (75)	1.1 ± 0.3 (75)	2.8 ± 0.2 (75)	3.1 ± 0.4 (75)
D. cauliflorum	0.25	1.0 ± 0.2 (74)	1.1 ± 0.3 (75)	3.4 ± 1.2 (74)	3.1 ± 0.4 (75)
D. caulostachyum	0.25	1.0 ± 0.2 (74)	1.1 ± 0.3 (75)	3.2 ± 0.9 (74)	3.1 ± 0.4 (75)
D. mollissimum	0.25	1.5 ± 0.6 (36)	1.1 ± 0.3 (75)	7.3 ± 0.5 (4)	3.1 ± 0.4 (75)
	0.05	1.4 ± 0.5 (75)	1.1 ± 0.3 (75)	3.5 ± 0.6 (75)	3.1 ± 0.4 (75)
D. parasiticum	0.25	1.1 ± 0.2 (75)	1.1 ± 0.3 (75)	3.3 ± 1.0 (75)	3.1 ± 0.4 (75)
Khaya ivorensis	0.25	1.1 ± 0.3 (74)	1.1 ± 0.3 (75)	3.0 ± 0.2 (74)	3.1 ± 0.4 (75)
K. senegalensis	0.25	1.0 ± 0.1 (75)	1.1 ± 0.3 (75)	2.7 ± 0.3 (75)	3.1 ± 0.4 (75)
Sandoricum koetjape	0.25	1.7 ± 0.5 (72)	1.5 ± 0.5 (66)	4.3 ± 0.9 (65)	3.9 ± 1.1 (62)
Swietenia candollei	0.25	2.3 ± 0.9 (61)	2.0 ± 0.6 (65)	6.4 ± 1.6 (41)	4.5 ± 1.1 (62)
S. macrophylla	0.25	2.6 ± 1.1 (67)	2.0 ± 0.6 (65)	6.7 ± 1.8 (38)	4.5 ± 1.1 (62)
S. mahagoni	0.25	2.2 ± 1.0 (60)	2.0 ± 0.6 (65)	6.4 ± 1.3 (36)	4.5 ± 1.1 (62)
Trichilia trijuga	0.25	1.7 ± 0.5 (16)	1.6 ± 0.5 (74)	6.4 ± 1.3 (9)	4.2 ± 0.6 (67)
	0.05	2.0 ± 0.2 (75)	2.0 ± 0.2 (74)	4.2 ± 0.8 (75)	4.1 ± 0.4 (74)

[1] SD: standard deviation; N: number of surviving larvae.
[2] All test larvae died in the second instar.

tenyl)-2-nor-bornanol from the fruits of *D. spectabile*. Isman *et al.* (1995) reported that extracts of two other species of *Dysoxylum*, i.e. *D. acutangulum* and *D. guadilaidianum*, were sufficiently active against the variegated cutworm, *Peridroma saucia* Hbn., but the active principles have not been studied.

In the USA and Canada, extracts of seeds and/or other parts of some other species of *Trichilia*, including *T. connaroides*, *T. glabra*, *T. hirta*, *T. prieureana*, *T. roka* and *T. trifolia*, were reported to have marked insect antifeedant and growth inhibiting activities (Mikolajczak & Reed 1987; Xie *et al.* 1994). Limonoids trichilins were identified as the main active compounds responsible for *T. roka* activity (Nakatani *et al.* 1981) and a limonoid hirtin was the main active principle in *T. hirta* twigs (Xie *et al.* 1994).

The test species which have been reported possessing insect control properties but do not seem promising in this study include *Aphanamixis grandifolia, Cipadessa baccifera, Khaya ivorensis, K. senegalensis, Sandoricum koetjape, Swietenia candollei, S. macrophylla* and *S. mahagoni* (Chiu 1985; Mikolajczak & Reed 1987; Champagne *et al.* 1989; Mikolajczak *et al.* 1989; Vanucci *et al.* 1992; Isman

et al. 1995). The discrepancy might be due to the difference in races of the source plants, test insects, methods of application and test concentrations. For instance, in this study the extracts were tested at a concentration of not more than 0.25%, whereas in many other studies it was not infrequently that the test concentrations were higher than 0.5%. Moreover, in many studies the test insects were exposed continuously to the test material in artificial diet for several days. The result from such test cannot always be repeated if the test insect is exposed to treated natural substrates for only 2 days.

In conclusion, among the test species which have not much been studied, *A. harmsiana*, *Dyso-xylum mollissimum*, *Trichilia trijuga* and *A. odora-tissima* stand out as promising sources of botanical insecticides or leads for development of new insecticides. Further studies on those species for isolating and identifying the active principles are worthwhile to be pursued.

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