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

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RINGKASAN

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

Penelitian ini bertujuan menguji aktivitas insektisida ekstrak biji 11 jenis tanaman Annonaceae terhadap ulat *Crocidolomia binotalis* Zeller. Pengujian dilakukan dengan metode residu pada daun brokoli terhadap larva instar III. Ekstrak aseton biji *Annona glabra* and *A. squamosa* menunjukkan aktivitas insektisida yang kuat terhadap larva *C. binotalis* dan lebih aktif daripada ekstrak akar tuba, *Derris elliptica.* Ekstrak air kedua jenis biji tersebut juga aktif terhadap larva uji. Perlakuan dengan ekstrak aseton tiga spesies *Annona* lainnya (*A. montana, A. muricata, A., reticulata*) pada konsentrasi 0,25% mengakibatkan tingkat kematian rendah sampai sedang, sedangkan ekstrak enam spesies lainnya tidak aktif. Perlakuan dengan ekstrak tiga spesies *Annona* tersebut memperlambat pembentukan kepompong selama hanya 0,20- 0,51 hari dan penundaan yang diakibatkan oleh ekstrak akar tuba adalah 1,42 hari. Pengaruh ekstrak *A. glabra* dan *A. squamosa* terhadap perkembangan larva uji tidak dapat ditentukan karena semua larva uji yang diberi perlakuan ekstrak tersebut telah mati pada hari ke-2.

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

ABSTRACT

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

Insecticidal activity of seed extracts of 11 species of Annonaceae was evaluated against *Crocidolomia binotalis* Zeller in a leaf-residue feeding bioassay. Acetone seed extracts of *Annona glabra* and *A. squamosa* showed strong insecticidal activity against *C. binotalis* and both extracts were more active than *Derris elliptica* root extract. Aqueous seed extracts of the two *Annona* species were also active against the test insect. Acetone extracts of three other *Annona* species (*A. montana, A. reticulata* and *A. muricata*) at 0.25% exerted only low to moderate lethal effect, whereas those of the other six species were inactive. The treatment with extracts of the latter three *Annona* species delayed pupation of *C. binotalis* larvae by only 0.20 - 0.51 day, whereas the delay caused by *D. elliptica* root extract was 1.42 days. Effect of *A. glabra* and *A. squamosa* extracts on development of *C. binotalis* larvae could not be determined since all test larvae had died at day 2.

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

INTRODUCTION

In addition to the cosmopolitan diamondback moth (DBM, *Plutella xylostella* [L.]), the cabbage head caterpillar (CHC, *Crocidolomia binotalis* Zeller) is a major pest of brassicaceous crops in some parts of Africa, Asia, Australia and the Pacific Islands (Rai & Chandra 1976; Brown & Hargreaves 1979; Kalshoven 1981). While the biological control of DBM has long been successfully implemented, there are no known effective natural enemies for CHC (Waterhouse & Norris 1987; Sastrosiswojo & Setiawati 1992). Consequently, brassica growers normally rely heavily on the use of insecticides to control CHC, and this in turn may interfere with the biological control of DBM. Therefore, it is necessary to find control agents that are

effective against the brassica pests but relatively safe to their natural enemies.

Powdered seeds of *Annona* spp. have long been used in traditional pest control (Burkill 1935; Watt & Breyer-Brandwijk 1962; Secoy & Smith 1983). Over the last 15 years, there have been heightened attempts worldwide to exploit insecticidal materials from *Annona* spp. and other annonaceous plants on a larger scale (Rupprecht *et al.* 1990; Mitsui *et al.* 1991; Ratnayake *et al.* 1992). In Indonesia, insecticidal activity of extracts of some *Annona* species (such as *A. glabra, A. muricata, A. squamosa* and *A. reticulata*) against CHC has been studied by some workers (Basana & Prijono 1994; Prijono *et al.* 1994). However, the study on the insecticidal activity of other annonaceous extracts against CHC is still very limited.

This paper reports the result of the testing on the insecticidal activity of seed extracts of 11 species of Annonaceae against CHC. The root extract of *Derris elliptica* (Fabaceae), an important source of rotenone, was included in the test for comparison.

MATERIALS AND METHODS

All experiments were conducted at the Laboratory of Insect Physiology and Toxicology (LIPT), Bogor Agricultural University (BAU), under ambient conditions (24.5 - 31°C, 65 - 85% RH, and ca. 12L:12D regime).

Seed Procurement

The seeds of *A. squamosa* were obtained from fruits collected in Solo, Central Java. Other annonaceous seeds and the roots of *D. elliptica* were obtained through Bogor Botanic Garden. All plant materials were air-dried and kept in refrigerator (≤4°C) until extracted.

Insect Rearing

Larvae of *C. binotalis* were obtained from a stock colony of the insect at the LIPT-BAU. The colony has been reared in the laboratory for more than 2 years. The larvae were maintained on pesticide-free broccoli leaves and the adults were fed 10% honey solution as previously described (Prijono & Hassan 1992).

Seed Extraction

In the initial test, acetone was used for extraction. Peeled or unpeeled seeds of Annonaceae and the roots of *D. elliptica* were ground in a blender and sieved through a 1-mm mesh sieve. The ground materials were extracted with acetone (1:10, w/v) by stirring for 24 h at room temperature. The extracts were filtered through No. 41 Whatman filter paper, the solvent was evaporated, and the solvent-free extracts were kept in refrigerator (≤4°C) until used.

The seeds that yielded active acetone extracts were also tested in the form of their aqueous extracts. The seeds were ground to ≤0.5 mm-sized particles and suspended in water containing 0.025% (w/v) detergent ("Rinso", Unilever Jakarta). The extracts were filtered through fine muslin cloth and the filtrate was used directly.

Extract Bioassay

In the initial test, each acetone extract was tested at a concentration of 0.25% (w/v), a concentration considered feasible for field application under high volume spraying (Prijono 1994). A known amount of each extract was dissolved in acetone-methanol (1:1) and diluted with water to a desired volume. The final concentration of acetone-methanol was 10%.

Parts of broccoli leaves (ca. 5 cm x 5 cm) were dipped in particular extract suspensions for ca. 10 seconds and air-dried. Control leaves were dipped in water containing 10% acetone-methanol. Treated or control leaf portions were placed singly in clear plastic cups (11 cm diam. x 6 cm) lined with moist filter paper, and 10 third-instar larvae of *C. binotalis* (within 10 hours after moulting) were put into each cup. A total of 60 larvae (10 larvae/cup) were used in the treatment with each extract or in the control. The number of dead larvae was recorded daily until pupation. The developmental time of the survivors from instar III to pupation was also recorded. Larval mortality in the treatment was corrected with control mortality using Abbott's (1925) formula.

Extracts which caused ≥90% mortality as assessed at pupation were tested further to determine their concentration-mortality relationship against CHC. Each extract was diluted to seven concentration levels covering a range that was expected to produce >0% and <100% larval mortality as determined in preliminary (range-finding) tests. Procedures for extract dilution and bioassay were the same as above. Larval mortality data at day 2, 3 and 6, and at pupation were subjected to probit analysis (Finney 1971).

For bioassay of the aqueous extracts, each extract was tested at six concentration levels. The aqueous extracts were prepared as described above just before

the treatment. Procedures for extract bioassay were the same as for acetone extracts. Larval mortality data at day 2 and 3 were analyzed by the probit method.

RESULTS

Among the annonaceous seed extracts tested, those of *Annona glabra* and *A. squamosa* showed strong insecticidal activity against CHC. Treatment with the two extracts at 0.25% yielded over 85% mortality at day 1 (the day of the treatment is designated as day 0) and the mortality reached 100% one day later. Both extracts were more active than *D. elliptica* root extract which caused only about 85% larval mortality at pupation (Table 1).

Extracts of the other three *Annona* species, i.e. *A. montana*, *A. muricata* and *A. reticulata*, exerted much weaker lethal effect than the above two extracts. Mortality of CHC in the treatment with these extracts increased gradually from day 1 to pupation, with the mortality at pupation ranging from only about 25% to 42%. Extracts of the other six species were inactive (0-1.8% mortality at pupation) (Table 1).

The treatment with four extracts, i.e. *A. montana*, *A. muricata*, *A. reticulata* and *Polyalthia lateriflora*, delayed the development of *C. binotalis* larval survivors from instar III to pupation by 0.51, 0.20, 0.20 and 0.17 days, respectively, and these delays were statistically significant as judged from the difference in mean ± SE values of the larval developmental time between the treatments and their respective controls (Table 1). These delays, however, may be biologically insignificant. The developmental time to pupation of larvae in the treatment with *A. glabra* and *A. squamosa* extracts could not be determined since all test larvae had died at day 2. The other test extracts did not have any effect on the development of *C. binotalis* larvae (Table 1).

In the further tests, acetone extracts of *A. glabra* and *A. squamosa* were tested at seven concentration levels ranging from 0.005 to 0.08% and from 0.006 to 0.1%, respectively, and their aqueous extracts were tested at six concentration levels bracketing a range of 0.625-5 and 1-5 g of ground seeds/l of water, respectively. At these concentrations, the extracts behaved as slow-acting toxicants. Mortality of the test larvae increased markedly at day 2 or day 3, and afterwards there were only slight increases in larval mortality (the data not shown). Therefore, probit analyses were done against larval mortality assessed at day 2 or later.

Results of probit analyses showed that the toxicity of *A. glabra* and *A. squamosa* seed extracts against *C. binotalis* larvae was of the same magnitude, with LC_{50}s of acetone extracts being in the order of 0.01 - 0.02% (Table 2) and those of aqueous extracts being as low as 2.5 - 3 g of seeds/l of water (Table 3). LC_{50}s of acetone extracts of both species assessed at day 6 did not differ significantly from that assessed at day 2 or day 3 (Table 2). This suggests that mortality of the test insect at day 2 or day 3 was sufficiently appropriate for the assessment of acute toxicity of the extracts. Likewise, LC_{50}s of aqueous seed extracts of the two *Annona* species at day 2 and day 3 were not significantly different, either between extracts or between assessment time (Table 3). LC_{50}s of acetone seed extracts of *A. glabra* and *A. squamosa* against pupation were about 65% and 75%, respectively, of those at day 3 (Table 2) suggesting that these extracts possessed some degree of insect growth inhibiting activity.

DISCUSSION

Insect control properties of *A. glabra* and *A. squamosa* seeds have long been known in most tropical countries (Burkill 1935; Watt & Breyer-Brandwijk 1962; Secoy & Smith 1983; Grainge & Ahmed 1988). This fact is supported by the results of this study which showed that both acetone and aqueous seed extracts of the two species had high insecticidal activity against *C. binotalis* larvae.

It is now known that the insecticidal activity of *A. glabra* and *A. squamosa* seed extracts is attributable to some acetogenin compounds, primarily asimicin (squamocin H) and squamocin (annonin I) which are among the most potent acetogenins isolated to date (Londershausen *et al.* 1991; Mitsui *et al.* 1991; Ohsawa *et al.* 1991; Sahai *et al.* 1994; Zafra-Polo *et al.* 1996). Acetogenins in *A. montana*, *A. muricata* and *A. reticulata* are insecticidally much less potent than those two acetogenins (Rupprecht *et al.* 1990; Zafra-Polo *et al.* 1996). This may explain, at least in part, the much lower insecticidal activity of seed extracts of the latter three *Annona* species than were the former two.

Some acetogenins including asimicin and squamocin were reported to inhibit insect mitochondrial respiration at complex I (NADH:ubiquinone oxidoreductase) (Londershausen *et al.* 1991; Lewis *et al.* 1993; Hollingworth & Ahammadsahib 1995). Interference in such system is not expected to cause a rapid knockdown effect if the interfering substances, like those present in *A. glabra* and *A. squamosa* extracts, are

Table 1. Mortality and developmental time of *C. binotalis* larvae fed broccoli leaves treated with 0.25% annonaceous seed extracts

Extract	Yield[1] (%)	% Mortality[2] at					Developmental time (days) Mean ± SE (N)[3]	
		Day 1	Day 2	Day 3	Day 6	Pupation	Treatment	Control
Annona glabra L.	44.5	86.7	100.0	-	-	-	-	6.02 ± 0.02 (55)
A. montana Macf.	42.0	6.7	15.0	18.3	32.2	42.4	6.53 ± 0.13 (34)	6.02 ± 0.02 (59)
A. muricata L.	36.8	0	3.3	6.7	15.3	25.4	6.22 ± 0.12 (44)	6.02 ± 0.02 (59)
A. reticulata L.	31.5	6.7	18.3	23.3	27.1	37.3	6.22 ± 0.09 (37)	6.02 ± 0.02 (59)
A. squamosa L.	33.5	88.3	100.0	-	-	-	-	6.02 ± 0.02 (55)
Desmos dasymaschala (Bl.) Saff.	18.2	0	0	0	1.7	1.7	6.02 ± 0.02 (57)	6.10 ± 0.04 (58)
Goniothalamus macrophyllus (Bl.) Hook. f. & Thoms.	10.5	0	0	0	0	0	6.00 ± 0.00 (58)	6.10 ± 0.04 (58)
Polyalthia lateriflora (Bl.) King	16.4	0	3.4	1.7	1.8	1.8	6.26 ± 0.12 (54)	6.09 ± 0.04 (55)
P. littoralis (Bl.) Boerl.	6.8	0	0	0	0	0	6.00 ± 0.00 (60)	6.00 ± 0.00 (60)
P. suberosa (Roxb.) Thwaites	8.7	0	0	0	0	0	6.02 ± 0.02 (60)	6.02 ± 0.02 (59)
Stelechocarpus cauliflorus (Scheff.) R. E. Fries	2.6	0	0	0	1.7	1.7	6.00 ± 0.00 (59)	6.00 ± 0.00 (60)
Derris elliptica (Wall.) Benth (Fabaceae) - root	12.5	35.0	70.0	75.0	75.0	84.7	7.44 ± 2.24 (9)	6.02 ± 0.02 (59)

[1] On the dry weight basis

[2] Mortality in the treatment with each extract was corrected with control mortality using Abbott's (1925) formula. Control mortality: day 1: 0%, day 2: 0-1.7%, day 3: 0-3.3%, day 6: 0-8.3%, pupation: 0-8.3%.

[3] Developmental time from instar III to pupation, N: number of survivors on pupation.

Table 2. Parameters of toxicity of acetone seed extracts of *A. glabra* and *A. squamosa* against *C. binotalis* larvae

Extract	Assessment time	Slope of probit regression ± SE	LC_{50} (95% FL) (%)
A. glabra	Day 2	2.299 ± 0.412	0.015 (0.009 - 0.022)
	Day 3	2.286 ± 0.388	0.014 (0.009 - 0.021)
	Day 6	2.214 ± 0.308	0.012 (0.008 - 0.016)
	Pupation	2.056 ± 0.225	0.009 (0.007 - 0.010)
A. squamosa	Day 2	2.166 ± 0.196	0.017 (0.015 - 0.020)
	Day 3	2.077 ± 0.199	0.016 (0.013 - 0.018)
	Day 6	2.026 ± 0.201	0.013 (0.011 - 0.015)
	Pupation	1.951 ± 0.200	0.012 (0.010 - 0.014)

Table 3. Parameters of toxicity of aqueous seed extracts of *A. glabra* and *A. squamosa* against *C. binotalis* larvae

Extract	Assessment time	Slope of probit regression ± SE	LC_{50} (95% FL) (g seeds/l water)
A. glabra	Day 2	1.993 ± 0.401	2.625 (1.795 - 5.049)
	Day 3	2.152 ± 0.257	2.525 (2.159 - 3.037)
A. squamosa	Day 2	4.109 ± 0.444	2.901 (2.645 - 3.202)
	Day 3	4.169 ± 0.458	2.755 (2.508 - 3.035)

applied by feeding. This reason explains the slow action of *Annona* extracts tested in this study.

The present data showed that *A. glabra* and *A. squamosa* seed extracts were much more active than *Derris elliptica* root extract. This is consistent with the fact that squamocin was about one order of magnitude more potent than rotenone in inhibiting mammalian respiration at complex I (Zafra-Polo *et al.* 1996). Moreover, the seeds of the two species gave high acetone extract yields, i.e. about 45% and 34% for *A. glabra* and *A. squamosa*, respectively (Table 1). Thus, those seeds can be considered as promising sources of botanical insecticides.

At sublethal levels, the test *Annona* extracts delayed the development of *C. binotalis* larvae from instar III to pupation. As argued earlier by Basana & Prijono (1994), the delay may be due to interference with respiration in organs involved in the regulation of insect development. The precise mechanisms leading to such developmental derangement, however, need to be further studied.

Like other insecticides, the potent *Annona* seed extracts should be handled with caution since the biochemical target of *Annona* active substances is also present in many other organisms. Sookvanichsilp *et al.* (1994) reported that four organic solvent extracts of *A. squamosa* seeds and leaves at 10% in propylene glycol caused a varying degree of irritation to rabbit eyes and skin, depending on the polarity of the solvent. Ethanol (the most polar) extracts exerted the mildest toxicity to rabbit eyes and no toxicity to rabbit ear skin. In an another study, Saxena *et al.* (1992) found that a methanolic extract of *A. squamosa* was toxic to fish, but not to other non-target aquatic organisms. The safety of *Annona* seed extracts against natural enemies of pests still needs to be studied.

This study shows that the potent *A. glabra* and *A. squamosa* seed extracts could be prepared in a simple way, i.e. by grinding the seeds in water containing household powdery detergent (0.25 g/l of water) followed by filtration of the extracts with muslin or some other kind of cheap cloth. This method can easily be adopted by resource-poor farmers in developing countries. Moreover, aqueous extracts are expected to be safer by contact to non-target organisms than organic solvent extracts. Thus, simple aqueous seed extracts of *A. glabra* and *A. squamosa* should be very appropriate to be incorporated into the REISA (reduced external input sustainable agriculture) system of some crops, particularly in developing countries.

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