Insecticidal Activity of Crude Seed Extracts of *Annona* spp., *Lansium domesticum* and *Sandoricum koetjape* Against Lepidopteran Larvae

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Crude ethanolic seed extracts of *Annona muricata*, *A. squamosa* (Annonaceae), *Lansium domesticum* and *Sandoricum koetjape* (Meliaceae) collected from different locations and years in Maluku, Indonesia, were screened for inhibition of larval growth against the polyphagous lepidopteran *Spodoptera litura* (Noctuidae). Extracts of *A. squamosa* were significantly more active (20-fold) than those of *A. muricata. A. squamosa* collected from Namlea yielded the extracts with the greatest inhibitory activity. There were significant differences among locations for both *A. squamosa* and *A. muricata* but not for *L. domesticum* and *S. koetjape*. Extracts of *A. squamosa,* collected from Namlea, inhibited larval growth in a dose-dependent manner, with a dietary EC_{50} (effective concentration to inhibit growth by 50% relative to controls) of 191.7 ppm fresh weight. Extracts of *A. squamosa* collected from individual trees in Namlea also varied in growth inhibitory effect against *S. litura* and *Trichoplusia ni* larvae. This species is a candidate for development of a botanical insecticide for local use in Indonesia.

KEY WORDS: *Annona squamosa*; *A. muricata*; Annonaceae; *Lansium domesticum*; *Sandoricum koetjape*; Meliaceae; *Spodoptera litura*; *Trichoplusia ni*; botanical insecticide.

INTRODUCTION

Botanical insecticides offer a more natural, 'environmentally friendly' approach to pest control than do synthetic insecticides. Screening of plant extracts for deleterious effects on insects is one of the approaches used in the search for novel botanical insecticides (4,10,31). The most promising botanicals for use at the present time and in the future are species in the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae (12).

The Meliaceae (mahogany) is a tropical family of woody plants comprising approximately 51 genera and 550 species (7). Seed (20,21) as well as foliar (5) extracts of several meliaceous species have been reported to have toxic and potent growth-reducing activity to insects. Many species of this family have been screened due to the outstanding bioactivity of azadirachtin, a limonoid from the neem tree (*Azadirachta indica*), which is both a potent antifeedant and an insect growth regulator (29,30). Limonoids (triterpene derivatives), natural products of Meliaceae, Rutaceae and other Rutales, have a wide range of biological activities including insect antifeedant and growth regulator, antifungal, bactericidal, antiviral and medicinal effects on animals and humans (6).

The Annonaceae (custard-apple family) is a large family of almost exclusively tropical trees and shrubs comprising about 130 genera and 2300 species (7). Plant parts of

Received May 12, 2003; accepted Aug. 15, 2003; http://www.phytoparasitica.org posting Dec. 1, 2003.
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some species of this family have been used traditionally as insecticides. For example, the powdered seeds and leaf juices of *Annona* spp. are used to kill head and body lice, and bark of *Goniothalamus macrophyllus* is used to repel mosquitoes (22,31). Annonaceous acetogenins extracted from tree leaves, bark and seeds have pesticidal and/or insect antifeedant properties (3,19,25,27). This group of $C_{32/34}$ fatty-acid-derived natural products, are among the most potent inhibitors of complex I (NADH: ubiquinone oxidoreductase) in the mitochondrial electron transport system (1,17,18,32). To date, nearly 400 of these compounds have been isolated from the genera *Annona, Asimina, Goniothalamus, Rollinia* and *Uvaria* (2,14). Their biological activities include cytotoxicity, and *in vivo* antitumor, antimalarial, parasiticidal and pesticidal effects (2,8,27).

The soursop (*Annona muricata*), sweetsop (*A. squamosa*) (Annonaceae), langsat (*Lansium domesticum*) and *Sandoricum koetjape* (Meliaceae) are abundant as fruit trees in Ambon (Maluku), Indonesia. These trees are sources of fresh fruit and/or fruit juices and could generate tons of waste seeds. These waste products might potentially be developed into simple, locally available botanical insecticides.

The insecticidal bioactivity of crude seed extracts of these four species against the Asian armyworm, *Spodoptera litura* and the cabbage looper, *Trichoplusia ni* was investigated in this study. Differences in bioactivity were compared from different locations in Ambon (Maluku), Indonesia and surrounding areas. *S. litura* is one of the major lepidopteran pests of tobacco in Sumatra, but it is an important pest also on other crops such as groundnuts, potatoes, onions and cabbage (15). *T. ni* is a polyphagous lepidopteran pest native to North America. This species is not present in Indonesia but other closely related species of looper such as *Plusia signata* are important pests on some vegetable crops (15). Screening for biological activity of the extracts was the purpose of this study. Therefore, it was important to test them on as many species as possible. The ease of rearing and availability of the insects determined the rationale for their use in this study.

MATERIALS AND METHODS

Plant extracts Thirty-eight plant samples (seeds) of *A. muricata*, *A. squamosa*, *L. domesticum* and *S. koetjape* were collected from different locations in different years (1996–99) in Maluku, Indonesia. Seeds were pooled from different trees from the same location. Voucher specimens are held in the Herbarium Bogoriense, Bogor, Indonesia. The seeds were air-dried, ground with a coffee grinder, and 100 g of each sample was extracted with 95% ethanol (5 $\times$ 200 ml) over 5 days by soaking. The extracts were vacuum-filtered (Whatman No. 1) and reduced *in vacuo* using a rotary evaporator. The dried extracts were resuspended in a small volume of 95% ethanol and transferred to pre-weighed vials. After evaporation of the ethanol in the fume hood, the vials were re-weighed to determine extract weight.

Insects Asian armyworms (*S. litura*) and cabbage loopers (*T. ni*) used in this study were obtained from laboratory cultures reared on artificial diet (F9796, Bioserv, Inc., Frenchtown, NJ, USA) and maintained at 22$\pm$1°C and a photoperiod of 16L:8D. A laboratory-reared colony of *S. litura* has been maintained at the University of British Columbia for 7 years. The original colony was started from insects provided by Hokkaido University, Japan, and has been supplemented with insects from Seoul National University, South Korea. A laboratory colony of *T. ni* has been maintained for over 12 years. The original colony was started with pupae provided by Safer Ltd. (Victoria, B.C., Canada).

Screening bioassays Extracts were screened for growth inhibitory effects on neonate *S. litura via* a chronic growth bioassay. Ethanolic seed extracts were incorporated into the artificial diet at concentrations of 0.025% f.wt (250 ppm) and 0.5% f.wt (5000 ppm) for *A. squamosa* and the others species, respectively, by the method of Isman and Rodriguez (11). Concentrations were determined from preliminary experiments. Control diets were treated with carrier solvent (ethanol) alone. Two newly hatched neonates were placed in an individual cell in a plastic assay tray with approximately 1 g of treated or control diet (n=20). Larvae were maintained in a growth chamber at 26°C and a photoperiod of 16L:8D. After 3 days, one of the two larvae was removed, leaving one larva per cell (20 larvae in total). This was to ensure that there was one, healthy larva per compartment. Larval weights were determined individually after 10 days and compared to larvae fed on control diet, the mean weight for each extract expressed as a percentage of controls. Seed extracts from five different trees of *A. squamosa* collected from Namlea in 1999 were tested for growth inhibitory effect against *S. litura* (0.025% f.wt or 250 ppm) as well as against *T. ni* (0.01% f.wt or 100 ppm), and this experiment was done twice.

Dose response bioassays Seed ethanolic extracts of *A. squamosa* collected from Namlea (1996), which showed the most inhibitory effect (Table 1), were used for dose response experiments. The chronic growth bioassay was carried out using a series of five different concentrations of extracts on each instar of *S. litura* to investigate whether different instars differ in their susceptibility to the extracts. Ethanolic seed extracts were incorporated into artificial diet at the following concentrations: 10, 25, 50, 100, 150 ppm for 1st and 2nd instars; 50, 100, 250, 500, 750 ppm for 3rd instars; and 250, 500, 750, 1000, 2000 ppm for 4th and 5th instars. Control diets were treated with carrier solvent (ethanol) alone. Bioassays were performed using neonates as described above. Bioassays with other instars (2nd, 3rd, 4th and 5th) were carried out as follows. Freshly molted insects were collected and the bioassays conducted as before, with one insect per cell (n=20). Each experiment proceeded until the control larvae reached late 5th/early 6th instar (this is the stage reached after 10 days, starting with neonates). For 2nd instars, this amounted to 7 days, 3rd instar - 6 days, 4th instars - 4 days and 5th instars - 3 days. Insects were weighed after this time and larval weights were compared to larvae fed on control diets. The EC_{50} (effective concentration to inhibit growth by 50% relative to controls) was calculated by extrapolating from the linear regression equation.

Data analysis Growth inhibitory effect data were subjected to Analysis of Variance (ANOVA) on the basis of the actual numbers observed if the variances of the sample means were determined to be homogeneous. Differences among treatment means were analyzed using the Least Significant Difference (LSD) test (SAS 1999), and dose response data were analyzed using linear regression in Microsoft Excel 1997.

RESULTS

Screening bioassays Extracts of both *A. squamosa* (sweetsop) and *A. muricata* (soursop) exhibited bioactivity against *S. litura*. Extracts of *A. squamosa* resulted in larval growth of 8–67% compared to controls (Table 1), whereas *A. muricata* showed larval growth of 18–96% (Table 2). Extracts of *A. squamosa* were screened at a dietary concentration of 250 ppm against *S. litura*, *viz.,* 20 times less than the concentration of *A. muricata* used. There were significant differences in growth inhibition among the extracts of both species

collected from different locations and years (Tables 1 and 2). *A. squamosa* collected from Namlea yielded extracts with the greatest inhibitory activity, but there was variation among three different years of collection (Table 1).

TABLE 1. Growth inhibitory effect of crude ethanolic seed extracts[z] of *Annona squamosa* (sweetsop) from different locations and years of collection on neonate *Spodoptera litura* (tested at 250 ppm = 0.025% f.wt; n=20)

Location (village, island, year)	Larval growth (% relative to control), mean±S.E.
Namlea,[y] Buru, 1996	8.3±2.8a[x]
Namlea, Buru, 1999	15.9 ± 2.4ab
Kate-Kate, Ambon, 1997	23.7 ± 4.9abc
Latuhalat, Ambon, 1996	32.4 ± 4.2bcd
Kudamati, Ambon, 1997	33.7 ± 6.9cd
Namlea, Buru, 1998	42.4 ± 7.5 de
Batugantung,[w] Ambon, 1997	42.8 ± 6.1 de
Batugantung, Ambon, 1998	45.6 ± 4.3 def
Batugantung, Ambon, 1999	47.2 ± 9.0 def
Tantui, Ambon, 1997	55.2 ± 6.1 efg
Batugantung, Ambon, 1996	59.8 ± 6.3 fg
Negeri Lama, Ambon, 1996	66.9 ± 7.2 g

[z] The extracts were from seeds of mixed tree origin.
[y] Seeds were collected from a different batch of trees each year at Namlea.
[x] Means followed by a common letter do not differ significantly at $P<0.05$ by the Least Significant Difference (LSD) test.
[w] Seeds were collected from the same trees each year at Batugantung.

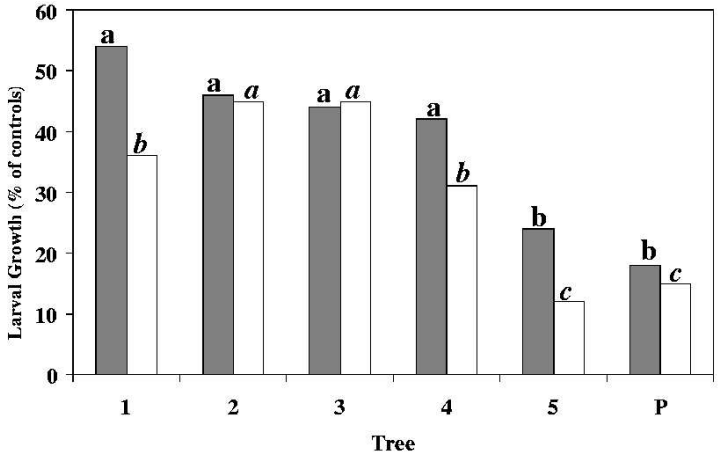

Fig. 1. Tree-to-tree variation in bioactivity of crude ethanolic seed extracts of *Annona squamosa* (Namlea, 1999) on larval growth of *Spodoptera litura* (250 ppm; solid columns) and *Trichoplusia ni* (100 ppm; open columns) (n=40). P indicates pooled extracts from trees 1–5. Within each species, means followed by the same letter do not differ significantly at $P<0.05$ by the Least Significant Difference (LSD) test.

There were also significant differences in larval growth for both *S. litura* and *T. ni* among extracts of *A. squamosa* collected from different trees at one location at the same time (Fig. 1). A pooled extract showed significantly more inhibitory effect (larval growth of 20%) than most single-tree extracts (larval growth of 45–55%) (Fig. 1). Extracts were tested against *S. litura* at 250 ppm, which was 2.5 times higher than the concentration tested against *T. ni*.

Extracts of *L. domesticum* and *S. koetjape* were relatively ineffective, resulting in 78–118% and 49–97% larval growth (Table 3), respectively. There were no significant differences between locations of collection for each species (Table 3).

Dose response bioassays Larval growth was significantly reduced in a dose-dependent manner, when different larval instars of *S. litura* were fed on artificial diet containing seed extracts of *A. squamosa*. We found that the first two instars were equally sensitive to the extract (EC_{50}s of 192 and 202 ppm, respectively). The 3rd and 4th instars were much less sensitive (EC_{50}s of 533 and 705 ppm, respectively) and the 5th instar was relatively insensitive (EC_{50} of 1708 ppm) (Table 4).

TABLE 2. Growth inhibitory effect of crude ethanolic seed extracts[z] of *Annona muricata* (soursop) from different locations and years of collection on neonate *Spodoptera litura* (tested at 5000 ppm = 0.5% f.wt; n=20)

Location (village, island, year)	Larval growth (% relative to control), mean±S.E.
Kusu-Kusu, Ambon, 1999	17.8 ± 2.8 a[y]
Mamala, Ambon, 1996	18.3 ± 4.2 a
Diponegoro, Ambon, 1999	20.4 ± 4.0 a
Amahusu, Ambon, 1999	20.7 ± 3.4 a
Hative Besar, Ambon, 1999	25.0 ± 4.2 ab
Amahusu, Ambon, 1996	31.1 ± 9.4 abc
Hative Besar, Ambon, 1996	34.2 ± 6.4 abc
Piru, Ceram, 1996	36.7 ± 5.9 abcd
Namlea, Buru, 1997	41.9 ± 9.1 bcd
Wasu, Haruku, 1997	46.7 ± 5.6 cd
Waii, Ambon, 1996	50.0 ± 6.7 cd
Latuhalat, Ambon, 1996	54.7 ±. 6.5 de
Kamarian, Ceram, 1997	69.2 ± 7.0 ef
Tuhaha, Ambon, 1996	69.4 ± 8.2 ef
Wakal, Ambon, 1996	75.8 ± 7.5 f
Kilang, Ambon, 1997	79.7 ± 6.7 fg
Batugantung,[x] Ambon,1997	83.5 ± 9.9 fg
Kayu Putih, Ambon, 1996	95.9 ± 8.5 g
Wainitu, Ambon, 1996	96.0 ± 9.1 g

[z] The extracts were from seeds of mixed tree origin.
[y] Means followed by a common letter do not differ significantly at $P<0.05$ by the Least Significant Difference (LSD) test.
[x] Seeds were collected from the same trees each year at Batugantung.

DISCUSSION

Screening of ethanolic seed extracts of two species of *Annona* from Indonesia showed that both possess bioactivity against *S. litura*. However, *A. squamosa* (sweetsop) was 20-fold more active than *A. muricata* (soursop) (Tables 1 and 2). Our results are comparable to

TABLE 3. Growth inhibitory effect of crude ethanolic seed extracts of *Lansium domesticum* and *Sandoricum koetjape* collected in 1997 from four different locations in Ambon on neonate *Spodoptera litura* (tested at 5000 ppm = 0.5% f.wt; n = 20)

Location	Larval growth (% relative to control), mean ± S.E.[z]
Lansium domesticum	
Kusu-kusu	77.6 ± 16.8
Kilang	112.2 ± 37.7
Amahusu	116.1 ± 32.4
Soya	117.7 ± 34.9
Sandoricum koetjape	
Tantui	48.8±7.0
Galala	73.4±14.2
Soya	97.4±33.2

[z] Within species, no significant difference at $P<0.05$ (LSD test) between means.

TABLE 4. Effect of crude ethanolic seed extracts of *Annona squamosa* incorporated into artificial diet on different larval instars of *Spodoptera litura*[z]

Larval instar	EC_{50} (ppm)[y]	*r* value of regression
1st	191.7	0.87
2nd	202.0	0.94
3rd	533.1	0.98
4th	704.9	0.70
5th	1707.5	0.94

[z] Regression lines were calculated from five points, n=20 for each point.
[y] EC_{50}= effective concentration to reduce larval growth by 50% relative to the control after 10 days of feeding.

those of Prijono *et al*. (24), which showed that acetonic seed extracts of *A. squamosa* were about 30-fold more active than those of *A. muricata* against the cabbage head caterpillar, *Crocidolomia binotalis*. The insecticidal activity of the seed extracts of *A. squamosa* is attributable to annonins (*i.e.*, annonin I = squamocin), adjacent bis-tetrahydrofuran (THF) ring acetogenins (28), whereas that of *A. muricata* is attributable to mono-THF ring acetogenins typified by annonacin (26). Structure-activity relationship (SAR) studies have shown that acetogenins having two THF rings are more potent than those having only one and the adjacent bis-THF acetogenins are the most potent ones (1,16,23). This SAR may explain the much lower activity of soursop compared to sweetsop seed extracts observed in this study. We did not analyze the seeds for their acetogenin contents due to difficulty in quantitation of these compounds by normal means, because they lack chromophores.

The sweetsop extracts collected from Namlea produced the most inhibitory effect but there was variation (five-fold) among three different years of collection (Table 1). Extracts collected from different trees at one location and at the same time also showed intraspecific variation with respect to inhibitory effect against *S. litura* and *T. ni*. Overall, *T. ni* was 2.5 times more susceptible than *S. litura* (Fig. 1). Isman (9) reported that different insects showed wide differences in their susceptibilities to the natural insecticide azadirachtin. Extracts from pooled trees were significantly more active than those from most single trees (Fig. 1). There was geographic variation among the extracts of both species (Tables 1 and 2). Similar variability was reported by Johnson *et al.* (13), who found between-tree variations in twig extracts of the paw-paw tree (*Asimina triloba*, Annonaceae) as well as monthly variations within a single tree. As natural products, these extracts are subjected

to environmental (*i.e.,* type of soil, soil nutrients, temperature, humidity) as well as genetic factors, which could be responsible for this variability. However, in the present study we did not collect specific environmental data from which any inferences regarding their effects on patterns of toxicity could be drawn.

Ethanolic seed extracts of *L. domesticum* and *S. koetjape* yielded minimal bioactivity at 5000 ppm against *S. litura* (Table 3). These results contrast with previous screening results (21) that showed that ethanolic seed extracts of *L. domesticum* and *S. koetjape* at 2000 ppm resulted in 99% larval growth inhibition in *Spodoptera frugiperda*. Variability among individuals of the same tree species and differences in sensitivity of test species used could account for these differences. Unlike sweetsop and soursop, there is limited local variation in bioactivity of seed extracts of both *L. domesticum* and *S. koetjape* (Table 3), but this may be due to the small number of different locations from which collections were made.

Extracts of *A. squamosa* collected from Namlea in 1996 and tested on different larval instars of *S. litura* showed negative growth correlated with dietary concentration. Dose-response experiments showed that the first two larval instars of *S. litura* were equally sensitive to the extracts. The 3rd and 4th instars were much less sensitive and the 5th was relatively insensitive to the extracts (Table 4). The age of the insects should therefore be considered when testing insecticidal activity of any compound or extract.

The present study showed that crude seed extract of *A. squamosa* is a promising candidate as a botanical insecticide. Simple methods for preparation of the extracts and their toxicity to the diamondback moth *Plutella xylostella* L. and natural enemies have been investigated, and the efficacy of aqueous extracts in the greenhouse against diamondback moth larvae have shown promising results (Leatemia and Isman, unpub. data).

ACKNOWLEDGMENTS

We are grateful to Nancy Brad for technical assistance and insect rearing, several persons for collecting seeds from different locations, and Dr. Joanne Wilson for critical reading of the manuscript. This study was supported by an Eastern Indonesia University Development Project (EIUDP-CIDA) scholarship to J.A.L. and Natural Science and Engineering Research Council (NSERC) of Canada grants to M.B.I.

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