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LARVICIDAL ACTIVITY OF LEGUMINOUS SEEDS AND GRAINS AGAINST *AEDES AEGYPTI* AND *CULEX PIPIENS PALLENS*

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ABSTRACT. Larvicidal activity of methanol extracts of 26 leguminous seeds and 20 grains against early 4th-stage larvae of *Aedes aegypti* and *Culex pipiens pallens* was examined. At 200 ppm of the extracts from *Cassia obtusifolia, Cassia tora,* and *Vicia tetrasperma,* more than 90% mortality was obtained in larvae of *Ae. aegypti* and *Cx. pipiens pallens.* Extract of *C. tora* gave 86.7 and 100% mortality in the larvae of *Ae. aegypti* and *Cx. pipiens pallens* at 40 ppm but 59.2 and 78.3% mortality against larvae of *Ae. aegypti* and *Cx. pipiens pallens* at 20 ppm, respectively. At 40 ppm, extract of *C. obtusifolia* caused 51.4 and 68.5% mortality of the 4th-stage larvae of *Ae. aegypti* and *Cx. pipiens pallens,* respectively. Larvicidal activity of extract of *C. obtusifolia* was significantly reduced when used at 20 ppm. Further studies of these plants as possible agents for mosquito control are warranted.

KEY WORDS Mosquito larvicide, leguminous seed, grain, *Aedes aegypti, Culex pipiens*

INTRODUCTION

Plants are a rich source of bioactive chemicals, and they may be an alternative source of mosquito control agents. Much effort has been focused on plant extracts or phytochemicals as potential sources of commercial mosquito control agents or as lead compounds (Arnason et al. 1989, Sukumar et al. 1991, Wink 1993). Little work has been done on the mosquito larvicidal activity of leguminous seeds and grains despite their excellent nutritional, pharmacological and industrial uses (Smith and Huyser 1987, Kim et al. 2001a). In the laboratory study described herein, we assessed the larvicidal activities of methanol extracts from 26 leguminous seeds and 20 grains against early 4th-stage larvae of *Aedes aegypti* (L.) and *Culex pipiens pallens* (Coquillett).

MATERIALS AND METHODS

Insects: The F21 laboratory strain of *Ae. aegypti* was obtained in 2000 from the National Institute of Health, Seoul, South Korea. Larvae of *Cx. pipiens pallens* were collected at Seoho Stream, Suwon (Kyunggi Province), South Korea. Adult mosquitoes were maintained on a 10% aqueous sucrose solution and blood from a live mouse; larvae were reared in plastic trays and fed a sterilized diet of chicken chow and yeast (80:20 mix). Mosquitoes were held at 28 ± 2°C, 70 ± 5% relative humidity, and a daily photoperiod of 16:8 h light:dark.

Sample materials and preparation: Leguminous seeds and grains were purchased from a local market in Seoul (Table 1). The samples were washed 3 times with 500 ml of distilled water and dried in an oven at 40°C for 2 days, then finely powdered. Each sample (100 g) was extracted twice with 400 ml of methanol at room temperature for 2 days and filtered. The combined filtrate was concentrated to dryness by rotary evaporation at 40°C. The yield of each methanol extraction is given in Table 1.

Bioassay: Concentrations of the test sample extracts were prepared by serial dilution of a stock solution of the extracts in ethanol. Each extract in ethanol was suspended in distilled water with Triton X-100 added at the rate of 10 mg/liter. Groups of 25 early 4th-stage larvae each of *Ae. aegypti* and *Cx. pipiens pallens* then were put into the paper cups (270 ml) containing each test solution (250 ml). The toxicity of each sample extract was determined with 4–7 concentrations ranging from 10 to 300 ppm. Controls received only ethanol–Triton X-100 solution. Treated and control larvae were held at the same conditions mentioned earlier. Larvicidal activity was evaluated 24 h after treatment. All treatments were replicated 4 times. No mortality was obtained in each control.

Statistical analysis: The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance. Treatment means were compared and separated by Scheffe's test at $\alpha = 0.05$ (SAS Institute 1990).

RESULTS

Significant differences were observed in the toxicity of the methanol extracts from 8 leguminous seeds and 1 grain against larvae of *Ae. aegypti* (Table 2), but the others (37 samples) caused no or little mortality at 300 ppm (not shown). At a concentration of 200 ppm, more than 90% mortality was observed in the methanol extracts from *Cassia obtusifolia, C. tora,* and *Vicia tetrasperma.* The methanol extracts from *C. obtusifolia* and *C. tora* gave 100% mortality at 100 ppm and more than 85% mortality at 50 ppm.

Comparable results were obtained with larvae of *Cx. pipiens pallens* (Table 3). At a dose of 300 ppm, more than 90% mortality was achieved with

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Table 1. Leguminous seeds and grains tested.

Species	Family	Yield (%)[1]
Amphicarpaea edgeworthii	Leguminosae	10.7
Arachis hypogaea[2]	Leguminosae	5.3
Avena sativa[2]	Graminales	11.5
Canavalia lineata[2]	Leguminosae	12.0
Cassia obtusifolia	Leguminosae	14.2
Cassia tora	Leguminosae	13.3
Coix lachryma-jobi var. *mayuen*[2]	Graminales	10.8
Dunbaria villosa[2]	Leguminosae	5.6
Elymus sibiricus[2]	Graminales	15.1
Fagopyrum esculentum[2]	Polygonaceae	9.7
Glycine max var. *bangkong*[2]	Leguminosae	5.4
Glycine max var. *chungtae*[2]	Leguminosae	11.1
Glycine max var. *geumdu*[2]	Leguminosae	4.8
Glycine max var. *hooktae*[2]	Leguminosae	6.6
Glycine max var. *mejukong*[2]	Leguminosae	7.1
Glycine max var. *solitae*[2]	Leguminosae	10.0
Glycine max var. *wooltalikong*	Leguminosae	1.9
Glycine max var. *yagkong*[2]	Leguminosae	5.5
Glycine soja[2]	Leguminosae	10.7
Hordeum vulgare var. *hexastichon*[2]	Graminales	16.8
Hordeum vulgare var. *nudum*[2]	Graminales	10.8
Ischaemum crassipes[2]	Graminales	12.3
Lathyrus japonica	Leguminosae	12.0
Oryza sativa var. *glutinosa*[2]	Graminales	8.9
Oryza sativa var. *japonica*[2]	Graminales	9.9
Panicum bisulcatum[2]	Graminales	14.6
Panicum miliaceum	Graminales	13.2
Perilla frutescens var. *japonica*[2]	Labiatae	11.4
Phaseolus multiflorus	Leguminosae	5.3
Phaseolus nipponensis[2]	Leguminosae	5.7
Phaseolus radiatus var. *aurea*[2]	Leguminosae	5.2
Phaseolus radiatus var. *geodu*[2]	Leguminosae	7.8
Pisum sativum[2]	Leguminosae	3.6
Rhynchosia volubilis	Leguminosae	5.3
Schizandra chinensis[2]	Magnoliaceae	10.6
Schizandra nigra	Magnoliaceae	15.8
Secale careale[2]	Graminales	10.6
Sesamum indicum[2]	Pedalidaceae	11.3
Setaria italica[2]	Graminales	7.9
Sorghum bicolor[2]	Graminales	9.4
Triticum aestivum	Graminales	10.2
Vicia angulasis[2]	Leguminosae	4.8
Vicia hirsute[2]	Leguminosae	11.8
Vicia sinensis[2]	Leguminosae	6.2
Vicia tetrasperma	Leguminosae	12.3
Zea mays[2]	Graminales	10.2

[1] (Weight of crude methanol extract/100 g of dried weight of test material) × 100.
[2] Samples caused no or little mortality at 300 ppm, and the mortality is not shown in Tables 2 and 3.

the methanol extracts from *C. obtusifolia, C. tora, Rhynchosia volubilis, Schizandra nigra,* and *V. tetrasperma.* At 200 ppm, the methanol extracts from *C. obtusifolia, C. tora, R. volubilis, S. nigra,* and *V. tetrasperma* caused 100, 100, 49.8, 48.7, and 100% mortality, respectively. At a concentration of 50 ppm, the methanol extracts from *C. obtusifolia, C. tora,* and *V. tetrasperma* caused 95.2, 100, and 76.2% mortality, respectively.

Because of high larvicidal activity of the extracts from *C. obtusifolia, C. tora,* and *V. tetrasperma* at 50 ppm, bioassays were performed with higher dilutions (Table 4). Extract of *C. tora* gave 86.7 and 100% mortality in larvae of *Ae. aegypti* and *Cx. pipiens pallens,* respectively, at 40 ppm, but 59.2 and 78.3% mortality in larvae of *Ae. aegypti* and *Cx. pipiens pallens,* respectively, at 20 ppm. Extract of *C. obtusifolia* caused 51.4 and 68.5% mortality of larvae of *Ae. aegypti* and *Cx. pipiens pallens,* respectively, at 40 ppm. The larvicidal activity of the extract of *C. obtusifolia* was significantly reduced when used at 20 ppm. Extract of *V. tetrasperma* caused 35.9% mortality of larvae of *Cx. pipiens pallens* at 40 ppm, but no mortality at the low concentrations (20 and 10 ppm) in larvae of *Ae. aegypti* and *Cx. pipiens pallens.*

Table 2. Mortality of early 4th-stage larvae of *Aedes aegypti* caused by methanol extracts of leguminous seeds and grains.[2]

Plant species	Concentration (ppm)[2]			
	300	200	100	50
Amphicarpaea edgeworthii	27.8 ± 4.4a	4.9 ± 1.1b	0c	0c
Cassia obtusifolia	100a	100a	100a	88.7 ± 2.5b
Cassia tora	100a	100	100a	94.5 ± 2.1b
Glycine max var. *wooltalikong*	43.8 ± 1.8a	0b	0b	0b
Lathyrus japonica	25.8 ± 2.9a	0b	0b	0b
Panicum miliaceum	58.4 ± 1.7a	17.3 ± 1.5b	0c	0c
Rhynchosia volubilis	39.2 ± 3.3a	9.1 ± 1.2b	0c	0c
Schizandra nigra	93.2 ± 2.9a	57.8 ± 3.2b	26.7 ± 2.8bc	0d
Vicia tetrasperma	100a	100a	67.4 ± 1.2b	15.9 ± 1.3c

[1] Values are percentages (mean ± SE; α = 0.05, Scheffe's test [SAS Institute 1990]). Samples showing no mortality are not presented.
[2] Values in the same row followed by the same lowercase letter are not significantly different.

DISCUSSION

Of the methanol extracts from 26 leguminous seeds and 20 grains belonging to the families Graminales, Leguminosae, Polygonaceae, Labiatae, Magnoliaceae, and Pedalidaceae, strong larvicidal activity against *Ae. aegypti* and *Cx. pipiens pallens* was observed in the families Graminales (1), Leguminosae (7), and Magnoliaceae (1). Many plant extracts and phytochemicals possess larvicidal activity against various mosquito species (Berenbaum 1989, Jacobson 1989, Sukumar et al. 1991). Yang et al. (2002) reported that the most promising botanical mosquito larvicides are in the families Apiaceae, Araceae, Magnoliaceae, Piperaceae, Rutaceae, and Zingiberaceae, whereas species of the family Annonaceae can be employed as economical and environmentally friendly mosquito larvicides. Various compounds, including phenolics, terpenoids, and alkaloids, exist in plants (Swain 1977, Wink 1993, Kim et al. 2001b). These compounds may jointly or independently contribute to generation of larvicidal activities of mosquito (Assabgui 1997, Hostettmann and Potterat 1997, Sukumar et al. 1991).

Larvicidal activities of the plant extracts vary according to the plant species, the parts of the plant, the geographical location where the plants were grown, and the application method (Sukumar et al. 1991). Crude extracts of the fruit from *Swartzia madagascariensis* produced higher mortality in larvae of *Anopheles gambiae* (Edwards) than in larvae of *Ae. aegypti* but were ineffective against larvae of *Culex quinquefasciatus* (Say) (Minijas and Sarda 1986). Sujatha et al. (1988) examined the larvicidal activity of 5 plants; extracts of *Acorus calamus* and *Bambusa arundanasia* were most effective against *Cx. quinquefasciatus* and *Anopheles stephensi*, respectively, whereas extract of *Citrus medica* affected only larvae of *An. stephensi* and extract of *Madhuca longifolia* was ineffective against this species. In our study, larvicidal responses varied according to mosquito and plant species. The methanol extracts of *C. obtusifolia, C. tora, R. volubilis, S. nigra,* and *V. tetrasperma* showed strong larvicidal activities, and the extracts of *Amphicarpaea edgeworthii, Glycine max* var. *wooltalikong, Lathyrus japonica,* and *Panicum miliaceum* showed only moderate activity against *Ae. aegypti* and *Cx. pipiens pallens.* These leguminous seeds and grains might form a new source for managing various mosquito larvae in field ecosystems, although their effects on nontarget organisms remain unknown.

Table 3. Mortality of early 4th-stage larvae of *Culex pipiens pallens* caused by methanol extracts of leguminous seeds and grains.[1]

Plant species	Concentration (ppm)[2]			
	300	200	100	50
Amphicarpaea edgeworthii	55.1 ± 1.4a	28.7 ± 2.3b	5.5 ± 2.3c	0d
Cassia obtusifolia	100a	100a	100a	95.2 ± 1.5b
Cassia tora	100a	100a	100a	100a
Glycine max var. *wooltalikong*	56.3 ± 2.1a	9.8 ± 2.4b	0c	0c
Lathyrus japonica	33.5 ± 2.7a	5.8 ± 2.8b	0c	0c
Panicum miliaceum	64.2 ± 1.3a	19.6 ± 2.5b	0c	0c
Rhynchosia volubilis	90.1 ± 2.5a	49.8 ± 3.1b	12.5 ± 2.8c	0d
Schizandra nigra	100a	48.7 ± 1.3b	14.7 ± 2.3dc	0d
Vicia tetrasperma	100a	100a	87.3 ± 1.5b	76.2 ± 1.2c

[1] Values are percentages (mean ± SE; α = 0.05, Scheffe's test [SAS Institute 1990]. Samples showing no mortality are not presented.
[2] Values in the same row followed by the same lowercase letter are not significantly different.

Table 4. Mortality of early 4th-stage larvae of *Aedes aegypti* and *Culex pipiens pallens* caused by the extracts of selected samples[1]

Plant species	Tested mosquito	Concentration (ppm)[2] 40	20	10
Cassia obtusifolia	*Aedes aegypti*	51.4 ± 3.1a	0b	0b
	Culex pipiens pallens	68.5 ± 3.2a	13.5 ± 2.9b	0c
Cassia tora	*Aedes aegypti*	86.7 ± 1.6a	59.2 ± 2.2b	38.6 ± 2.7c
	Culex pipiens pallens	100a	78.3 ± 2.3b	46.7 ± 2.2c
Vicia tetrasperma	*Aedes aegypti*	0a	0a	0a
	Culex pipiens pallens	35.9 ± 3.1a	0b	0b

[1] Values are percentages (mean ± SE; $\alpha = 0.05$, Scheffe's test [SAS Institute 1990]).
[2] Values in the same row followed by the same lowercase letter are not significantly different.

Variation in mosquito response to various plant species extracts has been studied. Among the steam-distilled oil of the whole plant from the *Tagetes* species (*T. erecta, T. minuta,* and *T. patula*), *T. minuta* has the greatest larvicidal activity (Green et al. 1991, Perich et al. 1994). In our study, the larvicidal activity against *Ae. aegypti* and *Cx. pipiens pallens* was much more pronounced in the extract of *Panicum miliaceum, Schizandra nigra,* and *V. tetrasperma* than in the extract of *Panicum bisulcatum, Schizandra chinensis, Vicia hirsute, V. angulasis,* and *V. sinensis.* These results suggest that chemical composition of extracts from the plant species may be different. We are sure that they are useful for managing mosquito larvae. Further works on these plant-derived constituents active against mosquito larvae is needed for developing them into effective formulations for control of mosquito larvae. Furthermore, further research to identify the biologically active substances in the extracts of *C. obtusifolia* and *C. tora,* which showed the most potent larvicidal activity, is in progress.

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