The Effects of *Acorus calamus* Linn. and *Stemona tuberosa* Lour. Extracts on the Insect Pest, *Plutella xylostella* (Linnaeus)

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Abstract

The effects of *Stemona tuberosa* Lour. and *Acorus calamus* Linn. ethanol extracts on the Diamondback Moth, *Plutella xylostella* (Linnaeus) was investigated. It was found that a 0.4 % *A. calamus* extract and 0.5 % *S. tuberosa* extract had the best activity against third-instar larvae of *P. xylostella* by the leaf dipping method. At this concentration *A. calamus* extract gave 63.3 % accumulated mortality with 23.33 mm^2 feeding sites per larva within 48 hours. Whereas *S. tuberosa* extract gave 53.3 % accumulated mortality, significantly with 3.67 mm^2 feeding sites per larva within 72 hours.

The study of the insecticidal substances from both plants revealed that the dichloromethane extracts exhibited a pronounced insecticidal activity by topical application to the third-instar larvae of *P. xylostella.* The fractionated compounds by TLC were tested for insecticidal activity. The R_f 0.49 band of the *A. calamus* extract showed 100 % accumulated mortality in 24 hours and the R_f 0.31 band of S. tuberosa extract 62.5 % in 72 hours. The structures of the active fractions are being identified.

INTRODUCTION

Phytoinsecticides are useful against several insect pests in yards and gardens such as ants, aphids, beetles, caterpillars, cockroaches, fleas, flies, leafhoppers and mosquitoes. Nicotine from the tobacco plant is an example of a phytoinsecticide. Pyrethrum, derived from the chrysanthemum, is another one.

The advantage of using phytoinsecticide is that they all break down into nontoxic compounds within hours or days when exposed to sunlight. The potential of these chemicals to contaminate groundwater is less than that of some synthetic insecticides.

These phytoinsecticides (or natural), even though they come from plants, are not always non toxic. Some are very toxic. In sufficiently high doses, nicotine causes convulsions and death in insects and humans. Ryania is 20 times more toxic to mammals than insects, (The Spokane Country Office of Washington State University, 1997).

Some of the currently recognized phytoinsecticides are limenene, neem, nicotine, pyrethrum, rotenone, ryania, and sabadilla. Some organic products contain combinations of these chemicals (The Spokane Country Office of Washington State University, 1997).

Acorus calamus Linn. is used to treat patients who have ingested toxins or poisons (Saralamp et al, 1980). *A. calamus* oil is a contact poison of adult *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.) and *Lasioderma serricorne* (F.) (Su, 1991) and the essential oil of Indian *A. calamus* has the potential for the control of stored-product insect pests (Mukerjea and Govind, 1960; Yadava, 1971; Agarwal et al., 1973; Saxena et al., 1976; Koul et al., 1997; Jilani et al., 1988; Schmidt and Streloke, 1994). Asarones (2,4,5-trimethoxypropenylbenzenes) isolated from the essential oil of *A. calamus* rhizomes, are potent growth inhibitors and antifeedants of the variegated cutworm,

Peridroma saucia Hubner (Koul et al., 1990).

In Thailand, various medicinal uses of *Stemona tuberosa* Lour. have been reported, such as for the treatment of respiratory diseases, and the roots are said to have insecticidal properties (Saralamp et al., 1980 and Burkill, 1996). *Stemona* is a small genus of tuberous herbs in the family Roxburghiaceae found in Southeast Asia and Australia. Ethanol and aqueous extracts of *S. kerrii* and *S. tuberosa* at room temperature were effective against larvae and adult flea beetles (Rodkvamtook, 1996). Palaharn (1996) found that the ethanolic crude extract from the rhizome of *S. tuberosa* showed insecticidal activity against the beet armyworm larvae, *Spodoptera exigua* (Hubner).

MATERIALS AND METHODS

Plant Material

The fresh roots of *Stemona tuberosa* were obtained from Maha Sarakarm province, Thailand, during May, 1999.

The dried rhizomes of *Acorus calamus* were obtained from a grocery in Chiang Mai province, Thailand.

Extraction and Separation of *A. calamus*

The dried rhizomes of *Acorus calamus* were ground and percolated with 95 % ethanol. After filtration and evaporation under reduced pressure, the crude extract was obtained. Concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4 % from 1 % of crude extract were prepared in ethanol.

The crude extract of *A. calamus* was separated by thin layer chromatography (TLC) using silica gel as the stationary phase and a mixture of toluene:ethyl acetate (93:7) as the mobile phase. Each fraction was tested for insecticidal activity on the 3rd-instar larvae of *Plutella xylostella* (Linnaeus) by topical application. The active compounds at R_f 0.49 (Table 2) were repurified twice by TLC using a mixture of toluene:ethyl acetate as mobile phase and the bands were detected with an UV-lamp at long wavelength. Each fraction was tested for insecticidal activity.

Extraction and Separation of *S. tuberosa*

The fresh roots of *Stemona tuberosa* were ground in a blender and percolated with 95 % ethanol. After filtration and evaporation under reduced pressure, the crude extract was obtained. Concentrations of 0.5, 0.75, 1.0, 1.25 and 1.5 % from 1 % of crude extract were prepared in ethanol.

S. tuberosa was also extracted with dichloromethane and dried with magnesium sulfate ($MgSO_4$) to give a crude extract. This was then separated by TLC using silica gel as the stationary phase, and a mixture of toluene:ethyl acetate:diethylamine (60:40:1) as the mobile phase; the bands were detected with Dragendroff's reagent. Each band was re-extracted with dichloromethane:methanol 1:1. The fractionated compounds extracted from the various bands were then tested on the 3rd-instar larvae of *P. xylostella* by topical application. The non-active fractions were discarded.

Methods of Testing Chemicals on Insects

Topical application (TA or contact poison) was achieved by dissolving the insecticide in a relatively nontoxic solvent, such as acetone or 10% ethanol and droplets of 0.05µl of the test compounds were applied at a chosen location on the body surface of the larva with a micropipette. The number of dead larvae were recorded after 24, 48, 72 and 94 hours.

The vegetable feed was changed every 24 hours after recording. Data were calculated in terms of percent of accumulated mortality (Janprasert, 1992).

Leaf dipping (LD) was carried out by dipping pieces of 4 x 4 mm of plant material into nontoxic solvent, 10 % ethanol (control) and different concentrations (0.4-1.5 %) of test insecticide. After drying, they were put into a box into which the test larvae were put.

The feeding sites were recorded at 24, 48 and 72 hours and the number of dead larvae was counted. The experiment was carried out using a Completely Randomized Design with 3 replications for LD and 2 replications for TA, 10 larvae were used for each replicate (Janprasert, 1992).

RESULTS AND DISCUSSION

Separation of *Acorus calamus* Extract

An active fraction at R_f 0.49 was obtained when the crude extract was purified by TLC using a mixture of toluene:ethyl acetate (93:7) as the mobile phase. Two of the three purified fractions (R_f 0.36 and R_f 0.58) were found to be active, exhibiting pronounced insecticidal activity. The R_f 0.58 fraction was further purified by TLC using a mixture of toluene:ethyl acetate (90:10) as the mobile phase to give two active fractions at R_f 0.62 and R_f 0.76. Fractions at R_f 0.36, R_f 0.62 and R_f 0.76 were brown liquids. The separation of *A. calamus* extract is shown in Figure 1.

Toxicity of *A. calamus* to the Third-Instar Larvae

Concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4 % were tested on the third-instar larvae of *Plutella xylostella* by using a leaf dipping method. At 0.4 % *A. calamus* extract had the best activity against the larvae. This concentration provided 63.3 % accumulated mortality with 23.33 mm^2 feeding sites per larva within 48 hours (Table 1).

The effects of *A. calamus* extracts on the insect pest by topical application are presented in Table 2. The experiments showed that the fraction at R_f 0.49 was toxic to the larvae at 100 percent of accumulated mortality in 24 hours. There were significant differences when compared with other fractions and the control. After separating the fraction with an R_f of 0.49, the R_f 0.36 and R_f 0.58 fractions were found to be toxic to the larvae with 93 and 100 % of accumulated mortality after 72 and 24 hours, respectively. There was a significant difference when comparing the fraction at R_f 0.22 and the control. After separating the R_f 0.58 fraction, the fractions at R_f 0.62 and R_f 0.76 were shown to be toxic to the larvae at 100 percent of accumulated mortality after 48 and 24 hours, respectively. There was a significant difference when compared with the fraction at R_f 0.38 and the control.

Separation of *Stemona tuberosa*

Separation of the crude dichloromethane extract of *S. tuberosa* using a mixture of toluene:ethyl acetate:diethylamine (60:40:1) as the mobile phase gave one active fraction (R_f 0.31) that exhibited a pronounced insecticidal activity. The fraction at R_f 0.31 was purified by using a mixture of toluene:ethyl acetate:diethylamine (40:60:1) to give 1 band at R_f 0.56 (Fig. 2)*. The R_f 0.56 fraction gave a yellow liquid after evaporation. The separation of *S. tuberosa* extract is shown in Figure 2.

*The R_f 0.56 fraction was not used to test for insecticidal activity against *P. xylostella*.

Toxicity of *S. tuberosa* to the Third-Instar Larvae

Concentrations of 0.5, 0.75, 1.0, 1.25 and 1.5 % were tested on the third-instar larvae of *Plutella xylostella* by the leaf dipping method. *S. tuberosa* extract at a concentration of 0.5 % gave the best activity against the larvae with 53.3 % accumulated mortality and 3.67 mm^2 feeding sites per larva within 72 hours (Table 1).

The data obtained from insecticidal test are presented in Table 3. The experiments showed that the fraction at R_f 0.31 gave 62.5 % of accumulated mortality in 72 hours, even though there was no significant difference when compared with other fractions. The percent accumulated mortality after the 96 hours of the R_f 0.31 fraction was obviously better than any other fraction and the control.

CONCLUSION

Insecticidal Activity of *Acorus calamus*

The result of this work corresponded with the report by Jilani et al. (1988) that various concentrations of sweet flag extract in petroleum ether showed high insecticidal activity and checked progeny emergence in the weevil. The fraction obtained from petroleum extract showing high insecticidal activity was not identified. Rahman et al (1999) tested the vapour of essential oils from *Acorus calamus* rhizomes collected from three countries, India, Russia and Yugoslavia on the adults and eggs of *Callosobruchus phaseoli* (Gyllenhal) reared on the seeds of *Lablab purpureus* (Medik.). They were found to be toxic to adults and eggs depending on the exposure period and dose. Longer exposure periods increased the differences in egg production of treated and untreated insects. The fertilities of the treated females was slightly affected. Newly-laid eggs were more susceptible than the older ones. However, the concentration used was not mentioned.

Insecticidal Activity of *Stemona tuberosa*

The result corresponded with the work reported by Rodkvamtook (1996). *S. tuberosa* and *S. kerrii* at different concentrations, 100 and 200 g at room temperature gave the best results on larvae and adult flea beetles. However the concentration of raw material used by Rodkvamtook (1996) cannot be compared with the % concentration of crude extract used in this experiment. Palaharn (1996) found that the ethanolic crude extract of *S. tuberosa* rhizome have insecticidal activity against the beet armyworm larvae, *Spodoptera exigua* (Hubner). Wichai (1977) (cited by Rodkvamtook, 1996) reported that the crude petroleum ether extract of the dried root of *S. tuberosa* mixed with acetone (1:5) showed 37.5% mortality on larvae; the exact concentration used was not reported.

The maximum mortality in this experiment was rather high (62.5 %) compared with that reported by Wichai (1977) (37.5 %). However the concentration of the extract used in this testing was rather high (50 %).

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Tables

Table 1. Percentage of accumulated mortality and feeding sites per larva of different concentrations of *Acorus calamus* and *Stemona tuberosa* extracts on *Plutella xylostella* by leaf dipping method (n=30)

Extract	**Concentration (%)**	**24 hours**		**48 hours**		**72 hours**	
		feeding sites per larva (mm^2)	% accumulated mortality	feeding sites per larva (mm^2)	% accumulated mortality	feeding sites per larva (mm^2)	% accumulated mortality
A. calamus	0.025	100.67	3.33	82.00	6.67	115.00	16.67
	0.05	61.33	10.00	25.67	13.33	77.00	10.00
	0.1	35.00	3.33	18.00	6.67	62.67	10.00
	0.2	29.00	3.33	29.00	6.67	59.33	20.00
	0.4	29.33	0.00	**23.33**	**63.33**	14.00	76.67
	control	69.33	0.00	36.33	0.00	56.00	0.00
S. tuberosa	0.5	18.33	23.33	10.00	36.67	**3.67**	**53.33**
	0.75	17.67	16.67	12.67	23.33	6.67	40.00
	1.0	55.33	3.33	40.00	13.33	36.67	26.67
	1.25	26.00	16.67	14.67	33.33	12.33	60.00
	1.5	55.33	13.33	25.33	36.67	17.67	53.33
	control	29.33	0.00	14.67	0.00	19.33	0.00

Table 2. Percentage of accumulated mortality of different fractions of *Acorus calamus* extract and control (10 % ethanol) on *Plutella xylostella* (95 %confidence) by topical application*

Tested fraction	**Concentration (%)**	**Percentage of accumulated mortality (n=20)**			
		24 hours	48 hours	72 hours	96 hours
Control	10	0^{a}	0^{a}	0^{a}	0^{a}
R_f 0.05	50	20^{ab}	20^{cd}	35^{cd}	50^{bcd}
R_f 0.14	50	15^{ab}	30^{cd}	45^{de}	55^{bcd}
R_f 0.24	50	0^{a}	5^{ab}	15^{ab}	30^{bc}
R_f 0.33	50	20^{ab}	35^{d}	60^{ef}	65^{cd}
R_f 0.49**	50	100^{d}	-	-	-
R_f 0.65	50	55^{c}	65^{e}	65^{f}	65^{cd}
R_f 0.72	50	30^{bc}	55^{e}	70^{f}	75^{de}
R_f 0.92	50	10^{ab}	15^{ab}	20^{bc}	35^{bc}

*Comparison between tested fractions in each column; the same letter indicates no significant difference by LSD (P=0.05)

** The active fraction

Table 3. Percentage of accumulated mortality of different fractions of *Stemona tuberosa* extract and control (10 % ethanol) on *Plutella xylostella* (95 % confidence) by topical application*

Tested fraction	**Concentration (%)**	**Percentage of accumulated mortality (n=20)**			
		24 hours	**48 hours**	**72 hours**	**96 hours**
Control	10	0^{a}	0^{a}	0^{a}	0^{a}
R_f 0.12	50	0^{a}	12.50^{ab}	31.25^{ab}	31.25^{b}
R_f 0.31**	50	12.50^{b}	43.75^{b}	62.50^{b}	62.50^{c}
R_f 0.40	50	6.25^{ab}	37.50^{ab}	43.75^{b}	50^{bc}
R_f 0.56	50	0^{a}	6.25^{ab}	31.25^{ab}	50^{bc}

* Comparison between tested fractions in each column; the same letter indicates no significant difference by LSD (P=0.05)

** The active fraction

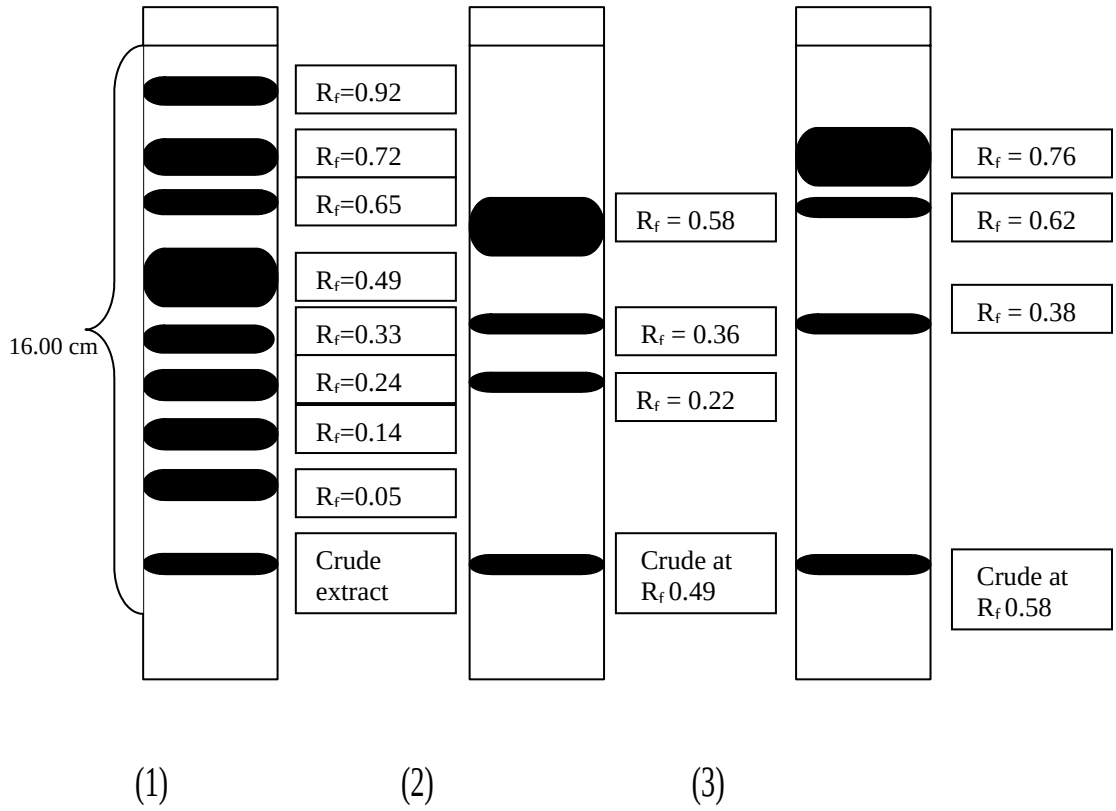

Fig. 1. TLCs of *A. calamus* extract (1), fraction at R_f 0.49 (2) and fraction at R_f 0.58 (3) with UV detection

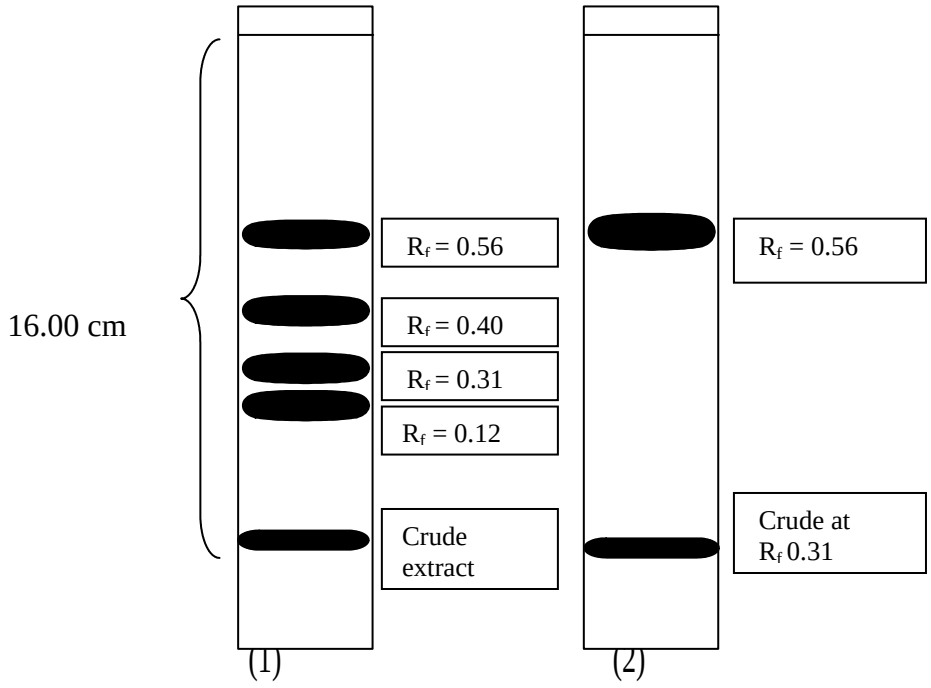

Fig. 2. TLCs of *S. tuberosa* extract(1) and fraction at R_f 0.31 (2) detected with Dragendroff's reagent