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Efficiency of extracts from indigenous herbs of northeastern Thailand in controlling the tobacco cutworm, Spodoptera litula (f.)

Saroj Charoensak[1], Jarongsak Pumnuan[2]* and Ammorn Insung[2]

[1]Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand.

[2]Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

*Author to whom correspondence should be addressed, email: kpjarong@kmitl.ac.th

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Abstract

The insecticidal, inhibiting growth and anti-feedant properties of hexane, acetone and ethanol extracts of three indigenous herbs of northeastern Thailand (*Anethum graveolens* Linn., *Oroxylum indicum* Linn. and *Polygonum odoratum* Lour.) were investigated on the 2nd instar larvae of tobacco cutworm, *Spodoptera litura* F. The leaf dipping method using various concentrations of extracts; 0.0 (10% Tween-20 as control), 2% (0.29 mg/cm^2), 4% (0.58 mg/cm^2), 6% (0.87 mg/cm^2), 8% (1.16 mg/cm^2) and 10% (1.45 mg/cm^2) were applied. The results showed that the hexane extract of *A. graveolens* and *P. odoratum* were highly effective in controlling tobacco cutworm. Extracts at concentrations of 10% (1.45 mg/cm^2) completely controlled the cutworm within 72 hours and showed the LD_{50} of 0.29 and 0.33 mg/cm^2 (w/v), respectively. These two extracts were effective at inhibiting the growth of the cutworm larval stage but could not inhibit the growth of pupae developing into adult. All extracts from the three test plant species had low anti-feedant efficiencies.

Keywords :

Introduction

There are several species of insect pests that attack vegetables, and among these, the tobacco cutworm, *Spodoptera litura* F. is an economically important species. It is able to destroy a vegetable crop and particularly prefers, vegetables within the cabbage family. The tobacco cutworm can quickly spread throughout the crop if it has a suitable environment (Theskayan, 2006). Traditional farmers have been used synthetic pesticides to eliminate *S. litura* but this pest has developed resistance against most of the commonly used pesticides. In addition, human health issues and environmental hazards caused by the indiscriminate use of chemical pesticides during past three decades have lead to scientists to examine the use of less persistent and biodegradable alternatives (Sahayaraj et al., 2003).

Botanical insecticides such as azadirachtin are often effective alternatives to organophosphate or other neurotoxins for pest control due to their multiple modes of action. These include toxicity, anti-feedant and anti-oviposition effects (Sutherland et al., 2002). Natural products containing secondary plant compounds such as terpenes, steroids, alkaloids, phenolics and cardiac glycosides effect insect behavior and are often toxic in some cases (Duke, 1990). These novel bioactive compounds isolated from the insecticides plants have been integrated in the Biological Integrated Pest Management (BIPM) program for many crops. Biological, physiological and biochemical impact of many insecticidal plants on different insect pests has been reported by many authors. Ethanol extract of *Pedalium munex* Linn. root has been used as insecticide against *S. litura* (Sahayajet et al., 2008).

It is the aim of this work to evaluate the efficacy of insecticide, inhibition growth and anti-feedant properties of hexane, acetone and ethanol extracts of some indigenous herbs of northeastern Thailand on the larvae of *S. litura*

.Materials and Methods

Insect culture

The tested insects were obtained from a culture *Spodoptera litura* F. maintained on Chinese cabbage leaves in the laboratory of Department of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The larval colony was originally collected from the field in Nakhonpathom province, Thailand. Insects were reared under laboratory conditions 25±2 °C, 70±5%RH. Prior to use larvae in the various bioassays, the larvae were starved for 6 hrs.

Extract preparation

Fresh leaves of three indigenous herbs of northeastern, Thailand (*Anethum graveolens* Linn., *Oroxylum indicum* Linn. and *Polygonum odoratum* Lour.) were air-dried and ground into finely powder in blender. Powder samples (0.5 kg) were extracted with 4 L of hexane using soaking method allowed to stand at room temperature about 7 days, then filtered through filter paper. After filtering, the hexane was removed at 60 °C using a rotary evaporator, to obtain the crude hexane extract. The remaining materials were then extracted with 4 L of acetone and ethanol respectively. By the same method, acetone and ethanol crude extracts were also obtained.

The final yield of crude extracts (5 g) were re-suspended in 20% Tween-20 in water to a volume of 20 mL, to make a 20% (w/v) stock solution and was kept at 10 °C for subsequent use. This stock solution was further diluted with water to obtain the 2, 4, 6, 8 and 10% extract solutions.

Bioassay

The oral toxicity of the crude extracts against *S. litura* larvae was investigated through no-choice bioassays using leaf discs (30 mm in diameter) prepared from Chinese cabbage leaves cut by a cork borer. Onto each leaf disc was dropped 125 µL with the extract solution 0 (10% tween-20 in water), 2, 4, 6, 8 and 10%. All discs were left at room temperature to air-dry, where after the leaf was placed in plastic Petri dish (10 cm diameter, top was cut and covered with fine cloth) and padded with moist filter paper marked on one side. Ten 2nd instar *S. litura* larvae (previously starved for 12 hrs) were then introduced in each plastic Petri dish containing a leaf disc. The percent mortality of the larvae was observed at 24, 48 and 72 hrs. In addition, the effects of continuous exposure of the plant extracts on larval development and survival during the 15 days treatment were also recorded.

In the choice tests, the same concentrations of extract solutions as referred in the no-choice tests were performed, but the treatments and controls were applied on each side of leaf disc to indicate the percentage leaf damage after 24 hrs. The percentage of the leaf area eaten of the treated leaf portion (T) and that of the control (C) were recorded. The percentage anti-feedant effect was calculated according to Antifeedant Index (AFI); ([(C-T)/(C+T)]×100), (Lewis and van Emden, 1986).

Statistical analysis

Data for the bioassay was corrected for mortality in the control using Abbott's formula (Abbott, 1925). The experiment was designed in three completely randomized replicates. The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). LD50 (median lethal dose) was calculated by the probit method.

Results and Discussion

The results show that the hexane extract of *P. odoratum* was highly effective in controlling 2nd instar larvae of *S. litura*. The hexane extract at various concentrations of 0.29, 0.58, 0.87, 1.19 and 1.45 µg/cm^2 at 24 hrs resulted in 11.1, 51.9, 70.3, 88.9 and 96.3% mortality, respectively. This compares to the results from the extract of *Anethum graveolens* Linn. which showed 33.3, 48.1, 55.6, 70.3 and 85.2% mortality, respectively. Extracts at the concentration of 1.45 µg/cm^2 completely controlled *S. litura* larvae within 48 hrs of treatment. Hexane extracts of *P. odoratum* showed LD_{50} of 0.58, 0.39 and 0.33 µg/cm^2, at 24, 48 and 72 hrs, respectively, whereas the hexane extract of *A. graveolens* showed LD_{50} of 0.64, 0.37 and 0.29 µg/cm^2, respectively (Table 1). A similar study by Chaona et al. (2005) examined the toxicities of *A. graveolens* and *P. odoratum* essential oils against *Callosobruchus maculatus* (F.) using the residual film technique. They showed LD_{50} at 48 hrs, of 3,003 and 18,530 ppm respectively, and impregnated filter paper test showed that *A. graveolens* essential oil was the most toxic, showed LD_{50} of 9,483 ppm (Chaona et al., 2005).

Hexane extracts of *P. odoratum* and *A. graveolens* were effective at inhibiting the growth of *S. litura* larval stage but could not inhibit the growth of pupae developing into the adult. The hexane extract of *P. odoratum* (at 0.87 µg/cm^2) and *A. graveolens* (at 1.16 µg/cm^2) completely inhibited the growth in the larval stage (Table 2). The extract of *P. odoratum* at the highest concentration also inhibited the growth rate of the Diamondback moth, *Plutella xylostella* Linn., followed by *O. indicum* and *A. graveolens*, respectively. The 10% (1.90 mg/cm^2) acetone extract of *P. odoratum* resulted in a 6.7% adult survival of *P. xylostella* (Pumnuan et al., 2008). In addition, Su (1989) showed that wheat grains surface-treated with an acetone

extract of dill (*A. graveolens*) seed and exposed to adults of curculionid, *Sitophilus oryzae* Linn. completely reduced the F1 generation.

Strong inhibitory effects of the crude extract against the *S. litura* larvae were observed. Under choice tests, the number of larvae fed on control leaves was more than that of the number of larvae fed on treated hexane extracts of *P. odoratum*, *A. graveolens* and acetone extract of *P. odoratum*, as indicated by the anti-feedant index (AFI) > 25% (Fig. 1). In no-choice tests with leaf discs, the consumptions leaf discs by the larvae treated with crude extracts were higher in the all treatment concentrations (Fig. 2). The acetone extract of *P. odoratum* showed the highest anti-feedant activity to Diamondback moth with 100% AFI at 4% (0.76 mg/cm^2) extract, followed by hexane extract of *A. graveolens*. The higher concentrations of plant extracts resulted in the lower insect feeding rates (Pumnuan et al., 2008).

Table 1. Percentage of mortality of *Spodoptera litura* F. after feeding with host plants treated with *Anethum graveolens* Linn., *Oroxylum indicum* Vent. and *Polygonum odoratum* Lour. extracts[1/].

Plant extract	Dose % (mg/cm^2)	% Mortality[1/]								
		A. graveolens			*O. indicum*			*P. odoratu*		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Hexane extract	0.0	0.0 d[2/]	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 d	0.0 c	0.0 c
	2 (0.29)	33.3 c	48.1 b	59.2 b	0.0 b	0.0 b	0.0 b	11.1 c	29.7 b	29.7 b
	4 (0.58)	48.1 bc	74.1 ab	85.2 ab	0.0 b	0.0 b	0.0 b	51.9 b	70.3 a	85.2 a
	6 (0.87)	55.6 bc	85.1 a	88.9 a	0.0 b	0.0 b	18.6 ab	70.3 ab	92.6 a	100.0 a
	8 (1.16)	70.3 ab	85.1 a	92.6 a	11.1 ab	22.2 a	29.7 a	88.9 ab	100.0 a	100.0 a
	10 (1.45)	85.2 a	100.0 a	100.0 a	14.8 a	33.3 a	40.8 a	96.3 a	100.0 a	100.0 a
LD_{50} (mg/cm^2)		0.64	0.37	0.29	2.76	1.77	1.52	0.58	0.39	0.33
Acetone extract	0.0	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b	0.0 c
	2 (0.29)	25.6 b	0.0 c	11.1 c	7.4 b	11.1 bc	14.8 bc	3.7 b	11.1 ab	18.6 ab
	4 (0.58)	37.0 b	44.4 b	48.1 b	33.3 a	40.8 a	40.8 a	7.4 b	14.8 ab	25.9 ab
	6 (0.87)	44.4 b	44.4 b	48.1 b	37.0 a	37.0 a	40.8 a	18.6 b	22.2 ab	29.7 a
	8 (1.16)	55.6 ab	55.6 ab	55.6 b	33.3 a	33.3 ab	37.0 ab	18.6 b	18.6 ab	40.8 a
	10 (1.45)	74.1 a	81.4 a	81.4 a	44.4 a	48.1 a	55.6 a	33.3 a	33.3 a	44.4 a
LD_{50} (mg/cm^2)		0.93	0.85	0.80	1.31	1.29	1.10	1.89	1.95	1.31
Ethanol extract	0.0	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 d	0.0 c	0.0 c	0.0 c
	2 (0.29)	0.0 c	0.0 c	7.4 b	0.0 c	0.0 c	3.7 c	0.0 c	0.0 c	3.7 c
	4 (0.58)	11.1 bc	14.8 b	18.6 b	11.1 b	14.8 b	25.9 bc	0.0 c	7.4 c	14.8 bc
	6 (0.87)	14.8 b	25.9 ab	44.4 a	18.6 b	22.2 b	37.0 ab	18.6 b	18.6 b	18.6 bc
	8 (1.16)	33.3 a	48.1 a	55.6 a	18.6 b	18.6 b	37.0 ab	33.3 ab	37.0 ab	51.9 ab
	10 (1.45)	40.8 a	48.1 a	55.6 a	44.4 a	51.9 a	59.0 a	44.4 a	48.1 a	63.0 a
LD_{50} (mg/cm^2)		1.53	1.25	1.05	1.47	1.45	1.15	1.44	1.38	1.14

[1/] Data was based on 2nd instars larvae, 10 larvae/ replication of 3 replications, [2/] Mean in column with the same plant extract followed by the same common letters are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

Table 2. Percentage of survival of *Spodoptera litura* F. after feeding with host plants treated with *Anethum graveolens* Linn., *Oroxylum indicum* Vent. and *Polygonum odoratum* Lour. extracts[1/].

Plant extract	Dose (mg/cm^2)	% Survival[1/]					
		A. graveolens		*O. indicum*		*P. odoratu*	
		pupae	adult	pupae	adult	pupae	adult
Hexane extract	0.0	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a
	2 (0.29)	30.0 b	30.0 b	73.3 b	60.0 b	36.7 b	30.0 b
	4 (0.58)	6.7 c	6.7 c	76.7 b	53.3 b	10.0 c	10.0 c
	6 (0.87)	6.7 c	6.7 c	60.0 b	60.0 b	0.0 c	0.0 c
	8 (1.16)	0.0 c	0.0 c	60.0 b	53.3 b	0.0 c	0.0 c
	10 (1.45)	0.0 c	0.0 c	53.3 b	53.3 b	0.0 c	0.0 c
Acetone extract	0.0	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a
	2 (0.29)	70.0 a	50.0 b	53.3 b	50.0 b	67.6 b	66.7 b
	4 (0.58)	36.7 b	36.7 bc	53.3 b	53.3 b	46.7 bc	46.7 bc
	6 (0.87)	43.3 b	40.0 bc	50.0 b	46.7 b	56.7 bc	50.0 bc
	8 (1.16)	30.3 bc	30.0 bc	43.3 b	40.0 b	40.0 c	40.0 c
	10 (1.45)	16.7 c	16.7 c	33.3 b	33.3 b	46.7 bc	46.7 bc
Ethanol extract	0.0	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a
	2 (0.29)	60.0 b	43.3 b	56.7 b	50.0 b	80.0 a	76.7 a
	4 (0.58)	56.7 b	40.0 b	60.0 b	53.3 b	66.7 a	66.7 ab
	6 (0.87)	40.0 b	36.7 b	46.7 b	43.3 b	60.0 ab	60.0 ab
	8 (1.16)	33.3 b	33.3 b	43.3 b	40.0 b	36.7 b	36.7 bc
	10 (1.45)	40.0 b	40.0 b	33.3 b	33.3 b	20.0 bc	20.0 c

[1/] Data was based on 2nd instars larvae, 10 larvae/ replication of 3 replications, [2/] Mean in column with the same plant extract followed by the same common letters are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

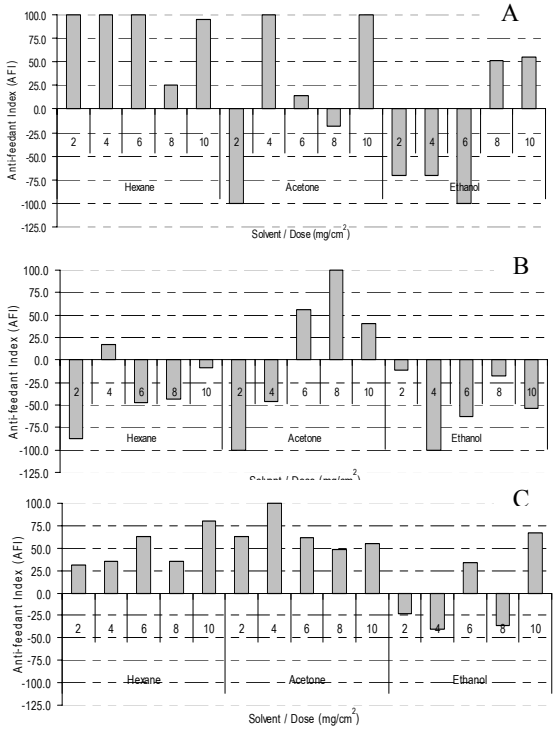

Figure. 1: Antifeedant Index (AFI) of *Spodoptera litura* F. after feeding with host plants treated with *Anethum graveolens* Linn.; (A), *Oroxylum indicum* Vent.; (B) and *Polygonum odoratum* Lour.; (C) extracts.

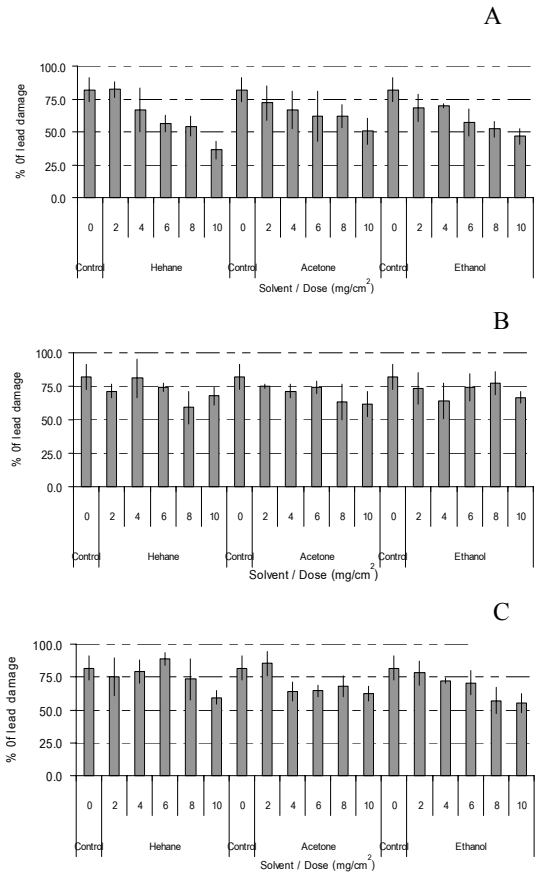

Figure 2: Percentage of leaf damage of *Spodoptera litura* F. after feeding with host plants treated with *Anethum graveolens* Linn.; (A), *Oroxylum indicum* Vent.; (B) and *Polygonum odoratum* Lour.; (C) extracts.

Conclusions

The hexane extract of *A. graveolens* and *P. odoratum* were highly effective in controlling tobacco cutworm. These extracts were effective at inhibiting the growth of the cutworm larval stage but could not inhibit the growth of pupae developing into adult. All extracts from the tested plant species including *O. indicum* had low anti-feedant efficiencies. This study demonstrated the potential of using crude extract from indigenous herbs for insect pest control.

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