

ANTIFEEDANT AND INSECTICIDAL ACTIVITIES OF SOME PHILIPPINE PLANT SPECIES

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ABSTRACT

Organic extracts from different parts of plants belonging to 15 Philippine species were screened for antifeedant activity adapting the dual choice antifeedant assay, and for other insecticidal activities through contact toxicity, using the Mexican bean beetle (*Epilachna varivestis*) as test insect. Materials with high antifeedant activity were extracts from *Derris philippinensis*, *Coleus amboinicus*, *Mikania cordata*, *Tithonia diversifolia*, *Aristolochia elegans* and *Agave cantala*, while those with lower antifeedant activity were from *Tinospora rumphii*, *Lantana camara*, *Dipterocarpus warburgii*, *Dioscorea hispida* and *Ceasalpinia pulcherrima*. Only *D. philippinensis* extract showed high insecticidal activity. Those with limited insecticidal action were extracts from *C. amboinicus*, *L. camara*, *M. cordata*, *T. diversifolia*, and *A. elegans*, while those that did not show any activity on *E. varivestis* were from *Piper betle*, *Donax cannaeformis*, *Leea aculeata* and *Entada phaseoloides*.

Keywords: contact toxicity, *Epilachna varivestis*, feeding inhibition, Philippine plants, botanical insecticides

Abbreviations: IGR – insect growth regulator, RFI – relative feeding inhibition, LSU – Leyte State University, UPLB- University of the Philippines Los Baños,

INTRODUCTION

There is hardly any plant which does not serve as food source for a particular pest species. As plants and herbivores co-evolve, the former have been developing defense mechanisms to protect them from the overgrazing of insects. Thus, many secondary plant metabolites are believed to have been synthesized as a result of evolutionary pressures from herbivores. Numerous defensive chemicals belonging to various categories (terpenoids, alkaloids, flavonoids, phenols, tannins,

polyacetylenes) which have behavioral and physiological effects on pests have already been identified. Some of these secondary metabolites are toxins, growth and development inhibitors, or repellents while others cause physiological abnormalities.

It is now well established that long-term use of organochlorines, carbamates, organophosphates and pyrethroids increases the number of pest species resistant to insecticides (Georghiou, 1990). This problem is further compounded by lack or unavailability of other control tactics against some pest species on certain crops. The important high yielding crop cultivars with improved biomass which have been developed often have also increased susceptibility to insect pests and diseases, highlighting the need for alternatives to pesticidal control tactics.

The use of antifeedant substances offers significant possibilities for management of insect pests of economic importance while keeping possible damage to the ecosystem at low level. Antifeedants are substances which cause cessation of feeding temporarily or permanently, depending upon the potency. A number of insecticidal compounds have been identified from plants and a lot more may be discovered. Plants showing minimal or no insect damage can be screened through feeding assay as the initial step towards isolation and identification of antifeedant and insecticidal compounds. These compounds may serve as templates in the formulation of more ecologically sound agrochemicals with low mammalian toxicity.

This paper reports on the screening of 15 Philippine plant species for antifeedant and insecticidal action undertaken to identify the most promising local materials among them.

MATERIALS AND METHODS

Selection of plants for screening

The strategies followed in selecting the plant species for antifeedant and pesticidal screening were: a) targeting plant families known to be rich in biologically active compounds based on literatures, and b) using the ethnobotanical approach whereby peoples' knowledge about the medicinal and pesticidal uses of the plants and their environment are considered. Surveys were conducted and local people were interviewed on plants they have been using for pest control. Selection was reinforced by the result of visual observation in the field on insect damage on pre-selected plants. Only those showing no feeding or minimal damage were finally selected. A total of 15 plant species belonging to 12 families were investigated (Table 1).

Collection and extraction of plant materials

The plant materials used in this study were collected inside the campuses of the University of the Philippines Los Baños (UPLB), College, Laguna and Leyte State University, (LSU), Baybay, Leyte and from the Bicol region. The plants collected from UPLB campus were *D. philippinensis*, *C. amboinicus*, *C. pulcherrima*, *A. elegans*, *A. cantala*, *T. diversifolia* and *P. betle*; from

Bicol were *E. phaseoloides*, *D. cannaeformis* and *L. aculeata*; and from LSU campus were *T. rumphii*, *L. camara*, *D. warburgii*, *M. cordata* and *D. hispida*. The collected plant materials were air dried and ground to fineness before extraction.

Extraction was done through the general procedure of using organic solvents of varying or increasing polarities. Direct extraction using methanol, a highly polar organic solvent, was done for leaves of *D. philippinensis*, *C. amboinicus*, *C. pulcherrima*; ethanol for *A. elegans* seeds; and chloroform for *A. cantala* trunks. Sequential extraction was done using solvents of increasing polarity, i.e. a) petroleum ether → diethyl ether → ethyl acetate → methanol for all plants coming from LSU campus and for *P. betle*, *D. cannaeformis*, *L. aculeata* and *E. phaseoloides* or b) hexane → dichloromethane → chloroform ethanol for *D. philippinensis*.

Table 1. Philippine plant species screened for antifeedant and insecticidal actions.

Plant Species	Family	Uses*
<i>Aristolochia elegans</i> Mast.	Aristolochiaceae	Ethnomedicine
<i>Agave cantala</i> Zucc.	Agavaceae	Ornamental
<i>Caesalpinia pulcherrima</i> (L.) Sw.	Fabaceae	Ornamental
<i>Coleus amboinicus</i> Lour.	Lamiaceae	Ethnomedicine Condiment
<i>Derris philippinensis</i> Merr.	Fabaceae	Insecticide Piscicide
<i>Dioscorea hispida</i> Dennst.	Dioscoreaceae	Bleaching agent Ethnomedicine
<i>Dipterocarpus warburgii</i> Foxworthy	Dipterocarpaceae	Lumber
<i>Donax cannaeformis</i> L.	Marantaceae	Ethnomedicine
<i>Enatada phaseoloides</i> (L.) Merr.	Fabaceae	Piscicide Molluscicide Hair tonic
<i>Lantana camara</i> (L.)	Verbenaceae	Ethnomedicine Food flavoring Insect repellent
<i>Leea aculeata</i> Blume	Leeaceae	Ethnomedicine
<i>Mikania cordata</i> (Burm. F.) B.L. Robinson	Asteraceae	Ethnomedicine
<i>Piper betle</i> L.	Piperaceae	Ethnomedicine
<i>Tithonia diversifolia</i> Hemmst.	Asteraceae	Ethnomedicine
<i>Tinospora rumphii</i> Miers	Menispermaceae	Ethnomedicine Insecticide

* References: Quisumbing (1975); De Guzman et al. (1986)

The decision on the type of solvent and extraction method to use was dependent on the availability of the solvent system during the collection process. When different organic solvents of varying polarities were not available, direct extraction with methanol was generally employed. Methanol is a good solvent commonly used in general plant extraction.

Rearing of test insect

The Mexican bean beetle, *Epilachna varivestis* Muls. (Coleoptera: Coccinellidae), was used as the test insect in this study. This species is the standard test insect being used in the bioassay-guided fractionation of plant materials at the Institute of Phytomedicine, University of Hohenheim, Stuttgart, Germany because of its short generation time, simplicity of the applicable mass-rearing technique, and the year-round availability of its host plant, *Phaseolus vulgaris*. Mated adults of the Mexican bean beetle were confined in rearing cages provided with 2-3 weeks old potted bean plants. The female adult beetles were allowed to oviposit on the host plants. The deposited eggs were collected daily and incubated in a petri dish lined with moist paper. Neonate larvae were transferred to another cage provided with the host plant. Mass-rearing was done in a room with constant temperature and relative humidity.

Dual-choice antifeedant assay

Leaves of suitable size were selected from one month old potted bean plants. One half of the nether surface of the leaf was painted with methano-dissolved extract starting at 5000 ppm and the other half with methanol. When no feeding was observed at 5 mg/mL, the concentration was further reduced.

Before introducing the insects, the treated leaves were allowed to dry and then placed on nylon mesh on top of the moistened filter paper laid on a 15 cm d. petri dish (lid plate). The bottom dish with a circular hole at the center (6 cm d) was laid on top of the leaf in such a way that the exposed leaf was divided into two equal parts, with the main leaf vein as demarcation line between the extract-treated and methanol-treated leaf surfaces. The methanol-treated leaf surface was used as the control. One day old fourth instar larvae of *E. varivestis* were collected and pre-starved for one to two hours before using them in the antifeedant assay. Two larvae were introduced into the 6.0 cm area and then the dish was finally covered with another lid plate. The larvae were allowed to feed for 24 hours and the relative feeding inhibition (RFI) was computed using the formula;

$$\text{RFI} = \frac{\% \text{ leaf area consumed in untreated part} - \% \text{ area consumed in treated part}}{\% \text{ leaf area consumed in untreated part} + \% \text{ area consumed in treated part}} \times 100 \%$$

The dead larvae were also counted and recorded. All tests in this study were conducted in five replicates.

Contact toxicity test

The semi-solid extracts were dissolved in acetone. The concentrations evaluated were: 0.001, 0.01, 0.05, 0.10, 0.50, 1 and 5 mg/mL. One microliter of each concentration was applied on the dorsal part of the body of one-day old fourth instar larva and on the ventral part of two- to three-week-old adult beetle. Two controls, acetone-treated and untreated, were used. All treated insects were placed in petri dish and fed with untreated bean leaves. Ten individuals were used per concentration of each extract, replicated three times.

Insect mortality was recorded at 24, 48 and 72 hours. Treated larvae which survived after 72 hours were continually fed with healthy bean leaves until they ceased feeding. Carry-over effect of the extracts on the growth and development of surviving individuals was noted. Abnormalities found on emerging adults were recorded.

No-choice feeding test for insect growth regulator activity

This test was designed to assess possible insect growth regulator (IGR) activity of the extracts. The nether surface of the leaf was painted with each semi-solid extract dissolved in methanol with the concentration causing 80-90% RFI. The set-up was exactly the same as that in the antifeedant test except that the test dish was smaller (15 mm x 90 mm) and the whole leaf surface was treated with the extract. The larvae were continually fed with treated leaves up to the prepupal stage. Larval and pupal abnormalities or deaths were recorded. Adults were allowed to emerge and abnormalities were noted.

RESULTS AND DISCUSSION

Antifeedant activity

Plant extracts exhibiting more than 80% RFI at concentrations lower than 1 mg/mL were rated as having high antifeedant activity, those at 1 mg/mL and higher have moderate level, those with RFI lower than 50% at 5 mg/mL were considered as having low antifeedant activity, while those that did not cause any feeding inhibition had no antifeedant activity. Among the 15 plants evaluated, *D. philippinensis*, *C. amboinicus*, *M. cordata*, *T. diversifolia*, *A. elegans* and *A. cantala* demonstrated antifeedant activity towards *E. varivestis* in decreasing order (Table 2). The non-polar and polar fractions of *D. philippinensis* showed the highest activity. Concentrations of 10-50 ppm in non-polar fractions (hexane and diethyl ether) and 0.05 – 0.10 mg/mL in the polar (ethanol and methanol) fractions caused RFI of more than 80%. Moderate antifeedant activity of *C. amboinicus* was manifested in methanol extract and its essential oil, however, phytotoxicity was observed in the latter. Moderate antifeedant activity was also found in the diethyl ether and ethyl acetate fractions of *M. cordata* and *T. diversifolia*, both of which belong to family Asteraceae. In addition to the antifeedant activity exhibited by *A. elegans*, stomach toxicity could be involved since the insects became moribund and died 24 to 48 h after ingesting the treated leaf. The chloroform extract of *A. cantala* also showed promising results.

Table 2. Antifeedant activity of different crude extracts of selected Philippine plant species on one day-old 4th instar larvae of *Epilachna varivestis*.

Plant Species	Plant Part	Extract	Conc'n (mg/mL)	RFI* (%)
<i>Derris philippinensis</i>	Root	Hexane	5	100.00
			1	99.00
			0.50	98.46
			0.10	91.96
			0.05	91.40
			0.01	91.65
			0.001	1.29
			0.001	1.29
		Chloroform	5	100.00
			1	100.00
			0.50	95.54
			0.10	87.81
			0.05	88.54
			0.01	58.97
			0.001	7.21
			0.001	7.21
		Ethanol	5	100.00
			1	100.00
			0.5	95.54
			0.10	87.81
			0.05	88.54
			0.01	58.97
			0.001	7.21
			0.001	7.21
Methanol	5	100.00		
	1	93.56		
	0.50	89.99		
	0.10	83.78		
	0.05	82.46		
	0.01	50.59		
	0.001	0.00		
	0.001	0.00		
<i>Coleus amboinicus</i>	Stem & Leaves ^a	Petroleum ether	5	30.00
			1	30.00
		Methanol	5	83.78
			1	83.78
		Volatile ^b Oil	5	94.60 ^p
			1	13.94

Table 2. cont'd.

Plant Species	Plant Part	Extract	Conc'n (mg/mL)	RFI* (%)
<i>Tinospora rumphii</i>	Vine	Petroleum ether	5	3.00
		Dichloromethane	5	2.00
		Ethyl acetate	5	37.00
		Methanol	5	1.00
<i>Lantana camara</i>	Leaf	Petroleum ether	5	4.3
		Dichloromethane	5	34.04
		Ethyl acetate	5	34.05
		Methanol	5	3.00
<i>Dipterocarpus warburgii</i>	Leaf	Petroleum ether	5	15.00
		Dichloromethane	5	35.00
		Ethyl acetate	5	39.9
		Methanol	5	3.16
<i>Mikania cordata</i>	Leaf	Petroleum ether	5	26.66
			5	26.66
			1	56.63
			0.5	34.00
		Ethyl acetate	5	98.93
			1	38.00
			0.50	36.00
			5	4.30
<i>Dioscorea hispida</i>	Tuber	Petroleum ether	5	19.00
		Dichloromethane	5	2.05
		Ethyl acetate	5	11.00
		Methanol	5	1.40
<i>Tithonia diversifolia</i>	Leaf	Petroleum ether	5	4.44
			5	88.14
			1	72.61
			0.50	57.84
			0.05	57.84
			0.01	12.30
	Leaf	Ethyl acetate	5	82.12
			1	58.97
			0.5	24.20
			5	0.00
Flower ^a	Methanol	5	0.00	

Table 2. cont'd.

Plant Species	Plant Part	Extract	Conc'n (mg/mL)	RFI* (%)	
<i>Piper betle</i>	Leaf	Petroleum ether	5	0.00	
		Dichloromethane	5	0.00	
		Ethyl acetate	5	0.00	
		Methanol	5	0.00	
	Vine	Petroleum ether	5	0.00	
		Dichloromethane	5	0.00	
		Ethyl acetate	5	0.00	
		Methanol	5	0.00	
<i>Donax cannaeformis</i>	Root	Petroleum ether	5	0.00	
		Dichloromethane	5	0.00	
		Ethyl acetate	5	0.00	
		Methanol	5	0.00	
<i>Leea aculeata</i>	Stem	Petroleum ether	5	0.00	
		Dichloromethane	5	0.00	
		Ethyl acetate	5	0.00	
		Methanol	5	0.00	
<i>Entada phaseoloides</i>	Vine	Petroleum ether	5	0.00	
		Dichloromethane	5	0.00	
		Ethyl acetate	5	0.00	
		Methanol	5	0.00	
<i>Caesalpinia pulcherrima</i>	Leaf ^a	Methanol	5	47.00	
	Yellow Flower	Flower ^a	Methanol	5	5.00
	Red Flower	Leaf ^a	Methanol	5	4.00
		Flower ^a	Methanol	5	44.00
<i>Aristolochia elegans</i>	Seed	Ethanol	5	100.00	
			1	70.76	
			0.5	24.00	
			0.10	22.50	
<i>Agave cantala</i>	Trunk	Choloroform	5	95.00	
			1	42.31	
			0.50	8.65	

*RFI – Relative Feeding Inhibition

^a Direct methanol extract^b With phytotoxic effect

Contact toxicity on adults and larvae of *E. varivestis*

All the extracts of the plants bioassayed for antifeedant activity (Table 2) were evaluated for contact toxicity. *D. philippinensis* was the most toxic to both adults and larvae of *E. varivestis* (Table 3). All the root extracts of *Derris* were highly toxic to both larva and adult (90 to 100% mortality) at 1 to 10 μg except for methanol extracts which caused 60% mortality only. The methanol leaf extracts caused zero to 20% mortality even at 50 mg/adult or larva. *Derris* fractions showed acute toxicity causing knockdown effect, convulsion and paralysis. The extracts of *T. rumphii*, *L. camara*, *D. warburgii*, *M. cordata*, *D. hispida* and *C. pulcherrima* showed weak toxicity (20-30% mortality) at 50 μg to both larvae and adult beetles. The other plant extracts were not toxic (0-10%) even at 50 μg /adult.

Table 3. Contact toxicity (by topical application) of the different crude extracts of *D. philippinensis* to larva and adult Mexican bean beetle at 24 hours after treatment.

Plant Part	Extract	Dosage $\mu\text{/adult}$	Percent Mortality	
			Adult	Larva
Root	Hexane	10	100	100
		5	100	100
		1	100	100
		0.5	10	60
		0.1	0	30
	Chloroform	10	100	100
		5	100	100
		1	50	100
		0.5	10	60
		0.1	0	30
	Ethanol	10	100	100
		5	90	100
		1	90	100
		0.5	50	90
		0.1	10	50
	Methanol	10	100	100
		5	90	100
		1	60	70
		1	60	70
		0.5	10	40
Leaf ^a	Methanol	50	20	10
	Chloroform	50	0	20
Control 1 (Acetone)			0	0
Control 2 (Water)		0	0	

^a Direct methanol extraction

Insect growth regulatory activity

Of the ten plant species bioassayed for insect growth regulatory (IGR) activity, abnormalities in emerged adult beetles were observed only on those from 4th instar larvae fed with bean leaves treated with sublethal doses of the extracts from *D. philippinensis*, *C. amboinicus*, *L. camara*, and *M. cordata*. However, only 10-30% of the test insects showed abnormalities and these were not typical for IGR activity, but rather for chronic toxic effect. On the other hand, the larval survivors from those topically treated with *Derris* extracts at 0.05 or 0.1 $\mu\text{g}/\text{larva}$ and were fed with bean leaves until adult stage showed varying degrees of abnormalities which included failure to undergo pupation, partial molting of the larvae, and pupae and adults with deformed wings.

Larvae fed with bean leaves treated with *A. elegans* extracts at 5 mg/mL died a few days after feeding, indicating stomach toxicity of the extract. Normal adults emerged from larvae fed with leaves treated with other plant extracts (*T. rumphii*, *D. hispida*, *C. pulcherrima*, *D. warburgii*, *A. cantala*) at 5 mg/mL. To ascertain the absence of IGR activity of the test plants, first or second instar larvae should be used in screening tests.

Compounds reported to be present in the plants evaluated

The insecticidal activity of *D. philippinensis* could be attributed to rotenoids present in the genus *Derris* (Dobouzet 1988). Studies on the insecticidal activity of *A. elegans* revealed non-preference by, and antifeedant activity against Asian corn borer *Ostrinia furnacalis* (Guenee) (Caasi-Lit and Morallo-Rejesus, 1989) and the common cutworm *Spodoptera litura* F. (Caasi-Lit and Morallo-Rejesus, 1990). Insecticidal activity of *A. elegans* was also observed by Caasi-Lit and Morallo-Rejesus, (1997) on papilionid butterfly *Troides rhadamanthus* Lucas. They suggested that the active compound responsible for the insecticidal activity of *A. elegans* was aristolochic acid found in the leaves and seeds of the plant. Other compounds in *A. elegans* are lignins, kaurene diterpenes, and alkaloids (El-Sebakhy and Waterman, 1984; El-Sebakhy et al, 1989) as well as sesquiterpenes in particular β -caryophyllene, isocaryophyllene, bicyclogermacrene and E-nerolidol (Vila et al, 1997). Based on the chemotaxonomic review, the antifeedant and insecticidal activity of other plants could be attributed to the different groups of secondary metabolites typical for the respective genera. The essential oil of *C. amboinicus* contains monoterpenes and sesquiterpenes (Pino et al, 1990; Bos and Hendriks, 1983). The compounds present in *D. warburgii* include sesquiterpenes, triterpenes, proanthocyanidins and flavonoids (Hegnauer, 1966a; Hegnauer, 1989). Several sesquiterpene lactones had been characterized and identified from the leaf of *T. diversifolia* (Cariño and Morallo-Rejesus, 1982; Baruah, et al., 1979; Schuster, et al, 1992; Bordoloi, et al, 1996). The tubers of *D. hispida* contain high concentration of tropane alkaloids and traces of saponins (Hegnauer, 1966b). Triterpenes, flavonoids, iridoids, phenylpropanoid lycosines and verbacosides are present in the leaf of *L. camara* (O'Neill et al, 1998; Misra et al, 1997). The antifeedant activity of *M. chordata* could be attributed to germacranolides (Aguinaldo et al, 1995).

CONCLUSION AND RECOMMENDATION

Based on the results of the study undertaken, it can be concluded that some local plant species are potential good sources of antifeeding compounds for possible use in insect pest management. It is worth noting that five of the six plant species which exhibited high antifeeding property have recorded use as ethnomedicine, suggesting safety to man. Aside from *D. philippinensis* which is a known pesticidal plant, no other test species showed high contact toxicity.

It is recommended that follow-up studies be conducted to determine if the plant extracts which showed antifeeding action against *E. varivestis* are effective also against the local species *E. philippinensis* and other coleopterous pests attacking vegetable crops. If found effective likewise, their use as antifeedant sprays should be a welcomed alternative to chemical pesticides most especially in organic and backyard vegetable production.

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