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EFFECTS OF *ARISTOLOCHIA* EXTRACTS ON THE DIAMOND-BACK MOTH, *PLUTELLA XYLOSTELLA* (L.)[1]

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ABSTRACT

Crude extracts of *Aristolochia tagala* Cham. and *A. elegans* Mast. were toxic, repellant, antifeedant and growth inhibitory to the diamond-back moth, *Plutella xylostella* (L.). Free choice and no-choice experiments were conducted to further demonstrate the repellant and antifeedant activity of the toxic extracts. Extracts of *A. elegans,* which was found to be more toxic to the test insects.

Key words: antifeedants, *Aristolochia tagala, Aristolochia elegans,* diamond-back moth, growth inhibitors, *Plutella xylostella*

INTRODUCTION

Crucifer production in the uplands is undoubtedly one of the biggest users of chemical pesticides. The evolution of resistance of major insect pests to pesticides and the development of secondary pests into primary pests were due to repeated and prolonged use of these chemicals. In the search for a humane and environmentally safer approach to insect pest control, the use of insecticidal plants is becoming a popular alternative (Morallo-Rejesus 1987). In the tropics, there is a vast array of plant sources with naturally occurring compounds that may be utilized for this purpose and one of them is the *Aristolochia* species.

Previous studies by Caasi-Lit & Morallo-Rejesus (1989 & 1990) showed that two species of *Aristolochia, A. tagala* and *A. elegans,* were repellent, antifeedant and growth inhibitory to the Asian corn borer, *Ostrinia furnacalis* (Guenee) and the common cutworm, *Spodoptera litura* (Fabricius). In addition, *A. elegans,* being a non-natural host plant proved to be toxic to the common birdwing, *Troides rhadamantus* Lucas (Caasi-Lit & Morallo-Rejesus, 1997). The common constituent in several species of Aristolochiaceae is believed to be aristolochic acid. This was first isolated and purified on *A. reticulata* and *A. indica* by Coutts *et al.* (1957) and *A. bracteata* by Rao *et al.* (1959). In the study of Morallo-Rejesus *et al.* (1988), aristolochic acid was isolated and identified from the leaves and seeds of *A. elegans.* They showed that *A. elegans* contained more of this toxic compound compared to *A. tagala,* which apparently confirms the results of previous studies in several insect pests tested.

The Aristolochiaceae are mostly herbs, vines and shrubs from the tropics and sub-tropics which are more commonly known as the Birthwort family. According to

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Ding Hou (1984) who revised this family in the Malesian floral region, there are 13 known species in the Philippines, eleven of these belong to the genus *Aristolochia* and 2 belong to *Thottea*. All but one are native to the Philippines, with 6 being endemic and found only in the Philippines. The only exotic species is *Aristolochia elegans* Mast., originally from South America and introduced as ornamental because of its beautiful, purplish and very attractive flowers.

Aristolochia species are also hosts of beautiful birdwing butterflies. An example of this is *A. tagala* which is also known in local languages as *altan, malaubi, parul-parolan, timbangan, timbang-timbangan* (Tagalog); *goan-goan* (Cebuano); *kamkamalau* (Igorot) and *nagerus* or *taoin-taoin* (Ilocano). This plant is a twining, suffrutescent and glabrous vine that flowers from March to December and found mostly at median altitudes.

On the other hand, *A. elegans,* also known as the Dutchman's pipe or Calico flower in English or *itik-itik* in Filipino, was found to be highly toxic to the Common birdwing, *Troides rhadamantus rhadamantus* (Lucas) (Jumalon *f.*, 1978; Caasi-Lit & Morallo-Rejesus 1997) and the Richmond birdwing, *Ornithoptera richmondi,* in Queensland, Australia (Sands & Scott 1997).

This study aims to compare the growth and development of the diamond-back month, *Plutella xylostella* (Linnaeus) fed with different concentration of the crude extracts of *A. elegans* and *A. tagala*. It will also further demonstrate the growth inhibitory, repellant and antifeedant activity of the extacts to DBM.

MATERIALS AND METHODS

Mass Rearing of Test Insects

Pupae of diamond-back moth were secured from Cyanamid Agricultural Research Foundation, Inc. (CARFI) through Dr. Mateo P. Ferino. The pupae were placed in a refrigerator to allow uniform emergence of the adult. To induce oviposition, a supply of 10% sugar solution was given. Eggs were collected and hatched as they reached the desired instar. Some of the adults were also allowed to lay eggs on live pechay (*Brassica pekinensis* L.) and cabbage plants. The ones used for the growth inhibitory experiment were live pechay leaves while cabbage leaves were used for the repellent and antifeedant experiments.

Mass Production of Aristolochia Species

Aristolochia elegans and *A. tagala* were grown and maintained following the same procedures as in Caasi-Lit & Morallo-Rejesus (1997). The plants were abundantly grown at the back of the Biological Science Building, Entomology Wing. Mature leaves of the two species were collected, air-dried and ground.

Aristolochia extracts and bioassay

The extraction process was cited in Caasi-Lit & Morallo-Rejesus (1989). Crude extracts of *Aristolochia tagala* and *A. elegans* were prepared. For determining the effects of *Aristolochia elegans* on the growth and development of the diamond-back moth, pechay leaves were used instead of cabbage as the latter. The leaves were

sprayed with *A. elegans* acetone extract with a concentation of 30, 60 and 100 mg/ml. Untreated leaves served as the control. Thirty third instar larvae were placed into the treated and untreated leaves and fed until the last stage of growth. Percent pupation was recorded based on the total number of larvae infested. Percent emergence of adults was taken based on the total number of larvae tested.

Repellant and Antifeedant Effects

Choice and no-choice tests were done to study the repellant and antifeedant properties of the *Aristolochia* extracts. The crude extract was dissolved in 50 ml of petroleum ether. One ml aliquot was diluted with nine ml acetone equivalent to 0.1 g/ml. From this, the acetone extract was sprayed into the cabbage leaves in concentration of 30, 60 and 100 mg/ml. After air-drying, the leaves were placed in a Petri dish and arranged in a circular manner including the control, untreated cabbage leaves. For the choice test, ten third instar larvae were released at the center of the dish. For the no-choice test, ten third instar larvae were placed directly on the surface of each leaf and only treated leaves were exposed to the larvae. Both tests were conducted in three replications.

The number of insects that visited each leaf was counted and their responses or behavior were observed for two days with the following specific hour readings: 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48.

Leaf consumption was also measured after 48 hours in both choice and no choice tests. Body weight gain or loss of larvae of DBM fed with cabbage leaves treated with either *A. elegans* and *A. tagala* were taken at 12, 24 and 48 h.

Statistical Analyses

Data were analyzed using Randomized complete block design (RCBD) and analysis of variance for the choice and no-choice tests while complete randomized design (CRD) was used for the effect on growth and development.

RESULTS AND DISCUSSION

Effects of *A. tagala* and *A. elegans* on Diamond-back moth

The toxic and growth inhibitory effects of *A. elegans* acetone extract to diamond-back moth were very apparent (Table 1, pupa and adult). Only 40% of the pupae survived when treated with 30 mg/ml extract and even a lower pupal percentage was observed when the concentrations of the extract were higher. No adults emerged at higher concentration and only 6.66% survived at 30 mg/mls. The larval mortality can be attributed to the antifeedant property of the plant. In this experiment, 75% of the larvae was observed to move away from the treated leaves and die due to non-feeding. Generally, some of those larvae that fed were able to pupate but failed to emerge as adults.

These results clearly indicate that *A. elegans* contain a growth inhibitory principle. This plant, acting also as toxic and antifeedant to *Troides rhadamantus* (Caasi-Lit and Morallo-Rejesus 1997) could be a potential source of natural insecticide which is shown to be effective against the test insects. The abnormalities observed were

Table 1. The rate of normal pupation and adult emergence of DBM larvae reared on pechay(*Brassica pekinensis*) leaves sprayed with *A. elegans* extract[a].

Concentration (mg/ml)	Normal Pupae[b] (%)	Normal Adults[b] (%)
Control	83.3 a	76.6 a
30	40.0 b	6.7 b
60	23.3 c	0.0 b
100	13.3 c	0.0 b

[a] Based on 30 third instar larvae.
[b] Means having a common letter are not significantly different at $\alpha = 0.05$ by DMRT

similar to those observed on corn borer, cutworm an diamond-back moth treated with *Attacus* juvenile hormone and triflubenzuron (Morallo-Rejesus, 1979). They indicated that the principle has a juvenile-like action disrupting the early stages of insect growth among the test insects.

Antifeedant and Repellent Effects of *Aristolochia*

The number of larvae that visited the leaves were much lower in the treated cabbage leaves as compared to the control (Figure 1). Only one or not even a single larva in the span of time they were allowed to visit, was observed on the treated leaves in extracts of *A. elegans*. On the other hand, five to ten larvae visited the leaves treated with 30 mg/ml extract of *A. tagala* and no larvae were observed on leaves with higher concentrations of the extract. Extracts of both *A. elegans* and *A. tagala* proved to be repellent to the DBM larvae.

In the no choice test where only treated leaves were exposed to the larvae, almost 75% of the test larvae moved away form the leaves treated with either 60 or 100 mg/ml extract (Figure 2). Fifty percent of the larvae remained on the leaves at lower concentation indicating that the extracts were less repellant at this level of concentration.

Significantly less feeding was observed in the treated leaves (Table 2). The data indicate that although there were insects that remained on the leaves as shown also in Figure 1, most of the larvae did not feed. Larval weights were much lower in the treated than those that fed on the control. The antifeedant effect is demonstrated further by the body weight loss observed after 24 and 48 h (Table 3). Larvae treated with either of *A. elegans* and *A. tagala* had loss weight even at the lowest concentration of 30mg/ml.

These results indicate that the extracts have repellent and antifeedant effects on DBM larvae. The extract had caused death by reducing food intake until the insect dies from induced starvation (antifeedant) and also repelled the insects after exposure to the treated leaves without necessarily feeding (repellent). Both *A. elegans* and *A. tagala* were feeding deterrents. In the no-choice test, feeding deterrent was more apparent where larvae remained on the treated leaves without feeding and eventually starved to death.

Both the choice and no-choice experiments clearly demonstrated the repellent and antifeedant effects of *Aristolochia* extracts to DBM. According to Wright (1963), a true antifeedant gives insects the opportunity to feed on the plant, but the food intake is reduced until the insects die from starvation while repellants drive the insects away after exposure to the plant without necessarily feeding.

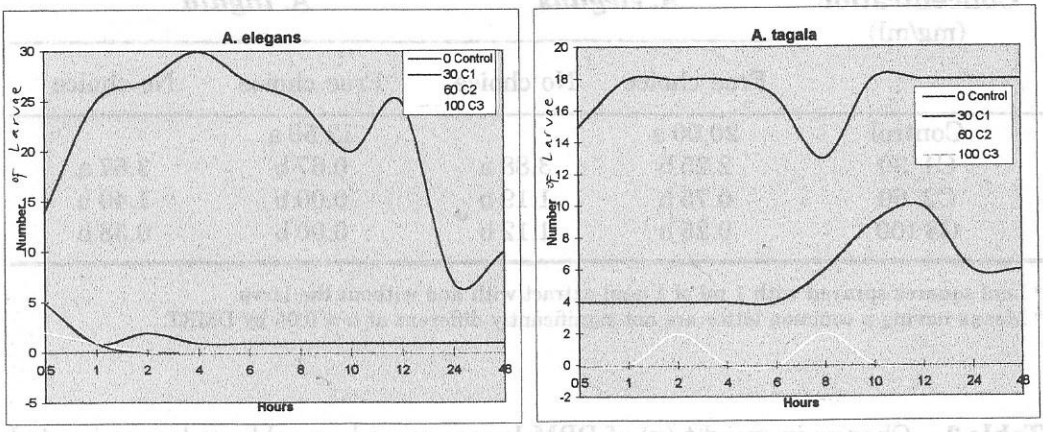

Figure 1. The number of diamond-back moth larvae that visited the diet treated with extracts of *Aristolochia elegans* and *A. tagala* in the choice test experiment.

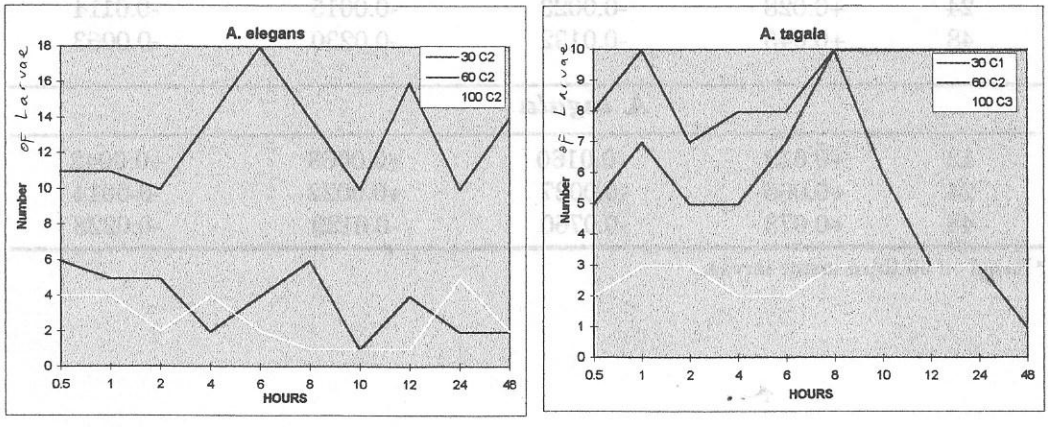

Figure 2. The number of diamond-back moth larvae that visited the diet treated with extracts of *Aristolochia elegans* and *A. tagala* in the no-choice test experiment.

Table 2. Consumption of *Aristolochia* extract-treated cabbage leaves by DBM larvae.[a]

Concentration (mg/ml)	Leaf area (cm^2) consumed per larva[b]			
	A. elegans		*A. tagala*	
	Free choice	No choice	Free choice	No choice
Control	20.00 a		12.50 a	
C1 30	2.25 b	3.88 a	0.67 b	3.67 a
C2 60	0.75 b	1.19 b	0.00 b	1.40 b
C3 100	0.25 b	1.12 b	0.00 b	0.38 b

[a] Leaf squares sprayed with 1 ml of 1 g/ml extract with and without the larva.
[b] Means having a common letter are not significantly different at $\alpha = 0.05$ by DMRT.

Table 3. Change in weight (g) of DBM larvae reared on cabbage leaves treated with *Aristolochia* extracts.[a]

Time (hr)	Weight gain (+) or loss (-) at concentration (mg/ml)			
	Control	30	60	100
		A. elegans		
12	+0.040	+0.0063	+0.0040	+0.0035
24	+0.028	-0.0023	-0.0015	-0.0114
48	+0.045	-0.0132	-0.0230	-0.0063
		A. tagala		
12	+0.013	+0.0160	+0.0063	+0.0042
24	+0.066	-0.0027	+0.0022	-0-0014
48	+0.073	-0.0760	-0.0129	-0.0228

[a] Based on 30 third instar larvae.

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