Philipp. Ent. 7 (6) 583 - 592 ISSN 0048-3753 October 1989

EFFECT OF *ARISTOLOCHIA* EXTRACTS ON THE ASIATIC CORN BORER, *OSTRINIA FURNACALIS* (GUENEE)[1]

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ABSTRACT

Crude extracts of *Aristolochia elegans* Mast. and *A. tagala* Cham., when tested on the Asiatic corn borer, *Ostrinia furnacalis* (Guenee), caused pronounced deformations and malformations in the larval, pupal and adult stages. Extracts of both species of *Aristolochia* revealed growth inhibitory, repellant and antifeedant properties.

Key words: *Aristolochia elegans* Mast., *Aristolochia tagala* Cham., antifeedants, growth inhibitors, Asiatic corn borer, *Ostrinia furnacalis* (Guenee)

INTRODUCTION

Aristolochia spp. (Aristolochiaceae) are hosts to several papilionid butterflies, such as species of *Atrophaneura*, *Trogonoptera* and *Troides*. However, a few *Aristolochia* species exert chemosterilant, antifeedant and/or toxic effects on other insects due to the presence of aristolochic acid, a substance common to the Aristolochiaceae. This compound has been isolated and purified by Coutts *et al.* (1957) from *Aristolochia reticulata* Nutt. and *A. indica* Linn. The same was confirmed by Rao *et al.* (1959) from *A. bracteata* Rotz. and by Pailer and Prackmayer (1969) from *A. clematitis* Linn.

In search for possible sources of botanical insecticides, we determined the effects of *Aristolochia* extracts on the Asiatic corn borer, *Ostrinia furnacalis* (Guenee), the primary pest of corn in the Philippines. For this purpose, two species viz., *A. tagala* Cham. and *A. elegans* Mast., were used. The former is known locally as "timbangan" or "mala-ubi" and is a twining, suffrutescent and nearly glabrous vine. It grows in thickets and flowers from March to October. It is widely distributed in the Philippines, Malaya and India. On the other hand, the latter, also a vine, is commonly known as the Dutchman's pipe or locally as "itik-itik" or "malakamote". It flowers from

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[1] Received August 1988; accepted July 1989. Portion of thesis presented in partial fulfillment of the degree of Bachelor of Science in Agriculture, U. P. at Los Baños, under the guidance of the second author.

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January to October and has heart-shaped leaves with a waxy material on the undersurface (Merrill, 1912).

MATERIALS AND METHODS

Extraction. Six to seven months old mature leaves were harvested, dried, and passed through a Wiley mill to obtain ground samples. Ground leaves (15 g) were soaked overnight in 95% ethanol and subsequently run through a Soxhlet extractor. Several batches of 15-g samples were needed to obtain a sufficient volume of extract. This procedure after Rao *et al.* (1959) was found to yield a slightly higher percentage ethanolic extract (0.7%) than mere maceration of 150 g ground samples in 600 ml 95% ethanol for 14 days (0.5%).

The alcohol extract was combined and concentrated *in vacuo* at reduced pressure in a rotary evaporator and yielded a blackish-green gummy concentrate. This crude extract was stored at O° C for subsequent use.

Mass Rearing of Test Insects. Corn borer larvae and pupae were collected from corn fields in the Central Experiment Station, UPLB University of the Philippines at Los Baños. They were reared to the adult stage in cages and allowed to oviposit on cut pieces of waxed paper ('Cutrite'). Egg masses were placed and reared to the desired larval stages in an artificial diet according to the methods of Camarao (1976).

Bioassay. To study the growth inhibitory effects on corn borer larvae, the crude extract was incorporated into the artificial diet in concentrations of 30, 60 and 100 mg/ml. Upon solidification and cooling, 10 first instar larvae were introduced into each treatment with four replications. Pupal and adult survival percentages were recorded, also noting the abnormalities in each stage.

The repellant and antifeedant effects were studied using choice and no-choice tests. Prior to testing, the crude extract was dissolved in 50 ml petroleum ether. One ml aliquot was diluted with nine ml acetone equivalent to 0.1 g/ml. Five ml of this solution was incorporated into the artificial diet. The solidified diet was then divided into cubes and these were arranged in a circular fashion inside an acrylic pan. The initial weights of the test larvae were taken.

For the choice test, third instar larvae were released at the center of the pan. This was done in three replications with 10 insects per replicate. Control (untreated) diet cubes were also provided.

For the no-choice test, the larvae were placed directly on the surface of the diet cube. Only treated cubes were exposed to the test insects.

The number of insects that visited each cube were counted and their behavior was observed for two days: at 30 minutes, one hour and two hours after introduction, and every two hours thereafter, up to the twelfth hour and at 24 and 48 hours. Final larval weights were also taken.

RESULTS AND DISCUSSION

Growth Inhibitory Effects of *A. elegans*

Larval feeding on artificial diet treated with *A. elegans* crude ethanol extract resulted in developmental abnormalities in the larval, pupal and adult stages. Some larvae that survived to the next instar showed no apparent increase in size and body shrinkage followed after some time (Figure 1). Leg paralysis was detected, followed by blackening of the thoracic portions of the body especially the hindlegs, and of the prolegs. This condition prevented molting to the succeeding instar (Figure 2). Larval-pupal intermediates were also produced (Figure 3). These intermediates had either the anterior portion larval in character and the remaining parts pupal or vice-versa. A few individuals exhibited adult abdomen and pupal head and thorax, hence pupal-adult intermediates. Pupae that survived resulted in abnormal adults (Figure 4). Abnormalities included deformities on the abdomen and wings. Wing deformities consisted of twisting, unevenness, incomplete development and disorientation of fore-and hindwings. In addition, their deformed adults failed to detach themselves from their pupal cases. Unusually large larvae, pupae and adults which were often abnormal were also observed (Figure 5).

Table 1. Percentage pupal and adult survival of corn borer reared on a diet treated with crude ethanol extract of *Aristolochia elegans* Mast.[a/]

Treatment (mg/ml)	Survival (%)			
	Pupa		Adult	
	Normal	Abnormal	Normal	Abnormal
Control	93.3	6.6	90.0	0.0
30	30.0	63.3	23.3	50.0
60	50.0	43.3	40.0	43.3
100	26.6	63.3	23.3	46.6

a/ Based on four replications, with 10 first instar larvae each.

Table 1 summarizes the percentage pupal and adult survival of corn borer when reared on a diet treated with *A. elegans* crude ethanol extract. The results showed the highest number of abnormal pupae and adults at 30 and 100 mg/ml. These clearly indicated that the extract is growth inhibitory to the Asiatic corn borer.

The developmental abnormalities were similar to those observed on corn borer and other insects treated with *Attacus* juvenile hormone and triflubenzuron (Morallo A Rejesus, 1979; Paguia and Morallo-Rejesus, 1977). Such a similarity suggests that the bioactive principle has probably a juvenile hormone-like mode of action.

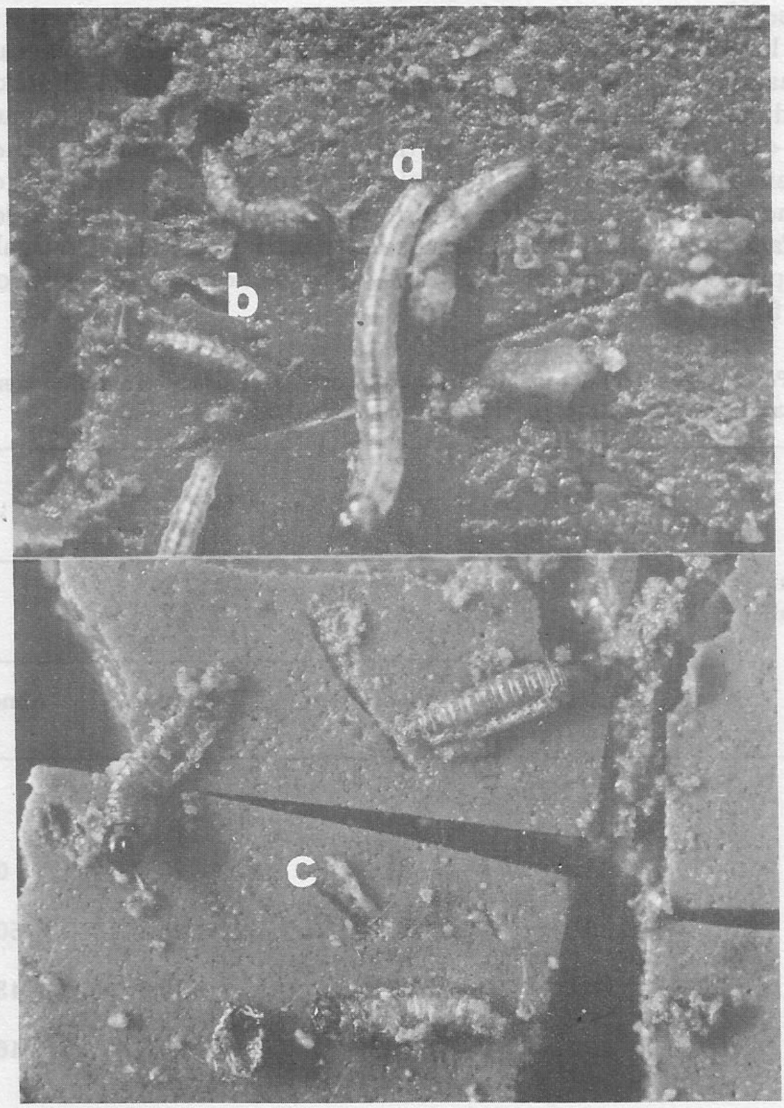

Figure 1. Selected examples of abnormal corn borer larvae obtained on diet treated with *A. elegans* crude extract. A. Normal larva. B. Molted but undersized larvae. C. Shrunk larvae.

Repellant and Antifeedant Effects of *A. elegans* and *A. tagala*

The repellant effect of *A. elegans* and *A. tagala* was measured by the number of insects visiting and initially feeding on the diet at different time intervals in the choice test (Figure 6). More insects were recorded in the control diet than in the diet treated with extracts at all time intervals for both species. The larvae that visited and initially fed on the treated diet tended to to wander afterwards. This showed that extracts of both species are repellant and antifeedant to the corn borer; *A. elegans* was evidently more so, as fewer larvae visited the treated diet and made fewer bites.

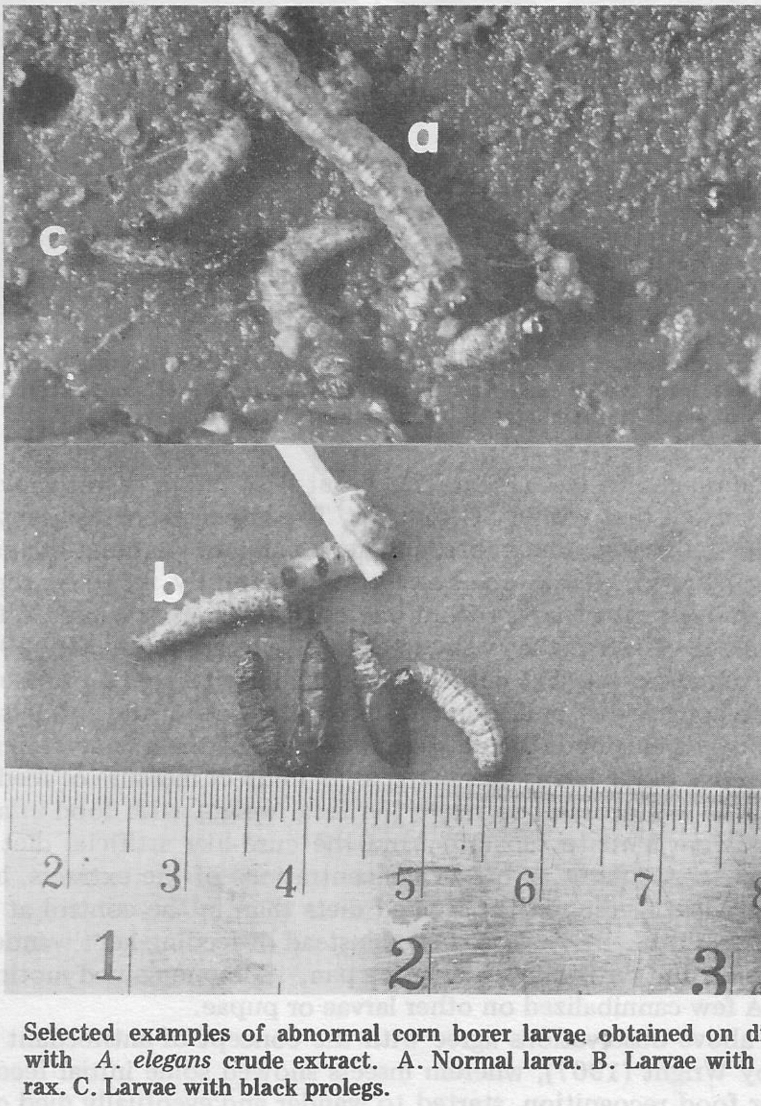

Figure 2. Selected examples of abnormal corn borer larvae obtained on diet treated with *A. elegans* crude extract. A. Normal larva. B. Larvae with black thorax. C. Larvae with black prolegs.

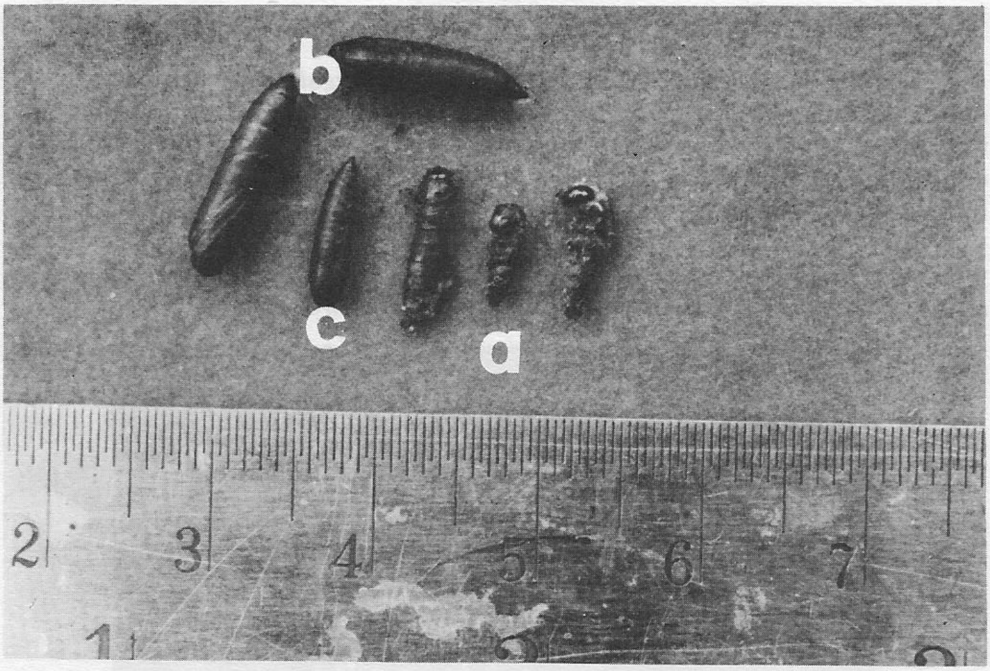

Figure 3. **Selected examples of developmental abnormalities of corn borer when larvae were reared on a diet treated with *A. elegans* ethanol extract. A. Larval-pupal intermediates. B. Giant pupae. C. Deformed pupae.**

In the no-choice test (Figure 7), about 25% of the larvae moved away from the treated diet within 30 minutes. The insects were disoriented when exposed to the treated diet cubes, and kept wandering around the pan. This wandering behavior in the no-choice tests apparently reflected repellency. Although movement of larvae from one cube to another caused differences in the number of larvae that visited the diets, yet approximately 50% of the larvae remained on the diet cubes. Their inability to feed on treated diet as much as on control diet indicated anti-feedant effects of the extracts.

To further elucidate the antifeedant effect of the extracts, weight gain or loss of corn borer larvae was taken as an indirect measure of feeding/consumption. It was difficult to relate the fecal weight with food intake as fecal pellets were hard to separate from the curd-like artificial diet. Larval weight loss, particularly at higher concentrations of the extracts, indicated significantly less feeding in the treated diets than in the control at both 12 and 24 hours (Table 2). Some larvae instead of feeding kept wandering on the top, sides and bottom of the rearing pan. Some remained motionless, as if dead. A few cannibalized on other larvae or pupae.

The above observations agree with the concept of antifeedant effect as defined by Wright (1967), wherein insects showed some initial feeding, and then after food recognition, started to wander and eventually died of starvation. The *A. elegans* crude extract was a stronger feeding deterrent as shown by the higher percent weight loss in 24 and 48 hours.

Growth Inhibitory and Antifeedant Principle

The substance causing growth inhibitory and antifeedant effects on the Asiatic corn borer is the aristolochic acid. The same substance was isolated and identified by Morallo-Rejesus *et al.* (1988) by spectrophotometric methods from the leaves and seeds of *A. elegans*. Probably the same toxic principle occurs in *A. tagala*.

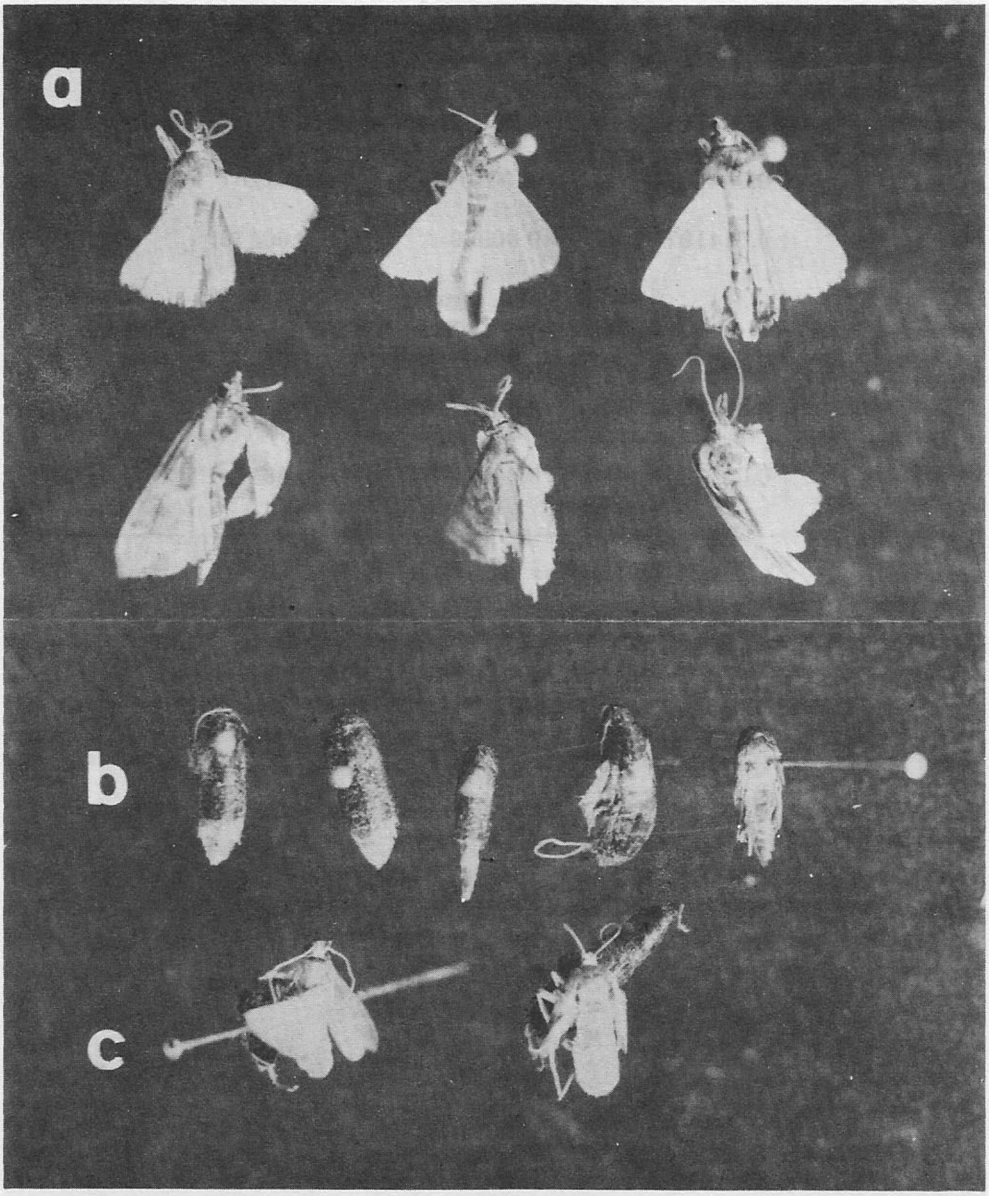

Figure 4. Selected examples of developmental abnormalities of corn borer when larvae were reared on a diet treated with *A. elegans* crude extract. A. Adults with crumpled, shortened wings or with hindwings curved upwards. B. Pupal A adult intermediates. C. Deformed adults with attached pupal cases.

Table 2. Weight gain (+) and loss (—) of corn borer larvae fed with artificial diet treated with either. *A. elegans* or *A. tagala* crude extract.[a/]

Time (hrs)	Concentration (mg/ml)			
	Control	30	60	100
		A. elegans		
12	+0.00076	−0.000021	+0.0039	−0.00021
24	+0.00410	+0.001075	−0.01167	−0.0103
48	+0.014187	+0.00959	−0.00470	−0.0077
		A. tagala		
12	+0.0036	+0.0014	+0.0072	−0.007
24	+0.0090	+0.0099	−0.0008	−0.0013
48	+0.0309	−0.0009	−0.0015	−0.0041

a/ Average of 10 larvae per treatment in three replications.

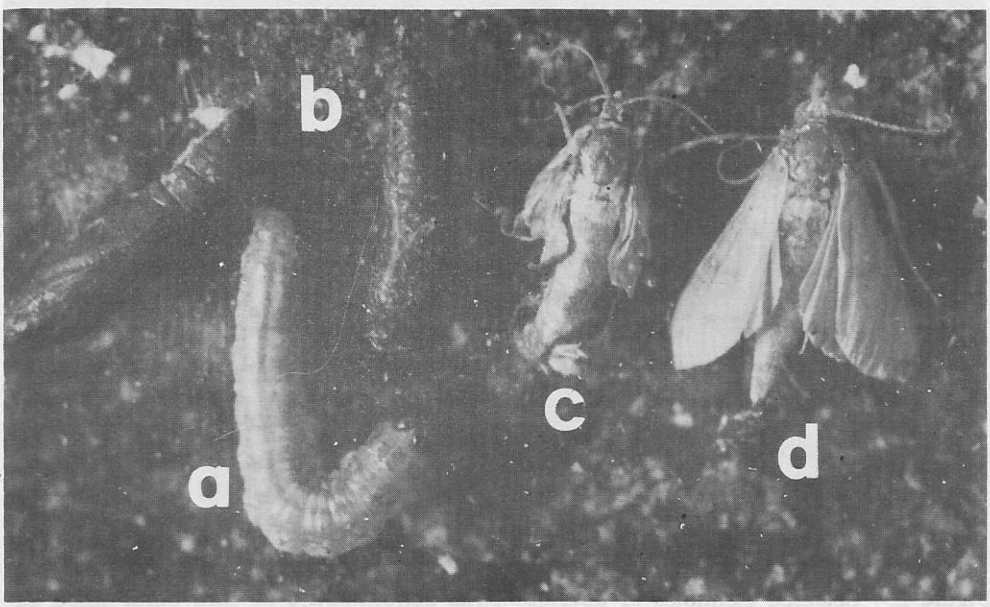

Figure 5. Selected examples of developmental abnormalities of corn borer when larvae were reared on a diet treated with *A. elegans* crude extract. A. Giant larva . B. Giant pupa. C., D. Giant but abnormal adults

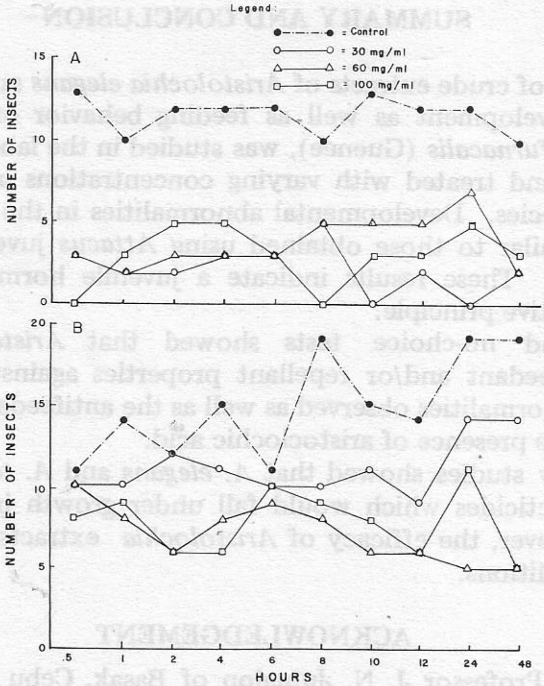

Figure 6. The number of corn borer larvae that visited the diet treated with extracts of *Aristolochia elegans* (A) and *A. tagala* (B). (Choice test).

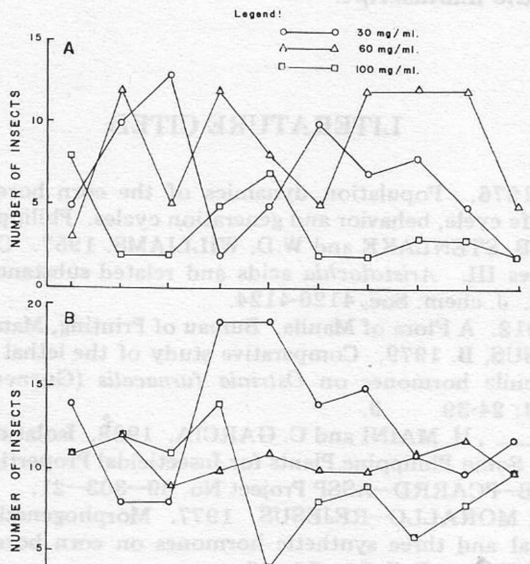

Figure 7. The number of corn borer larvae that visited the diet treated with crude extracts of *A. elegans* (A) and *A. tagala* (B). (No-choice test).

SUMMARY AND CONCLUSION

The effect of crude extracts of *Aristolochia elegans* and *A. tagala* on the growth and development as well as feeding behavior of the Asiatic corn borer, *Ostrinia furnacalis* (Guenee), was studied in the laboratory. Artificial diet was used and treated with varying concentrations of extracts of both *Aristolochia* species. Developmental abnormalities in the larvae, pupae and adults were similar to those obtained using *Attacus* juvenile hormone and triflubenzuron. These results indicate a juvenile hormone-like mode of action of the active principle.

Choice and no-choice tests showed that *Aristolochia* extracts possessed antifeedant and/or repellant properties against the Asiatic corn borer. The abnormalities observed as well as the antifeedant properties were attributed to the presence of aristoclochic acid.

Laboratory studies showed that *A. elegans* and *A. tagala* are potential sources of insecticides which would fall under growth inhibitors and antifeedants. However, the efficacy of *Aristolochia* extracts remains to be tested in field conditions.

ACKNOWLEDGEMENT

We thank Professor J. N. Jumalon of Basak, Cebu City for providing seeds of *Aristolochia elegans* and *A. tagala* from his butterfly sanctuary and for information on other places where these species might be collected. We also thank Mr. Ireneo L. Lit, Jr. for his criticism and suggestions for the improvement of the manuscript.

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