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Efficacy of three plant species on the mortality and food consumption of *Epilachna vigintioctopunctata*

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Abstract

The Evaluation of *Azadiracta indica* (Neem), *Annona reticulata,* (Custard apple) and *Annona squamosa* (Sugar apple) under laboratory conditions showed their potential as antifeedants and insecticides for the control of *Epilachna vigintioctopunctata.* Methanol extracts of all three plants elicited a highly significant reduction in food consumption from the *Epilachna* larvae. When compared with the other two plant extracts, *.A. squamosa* produced a very strong antifeedant effect on the larvae at all concentrations tested. At the highest concentration (20.0g/l)., none of the larvae consumed leaf disks treated with *A. squamosa* extract at all, but all larvae died immediately after they were introduced to the test medium, indicating a strong contact toxic effect on the larvae. With all the plants, the food consumption decreased with the increase of the concentration. Among the three plants tested, *A. squamosa* was the most effective against the *Epilachna* larvae causing significantly very high mortality even after 24 hours. *A. indica* extract on the other hand, elicited a much more delayed toxic action in the larvae. When treated with extracts of *A. indica,* a positive relationship was observed between larval mortality and concentration of extracts and also between larval mortality and time. Larval mortality increased with the increase of concentration and also with time. However, no larval deaths were recorded for 4 days with any concentration tested and 100% mortality was recorded on the 12th day after treatment at the highest concentration (20.0g/l).

Key Words: *Epilachna vigintioctopunctata,* Plant extracts, Larval mortality, Antifeedant activity

Introduction

Epilachna vigintioctopunctata, commonly known as the Epilachna beetle or cucurbit beetle is a serious pest of cucurbitaceous and solanacaeous crops in Sri Lanka. Both adult and larva feed on epidermal tissues of leaves leaving veins and sometimes the leaf is completely stripped to the midrib causing plant death. The life cycle of the Epilachna beetle takes about twenty five days under local conditions and a few generations can occur during a cropping period causing severe damage to the vegetable crop, which may result in an economic loss to the farmer.

Synthetic insecticides are widely used in most developing countries to control insect pests of food crops. In Sri Lanka, it is reported that more potent wide spectrum insecticides are applied frequently by vegetable farmers without considering the pre-harvest intervals as they aim to obtain absolutely pest free crop harvest (Jayathilake and Bandara, 1989). The enormous quantity of synthetic pesticides used over the last few decades has created serious environmental and health problems (Ahmed et al, 1983; Georgiou and Taylor, 1977: Pimental, 1977). As an alternative to synthetic pesticides, many researchers are now focusing their interest in developing environmental friendly pesticide formulations from plant species with insecticidal properties after the successful exploitation of neem (Schmutterer, 1990; Del Bene, et. al., 2000). Traditionaly farmers in Sri Lanka have been using plant products for pest control. Many Sri Lankan researchers also concentrate their studies on locally available plants with the aim of using them as natural pesticides. According to Vijay Kumar (1987) over 800 plant extracts have been screened and about 16% have shown strong or moderate insecticidal activity. The present study was therefore carried out with the view of investigating the antifeedant and insecticidal effects of the seed extracts of three plant species, *Azadiracta indica* (Neem), *Annona reticulata* (Custard apple) and *Annona squamosa* (Sugar apple) on *Epilachna vigintioctopunctata*

Materials and Mehods

Host plant culture

Bittergourd plants were grown in plastic pots ((one plant/pot) under plant house conditions and in the field in small plots. These plants were watered daily and maintained by applying recommended quantities of fertilizer (Urea and NPK) once in two weeks.

Insect Culture

Epilachna beetles collected from infested field cultivation were introduced into potted cucurbit plants and allowed to mate and oviposit inside fine wire mesh cages (45cm×35cm × 60cm) under laboratory conditions at

28±2°C and 81±2% RH, in order to provide a continuous food supply (i.e. fresh leaves) for adults and larvae, damaged plants were replaced by fresh plants throughout the research period.

Mated female Epilachna beetles were placed in potted plants in cages covered with cylindrical nylon mesh (60cm height & 22.5cm diameter). After oviposition, the adults were removed from the plants. The eggs on the plants were left undisturbed for hatching. Immediately after hatching, the larvae were transferred on to fresh and healthy host plant leaves (1 larva per leaf) by using a soft brush. The cut end of each leaf petiole was wrapped with a wet cotton wad to prevent water loss from the leaves. Each leaf with a single larva was then placed in a Petridish (9 cm diameter) and covered with a fine net. Once a leaf was consumed by the larva, it was replaced by a fresh leaf. The larvae were reared up to adult emergence and the number of instars was determined by their shed skin. This procedure was used to obtain a large number of larvae of the same instar (4^{th}) at the same time for experiments.

Plant extracts

Ripened fruits of *Azadiracta indica* (Meliaceae), *Annona reticulata* and *Annona* squamosa (Annonaceae) were collected from home gardens. Seeds from the fruits were washed with running tap water and dried in an oven at 38°C for two days. These were then finely powdered using a mortar and pestle. Samples were extracted by mixing with methanol (500ml) at room temperature for 24 hours. Each sample was filtered using a Whatman No 1 filter paper. The filtrate was concentrated at 35°C using a rotary evaporator. The crude extract was then dissolved in 95% methanol and a series of concentrations were prepared to test their antifeedant and toxicity effects on Epilachna.

Food Consumption

Feeding response of Epilachna larvae to different plant extracts were determined by using the leaf dipping method. A series of concentrations (1, 2.5, 5, 10, 15,20g/l) were prepared from the three plant species. Leaf disks (3.5cm) cut out from the same position on bittergourd leaves of same age were used in the bioassays. Each of the leaf discs was then dipped in 10ml of the respective extract solutions and allowed to dry at room temperature for 30 minutes. Leaf discs dipped in 95% methanol were used as the control. Subsequently, treated leaf discs were placed on a filter paper in small transparent plastic containers (one leaf disc/container). Fourth instar larvae which were previously starved for three hours were then introduced individually into containers with treated leaf discs (one larva/container) and allowed to feed on leaf discs for six hours. At the end of the test period, the area of the discs consumed by the larvae was measured by using the grid method. This experiment was replicated twenty four times.

At the end of the experiment, percentage antifeedant activity (AA %) was calculated using the following equation.

$$AA\% = \frac{C-T}{C+T} \times 100$$

Where,

C - Leaf area consumed in the control

T - Leaf area consumed in the treatment

Antifeedant activity was classified as follows;

Strong activity	>80%	+++
Moderate activity	61-80%	++
Weak activity	40-60%	+
Little activity	<40%	-

(Kwon et. al., 1994)

Toxic Effects

Toxic effects of the plant extracts were also determined by the leaf dipping method using fourth instar larvae. Seven concentrations (1.0, 2.5, 5.0, 7.5 10.0, 15.0, 20.0g/l) of three plant species were used for the bioassay with five replicates for each treatment.

The experimental setup was similar to the previous bioassay and five fourth instar larvae each were introduced into each container. Mortality counts of larvae were recorded 24 and 48 hours after the introduction of larvae into containers wirh leaves treated with *Annona* extracts. However, with the neem extract, observations were recorded only 4, 8, 12, 16 and 20 days after the introduction of larvae as no larval deaths were observed until 4 days for any concentration tested.

Statistical Analysis

Data were analyzed using MINITAB 11 for windows. One-way analysis of variance (1-way ANOVA) was used to test the significance of different treatments. Whenever the ANOVA indicated a significant difference among them, Tukey test was carried out at p=0.05 level of significance to identify the significant treatments.

Results

Food Consumption

Food consumption of larvae at all concentrations for three plant extracts was significantly lower (p=0.05) than that of the control (Table 1).

Table1. Antifeedant activity (AA %) of third instar larvae of *E. vigintioctopunctata* on leaf discs treated with three plant extracts

Concentration (g/l)	*Annona squamosa* (%)	*Annona reticulata* (%)	*Azadirachta indica* (%)
1.0	64.8 **a**	28.1 **a**	35.1 **a**
2.5	83.4 **b**	49.9 **b**	50.6 **ab**
5.0	92.3 **c**	74.3 **c**	57.6 **b**
10.0	95.9 **cd**	78.9 **c**	77.6 **cd**
15.0	95.9 **cd**	94.3 **d**	88.9 **d**
20.0	100.0 **d**	97.6 **d**	94.3 **d**

Means in each column followed by the same letter are not significantly different at p = 0.05.

The reduction of the food intake was found to be dependant on the concentration and the plant species. Of the three plant species tested, *A. sqauamosa* extract elicited significantly a very strong antifeedant activity (>80%) at all concentrations except at 1.0g/l, where moderate activity (64.8%) was observed (Table 2). On the other hand, *A. reticulata and A. indica* both exhibited very low to moderate activity at 1.0, 2.5, 5.0 and 10.0g/l, while strong activity was observed only at 15.0 and 20g/l concentrations.

Toxic Effects

Toxic effects of two *Annona* extracts on *Epilachna* larvae are shown in Table 3. *A. sqauamosa* compared to *A. reticulata* was found to be the most effective in toxicity tests against Epilachna larvae causing 100% mortality at 7.5g/l (24 hrs after treatments). It also caused high percentage of mortality (80%) even at 5g/l. However, *A. reticulata* at 1.0, 2.5 and 5.0g/l doses were not toxic to larvae. Also, only a 13.3% larval mortality resulted at 7.5g/l and this was not significantly different from that of the control. The highest larval mortality with *A. reticulata* was only 40% and it was significantly higher than those of other concentrations including the control. After 48 hours of the introduction of larvae, 100% mortality was recorded at all concentrations above 5g/l with *A. sqauamosa.* This was significantly higher than those of all the

Table2. Classified Antifeedant Activity (AA %) of third instar larvae of *E. vigintioctopunctata* on leaf discs treated with three plant extracts

Concentration (g/l)	*Annona sqauamosa*	*Annona. reticulata*	*Azadirachta indica*
1.0	++	-	-
2.5	+++	+	+
5.0	+++	+	++
10.0	+++	++	++
15.0	+++	+++	+++
20.0	+++	+++	+++

Strong activity	>80%	-	+++
Moderate activity	61-80%	-	++
Weak activity	40-60%	-	+
Low or no activity	<40%	-	-

other treatments. Also, even at 2.5g/l, 53.3% mortality was recorded. *A. reticulata* was not toxic to the larvae at 1.0 and 2.5g/l concentrations (0%) after 48 hours and only 80% mortality was attained at the highest concentration (20.0 g/l) tested.

No larval deaths were observed when they were exposed to different concentrations of neem extract until the fourth day (Table 4). However, with 20g/l concentration, a 33.3% mortality which was significantly different from the rest of the treatments was observed. Eight days after the larval introduction, larval mortality with neem extracts was 22.2% at 7.5g/l and 66.7% at 20g/l. However, 100% mortality was observed after 12 days at 20g/l. and after 16 and 20 days at 10g/l and above.

From the results it is quite evident that the insecticidal activity of neem on larvae is considerably slower than that of the two *Annona* sp., but the toxic effect of neem increased gradually with time, finally reaching up to 100% at 20g/l after 12 days.

Table3. Effects of *Annona sqauamosa* and *A. reticulata* extracts on the mortality of *E. vigintioctopunctata* larvae, after 24 and 48 hrs of treatment

Concentration (g/l)	% Larval Mortality			
	Annona squamosa		*Annona reticulata*	
	24 h	48 h	24 h	48 h
Control	0 a	0 a	0 a	0 a
1.0	6.7 a	13.4 a	0 a	0 a
2.5	40.0 b	53.4 b	0 a	0 a
5.0	80.0 bc	100 c	0 a	13.3 ab
7.5	100 c	100 c	13.3 ab	26.7 b
10.0	100 c	100 c	20.0 b	53.4 c
15.0	100 c	100 c	26.7 b	66.7 cd
20.0	100 c	100 c	40.0 c	80.0 d

Means in each column followed by the same letter are not significantly different at p = 0.05.

Discussion

The study using neem and two species of *Annona* extracts indicated a highly significant reduction in food consumption by the *Epilachna* larvae. With all the plant extracts, the food consumption decreased with the increase of concentration. However, Antifeedant activity of the larvae on leaf discs treated with *Annona squamosa* was considerably higher at all concentrations when compared with that of the other two plant species. Also, a 100% antifeedant

Table 4: Effects of *Annona indica* extract on the mortality of *E. vigintioctopunctata* larvae at different time intervals

Concentration (g/l)	% Mortality				
	4 days	8 days	12 days	16 days	20 days
Control	0 a	0 a	0 a	0 a	0 a
1.0	0 a	0 a	22.2 b	33.3 b	44.4 b
2.5	0 a	0 a	33.3 b	66.7 c	77.8 bc
5.0	0 a	0 a	33.3 b	66.7c	77.8 bc
7.5	0 a	22.2 b	33.3 b	55.6 c	88.9 cd
10.0	0 a	22.2 b	55.6 c	100 d	100 d
15.0	0 a	33.3 bc	77.8 cd	100 d	100 d
20.0	33.3 b	66.7 c	100 d	100 d	100 d

Means in each column followed by the same letter are not significantlydifferent at p = 0.05.

activity was recorded with A. sqauamosa extracts at the highest concentration (20.0g/l) tested. It is apparent by the results that consumption index (AA %) reflects the degree of acceptability of food by the larvae. All the three plants deterred feeding by the Epilachna larvae as from the concentration of 1.0g/l. Thorsteinson (1960) reported that feeding inhibitors are of primary importance in determining which plants are eaten and the extent to which they can be consumed. Antifeedants which retard feeding activities of insect pests and reduce their damage offer considerable scope in crop protection. These substances could be applied to crop plants in a similar manner as insecticides.

Among the three plant extracts tested, *A. squamosa* was the most effective against Epilachna beetles causing significantly very high larval mortality at concentrations above 5.0g/l. It is of great interest to note that larvae treated with the highest concentration (20.0g/l) of *A. squamosa* extract turned a black colour and died within only a few hours after their exposure to the extract. Although the larval mortality increased with the increase of concentration (for both *Annona* species), any distinct morphological defects of larvae were not observed. On the contrary, the toxic action of Neem on Epilachna larvae was found to be comparatively very much slower than those of *Annona* and a 100% larval mortality was observed only on the 12th day after treatment.

Several biologically active compounds have been isolated from different parts of the neem tree. Azadiractin is found to be the most potent compound, (Butterworth and Morgan, 1968). Neem formulations are said to be very effective for the control of many pests including brown planthopper *Nilaparvata lugens*, rice leaf folder *Cnaphalocrocis medinalis,* rice weevil *Sitophilus oryzae*, white-backed planthopper, *Sogatella furcifera*, brinjal fruit borer *Leucinodes orbonallis* and common cutworm *Spodoptera litura* (Ahmed et al., 1983). Similarly, insecticidal properties if *Annona* species are attributed to acetogenins which are the active principles in a large number of Annonaceous plants (Rupprecht, et al., 1990). Jacobson (1989) stated that the biological effect of *Annona* is direct toxicity to insects, However, Antifeedant effects have also been observed in the present study, although low feeding may be associated with the toxic effects of the chemicals on insect activity (Sharma et al., 1999). In a survey on the use of natural plant products in pest control under traditional farming systems, Ahmed et al, (1983) reported that extracts of *A. reticulata* and *A. sqauamosa* have been used for the control of several crop pests. It is also reported that oil from *Annona* sp. is used by farmers in Vietnam for protecting rice crops from leafhoppers and planthoppers (Saxena, et al., 1983).

Pesticides developed from plants have the potential to play a major role in insect pest management in sustainable agriculture production. Effective control of crop pests by the small-scale farmer in developing countries like Sri Lanka has been limited because of the high cost of insecticides. On the other hand,

plants such as sugar apple, custard apple, and neem can be grown by farmers in developing countries with minimal maintenance and extracts can be made using simple devices. Effectiveness against pests, low cost, environmental safety, and availability of these plants emphasize that extracts or their derivatives should be considered seriously for insect pest control.

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