

Effect of *Azadirachta indica* and *Pongamia glabra* leaf extracts on food utilization and modulation of efficiency of digestive enzymes in *Euproctis fraterna* (Lepidoptera: Lymantridae)

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Abstract. The effect of application of *Azadirachta indica* and *Pongamia glabra* leaf extracts on food consumption and utilization in fifth instar larvae of *Euproctis fraterna*, a serious lepidopterous pest on castor, *Ricinus communis*, is assessed. Reduction in food consumption by *Euproctis fraterna* feeding on castor leaves treated with extract of *Azadirachta indica* was 11% higher than that recorded for castor leaves treated with *Pongamia glabra*. Consumption, assimilation, production and their rates showed a negative correlation with the concentration of the extracts used.

The effect of administration of these two leaf extracts on efficiency of the digestive enzymes invertase, amylase and protease of *Euproctis fraterna* was also assessed. A reduction in activity of these enzymes with the administration of increasing concentration of extracts was also observed.

Leaf extracts of neem and *Pongamia* appear to be most efficacious in controlling *Euproctis fraterna* especially when they are administered along with castor leaves in the fifth instar providing a suitable alternative to synthetic pesticides.

Keywords. Leaf extract; *Azadirachta indica*; *Pongamia glabra*; food consumption; food utilization; enzyme activity; *Euproctis fraterna*; *Ricinus communis*.

1. Introduction

It is being increasingly realised that the potential of several secondary chemical compounds in plants to disrupt or interrupt specific mechanism involving metamorphosis, nutrition, reproduction and behaviour of insects could be exploited in the control of insect pest, offering a safer alternative to the conventional pesticide use (Ananthakrishnan 1987). In this context, use of extracts of such plants like *Azadirachta indica* is gaining momentum since azadirachtin (Aza) is a feeding inhibitor and growth disrupting compound for most insect orders (Garcia *et al* 1986). It affects growth and development (Leuschner 1972; Meisner *et al* 1976, 1978), reduces oviposition (Jacobson *et al* 1978) or interferes with the insect's endocrine system (Sieber and Rembold 1983). Whereas some investigations have been carried out on the efficacy of extracts in seeds and roots of *Azadirachta* and *Pongamia* respectively (Butterworth and Morgan 1971; Srimannarayana *et al* 1987) on the control of insect pests. There is paucity of information on the role of leaf extracts of these plants as an effective antifeedant (Hussain and Masood 1975; Eganjobi and Afolami 1976; Rossner and Zenbity 1986). Hence the present study aims at assessing the effect of application of *A. indica* and *P. glabra* leaf extracts on the food consumption and utilization in *Euproctis fraterna*, a serious lepidopterous pest on castor, *Ricinus communis*. Consumption, digestion and utilization of food plants by herbivorous insect pests are important since consumption indices are

considered to be indirect measurement of the relative susceptibilities of different varieties of crops to pest infestation (Dandapani and Balasubramanian 1980).

Digestive enzymes in insects are generally adapted to the diet on which the species feed (Wigglesworth 1965). Nutritional and environmental factors affect digestive enzymes (Waldbauer 1962; Soo Hoo and Fraenkel 1966; Nalinasundari *et al* 1987). In some cases digestive enzymes can be used as parameters for assessing antifeeding activity (Ishaaya *et al* 1974, 1977, 1980, 1982). Despite availability of ample information concerning biochemical properties of digestive enzymes in various insects (House 1974; Wigglesworth 1974), relatively little is known about their role in insect feeding and insect-host compatibility. Hence, an attempt has been made to assess the effect of administration of *A. indica* and *P. glabra* leaf extracts on efficiency of digestive enzymes invertase, amylase and protease of *E. fraterna*.

2. Materials and methods

The larvae of *E. fraterna* were collected from Thiagarajar College campus and were reared in the laboratory on the leaves of castor (LD 12:12; temp. $30 \pm 1^\circ\text{C}$ at 80% RH). Acetone extract of *A. indica* and *P. glabra* leaves (25 g) were taken in soxhlett for 8 h at $65 \pm 2^\circ\text{C}$ and were evaporated to dryness (taken as 100% concentration). Different concentrations (ppm) were prepared from the above extract by weighing 25 mg of neem and *Pongamia* leaf extracts and dissolving it in 2 ml of acetone and 98 ml of distilled water was added to this to make 100 ml. This gives 250 ppm concentration. Likewise 500, 750 and 1000 ppm were prepared by weighing 50, 75, 100 mg of both extracts and dissolving it in the same procedure.

Third instar larvae of *E. fraterna* were selected and fed with castor leaves soaked in different concentrations of extracts and ED_{50} values were assessed. Growth inhibition at different concentrations were plotted and ED_{50} values were found for the two extracts (figure 1 and table 1).

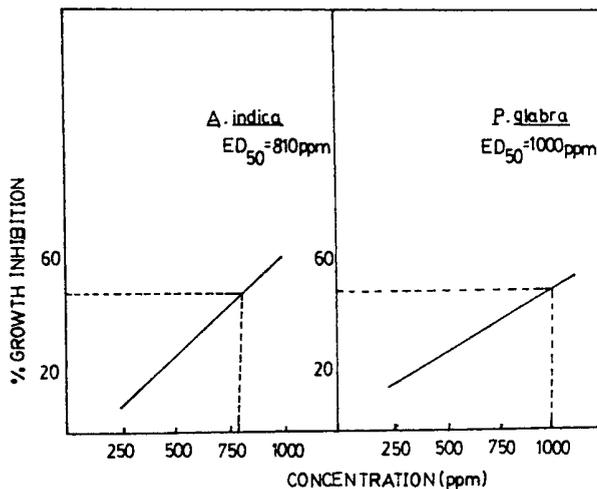


Figure 1. ED_{50} value of third instar larvae of *E. fraterna* treated with different doses of *A. indica* and *P. glabra* leaf extracts.

Table 1. ED₅₀ value for third instar larva of *E. fraterna*.

Plant extract used	ED ₅₀ * value (ppm)
<i>A. indica</i>	810
<i>P. glabra</i>	1000

*Dose required to produce 50% inhibition in growth.

For oral administration experiment, fifth instar larvae were allowed to feed on treated castor leaves and in control experiments, larvae were fed on untreated castor leaves. The effect of plant extracts was assessed on conversion and utilization efficiencies of this pest fed on castor leaves.

2.1 Calculation of feeding budget

The scheme of feeding budget followed in the present work is that of the IBP formula (Petrušewicz and Macfadyen 1970) usually represented as $C = F + U + R + P$, where C is the food energy consumed, F the energy of faeces egested, U the nitrogenous waste excreted, R the energy spent on metabolism and P the growth. Rates of feeding, conversion and metabolism as well as assimilation efficiency and conversion efficiency of the larva were calculated by the following formulae:

$$\text{Conversion rate (Cr)} = \frac{C \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}$$

$$\text{Assimilation rate (Ar)} = \frac{A \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}$$

$$\text{Production rate (Pr)} = \frac{C \text{ (mg/individual)}}{\text{Mid body wt. (g)} \times \text{duration (day)}}$$

$$\text{Assimilation efficiency (AD)} = A/C \times 100.$$

$$\text{Gross production efficiency (ECI)} = P/C \times 100.$$

$$\text{Net production efficiency (ECD)} = P/A \times 100.$$

2.2 Enzyme bioassay

2.2a Preparation of larval enzyme solution and enzyme assays: Extracts of *A. indica* and *P. glabra*-treated castor leaf-fed fifth instar larvae were anaesthetized with cotton pads soaked in chloroform. The gut of each fifth instar larva was dissected free from other tissues by cutting off the larval head with a razor blade and removing the alimentary canal on a wet filter paper using a fine forceps assisted by a slight pressure at the posterior end of the body. The gut was homogenized for 3 min at 3°C in ice-cold citrate-phosphate buffer (pH 6.8) using a chilled tissue

grinder. Brei of the gut was suspended in ice-cold buffer and made up to 1 ml. The homogenate was spun at 12,000 *g* for 15 min. The supernatant was used as enzyme source. Amylase and invertase activities were determined adopting the method of Ishaaya and Swirsky (1970) and protease by the method of Birk *et al* (1962).

3. Results

3.1 Consumption and utilization

The quantity of food consumed by *E. fraterna* was found to be lower when fed on castor leaves treated with leaf extracts of *A. indica* and *P. glabra*. Reduction in food consumption by *E. fraterna* feeding on castor leaves treated with extract of *A. indica* was 11% higher than that recorded for castor leaves treated with *P. glabra* (table 2). This variation is highly significant as shown by *t*-test value (table 2). The concentration of extracts and consumption are significantly correlated negatively (figure 2). Consumption rate (*Cr*) is an index of damage inflicted by insect pests on plants. There was a significant reduction in the rate of food consumption in the later stages of development when compared to control reflecting the level of damage to the tune of 84.325 mg/g/body wt./day in *A. indica* extract-treated insect and 97.843 mg/g/body wt./day in *P. glabra*-treated insect. Whereas the value for the control experiment was as high as 120.338 mg/g/body wt./day (table 2). Two-way analysis of variance reveal that concentration of the extracts and age of the larva significantly alter the consumption rate of the pest ($F = 1236, P < 0.001$).

3.2 Food conversion

A reduction in the quantity of food converted into body weight was observed in *E. fraterna* fed on castor leaves treated with leaf extracts of *A. indica* and *P. glabra*. Percentage of reduction in food converted by *E. fraterna* feeding on castor leaves treated with leaf extracts of *A. indica* was higher (22.66) than that recorded for the food treated with *P. glabra* (17.13) (table 2). This variation is highly significant as shown by '*t*' test value. Apparently there is a significant reduction in the rate of conversion (10.206 mg/g/body wt./day fed on *A. indica*-treated castor leaf and 10.166 mg/g/body wt./day fed on *P. glabra*-treated castor leaf) when compared to control (12.152 mg/g/body wt./day). Two-way analysis of variance reveals that concentration of the extract and age of the larva significantly alter the conversion rate ($F = 30.02, P < 0.001$ with reference to *A. indica*-treated food and $F = 179.17, P < 0.001$ with reference to *P. glabra*-treated food).

3.3 Assimilation and growth efficiencies

Assimilation efficiency (*AD*) showed a decline when insects were fed on leaves treated with extracts of *A. indica* and *P. glabra*. Percentage of decline in assimilation efficiency compared with control was around 15 when larvae were fed on a maximum quantity of 1000 ppm extract (table 2). Two-way ANOVA for both the extracts reveals that the approximate digestibility was significantly influenced by the concentration of the extract and age of the larva ($F = 1467, P < 0.001$ with reference

Table 2. Effect of *A. indica* and *P. glabra* leaf extracts on the feeding parameters of fifth instar larva of *E. fraterna*.

Concentration (ppm)	C	A	P	M	Cr	Ar	Pr	Mr	AD	ECl	ECD
<i>A. indica</i>											
Control	46.517 ± 0.490	36.000 ± 0.560	4.355 ± 0.449	31.645 ± 0.792	120.338 ± 0.439	94.04 ± 0.154	12.152 ± 0.168	81.268 ± 0.295	78.068 ± 0.130	10.545 ± 0.456	13.248 ± 0.463
250	42.537 ± 0.487	30.255 ± 0.595	4.482 ± 0.422	25.773 ± 0.587	108.500 ± 0.488	77.00 ± 0.575	12.285 ± 0.306	65.183 ± 0.374	71.35 ± 0.685	11.445 ± 0.358	15.200 ± 0.434
500	38.172 ± 0.452	27.352 ± 0.514	4.155 ± 0.324	22.000 ± 0.778	99.168 ± 0.363	71.00 ± 0.475	12.355 ± 0.288	59.47 ± 0.416	72.498 ± 0.451	12.100 ± 0.193	17.200 ± 0.428
750	33.115 ± 0.383	23.000 ± 0.565	4.227 ± 0.288	18.773 ± 0.224	86.380 ± 0.416	60.32 ± 0.434	12.183 ± 0.133	48.052 ± 0.862	69.488 ± 0.458	14.278 ± 0.358	20.300 ± 0.598
1000	31.05 ± 0.485	19.600 ± 0.527	3.368 ± 0.414	16.292 ± 0.453	84.323 ± 0.425	53.54 ± 0.382	10.206 ± 0.360	43.077 ± 0.628	63.405 ± 0.507	10.847 ± 0.503	17.131 ± 0.595
	$t = 47.52$	$t = 47.52$	$t = 31.61$								$t = 5.727$
	$P < 0.001$	$P < 0.001$	$P < 0.01$								$P < 0.001$
<i>P. glabra</i>											
Control	46.517 ± 0.490	36.000 ± 0.560	4.355 ± 0.449	31.645 ± 0.792	120.338 ± 0.439	94.04 ± 0.154	12.152 ± 0.168	81.268 ± 0.295	78.068 ± 0.130	10.545 ± 0.456	13.248 ± 0.463
250	46.100 ± 0.260	34.200 ± 0.159	4.125 ± 0.554	30.075 ± 0.654	119.00 ± 0.741	87.000 ± 1.000	12.323 ± 0.489	75.000 ± 0.453	72.383 ± 0.962	10.195 ± 0.640	14.186 ± 0.680
500	45.053 ± 0.131	29.163 ± 0.325	4.268 ± 0.350	24.895 ± 0.625	118.000 ± 0.602	77.347 ± 0.736	12.387 ± 0.481	65.113 ± 0.725	65.255 ± 0.725	10.140 ± 0.366	16.314 ± 0.760
750	41.420 ± 0.329	27.220 ± 0.257	4.150 ± 0.313	23.070 ± 2.348	110.000 ± 0.921	73.294 ± 0.470	11.658 ± 0.936	60.277 ± 0.727	66.069 ± 0.784	11.082 ± 0.648	16.865 ± 0.563
1000	36.113 ± 0.515	23.118 ± 0.260	3.609 ± 0.345	19.509 ± 1.251	97.843 ± 0.987	63.000 ± 0.732	10.166 ± 0.700	85.000 ± 0.956	64.115 ± 0.619	9.993 ± 0.667	15.611 ± 1.036
	$t = 40.79$	$t = 46.67$	$t = 3.00$								$t = 3.744$
	$P < 0.001$	$P < 0.001$	$P < 0.01$								$P < 0.01$

Mean ± SD represents an average of 5 replicates.

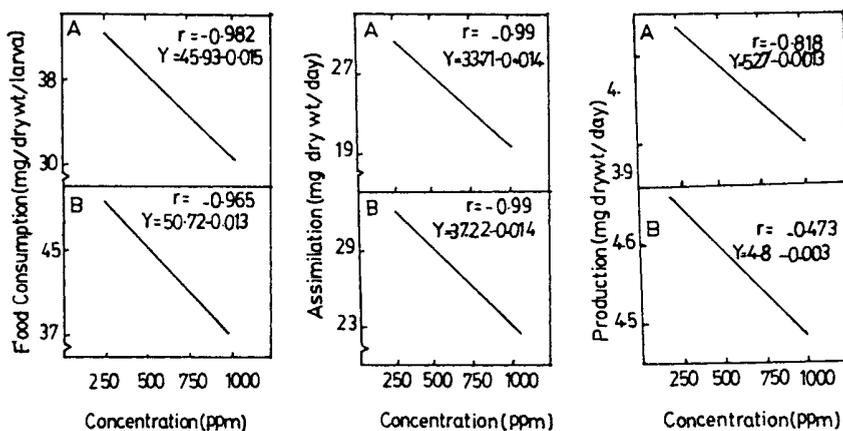


Figure 2. Relationship between food consumption, assimilation and production of fifth instar larva of *E. fraterna* and concentration of the leaf extracts of *A. indica* (A) and *P. glabra* (B).

to *A. indica*-treated food and $F = 103$, $P < 0.001$ with reference to *P. glabra*-treated food). However, growth efficiency of the pest was not affected by these two treatments with extracts whereas this pest developed a compensatory mechanism by increasing its growth efficiency which is statistically significant.

3.4 Impact on enzyme activity

Digestive enzymes can be used as parameters for assessing antifeedant activity. Treatment of fifth instar larvae of *E. fraterna* with extracts of *A. indica* and *P. glabra* along with castor food showed a decreased activity of digestive enzymes invertase, amylase and protease (table 3). A greater inhibition was found in larvae consuming *A. indica* extract-treated food than in *P. glabra* extract-treated food. This variation is highly significant as evidenced by 't' test value. The concentration of the extracts and activity of invertase, amylase and protease are significantly correlated negatively. Two way ANOVA for both the extracts reveals that the activity of the enzymes invertase, amylase and protease was significantly influenced by the concentration of the extracts and age of the larvae. The following F values were calculated:

For invertase

$F = 5.26$ $P < 0.01$ (*A. indica*-treated food).

$F = 3.12$ $P < 0.01$ (*P. glabra*-treated food).

For amylase

$F = 5.50$ $P < 0.01$ (*A. indica*-treated food).

$F = 3.48$ $P < 0.01$ (*P. glabra*-treated food).

For protease

$F = 12.22$ $P < 0.001$ (*A. indica*-treated food).

$F = 30.74$ $P < 0.001$ (*P. glabra*-treated food).

Table 3. Effect of *A. indica* and *P. glabra* leaf extracts on the enzyme activity of invertase μg glucose/reaction, amylase μg maltose/reaction and protease μg tyrosine/reaction in fifth instar larva of *E. fraterna*.

Concentration (ppm)	Invertase $\mu\text{g}/\text{glucose}$ activity	Amylase $\mu\text{g}/\text{maltose}$ activity	Protease $\mu\text{g}/\text{tyrosine}$ activity
<i>A. indica</i>			
Control	2000.00 \pm 319.94	5090.90 \pm 562.45	362.50 \pm 17.62
250	1520.27 \pm 37.56	4064.50 \pm 83.00	322.22 \pm 17.11
500	1077.92 \pm 25.62	3553.84 \pm 242.64	289.99 \pm 63.00
750	988.09 \pm 17.03	3043.47 \pm 52.50	236.36 \pm 3.28
1000	874.41*	2000.00*	205.26*
	$t = 143.706$ $P < 0.001$	$t = 272.78$ $P < 0.001$	$t = 8.365$ $P < 0.001$
<i>P. glabra</i>			
Control	2000.00 \pm 319.94	5090.90 \pm 562.45	362.50 \pm 17.62
250	1092.60 \pm 27.09	4554.16 \pm 48.86	348.00 \pm 9.80
500	1064.10 \pm 26.00	4000.00 \pm 295.32	322.22 \pm 15.81
750	1000.00 \pm 67.08	3000.00 \pm 269.80	251.61 \pm 5.75
1000	943.18* \pm 84.38	2876.74* \pm 23.50	216.66* \pm 7.81
	$t = 147.97$ $P < 0.001$	$t = 251.75$ $P < 0.001$	$t = 8.618$ $P < 0.001$

*Control vs 1000 ppm.

Mean \pm SD represents average performance of 5 individuals.

A direct significant correlation exists between the amount of food consumed and the activity of the 3 enzymes invertase, amylase and protease ($P = < 0.001$) (figure 3).

3.5 Morphological deformities

Varying degrees of deformities such as 'larval-pupal' intermediates were produced when treated with 1000 ppm of neem and *Pongamia* extracts. The emerging adult was quite deformed.

4. Discussion

Neem extracts contain azadirachtin which is tetranortriterpenoid (Garcia *et al* 1984). This azadirachtin from *A. indica* as well as karanjin, pongapin, 3-methoxy pongapin and globachrome from *P. glabra* in general inhibit the physiological functions of the insects and have pronounced antifeedant effect (Butterworth and Morgan 1971; Zanno *et al* 1975; Parmar *et al* 1976; Rembold *et al* 1982; Steffens and Schmutterer 1982; Dorn *et al* 1986; Srimannarayana *et al* 1987). In the present study *E. fraterna* consumed less quantity of food when treated with *A. indica* and *P. glabra* extracts and this reduction in consumption led to diminished feeding rate in both cases. Earlier reports in support of the present findings indicate that in *E. fraterna* the extracts of *Datura metal* caused greater reduction in food consumption and in its feeding rates (Chockalingam *et al* 1983; Nalinasundari 1988). This reduced food consumption might be explained by a behavioural antifeedant effect due to perception by the insects peripheral chemoreceptors

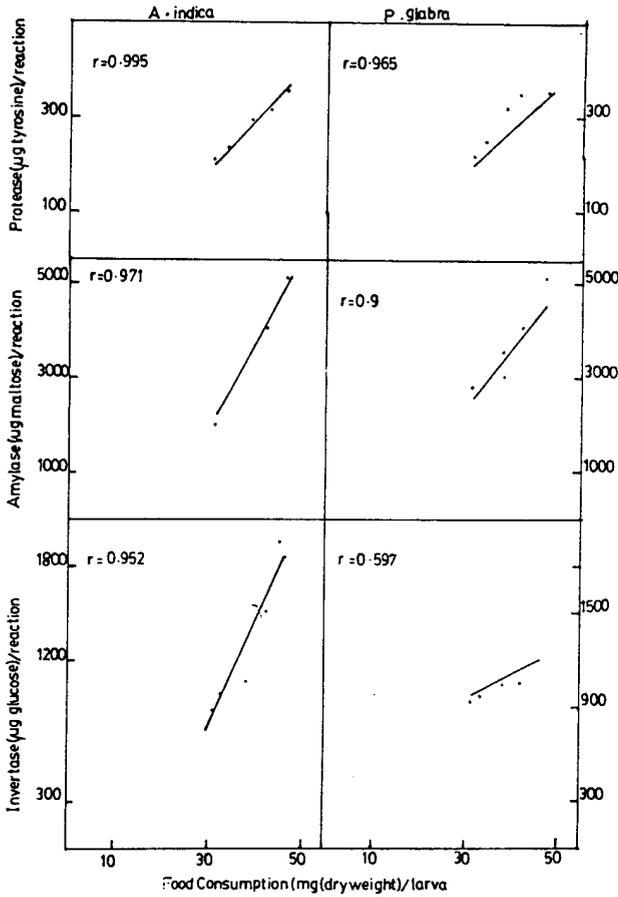


Figure 3. Relationship between food consumption and activity of digestive enzymes treated with extracts of *A. indica* and *P. glabra* to fifth instar larva of *E. fraterna*.

(Bernays 1981). Consumption, assimilation, production and their rates showed a negative correlation with the concentration of the extracts used. This is in conformity with the findings on the use of *Eucalyptus globulus* on food utilization of *E. fraterna* (Nalinasundari 1988). Plant products possess antiherbivore chemicals like tannins, phenol (Hillies 1966) and essential oils (Penfold and Willis 1961) which adversely affected the growth of the insect and its metabolic efficiency (Feeny 1970; Levin 1971; Morrow and Fox 1980).

The polyphagous insect, *E. fraterna* was unable to gain a significant increase in weight compared to control when this insect was subjected to the toxic stress of the extracts of *A. indica* and *P. glabra* leaves (table 2). Studies on the effect of neem oil extractive (NOE) on the food utilization efficiency in *Spodoptera litura* led to the conclusion of the fact that the azadirachtin compound of the NOE might have inhibited proper growth and growth rate (Chockalingam *et al* 1983). This fact has also been substantiated by the present study. Dryer *et al* (1979) showed that pinitol, a fatty acid extract from soya bean leaves reduced the weight gained by the larvae of *Heliothis zea*. Since NOE contains 53% of fatty acid (Attri and Prasad 1980) it is

likely that this higher concentration of fatty acid might have been responsible in blocking the pores of the cellular membrane of the alimentary canal of *E. fraterna* fed on neem extract-treated castor leaf resulting in reduction in growth and growth rates. Deterrence is also reflected by decreased weight gain (Fagoone 1983). Meisner *et al* (1981) also reported small weight gains in *Spodoptera littoralis* larvae with fairly high concentration (0.002% and above) of azadirachtin.

Reduction in assimilation efficiency of *E. fraterna* fed on castor leaves treated with neem and *Pongamia* extracts might be due to reduced activities of digestive enzymes. The hypothesis put forward by Singleton and Kratzer (1973) implicates direct inhibition of digestive enzymes by the extracts. Growth efficiency of the insect appears to have been enhanced by increasing the dosage of both the plant extracts (table 2). A similar pattern of enhanced growth efficiency was also reported in the larvae of *Crocidolomia linotalis* suggesting the fact that this insect is capable of detoxifying to some extent (Fagoone 1983). This explanation may hold good with regard to the enhanced growth efficiency noticed in the present study on *E. fraterna*.

Gut invertase, amylase and protease activities in fifth instar larvae of *E. fraterna* fed on *A. indica* and *P. glabra*-treated castor leaves were found to be inhibited. Ishaaya and Casida (1975) reported that inhibition of digestive enzymes of *Tribolium castaneum* larvae by phenyltin compound which are antifeedants. They also confirmed the fact that inhibition of protease activity due to the antifeedant compound may be a direct probably acting on a physiological system affecting protease activity. Direct inhibition of the 3 digestive enzymes due to the impact of antifeedant compounds azadirachtin found in *A. indica* and Karanjin, 3-methoxy pongapin and globachrome found in *P. glabra* noted in the present investigation is in conformity with the observations of Ishaaya *et al* (1974) and Ishaaya and Casida (1975).

A direct correlation was obtained between decreased food consumption and the activity of invertase, amylase, protease in the present investigation. This lowered food consumption may be due to the presence of antifeedant compound in the diet which in turn inhibits digestive enzymes. Ishaaya and Casida (1975) also suggested that retardation in larval growth may result from a lower feeding level caused by the reduced activity of the larval digestive enzymes.

The results of the present investigation reinforce the earlier findings that extracts of neem and *Pongamia* appear to be most efficacious in the control of *E. fraterna* especially when they are administered along with castor leaves in the fifth instar thereby paving the way for effective pest management and providing suitable alternative to synthetic pesticides.

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