Insect growth regulatory activity of the crude and purified fractions from *Solanum melongena* L., *Lycopersicum esculentum* Mill. and *Capsicum annuum* L.

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ABSTRACT

The biological activities of acetone crude extracts from the leaves of *Solanum melongena* L., *Lycopersicum esculentum* Mill., *Capsicum annuum* L. and fruit extract of *S. melongena* plants, were evaluated against two major pests, cluster caterpillar, *Spodoptera litura* Fab. and castor semilooper, *Achaea janata* L. The crude extracts were purified in a column and its purified fractions were assessed for its antifeedant and growth inhibitory activities by oral feeding method. The leaf extract of *C. annuum* and fruit extract of *S. melongena* showed strong antifeedant activity against *S. litura* and *A. janata* in leaf disc bioassays with a range of EC_{50} 31.4- 34.7 mg/10cm² as compared to other extracts tested. The fruit extract of *S. melongena* and leaf extract of *L. esculentum* caused significant larval growth inhibition after seven days of feeding to both the larvae by oral feeding assay. The fruit extract of *S. melongena* and leaf extracts of *L. esculentum* and *C. annuum* interfered with the molting process and produced morphological abnormalities by oral ingestion. Methanol eluted purified fraction from *S. melongena* fruit extract produced more potent antifeedant and larval growth inhibitory activity against test larvae than the other eluted test fractions. The effect of these plant extracts on proteolytic activity of the midguts of the two lepidopteran larvae were analyzed using azocasein/BApNA/SAAPFpNA as substrates. The serine protease activity was inhibited in all midguts fed with *S. melongena* fruit extract, when compared to the other treated and controlled midguts. Fruit extract from *S. melongena* showed more potent activity against both lepidopteran larvae.

Key words: Solanaceae plants, Lepidoptera, antifeedant, growth inhibitory activity, proteolytic activity, morphological abnormalities.

INTRODUCTION

Spodoptera litura Fab. (Lepidoptera: Noctuidae) is an extremely dangerous pest of many economically important crops, it is a major pest of groundnut in India particularly Andhra Pradesh (Murali Krishna *et al*., 2008). It damages numerous vegetables and field crops in China and many other Asian countries (Shivayogeshwara, 1991). In India, it was estimated that in tobacco also 4 to 8 larvae/ plant reduced the yield up to 50.4% (Patel *et al.*, 1971). *Achaea janata* L. (Lepidoptera: Noctuidae) caterpillars feed on leaves of castor (*Ricinus communis* L.), larvae consume most of the foliage leaving just the veins and petioles (Ronald and Jayma, 2007; Budatha *et al.*, 2008). In *A. janata*, duration of pupal stage is influenced by temperature with warmer temperatures shortening the development time (Muthukrishman and Pandian, 1984). The maximum damage caused by *A. janata* is a season between August to November in South Indian region. Owing to their high fecundity both the pests occur in vast number and cause severe damage to the crop and reduce the yield. Several chemical insecticides are effective against these caterpillars, particularly pyrethroids insecticides are more effective than natural pyrethrins (Singh *et al.*, 1987). Neem seed kernel suspensions are also effective in feeding deterrents on castor (Chari and Muralidharan, 1985) against *A. janata*.

Botanical products are one of the most prominent alternatives for pest control in current and future requirements (National Research Council, 2000). Survey on different plant families (Isman 1994; Regnault-Roger *et al.*, 2002) since the past two decades have promoted new sources for botanical insecticides that could possibly meet some of these demands. Numerous plant species have been identified, with the most promising

insecticides belonging to the families: Meliaceae, Rutaceae, Annonaceae, Asteraceae, Labiatae and Piperaceae (Jacobson, 1989; Schmutterer, 1992). Many of these plant extracts have been shown to affect insect growth and behaviour, acting as insect growth regulators, antifeedants and toxicants (Champagne *et al.*, 1992).

Solanum melongena L., *Lycopersicon esculentum* Mill. and *Capsicum annuum* L. (Solanaceae) are widely cultivated in India and other parts of the world. Few reports are available using *C. annuum* fruit powder (Ashouri and Shayesteh, 2009) and *L. esculentum* and *C. annuum* leaf powder against stored pests control (Usha Rani *et al.*, 2008). Nenaah (2011) reported the presence of toxic effects of glycoalkaliods isolated from *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill. against red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). In the present paper, we report the antifeedant, growth inhibitory and gut proteolytic effects of three Solanaceae plant extracts against two lepidopteran vegetables and crop pests such as *S. litura* and *A. janata*.

MATERIALS AND METHODS

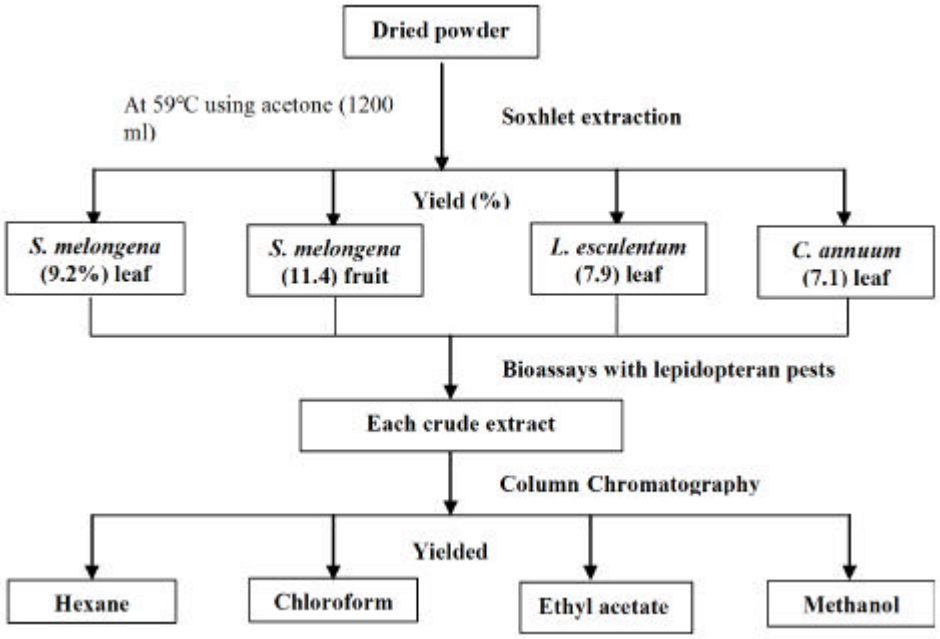
Insects and plant materials

Spodoptera litura and *Achaea janata* larvae used in this study were obtained from Entomology Division, Directorate of Oil Seed Research, Hyderabad. The culture was continuously maintained on castor bean leaves (*Ricinus communis* L.) at room temperature 28 ± 2º C, 65 ± 5% RH and 16:8 h L: D photo period in the laboratory, Indian Institute of Chemical Technology, Hyderabad. Healthy plants of *Solanum melongena* L. (leaves and fruits), *Lycopersicon esculentum* Mill. (leaves) and *Capsicum annuum* L. (leaves) were collected from the Vegetable Section, Acharya N. G. Ranga Agricultural University, Rajendar Nagar, Hyderabad.

Preparation and extraction of plant material

The leaves after collection were washed with distilled water to remove dust and other contaminants. The clean leaves were air dried for 4-6 days at room temperature at 30 ± 2ºC until all the moisture content was evaporated. The dried material approximately 600 g of leaves was milled to 4.0 mm particle size in an electric grinder. The ground leaves were subjected to extraction in soxhlet apparatus using acetone (1200 ml) as a solvent. The extraction went up to 15-18 hrs and the solvent was evaporated under reduced pressure in a rotary evaporator (Heidolph Laborota 4000) at 40-45ºC. The crude extracts were diluted in acetone (analytical grade) (w/v) to 500 mg/ml concentration denoted as 'crude' extract and was employed in all the experiments (Fig. 1).

Figure 1. Extraction process of plant crude extracts



Fractionation by chromatography

The crude extract was chromatographed on a silica gel column (50 cm length and 4 cm diameter), with chloroform [(100%) (Fraction 1)], ethyl acetate [(100%) (Fraction 2)]; methanol [(100%) (Fraction 3)] as eluents. Each eluted material was further concentrated using a rotary vacuum evaporator (Heidolph Laborota 4000) to remove excess solvent and kept at -20ºC till further use in bioassays.

Antifeedant assay

Antifeedant activity of Solanaceae plant leaf extract was assessed against two lepidopteran agricultural pests, *S. litura* and *A. janata*. The experiments were conducted according to the classical no-choice leaf-disc method (Sreelatha *et al.*, 2010). A small circular disc (10 cm^2) was cut from the fresh castor leaves. All crude extracts at different concentrations (5, 10, 20, 30, 40 and 50 mg/ 10cm^2) and purified fractions (1, 4, 6, 8 and 1 mg/ 10cm^2) were applied separately on the upper surface of the leaf disc with the aid of a glass atomizer. The solvent was allowed to evaporate by air drying briefly for a couple of minutes and the discs were placed individually inside a plastic petridish (9 cm dia), lined with moist absorbent cotton.

In each petri dish, a single pre-starved healthy 3rd instar larva of *S. litura* and *A. janata* was introduced separately for assessing antifeedant activity. The progress of the consumption of the leaf area was measured at 6, 12 and 24 hrs in both treated and control leaf discs. The leaf area consumed was measured at every 6 hrs using AM-300 leaf area meter (ADC, Bioscientific Limited, England, UK). The antifeedant index (AFI) was then calculated as (C-T)/(C +T) x 100, where C is the consumption of control discs and T, the consumption of treated discs (Belles *et al.*,, 1985). For each dose, 15 experimental sets were assayed. Each test was replicated five times (N= 150).

Larval growth inhibitory studies

The effect of plant extract on growth inhibitory of *S. litura* and *A. janata* activity was evaluated by the oral feeding method. Different concentrations of crude extract (5, 10, 20, 30 and 40 mg/ 100µl) andpurified fractions (1, 2, 4, 6 and 8 mg/ 100µl) were sprayed on the upper surface of the castor leaf discs separately. Control discs were sprayed with the carrier solvent alone. Two pre-weighed third-instar larvae of *S. litura* (average weight, 350±50 mg/larva) and *A. janata* (average weight, 395±40 mg/ larva) were released separately into each container. The treatment was replicated 30 times and there were a total of 60 insects exposed to the treatments (N= 60). After a period of 24hrs feeding, the larvae were transferred to a normal diet in a separate container. Everyday, the left-over leaf discs, if any and excreta of the insects were removed and fresh and clean leaves (untreated) were provided. The growth inhibition (%) was calculated by using the following formula (El-Aswad *et al.*, 2003): Growth inhibition (%) = $[(C_L-T_L)/C_L)]$ x 100 Where C_L is the larval weight gained in the control and T_L is the larval weight gained in the treatment.

Effect of test plant extracts on pest development

In another set of experiments, effects of three Solanaceae plant extracts on pupal and adult development after post-ingestion were identified by the oral feeding method. Freshly molted fourth-instar larvae were left to feed on the diet incorporated with crude extracts (at two concentrations 30 and 40 mg/ larva) till adult emergence. After molting, the larvae were transferred to fresh diet (untreated) and reared to adult development. The pupal weight (milligrams), pupal mortality (percent), adult abnormality (percent) and adult emergence (percent) were recorded. Each experiment was replicated 30 times with a total number of 60 insects/treatment (2 larva / replicate).

Midgut Proteolytic Activity

Isolation of protease enzyme

Fourth instar larvae (*S. litura* and *A. janata*) were left to feed on the diet (castor leaf) sprayed with all the plant extracts for 6 hrs at a concentration of 20 mg/larva, because at this concentration larval feeding was observed. Diet sprayed with acetone served as controls. After 6 hrs feeding, all the larvae were dissected and their guts were removed over ice cold normal saline and homogenized immediately in 50mM Tris-Cl, pH 8.0 [1:1 ratio (w/v)]. The crude gut homogenate was centrifuged at 14,000g for 15 min at 4ºC. The clear supernatant was filtered through a Whatman No.1 filter paper and transferred to a pre-chilled eppendorf tube. The samples were stored at -20ºC until further use. Protein analysis in the gut contents was carried out according to the procedure described by Bradford (1976) with BSA as a standard protein.

Enzyme assay

Proteolytic activity was carried out as described by Marchetti *et al.* (1998) with slight modifications using 2% (w/v) azocasein as a substrate. Typically, the sample (20 µL containing 10-15 µg protein) and 0.1 M Tris buffer, pH 10.0 (500 µL), was pre-incubated for 5 min at 30ºC before the addition of 25 µL 2% azocasein (w/v, in glass-distilled water). After 20 mins of incubation, the reaction was stopped using 400 µL 10% trichloroacetic acid (w/v). Tubes were kept on ice for 10 min and then centrifuged at 5,000 *g* for 5 min; 500-µL aliquots of the supernatant were withdrawn and mixed in a cuvette with 500 µL 1 M NaOH and absorbance at 420 nm was determined.

Blanks (test tubes without samples) were run in all cases. One unit of proteolytic activity is the amount of enzyme that causes the formation of 1 µg of TCA-soluble positive material per minute. Each experiment was replicated five times (N= 50). The specific activity of total protease activity (U) was calculated as:

$$\frac{\text{Absorbance value at 420nm (test)- Absorbance value at 420nm(blank)}}{\text{generated by an Adobe application 30 min X mg protein}}$$

Assays to quantify specific serine protease activities were conducted using paranitroanalide-conjugated peptide substrates in 96-well micro-titre plates. Trypsin-like activity was detected using BApNA (*N*-benzoyl DL-arginine *p*-nitroanilide) and chymotrypsin-like activity with SAAPFpNA (*N*-succinyl-alanine-alanine-pro-phe-*p*-nitroanilide) substrates. Briefly, 0.1 M Tris buffer, pH 10.0 (1.35 mL), and the sample (15 µL containing 5-15 µg protein) were pre-incubated for 5 min at 30ºC before the addition of 0.2 mL 7.8 mM BApNA/SAAPFpNA in 13% dimethyl sulfoxide; 1 mM final concentration) to start the reaction. After 10 min of incubation, the reaction was stopped with 0.75 mL 30% acetic acid and absorbance was measured at 410 nm. Assays were carried out in triplicate and appropriate blanks were run in all cases. The molar extinction coefficient (M^{-1} cm^{-1}) for pNA at 410 nm equals to 8800 (Erlanger *et al.*,, 1961) was taken in to account to calculate trypsin and chymotrypsin-like activity (BApNA/SAAPFpNA units /mg protein) using the formula.

$$\frac{\text{Absorbance value at 410nm/min x 1000 x volume of reaction mixture}}{\text{generated by an Adobe application 8800 x mg protein in the assay}}$$

Statistical analysis

Antifeedant and growth inhibitory activities were calculated using five different concentrations of each extract. The data was subjected to probit analysis (Finney, 1971) to determine the EC_{50} values representing the concentrations that caused 50% feeding deterrence and growth inhibition along with 95% confidence intervals. Results from the growth abnormalities (pupal and adult) were analyzed by one-way ANOVA using statistical software Sigma Stat ver 3.5. The post hoc testing was carried out using the Tukey's test. A significant level of 0.05 was used for all statistical tests.

RESULTS

Antifeedant activity of test plant extracts

The Solaneceae plant extracts showed a significant deterrence of food consumption at different concentrations. The antifeedant index percentages and EC_{50} values of the test extracts after 24 hrs of feeding are shown in Table 1. The fruit extract of *S. melongena* and leaf extract of *C. annuum* exhibited strong antifeedant activity at 50mg/ 10 cm² concentration, when compared with other plant extracts. The leaf extract of *C. annuum* was the most active antifeedant with EC_{50} = 31.4, 34.7 mg/10 cm² against *S. litura* and *A. janata* respectively. In comparison *S. melongena* fruit extract showed strong antifeedant index, with EC_{50} = 33.9 and 32.6 mg/10 cm² against two lepidopteran pests respectively. The extracts of the remaining plants showed less antifeedant activity. Therefore, it could be concluded that the fruit extract of *S. melongena* and leaf extract of *C. annuum* exhibited effective antifeedant activity against *S. litura* and *A. janata*.

Table 1. Antifeedant activity of certain plant extracts against third instar larvae of *S. litura* and *A. janata* by leaf disc method.

Test plants[a]	*Spodoptera litura*		*Achaea janata*	
	AI ± SD[b] (50 mg/ 10cm²)	EC_{50} (95% CL) (mg/ 10cm²)[c]	AI ± SD[b] (50 mg/ 10cm²)	EC_{50} (95% CL) (mg/ 10cm²)[c]
S. melongena	67.8 ± 2.8	40.2 (38.1-48.2)	68.3 ± 4.8	43.8 (40.3-46.2)
S. melongena	75.8 ± 4.2	33.9 (32.1-35.7)	82.8 ± 5.1	32.6 (30.9-34.4)
L. esculentum	72.6 ± 3.2	36.0 (34.6-38.0)	70.1 ± 2.1	38.1 (31.2-40.6)
C. annuum	81.0 ± 3.3	31.4 (29.7-33.2)	79.4 ± 3.4	34.7 (32.9-36.5)

[a] Antifeedant activity of plant extracts, at five different concentrations (10 – 50 mg range), N= 60.
[b] Means of Antifeedant Index (AI) are significantly different (ANOVA, P< 0.05, Turky HSD test).
[c] From the concentration-response curves, EC_{50} values were calculated by probit-log analysis.

Table 2. Antifeedant activity of eluted chromatographic fractions against third instar larvae of *S. litura* and *A. janata* by leaf disc method.

Test fractions[a]	*Spodoptera litura*		*Achaea janata*	
	AI ± SD[b] (10 mg/ 10cm^2)	EC_{50} (95% CL) (mg/ 10cm^2)[c]	AI ± SD[b] (10 mg/ 10cm^2)	EC_{50} (95% CL) (mg/ 10cm^2)[c]
S. melongena				
F1	14.0 ± 3.8	>10	12.2 ± 2.9	>10
F2	37.6 ± 4.7	>10	30.4 ± 2.7	>10
F3	18.8 ± 4.4	>10	19.8 ± 4.1	>10
S. melongena				
F1	22.2 ± 2.4	>10	22.8 ± 3.3	>10
F2	59.4 ± 2.8	8.5 (8.0- 9.0)	51.6 ± 2.8	8.9 (8.3- 9.9)
F3	81.6 ± 4.3	5.4 (5.3- 6.4)	90.2 ± 3.2	5.7 (4.6- 6.3)
L. esculentum				
F1	32.0 ± 1.9	>10	37.8 ± 2.8	>10
F2	67.6 ± 3.0	7.1 (6.5- 7.6)	71.4 ±2.2	6.9 (6.4- 7.4)
F3	42.0 ± 2.9	> 10	49.0 ± 2.7	>10
C. annuum				
F1	29.8 ± 2.5	>10	29.4 ± 3.0	>10
F2	71.8 ± 2.5	6.8 (6.3- 7.3)	79.4 ± 2.9	5.2 (5.1- 6.2)
F3	66.0 ± 4.1	8.3 (7.8- 8.9)	70.8 ± 3.5	7.5 (7.1- 8.0)

[a] Antifeedant activity of eluted fractions, at five different concentrations (1 – 10 mg range), N= 60.
[b] Means of Antifeedant Index (AI) are significantly different (ANOVA, P< 0.05, Tukey HSD test).
[c] From the concentration-response curves, EC_{50} values were calculated by probit-log analysis.
F1- Chloroform; F2- Ethyl acetate; F3- Methanol fractions

Antifeedant activity of chromatographic eluted purified fractions from Solanaceae plant extracts were also tested against two lepidopteran pests by oral feeding method and the results are presented in Table 2. Methanol eluted fraction from *S. melongena* fruit extract produced significant ($p< 0.001$) antifeedant activity against *S. litura* and *A. janata* larvae with an EC_{50} of 5.4 and 5.7 mg/10 cm² respectively (Table 2). The crude leaf extract of *C. annuum* eluted with ethyl acetate and methanol fractions exhibited antifeedant activity with an EC_{50} of 5.2 to 8.3 mg/10 cm² against the above test insects. In comparison, ethyl acetate eluted fraction from *L. esculentum* produced >60% antifeedant activity against both the larvae, with an EC_{50} of 6.9 and 7.1 mg/10 cm². However, from the results, we found that the fruit extract of *S. melongena* eluted with methanol showed more potent activity than the other test extracts.

Effect of test plant extracts on larval growth

The larval growth-inhibitory activity of these Solanaceae plant extracts on *S. litura* and *A. janata* are shown in Table 3. All the extracts tested inhibited the larval growth in a concentration-dependent manner after 7 days of feeding on the treated leaf discs. The fruit extract of *S. melongena* was the most potent larval growth inhibitor among the extracts tested. Significant larval growth inhibition against *S. litura* (df-59; $p< 0.001$) with an EC_{50} value of 22.6 mg/larva and *A. janata* (df-59; $p< 0.001$) with an EC_{50} value of 22.6 mg/larva was observed. In comparison, *L. esculentum* leaf extract produced >60% of larval growth inhibition with an EC_{50} value of 31.1-34.0 mg/larva against both the larvae respectively by the oral ingestion. The rest of the extracts exhibited moderate inhibition of insect growth (Table 3).

The effects of column eluted fractions from the Solanaceae plant extracts on *S. litura* and *A. janata* larval growth was studied and data shown in Table 5. Methanol eluted fraction from *S. melongena* fruit extract and ethyl acetate eluted *C. annuum* and *L. esculentum* fractions inhibited larval growth after 7 days of treatment in oral feeding assay. Methanol eluted fraction from *S. melongena* was the most potent growth

Table 3. Insect growth inhibitory activity of the Solanaceae plant extracts against third instar larvae of *S. litura* and *A. janata* after 7 days of exposure.

Test plants [a]	*Spodoptera litura*		*Achaea janata*	
	GI (%)[b] (40 mg/ larva)	EC_{50} (95% CL) (mg/ larva)[c]	GI (%)[b] (40 mg/ larva)	EC_{50} (95% CL) (mg/ larva)[c]
S. melongena	24.2 ± 3.0	>40	38.6 ± 4.0	>40
S. melongena	80.4 ± 2.8	25.2 (23.5- 27.0)	84.2 ± 3.6	22.6 (20.1- 27.3)
L. esculentum	68.8 ± 2.9	31.1 (29.2- 32.9)	60.4 ± 3.4	34.0 (28.9- 37.2)
C. annuum	55.4 ± 3.7	37.5 (35.1- 39.9)	50.2 ± 3.5	39.8 (36.3- .40)

[a] Growth inhibitory activity (mean ± SD) of plant extracts, at five different concentrations (5 – 40 mg range).
[b] Means within the column are significantly different (ANOVA, P< 0.05, Turky HSD test, N= 60).
[c] EC_{50} values was calculated by probit-log analysis. GI- Growth Inhibition

Table 4. Growth inhibitory activity of chromatographic fractions against third instar larvae of *S. litura* and *A. janata* by leaf disc method.

Test plants [a]	*Spodoptera litura*		*Achaea janata*	
	GI ± SD[b] (10 mg/ 10cm^2)	EC_{50} (95% CL) (mg/ 10cm^2)[c]	GI ± SD[b] (10 mg/ 10cm^2)	EC_{50} (95% CL) (mg/ 10cm^2)[c]
S. melongena				
F1	14.0 ± 3.5	> 8	18.2 ± 2.5	> 8
F2	29.0 ± 2.7	> 8	30.2 ± 2.0	> 8
F3	29.6 ± 2.2	> 8	26.8 ± 2.5	> 8
S. melongena				
F1	40.4 ± 2.8	> 8	44.4 ± 4.3	> 8
F2	50.6 ± 1.7	7.5 (7.0- 8.0)	51.2 ± 2.4	7.3 (6.8- 7.8)
F3	71.6 ± 3.8	5.0 (4.6- 5.4)	81.6 ± 2.4	4.5 (5.5- 6.3)
L. esculentum				
F1	32.4 ± 4.0	> 8	23.0 ± 3.6	> 8
F2	57.2 ±1.9	6.6 (6.2- 7.1)	51.2 ± 3.6	6.0 (5.8- 6.7)
F3	21.6 ± 3.0	> 8	44.2 ± 2.9	> 8
C. annuum				
F1	24.2 ± 3.0	> 8	20.0 ± 3.4	> 8
F2	65.0 ± 3.2	6.2 (5.7- 6.6)	69.4 ± 3.0	5.7 (5.5- 6.3)
F3	40.2 ± 2.1	> 8	41.0 ± 2.1	> 8

[a] Growth inhibitory activity (mean ± SD) of fractions, at five different concentrations (1- 8 mg range), N= 60.
[b] Means within the column are significantly different (ANOVA, P< 0.05, Turky HSD test).
[c] EC_{50} values was calculated by probit-log analysis. GI- Growth Inhibition
F1- Chloroform; F2- Ethyl acetate; F3- Methanol fractions

Table 5. Effect of Solanaceae plant extracts on pupal development of *S. litura* and *A. janata* by oral feeding method.

Plant extracts [a]	*Spodoptera litura*[b]		*Achaea janata*[b]	
	30 (mg/larva)	40 (mg/larva)	30 (mg/larva)	40 (mg/larva)
Pupal weight (mg)				
S. melongena	372.2 ± 11.2	312.6 ± 7.2	665.0 ± 14.1	638.2 ± 9.3
S. melongena	306.0 ± 6.6	243.7 ± 4.1	591.8 ± 9.6	545.1 ± 8.1
L. esculentum	315.4 ± 8.9	293.3 ± 12.0	659.4 ± 10.9	606.5 ± 11.6
C. annuum	300.9 ± 11.0	290.5 ± 8.8	620.3 ± 11.4	580.2 ± 10.2
Control	384.6 ± 8.3	384.6 ± 8.3	689.3 ± 10.1	689.3 ± 10.1
Pupal Toxicity (%)				
S. melongena	0 ± 0.0	12.0 ± 2.0	0 ± 0.0	2.0 ± 0.4
S. melongena	21.6 ± 1.9	40.4 ± 3.5	29.4 ± 3.2	42.8 ± 2.9
L. esculentum	18.2 ± 2.0	23.3 ± 3.0	21.4 ± 2.3	30.6 ± 1.2
C. annuum	13.1 ± 1.8	10.4 ± 2.2	10.3 ± 2.0	14.2 ± 2.8
Control	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

[a] Each plant extract at two concentrations of 30 and 40 mg/larva, N= 60.
[b] Each datum represents mean ± SD, are significantly different (ANOVA, P < 0.05, Turky HSD test).

inhibitor among the eluted extracts, with an EC_{50} value of 5.0 and 4.5 mg/larva against *S. litura* and *A. janata* respectively (Table 4). Whereas, leaf extract of *S. melongena* eluted with all three solvents fail to produce growth inhibition. However, ethyl acetate eluted fractions from *C. annuum* and *L. esculentum* showed significant ($p< 0.001$) larval growth inhibition against both the test lepidopterans.

Effects of test plant extracts on pest development

The insect growth deformities were mostly in the form of reduction in pupal-weight, percentage of pupal-toxicity, abnormal and normal adults emergence. *S. litura* and *A. janata* larvae which fed on fruit extract of *S. melongena* showed significant ($p< 0.001$) reduction in pupal weight of *S. litura* and *A. janata*, when compared to other extracts respectively (Table 5). Apart from this, potent pupal toxicity was also observed with fruit extract of *S. melongena* at 40 mg/larva in both the larvae respectively (Table 5). Treated larvae inhibited their normal development, most of these larvae molted into defective or malformed pupae. Though some of these pupae metamorphosed into the next stage, the resulting adults were also abnormal having crumpled wings and malformed body etc (Table 6). Due to precocious pupation, the insect might have failed to complete its development and they were unable to emerge into normal adults. The fruit extract of *S. melongena* was effective in producing deformed adults (38.6 and 44.2% respectively) at active concentration of 40 mg/larva. The leaf extracts of *L. esculentum* and *C. annuum* also produced morphological changes in both the larvae at same concentration. The percentage of emergence of adults from the pupae was drastically affected by fruit extract of *S. melongena* in *S. litura* and *A. janata* at 40 mg/larva (Table 6). *S. melongena* leaf extract caused normal emergence without any deformity equivalent to control adults. From the results, we noted that *A. janata* showed more susceptibility to test compounds than *S. litura*.

Regulation of midgut proteolytic activity

The midgut proteolytic activity was determined by using azocasein as a substrate with respect to three solanaceae plant extracts. Changes were observed in proteolytic activity of both *S. litura* and *A. janata* larvae, using these extracts. Total proteolytic activity of midgut extract in terms of azocasein hydrolysis inhibited with fruit extract of *S. melongena* was incorporated into the diet at a concentration of 20 mg/larva. Proteolytic activity was decreased in *S. litura* and A. *janata* when compared to control gut extracts respectively with *S. melongena* fruit extract at 20 mg/larva

Table 6. Effect of Solanaceae plant extracts on *S. litura* and *A. janata* adult development by oral feeding method.

Plant extracts [a]	*Spodoptera litura* [b]		*Achaea janata* [b]	
	30 mg	40 mg	30 mg	40 mg
Adult deformity (%)				
S. melongena	0 ± 0.0	5.4 ± 2.1	0 ± 0.0	9.4 ± 2.9
S. melongena	34.6 ± 4.1	38.6 ± 3.5	24.4 ± 3.4	44.2 ± 5.7
L. esculentum	22.6 ± 1.7	26.8 ± 2.7	19.4 ± 3.2	33.6 ± 3.4
C. annuum	27.4 ± 3.0	34.6 ± 3.0	21.0 ± 2.3	30.4 ± 2.8
Control	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Adult emergence (%)				
S. melongena	100 ± 0.0	74.0 ± 3.2	100 ± 0.0	80.2 ± 3.6
S. melongena	34.6 ± 3.1	11.2 ± 4.8	33.2 ±2.5	5.6 ± 1.9
L. esculentum	50.8 ± 5.0	43.0 ± 3.9	48.6 ± 3.2	29.0 ± 4.6
C. annuum	49.6 ± 3.6	47.2 ± 2.6	59.0 ± 3.1	52.8 ± 3.5
Control	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

[a] Each plant extract at a concentration of 30 and 40 mg/larva, N= 60.
[b] Each datum represents mean ± SD, are significantly different (ANOVA, $P < 0.05$, Turky HSD test).

(Fig 2). In comparison, leaf extracts of *L. esculentum* and *C. annuum* ingested larval gut extracts showed increase in proteolytic activity (*S. litura*, 18.8-21.1 and *A. janata*, 23.1-30.2 units of $min^{-1}mg^{-1}$ protein) when compared to control gut extracts respectively. From the results, the Solanaceae plant extracts showed over a very good regulation of the digestive proteolytic activity of both the lepidopteran larval midgut extracts (*in vivo*). The inhibition of proteolytic activity by *S. melongena* may be a consequence of interaction with the substrate, since at 20 mg concentration of fruit extract inhibited azocasein hydrolysis. *L. esculentum* and *C. annuum* did not inhibit azocasein hydrolysis to a great extent, but increased in proteolytic activity suggesting increased enzyme activity in midgut extracts (Figure 2).

The main proteolytic activity in larval midgut extracts was classified as serine protease type. Using synthetic substrates specific for different proteases, two types of serine protease activity were found to be present in the extracts from the larval midguts of *S. litura* and *A. janata.* When synthetic substrate containing a single amino acid residue was used, only trypsin like activity was detected. The substrate BApNA was hydrolysed with maximum hydrolysis at the pH of 11.0, whereas, substrate (SAAPFpNA) containing more than one amino acid residue was used to detect the chymotrypsin-like activity at same pH conditions. The test larvae, *S. litura* and *A. janata* fed with fruit extract of *S. melongena* showed significant reduction in trypsin and chymotrypsin-like activities when compared with control gut extracts at 20 mg/larva respectively (Fig 3), whereas, gut extracts from *S. litura* and *A. janata* fed with leaf extracts from *C. annuum*, *L. esculentum* and *S. melongena* showed increase in serine protease activity by maximum hydrolysis of respective substrates in comparison with control (Figure 3).

DISCUSSION

The leaf extracts of *C. annuum*, *L. esculentum* and the fruit extract of *S. melomgena* and its chromatographic fractions showed conspicuous antifeedant and growth inhibitory effects on the larvae of *S. litura* and *A. janata*. The behavior of the treated insects showed that the treated larvae frequently sampled the treated food, suggesting reduced feeding on treated food or of rejection. Antifeedant substances are customarily classified into repellents and deterrents (Schoonhoven, 1982). Based on these, it was found that the test extracts had deterrent properties. The validity of using artificial diet in antifeedant assays has sometimes been questioned (Jermy 1990). Results obtained from the leaf disc bioassays may be more reliable because the quality of plant's

Figure 2. The effect of Solanaceae plant extracts on activity of protease extracted from the midgut of 4th instar larvae of *S. litura* and *A. janata* using azocasein as a substrate.

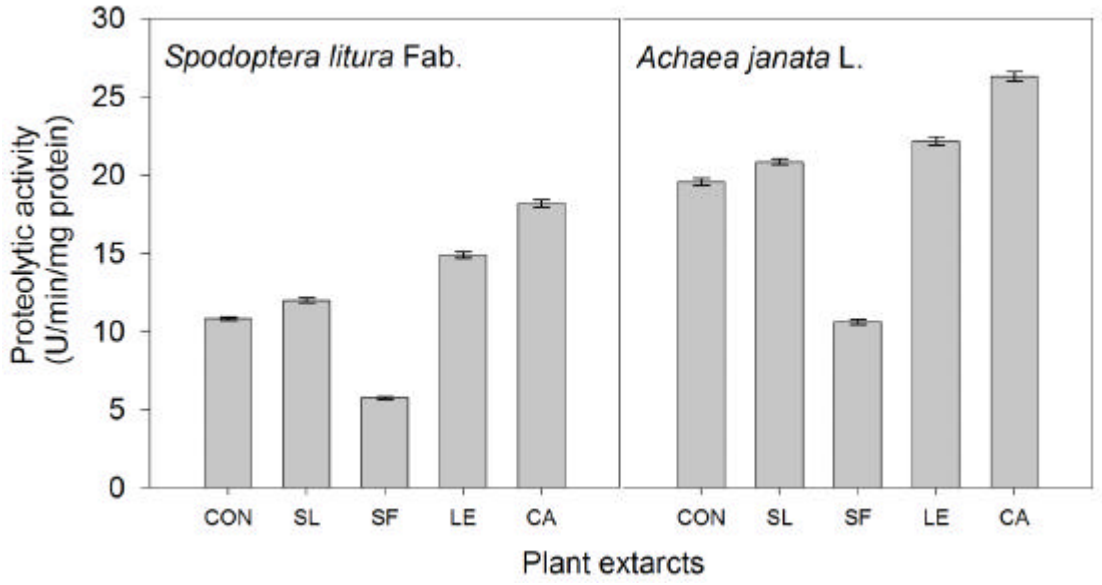
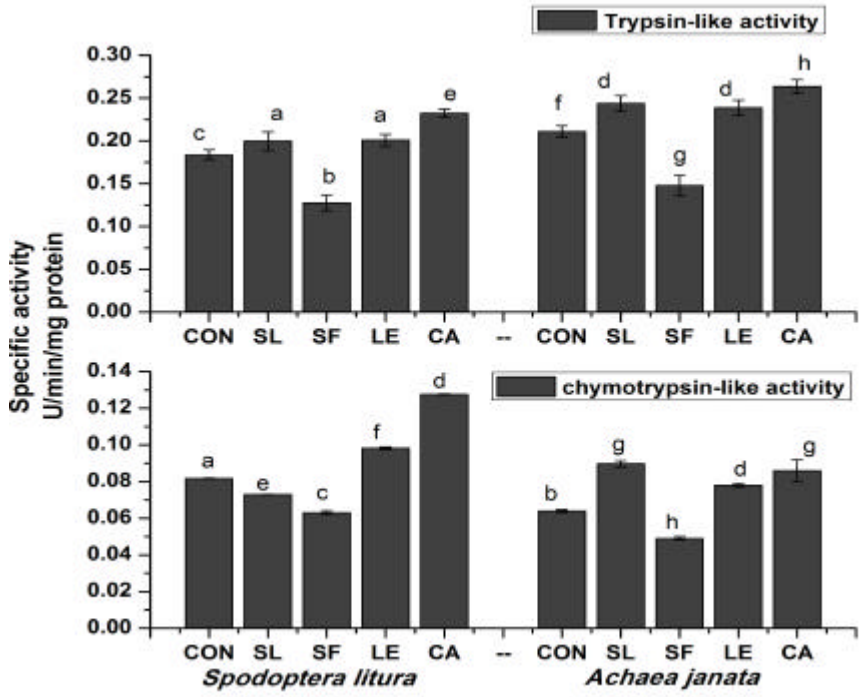


Figure 3. The effect of Solanaceae plant extracts on trypsin and chymotrypsin-like midgut proteases of fourth instar larvae of *S. litura* and *A. janata* using BApNA/ SAAPFpNA substrates



Note: Each data represents the mean ± SD of 5 replicates (N= 50) at 20 mg/larva
CON-Control (Acetone); SL- *S. melongena* leaf extract; SF- *S. melongena* fruit extract; LE- *L. esculentum*; CA- *C. annuum*.

surface plays a crucial role in determining the acceptance or avoidance (Chapman and Bernays 1989; Lin *et al.*, 1998).

The consumption rate of the lepidopterans, *S. litura* and *A. janata* was affected by the presence of plant extracts in the diet, with the increasing concentrations of *S. melongena* and *C. annuum* having a greater effect than other extracts. The results of feeding experiments indicate that both the lepidopteran species showed a significant increase in feeding deterrent response to the fruit extract of *S. melongena* and leaf extract of *C. annuum*. Also, methanol eluted fraction from fruit extract of *S. melongena* and ethyl acetate fraction from the leaf extract of *C. annuum* showed excellent feeding deterrent activity against both the test larvae. Several investigators have already reported that botanicals offer antifeedant activity against *S. litura* (Ulrichs *et al.,* 2008; Sreelatha *et al.,* 2009) and *A. janata* (Devanand and Usha Rani, 2008). For example, Pavunraj *et al.* (2011) stated that leaf extract and its column eluted ethyl acetate fraction from *Pergularia daemia* (Forssk) Choiv., exhibited good antifeedant activity against *Helicoverpa armigera* (Hub.) and *S. litura*. The root extracts of *Pedalium murex* L. exhibited good antifeedant activity against *S. litura* (Sahayaraj *et al.,* 2003). The extract of *Adhatoda vasica* leaves was found to have feeding deterrent properties when applied on leaf discs method (Sadek, 2003). Another report from Mikolajczak and Reed (1987) stated that the seed extracts of *Trichilia prieureana, T. roka* and *T. connaraides* exhibit high levels of antifeedant activity in leaf disc method against *Spodoptera frugiperda*. Devanand and Usha Rani (2008) reported that acetone extracts of 15 plant leaves showed excellent antifeedant and toxic properties against *S. litura* and *A. janata*.

In addition to its antifeedant activity, these solanaceae plant extracts exhibited growth inhibitory activity against both the test larvae. Our results indicate that acetone extracts of *S. melongena* and *L. esculentum* were potent growth inhibitors to *S. litura* and *A. janata* among the plant extracts tested. These extracts were quite effective in reducing growth of two lepidopteran larvae in the oral feeding bioassay. The growth inhibition activities of the extracts of several Meliaceae plants such as *Azadirachta indica* (Agrawal and Mall, 1988), *Melia azedarach* (Al-Sharook *et al.,* 1991), *Melia toosendan* (Chen *et al.,* 1995) and *Aglaia* species (Koul *et al.,* 1997) have been extensively evaluated on several insect pests. Ethyl acetate extract from *Syzygium lineare* Wall (Myrtaceae) (Jeyasankar *et al.,* 2010), methanol extract of *Melia dubia* (Meliaceae) (Koul *et al.,* 2000) showed growth inhibitory activity against *S. litura*. In our experiments, we also observed the larval growth inhibitory activity of methanol and ethyl acetate eluted fractions from *S. melongena* and *C. annuum* extracts. Predominantly, fruit extract of *S. melongena* eluted with methanol produced most potent growth inhibitor against *S. litura* and *A. janata* by oral feeding assay. According to Janprasert *et al.* (1993) report, isolated fractions and compounds from *Aglaia odorata* have feeding inhibition and growth regulating activity against *S. littoralis*. Sreelatha *et al.* (2010) reported that the new benzil derivative from *Derris scandens* exhibited growth inhibitory efficacy against *A. janata* larva by the oral feeding method.

The results on insect development revealed that the fruit extract of *S. melongena* and leaf extracts of *L. esculentum* and *C. annuum* disrupted developmental cycle of larvae after feeding by reducing the pupal weight or causing of pupal mortality and formation of pupal-adult intermediates. In several cases, the application of the extracts resulted into the emergence of deformed adults from the treated pupa. A reduction in pupal weight and emergence of deformed adults suggest that the extracts of *S. melongena, L. esculentum* and *C. annuum,* interferes with mechanisms under-lying development which are hormonally regulated. This type of delayed development and appearance of malformations were reported from azadirachtin treated *S. litura* (Rao and Subrahmanyam, 1987), plumbagin treated *Helicoverpa armigera* (Krishnayya and Rao, 1995) and new benzil derivative from plumbagin against *A. janata* (Sreelatha *et al.,* 2010). Larva-pupal intermediates had a pupal cuticle which is usually tanned on the abdomen, and on the dorsal region of the head and thorax. The remaining parts became darker when the insect was close to death. However no moult occurred and the body was still covered by the larval exuvium. None was able to emerge. Similar results were obtained when last-instar larvae of *S. litura, S. mauritia, Ephestia kuehniella* Zell. and *Manduca sexta* were subjected to azadirachtin (Jagannadh and Nair, 1992). In the present report we found the regulation in growth and development of two major lepidopteran pests such as *S. litura* and *A. janata* by fruit extract of *S. melongena* by oral ingestion method. The reduction in the pupal weight in the present study might be due to the ingested treated diet, disturbed digestive physiology of the larvae after treatment. This disturbance led to reduction in larval and pupal weight, poor growth and development and production of more deformities and emergence of adults. Wheeler and Isman (2001) reported that ethanolic extracts of *Annona squamosal* seeds exhibited reduction in pupal weight against *S. litura*.

In the present study, we also reported the proteolytic activity of the two lepidopteran larvae and their sensitivity to solanaceae plant extracts. Houseman *et al.* (1989) stated that the extracts from the digestive tracts of insects from many families, particularly those of lepidoptera contain serine proteases. These serine proteases are responsible for protein

digestion and consequently for the supply of amino acids needed for development. Serine proteases, as a group of digestive enzymes, have also been detected in guts of other *Spodoptera* species (Jongsma *et al*., 1996). The serine classes of proteinases such as trypsin and chymotrypsin, which belong to a common protein superfamily, are responsible for initial digestion of proteins in the gut of plant herbivores (GarciaOlmedo *et al.*, 1987). These proteinases involved in cleavage of polypeptide chains into short peptides which are then cleaved by exopeptidases to generate amino acids, the end products of protein digestion (Lawrence and Koundal, 2002).

In this study, our results shows the regulation of proteases in the midgut of *S. litura* and *A. janata*, which could be targeted with natural products to active insect control. Midgut trypsin and chymotrypsin showed diverse level of susceptibility towards crude extracts from the leaves and fruit of Solanaceae plants. Trypsin has been found to be the predominant and most active protease enzyme in lepidopterous larvae, and chymotrypsin activity was also found, but its activity is lower than that of trypsin (Broadway and Duffey, 1986). Gut proteases like trypsin and chymotrypsin activities were significantly inhibited by fruit extract of *S. melongena*, which lead to the growth retardation in the both larvae due to the reduction in protein metabolism. In comparison, larvae fed on diet containing leaf extracts of *C. annuum* and *L. esculentum* showed higher proteolytic activity in midguts as compared to those fed with fruit extracts of *S. melongena* and control. Many insects and poly-phytophagous insects, adapt easily to exogenous protease inhibitor chemical in their diet, because these insects generally have complex digestive biochemistries and compensate for the loss of proteolytic activity by either increasing affected proteases or by expressing novel proteases insensitive to the ingested protein inhibitors (Broadway, 1997; Gatehouse *et al.*, 1997; Brousseau *et al.*, 1999). From the results we noticed that the hyper-production of proteases in response to ingested compounds leads to an extra load on the insect for essential amino acids, resulting in retardation of insect growth was observed. From the results we conclude that the fruit extract of *S. melongena* and leaf extracts of *C. annuum* and *L. escculentum* have the potential to regulate the gut proteolytic activity of the two lepidopteran pests *S. litura* and *A. janata* by oral ingestion.

A large number of plant secondary metabolites are known to affect insect growth, development and reproduction; their exact mechanisms of action remain to be established. The regulatory activities of these plants *S. melongena*, *L. esculentum* and *C. annuum* extracts also suggest the possibility of a future exploitation of these materials into potential insect management chemicals with a minimum environmental impact and crop protectant against *S. litura and A. janata* larvae.

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