Biological potency of certain plant extracts in management of two lepidopteran pests of *Ricinus communis* L.

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ABSTRACT

Antifeedant and toxic effects of leaf extracts of *Syzygium cumini* L., *Ocimum basilicum* L., *Luffa aegyptica* Mill., *Eichhornia crassipes* Mart., *Tamarindus indica* L., *Terminalia catappa* L., *Limonia acidissima* L., *Murraya koenigii* L., *Breynia retusa* (Dennst.)., *Jatropha curcas* L., *Tectona grandis* L., *Momordica charantia* L., *Mangifera indica* L., *Ricinus communis* L., and the seed extract of *Madhuca indica* Gmelin., were evaluated in the laboratory and the results were reported. The crude acetone extracts were tested against the third instar larvae of two lepidopteran pests i.e. the tobacco cut worm, *Spodoptera litura* Fab. and the castor semilooper, *Achaea janata* L. (Noctuidae: Lepidoptera). Antifeedant activity of the extracts was assessed through leaf disc choice tests. The leaf extracts of *M. charantia, T. grandis, M. indica* and *T. indica* exhibited strong antifeedant activity (> 85%) in leaf disc bioassays at a dosage of 100-mg/ 21cm^2. Test extracts of *T. grandis, M. indica* and *M. charantia* produced higher toxicity (> 80%) to *S. litura* and *A. janata* at 20-100 mg/21 cm^2/ larva doses were applied, and LD_{50} values of (47.85- 72.60 mg/21 cm^2). The acetone extracts of *M. charantia, T. grandis* and *M. indica* were found to exhibit both feeding deterrent and toxic activities. The other plants which were tested had moderate effects towards these pests. Based on their feeding deterrent and toxic properties, some of these plant extracts have potential for use as alternative crop protectants against certain pest species.

Key words: Plant products, Antifeedant, *Spodoptera litura* Fab., *Achaea janata* L., toxicity.

INTRODUCTION

The use of conventional insecticides has raised some concern about their threat to the environment and development of insecticide resistance in insects (Huang *et al.*, 1998), there is a imperative need for the development of safer, alternative crop protectants such as botanical insecticides and antifeedants. Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Crude plant extracts often consist of complex mixtures of active compounds, they may show greater overall bioactivity compared to the individual constituents (Berenbam *et al.*, 1991; Chen *et al.*, 1995). The deleterious effects of crude plant extracts on insects are manifested in several ways, including toxicity (Hiremath *et al.*, 1997), feeding inhibition (Klepzig and Schlyter, 1999; Wheeler and Isman, 2001).

The search for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants, growth inhibitors) often begins with the screening of plant extracts (Ho *et al.*, 1997; Kelm *et al.*, 1997). Certain plant families, particularly Meliaceae, Rutaceae, Asteraceae, Labiateae, Piperaceaea and Annonaceae are viewed as exceptionally promising sources of plant-based insecticides (Jacobson, 1989; Schmutter, 1990, Isman 1995). Two antifeedant compounds have been isolated from the petroleum ether extract of *Clausena anisata* (Rutaceae), against the larva of African armyworm (*Spodoptera exempta*), the compounds identified as the coumarins imperatorin and xanthoxyletin (Gebreyesus and Chapya, 1983). *Melia volkensii* contains limonoids related to azadiractin, crude *M. volkensii* fruit extract is toxic to a broad range of insects including dipterans, lepidopterans, and coleopterans (Mwangi and Rembold, 1987, 1988). Limonoids from *Khaya senegallensis* having feeding deterrent and growth inhibitory properties against the cotton leafworm, *Spodoptera littoralis* (Aswad *et al.*, 2003). Bio-pesticidal property of *Chrystella parasitica* and *Ipomoea carnea* on *Achaea janata* has been reported by Sahayaraj *et al.* (2003). Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Sadek, 2003).

In the present study we report the antifeedant and toxic activity of crude leaf extracts of Jamun (*Syzygium cumini* L.), Basil (*Ocimum basilicum* L.), Sponge Gourd (*Luffa aegyptica* Mill.), Water Hyacinth (*Eichhornia crassipes* Mart.), Tamarind Tree (*Tamarindus indica* L.), Indian almond (*Terminalia catappa* L.), Musk Deer Plant (*Limonia acidissima* L.), curry leaf plant (*Murraya koenigii* L.), berry Tree (*Breynia retusa* Dennst.), Moghul Castor Oil Plant (*Jatropha curcas* L.), castor-Oil (*Ricinus communis* L.), Indian Oak Teak (*Tectona grandis* L.), Bitter

Table 1. Plant species tested for the management of *Ricinus* pests

Family	Plant Name	Parts Used	Yield (%)
Myrtaceae	*Syzygium cumini* L.	Leaf	8.7
Lamiaceae	*Ocimum basilicum* L.	Leaf	10.2
Cucurbitaceae	*Luffa aegyptica* Mill.	Leaf	7.9
Pontederiaceae	*Eichhornia crassipes* (Mart.)	Leaf	6.9
Caesalpiniaceae	*Tamarindus indica* L.	Leaf	8.8
Combretaceae	*Terminalia catappa* L.	Leaf	10.1
Sapotaceae	*Madhuca indica* Gmelin	Seed cake	14.6
Rutaceae	*Limonia acidissima L.*	Leaf	7.9
Euphorbiaceae	*Breynia retusa* (Dennst.)	Leaf	6.3
Euphorbiaceae	*Jatropha curcas L.*	Leaf	11.0
Cucurbitaceae	*Momordica charantia L.*	Leaf	9.0
Verbenaceae	*Tectona grandis L.*	Leaf	12.0
Anacardiaceae	*Mangifera indica* L.	Leaf	6.5
Rutaceae	*Murraya koenigii L.*	Leaf	9.2
Euphorbiaceae	*Ricinus communis L.*	Leaf	7.1

Gourd (*Momordica charantia* L.), Mango (*Mangifera indica* L.) and seed cake extract of Mahua (*Madhuca indica* Gmelin), against tobacco caterpillar, *Spodoptera litura* Fab. and castor semilooper *Achaea janata* L.

MATERIALS AND METHODS

Insects

Spodoptera litura and *Achaea janata* larvae used in this study were obtained from a laboratory colony of Department of Entomology, Directorate of Oil Seed Research, Rajendra Nagar, Hyderabad. The culture has been continuously maintained on castor bean leaves (*Ricinus communis* L.) at room temperature (25 ± 2º C), 65 ± 5% RH and 16:8 L:D photo period in the laboratory, Indian Institute of Chemical Technology, Hyderabad.

Plant material

Healthy plants of *Syzygium cumini* L., *Ocimum basilicum* L., *Luffa aegyptica* Mill., *Eichhornia crassipes* Mart., *Tamarindus indica* L., *Terminalia catappa* L., *Limonia acidissima* L., *Murraya koenigii* L., *Breynia retusa* (Dennst.)., *Jatropha curcas* L., *Ricinus communis* L., *Tectona grandis* L., *Momordica charantia* L., *Mangifera indica* L. were collected from Vegetable section, Acharya N G Ranga Agricultural University, Rajendar Nagar, Hyderabad, and *Madhuca indica* Gmelin seed cake was supplied by Center for People's Forestry (CPF), Hyderabad, India. The plant material was collected in the morning hours.

Preparation and Extraction of plant material

Plant material was prepared in the laboratory by harvesting leaves from all plant species and separated from their petioles at the base of the leaf blade. The leaves after collections were brought to the laboratory, washed with distilled water to remove dust and other contaminants. The clean leaves were air dried for 4-5 days at room temperature (28 ± 2ºC) until all the moisture content was evaporated. The dried material approximately 300 g of leaves was milled to 4.0 mm particle sizes in an electric grinder. The ground leaves were subjected to extraction in soxhlet apparatus using acetone (900 ml) as a solvent for each test material. The extraction proceeded up to 15-18 hours at room temperature and the solvent was evaporated under reduced pressure in a rotary evaporator at 56ºC. The yield of each extraction is shown in Table 1. *Madhuca indica* [dry weight of acetone extract / dry from of seed cake weight X 100], and other plants [dry weight of acetone extract / dry weight of test plant X 100] extraction values were calculated. The crude was diluted in analytical grade acetone (w/v) to 500 mg/ml concentrations was employed in all the experiments denoted as 'crude extract'.

Antifeedant assay

Leaf disc bioassays were carried out to determine antifeedant activity of crude extracts of different aromatic plants. The third instar larvae of *S. litura* and *A. janata* were starved for three hours. Leaf discs (21 cm^2) were

Table 2. Toxic effects of plant extracts against third instar larvae of *S. litura* and *A. janata* by oral feeding.

Test plants [a]	*Spodoptera litura* [b]		*Achaea janata* [b]	
	Mean (%) Toxicity ± SD [c]	LD_{50} (95% CL) (mg/ 21cm^2/ larva) [d]	Mean (%) Toxicity ± SD [c]	LD_{50} (95% CL) (mg/ 21cm^2/ larva) [d]
S. cumini	42.5 ± 5.0	>100	45.0 ± 3.4	>100
O. basilicum	41.4 ± 4.2	>100	51.8 ± 3.7	>100
L. aegyptica	33.3 ± 3.6	>100	24.4 ± 5.0	>100
E. crassipes	56.3 ± 4.1	93.0 (83-6-101.5)	28.1 ± 3.5	>100
T. indica .	50.9 ± 3.6	>100	51.9 ± 3.9	>100
T. catappa .	61.0 ± 3.6	90.0 (83.6-102.5)	35.5 ± 3.9	>100
M. indica	95.9 ± 3.6	47.85 (45.3-50.3)	90.2 ± 2.9	55.4 (49.9-60.8)
L. acidissima .	28.3 ± 3.6	>100	22.5 ± 3.5	>100
B. retusa	23.1 ± 3.6	>100	23.6 ± 3.9	>100
J. curcas.	36.6 ± 4.7	>100	60.6 ± 4.2	90.1 (82.6-97.6)
M. charantia	87.6 ± 4.4	72.6 (66.3-79.0)	83.9 ± 4.2	71.0 (65.6-76.5)
T. grandis	88.9 ± 4.4	71.9 (66.3-79.0)	82.4 ± 4.4	66.7 (61.1-72.3)
M. indica	21.7 ± 4.6	>100	25.4 ± 4.0	>100
M. koenigii	36.0 ± 3.6	>100	38.3 ± 4.0	>100
R. communis	19.5 ± 3.6	>100	18.3 ± 4.0	>100

[a] Toxicity data (Mean (%) toxicity) of all extracts tested at 100 mg /21cm^2 / larva

[b]Feeding deterrent experiments were conducted with *S. litura* and *A. janata* larvae (n= 60 for each treatment) that were feed on an diet with five different concentrations of the tested plant extracts (20 – 100 mg range), after 4-6 days of exposure.

[c]Toxicity percentages are significantly different (ANOVA, P< 0.05, Tukey HSD test).

[d]From the concentration-response curves, LD_{50} values was calculated by probit-log analysis.

punched from castor bean leaves and sprayed separately with different dosages of 20, 30, 50, 75 and 100 mg of crude extracts in acetone. The treated discs were air dried for about 3 min to evaporate the solvent. Control discs were sprayed with acetone. A choice test was performed in a 14 cm dia. perti dish lined with moistened Whatman (No.1) filter paper. In choice test, the area was divided in to equal quadrants, each quadrant containing a treated or control disc placed alternately. Two third-instar larvae of each like *S. litura* and *A. janata* were placed separately in the center of the dish. There were ten replicates for each treatment and all the treatments were repeated on 3 different days. The percentage antifeedant index was calculated from the following formula (Lewis and Van Emden, 1986): Antifeedant index (AFI) = [(C - T) \ (C + T)] x 100 Where C is the weight of leaf disc consumed in the control and T is the weight of leaf discs consumed in the treatment.

Toxicity studies

The toxic effects produced by various plant extracts due to the contact in *A. janata* and *S. litura* were measured by treating a set of 60 insects in the same manner as above, except that after exposure of the insects to the treated leaf disc for 24 hrs, the insects were transferred to the normal diet in a separate container. Mortality and other toxic effects like knock down etc were observed and recorded after every 24 hrs. The data obtained revealed the contact toxicity of the extract. The oral toxicity of the compounds was evaluated along with the antifeedant activity. In the experiments with antifeedance some of the treated leaf discs were partially consumed by the test insects. These insects were transformed to the normal diet in a separate container and were maintained and observed for the mortality, knock down etc. the data obtained gave the oral toxicity of various plant extracts and their effects upon ingestion by lepidopteron larvae.

Data analysis

Statistical analysis of the experimental data was performed using probit analysis to find out the LD_{50} (Finney 1971). The data was analysed by completely randomized, one-way Analysis of Variance (ANOVA) and the means were separated using Tukey HSD test (Sigmastat v3.1). All figures were plotted using origin plot software.

RESULTS

Antifeedant activity

The antifeedant activity of the plant extracts was tested against the third-instar larvae of *S. litura* and *A. janata*

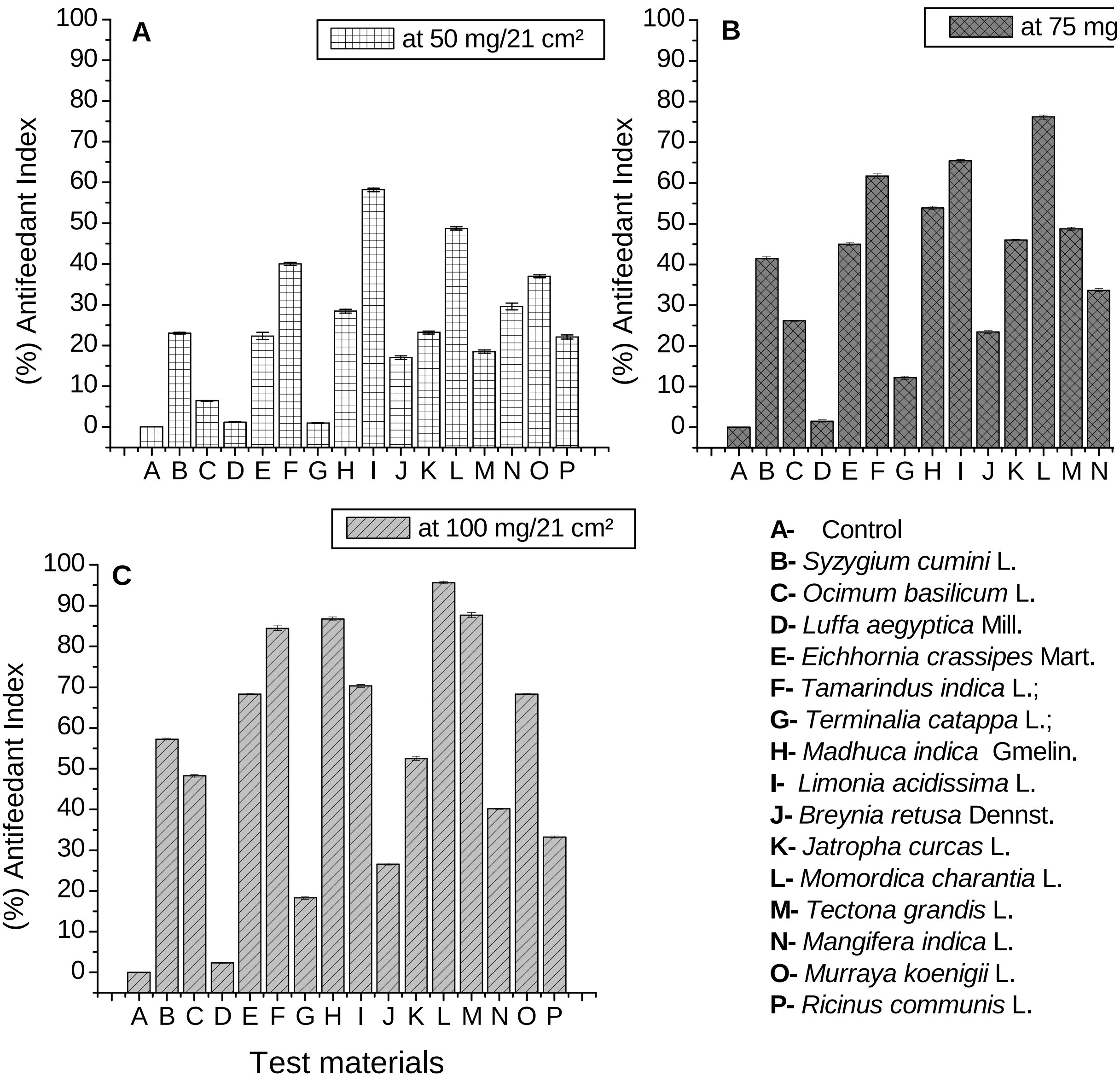

Figure 1. Feeding deterrent effect of plant crude extracts on Antifeedant Index (AFI) of third instar larvae of *S. litura* at dosages of A) 50 mg/ 21 cm^2 B) 75 mg/21 cm^2 C) 100 mg/21 cm^2 after 24 h application.

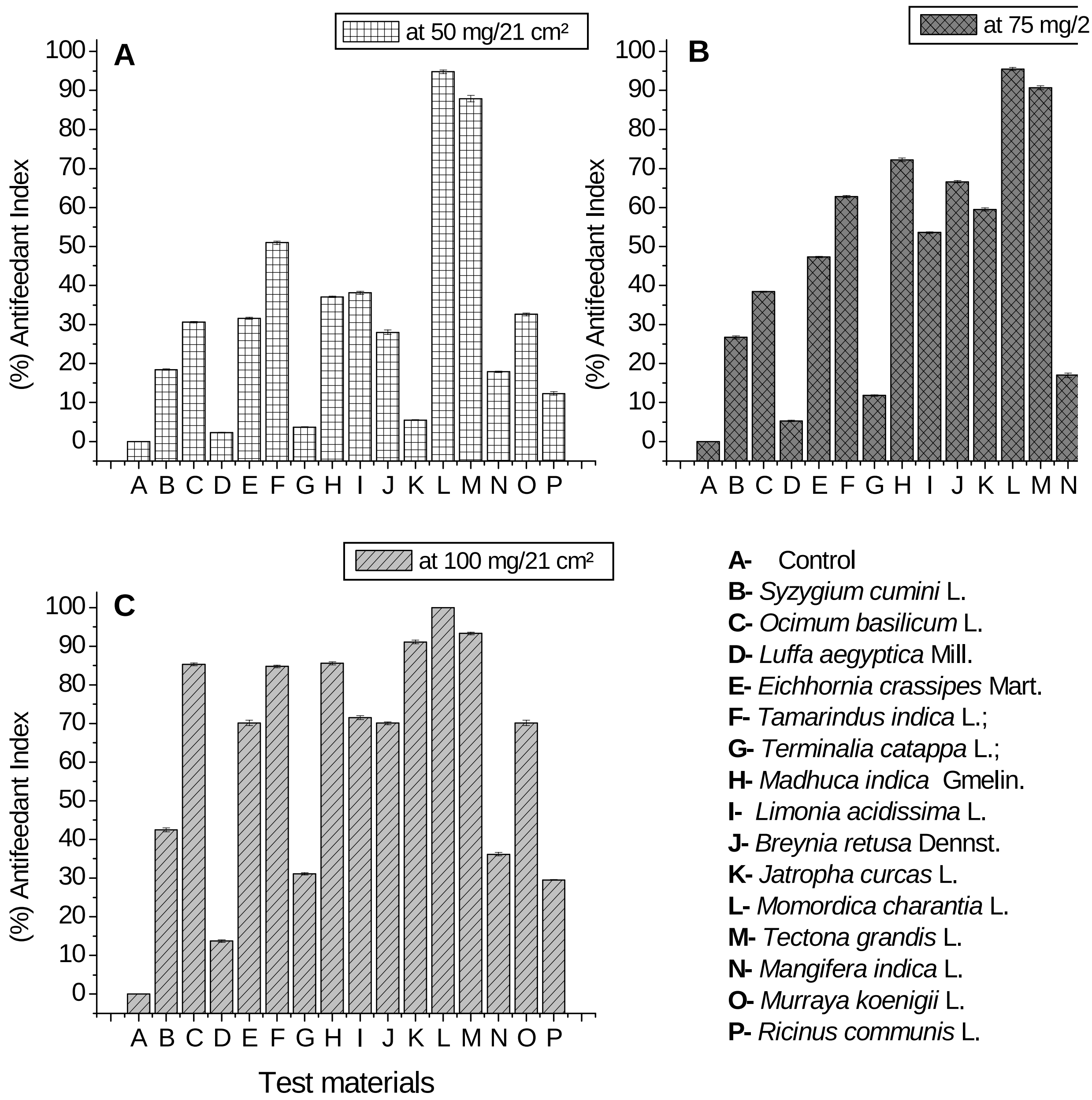

Figure 2. Feeding deterrent effect of plant crude extracts on Antifeedant Index (AFI) of third instar larvae of *A. janata* at dosages of A) 50 mg/ 21 cm^2 B) 75 mg/21 cm^2 C) 100 mg/21 cm^2 after 24 h application.

by leaf disc method. The extracts showed a significant deterrence of food consumption at different doses. The results on antifeedant activity of foliar extracts in plants tested are presented in Figures 1 and 2. Among the plant materials evaluated, *M. charantia* and *T. grandis* showed highest antifeedant potential with significant ($P < 0.05$) AFI (100%) followed by *J. curcas* (> 90%), *O. basilicum, T. indica* and *M. indica* extracts (> 80%). Except other plant extracts were showed the weakest antifeedant activity (Figures 1 and 2).

Toxic effects of plant extracts

It is interesting to note that *S. litura* was susceptible to certain treatments like, *M. indica, M. charantia* and *T. grandis* than *A. janata* as shown in Table 2. Test extracts caused a significant increase on mortality of *S. litura* and *A. janata* which intensified during the development of the insects. As shown in Table 2, a significant ($P < 0.05$) increase in mortality (ranging from 82.4 to 95.2) was observed in diets supplemented with *M. indica*, *M. charantia*, and *T. grandis* extracts at higher dose (100 mg/21 cm²) and LD_{50} (47.85 to 71.90 mg/21 cm²), comparative other plant extracts (LD_{50} > 100 mg/21 cm²) tested. Toxicity increased as dose increased, this corresponded to a decrease in consumption. It is likely that this decrease in consumption rate is due to antifeedant nature of the extract and this indicates that the toxicity of this larvae is not entirely due to starvation; some other toxic effect of the ingested extract.

DISCUSSION

The present work revealed the effects of plant extracts on major agricultural pests like, *Spodoptera litura* Fab. and *Achaea janata* L. Significant antifeedant activity and toxicity against *S. litura* Fab. and *A. janata* larvae, was observed with crude acetone extracts of *T. grandis*, *T. indica*, *Madhuca indica*, *M. charantia* and *J. curcas* except other plants tested. Champagna *et al.* (1992), Senthil Nathan (2006) reported that majority of limnoid compounds showed antifeedant activity. Ethanol extracts of the seeds of *Trichilia prieureana*, *T. roka* and *T. connaraides* exhibit high levels of antifeedant activity in leaf disc method against *Spodoptera frugiperda* (Mikolajczak and Reed, 1987), and Xie *et al.* (1994) reported antifeedant effects of *T. connaraides* extracts to *S. litura*. The crude seed extracts of *Annona squamosa* having antifeedant potential against lepidopteran pests like, *Plutella xylostella* L. and *Trichoplusia ni* (Leatemia and Isman, 2004). The extract of *Adhatoda vasica* leaves was found to have feeding deterrent properties when applied on leaf discs method (Sadek, 2003). Once antifeedant effects were observed, experiments were carried out to investigate other activity like toxicity. The most active extracts were *M. charantia*, *T. indica*, *T. grandis* and *Madhuca indica* showed fabulous antifeedant and toxic activity to both the pests. Experiments on the toxicity of crude extracts of *M. charantia*, *T. grandis* and *Madhuca indica* showed fabulous toxic activity (> 80%) to larvae, *S. litura* and *A. janata* than other extracts tested. Martin Rathi and Gopalakrishnan (2005) reported that the toxic effects of methanol extracts of *Synedrella nodiflora* against *Spodoptera litura*. Ethanol extracts of *Aesculus hippocastanum* L. showed toxic effect against *Thaumetopoae solitaria* Frey. larvae (Erturk, 2006).

These results suggest that there may be different compounds in extracts possessing different bioactivities, particularly antifeedant and toxicity of insect pests. We can conclude that this study suggest that acetone extracts of *M. charantia, T. grandis, T. indica, Madhuca indica, M. charantia*, and *J. curcas* plants belonging to families taxonomically possesses toxic principles with significant antifeedant effects and could be a potential crop protectents against *S. litura and A. janata*.

It is interesting that certain plant extracts employed in the present study possess antifeedance and toxicity in the lepidopteran pest species studied. It appears that these plants contain different chemicals that act upon target cells effectively. A through chemical analysis of the active plants is underway and we hope to reveal some interesting similarities between the chemicals isolated as well as their bioactivities. The activity of these extracts also suggests a future exploitation of the materials in to potential insect management chemicals with a minimum environmental impact. It is advantageous, as the extracts at higher doses act as antifeedant, while the lower dilution of the same plant is oral toxicant. The results implying the dual role of a single plant material in ricinus pest management by chosen plant extracts. It also suggests that by a single application of these compounds a complete success of the insect control can be achieved.

ACKNOWLEDGEMENT

The authors acknowledge Dr. J.S. Yadav, Director, Indian Institute of Chemical Technology, Hyderabad.

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