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Research Article

Screening of Plant Extracts for Antifeedant Activity Against *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

Background and Objectives: Pesticidal properties of plants against economically important pests are searched. The objective of study was to investigate, biological screening of crude extracts from 26 different plant species and then screened different crude extracts for their antifeedant activity against the fourth instar larvae of *Spodoptera litura* (*S. litura*) and *Helicoverpa armigera* (*H. armigera*). **Methodology:** Screening bioassay method was used for antifeedant activity and after 24 h, consumed leaf area was calculated as percentage of antifeedant. **Results:** Twenty six plants screened for antifeedant activity, 3 plants showed significant antifeedant activity viz., *Pseudocalymma alliaceum* (81.55 and 79.44 %), *Solanum pseudocapsicum* (76.32 and 74.66%) and *Barleria buxifolia* (73.23 and 70.66%) in ethyl acetate extracts against *Spodoptera litura* and *Helicoverpa armigera*, respectively. **Conclusion:** This study proposed plant chemicals from *Pseudocalymma alliaceum*, *Solanum pseudocapsicum* and *Barleria buxifolia* by the green chemistry approach using controlling the economically important pests alternate to synthetic pesticides.

Key words: Insect pests, botanical pesticides, antifeedant, synthetic pesticides, screening bioassay

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Man suffers extensively due to the nuisance of insect populations both in agriculture and health. In agriculture, insects affect directly the growing part of the crop and causes severe damage, resulting in revenue loss. Crop loss due to insect pests is estimated between 10 and 30% for major crops[1]. In a tropical country like India, owing to climatic conditions and its particular environment, agriculture is suffering from severe losses due to pests. Considering the agro-ecosystems with an increase in population and dwindling land resources there is worldwide demand for natural insecticides to increase the agriculture production. Due to these problems, a search is going on to discover new, less damaging pest management tools[2]. Chemical pesticides have been used for several decades in controlling pests as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance to pesticides, resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affected human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment. Under such alarming situations, plants and plant derived products offered a tremendous advantage over synthetic pesticides in use as control agents for the pests of agriculture, veterinary and public health since plant kingdom is the most efficient producer of chemical compounds, synthesizing many products that are used in defense against insects[3]. However, the screening of plant extracts against insects are still continuing throughout the world to find out different kinds of effects of botanicals to obtain an ecofriendly and economical biopesticide. *Helicoverpa armigera* (Hub.) has gained increased attention in many parts of the world. *Helicoverpa armigera* is highly polyphagous pest, infest more than 500 plant species and is a serious pest in India. The greatest damage is caused to cotton, tomatoes, maize, chick peas, alfalfa and tobacco etc.[4,5]. Very few reports were present pertaining to the antifeedant activity of plant extracts against *Spodoptera litura* and *Helicoverpa armigera*[6,7]. Therefore, the present study deals with screening of various plant extracts for their antifeedant activity against *Spodoptera litura* and *Helicoverpa armigera*.

MATERIALS AND METHODS

Collection of plant materials: In the present study, total of 26 plants was belonging to diverse families and genera collected from Puliansolai, Kolli hills, Namakkal district, Tamil Nadu, India. Plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St' Joseph's College, Tiruchirappalli, Tamil Nadu, India. The voucher specimen was prepared and deposited at PG and Research Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India. The plant materials were thoroughly washed with tap water and air dried under room temperature (28±2°C) and relative humidity (RH, 75±5) at Department of Zoology, Government Arts College, Musiri (Table 1).

Extraction methods: After complete drying the plant materials were powdered using electric blender and sieved through kitchen strainer. The extracts were prepared by soaking 200 g of dried powder in 600 mL of hexane, chloroform and ethyl acetate sequentially with increasing polarity of solvents successively for 24 h by cold extraction methods. The extracts were filtered through Whatman's No. 1 filter paper. The solvent from the crude extracts were evaporated to air dryness at room temperature. The crude extracts were collected in clean Borosil vials and stored in the refrigerator at 4°C for subsequent bioassay against *S. litura* and *H. armigera*.

Rearing of test insects

***Spodoptera litura*:** Egg masses of *S. litura* were collected from caster field at Anaipatti near Arignar Anna Govt. Arts College Musiri, Tiruchirappalli district, Tamil Nadu, India. Collected leaves with egg masses were transferred on the filter paper and kept in petri dishes under laboratory condition (28±2°C) temperature and (RH 75±5%). Newly hatching larvae were reared on leaves of castor (*Ricinus communis*) till pre-pupal stage and sterilized soil was provided for pupation. After pupation, the pupa were collected from soil and placed inside the oviposition champers (46×40.5×40.5, 61×45.6×45.6). After adult emergence, cotton soaked with 10% sugar solution with few drops of multi-vitamins was kept inside oviposition cage for adult feeding. After hatching the larvae were provided castor leaf for feeding. These laboratory reared larvae were used for bioassay, at room temperature (28±2°C) and (RH 75±5%).

***Helicoverpa armigera*:** *Helicoverpa armigera* larvae will be collected from bhendi field near Arignar Anna Government Arts College, Musiri and will be reared in bhendi individually in a plastic container (for avoiding cannibalism) till they attain pupal stage under laboratory conditions (28±2°C and 80±5% RH). Sterilized soil will be provided for pupation. After pupation, the pupa were collected from soil and placed inside

Table 1: List of plants utilized in study

Scientific name	Vernacular name	Family	Growth habitat	Reference for gamble book pp No.
Asparagus racemosus Willd.	Shatavari	Liliaceae	Scandent	Vol-3: pp1516-1517
Atalantia monophylla Correa	Kattue lumeachi	Rutaceae	Small thorny tree	Vol-1: pp158-159
Barleria buxifolia Linn.	Kattimullu, rosmullippuntu	Acanthaceae	Prickly Hairy shrub	Vol-2: pp1055-1059
Barleria montana Nees	Makavannakkurinci	Acanthaceae	Under shrub	Vol-2: pp1055-1061
Cleome gynandra L.	Shona cabbage	Euphorbiaceae	Herb	Vol-1: pp40-41
Cyclea peltata Hook. f. and Thoms	Malaitanki, ponmucuttai	Menispermaceae	Climbing shrub	Vol-1: pp30-31
Gloriosa Superba Linn.	Kallappa kilangu	Liliaceae	Climbing herb	Vol-3: pp1519
Glycosmis mauritiana Correa	Orangeberry	Rutaceae	Unarmed shrub	Vol-1: pp153
Grewia bicolor Juss.	False brandy bush	Tiliaceae	Climbing shrub	Vol-1: pp114-119
Hugonia mystax Linn.	Mothirakanni	Linaceae	Scandent shrub	Vol-1: pp126
Hyptis suaveolens (L.) Poit	Pignut	Lamiaceae	Tall herb	Vol-2: pp1128-1129
Jatropha integerrima Jacq.	Peregrina	Verbenaceae	Tree	Vol-2: pp1339-1340
Lepidagathis fasciculata Retz. Nees	Striped lepidagathis	Acanthaceae	Small perennial herb	Vol-2: pp1064-1068
Murraya paniculata L. Jack.	Kattu karuveppilai	Rutaceae	Evergreen tree	------
Pavonia odorata Willd.	Pavattai	Malvaceae	Small shrub	Vol-1: pp92-93
Premna latifolia Roxb.	Arani	Verbenaceae	Small tree	Vol-2: pp1093-1096
Pseudocalymma alliaceum Lam. Sandwith	Garlic creeper	Bignoniaceae	Shrub	------
Psychotria octosulcata W.A. Talbot	Sore mouth bush	Rubiaceae	Shrub	Vol-2: pp637-642
Rhus mysorensis Heyne	Chippamaram, neyyi kiluvai	Anacardiaceae	Small shrub	Vol-1: pp263-264
Scutellaria violacea Heyne	Novupacchilai, violet skullcap	Capparaceae	Herb	Vol-2: pp1141-1142
Sebastiania chamaelea L. Mull. Arg.	Snakes tongue	Euphorbiaceae	Herb	Vol-2: pp1343-1344
Solanum pseudocapsicum Linn	Jerusalem cherry	Solanaceae	Herb	------
Tarenna asiatica	Tharani, kottam	Rubiaceae	Small tree	------
Tiliacora acuminata Lam.	Vallikanjiram	Menispermaceae	Scandent shrub	Vol-1: pp27-28
Tragia involucrata Linn.	Kanchori	Euphorbiaceae	Climbing hispid herb	Vol-2: pp1331-1332
Ziziphus oenoplia L. Mill.	Suraimullu	Rhamnaceae	Climbing shrub	Vol-1: pp218-220

the oviposition champers (46×40.5×40.5, 46×40.5×40). After adult emergence, cotton soaked with 10% sugar solution with few drops of multivitamins was kept inside oviposition cage for adult feeding. After hatching newly emerged larvae will be providing bhendi for feeding. These laboratory reared larvae were used for bioassay, at room temperature (28±2°C) and (RH 75±5%).

Bioassay

Antifeedant activity: Antifeedant activity of crude extracts studied using leaf disc method. The stock concentration of crude extracts (5%) was prepared by dissolving in acetone and mixing with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was used as emulsifier. Fresh cotton leaf (for *H. armigera*) and castor leaf (for *S. litura*) discs of 4 and 3cm diameter were punched using cork borer and dipped in 0.625, 1.25, 2.50 and 5.00%, respectively concentration of crude extracts separately and air dried for 5 min. After air drying, treated leaf discs were kept inside the petri dishes (15×90 mm diameter) separately containing wet filter paper to avoid drying of the leaf disc and single 2 h pre-starved fourth instar larva of *H. armigera* and *S. litura* was introduced on each treated leaf disc. Leaf discs treated with acetone were considered as control. Ten replications were maintained for each treatment. Progressive consumption of leaf area by the larva in 24 h period was recorded in control and treatments using leaf area meter (systronics 211). Leaf area consumed in plant extract treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Ben Jannet *et al.*[8]:

$$\text{AFI} = \frac{\text{C} - \text{T}}{\text{C} + \text{T}} \times 100$$

Where, C and T represent the amount of leaf eaten by the larva on control and treated discs, respectively.

RESULTS

Antifeedant activity of crude plant extracts was assessed based on antifeedant index. Higher antifeedant index normally indicate decreased rate of feeding. In the present study, the antifeedant activity varied significantly based on the solvents used for extraction. Antifeedant effects of different plant extracts were evaluated based on leaf area consumed by *Spodoptera litura* and *Helicoverpa armigera* (Table 2) lists out the antifeedant effect of various plant species tested. Increase in number of plus signs against the extracts of a plant reflects the degree of antifeedant activity. Among the 26 plant species tested, the extracts of *Pseudocalymma alliaceum*, *Barleria buxifolia*, *Solanum pseudocapsicum* were found to be effective against the 4th instar larvae of *S. litura* and

Table 2: Screening of antifeedant activity of twenty six plants extracts against *Spodoptera litura* and *Helicoverpa armigera*

		Different solvent extracts at 5% concentrations (Mean±SD)					
		Spodoptera litura			*Helicoverpa armigera*		
Plant names	Parts used	H	CFM	EA	H	CFM	EA
Asparagus racemosus Willd.	Leaves	+	+	+	++	+	+
Atalantia monophylla Correa	Leaves	++	+++	++	+	++	++
Barleria buxifolia Linn.	Leaves	++	+++	+++	++	+++	+++
Barleria montana Nees	Leaves	+	+++	++	++	+	++
Cleome gynandra L.	Leaves	+	++	+	+	++	+
Cyclea peltata Hook. f. and Thoms	Leaves	+	++	++	++	+	++
Gloriosa Superba Linn.	Leaves	-	+	++	-	+	+
Glycosmis mauritiana Correa	Leaves	+	+++	++	+	+++	++
Grewia bicolor Juss.	Leaves	+	++	++	+	++	++
Hugonia mystax Linn.	Leaves	+	++	++	+	++	++
Hypotis suaveolens (L.) Poit	Leaves	++	++	++	++	++	+++
Jatropha integerrima Jacq.	Leaves	++	+	+++	+	++	++
Lepidagathis fasciculata Retz.	Aerial	+	-	+	+	-	+
Murraya paniculata L. Jack.	Leaves	+	++	+++	++	+	++
Pavonia odorata Willd.	Leaves	++	++	+	++	++	+
Premna latifolia Roxb.	Leaves	++	++	+	++	+	++
Pseudocalymma alliaceum Lam.	Leaves	++	+++	++++	++	+++	++++
Psychotria octosulcata W.A. Talbot	Leaves	++	++	++	++	+++	++
Rhus mysorensis Heyne	Leaves	+	+	-	+	+	-
Scutellaria violacea Heyne	Leaves	++	+	+	++	+	+
Sebastiania chamaelea L. Mull. Arg.	Leaves	+	++	++	+	++	++
Solanum pseudocapsicum Linn	Aerial	++	+++	++++	++	+++	+++
Tarenna asiatica	Leaves	++	++	+++	+	++	+++
Tiliacora acuminata Lam.	Leaves	+	+	++	+	+	++
Tragia involucrata Linn.	Leaves	+	++	+++	+++	++	++
Ziziphus oenoplia L. Mill.	Leaves	+	++	++	+	++	++

No activity, +: Below 25% activity, ++: Above 25% but below 50% activity, +++: Above 50% but below 75% activity, ++++: Above 75% activity

H. armigera. The maximum antifeedant activity was recorded in 5% concentration of ethyl acetate extract of *Pseudocalymma alliaceum* (81.55 and 79.44%), *Solanum pseudocapsicum* (76.32 and 74.66%), *Barleria buxifolia* (73.23 and 70.66%) and chloroform extract of *Pseudocalymma alliaceum* (68.33 and 63.77%), *Solanum pseudocapsicum* (61.55 and 57.12%), *Barleria buxifolia* (54.80 and 48.46%),) whereas, minimum in hexane extracts of *Pseudocalymma alliaceum* (39.46 and 31.17%), *Solanum pseudocapsicum* (31.11 and 29.40), *Barleria buxifolia* (28.99 and 21.55%), (Table 3).

DISCUSSION

Plants have more numbers of naturally occurring phytocompounds that possess plant protection properties against insect pests and diseases. These natural products in insect pest management programs are received much attention in recent years due to environmental pollution, pest resistance, resurgence and undesirable effects to the non target organisms caused by unsystematic use of synthetic pesticides. Antifeedant activity of botanicals against insects has been studied in many countries. Higher antifeedant index normally indicate decreased rate of feeding. Antifeedant is a chemical that inhibits the feeding without killing the insect pests directly, while it remains near the treated foliage and dies through starvation[9-10].

Antifeedant activities of rhein isolated from *Cassia fistula* flower against lepidopteran pests *Spodoptera litura* and *Helicoverpa armigera* with significant antifeedant activity at 1000 ppm concentration[11] stated that leaf extract and its column eluted with ethyl acetate fraction from *Pergularia daemia* exhibited good antifeedant activity against *Spodoptera litura*. Due to the toxic effect of plant extracts, maximum number of treated larvae died inspite of less food consumption[12]. Earlier, the maximum larval mortality was found in the essential oil of *Zingiber officinales* tested against armyworm, *S. litura* an agricultural important lepidopteron pest[13]. This indicates that the active principles present in the particular solvent extracts inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on

Table 3: Antifeedant activity of *P. alliaceum*, *S. pseudocapsicum* and *B. buxifolia* plant extract against *S. litura* and *H. armigera*

		Concentrations							
		Spodoptera litura				*Helicoverpa armigera*			
Plants	Solvent	0.625%	1.25%	2.5%	5%	0.625%	1.25%	2.5%	5%
Barleria buxifolia	Hexane	9.77±2.99	13.22±6.72	19.52±3.88	28.99±3.43	7.76±8.29	11.83±7.84	18.11±5.58	21.55±2.77
	Chloroform	17.33±4.00	21.82±1.66	32.77±4.90	54.80±4.55	14.33±4.92	20.37±6.58	26.46±2.21	48.46±4.22
	Ethyl acetate	23.81±7.99	50.22±7.88	64.33±2.00	73.23±3.66	23.77±8.29	50.02±9.75	59.34±7.86	70.66±4.66
Solanum pseudocapsicum	Hexane	10.33±3.29	17.25±5.99	22.11±1.88	31.11±2.22	8.30±6.92	16.32±4.53	20.21±3.77	29.40±5.44
	Chloroform	16.33±6.33	27.54±5.34	45.38±6.33	61.55±6.53	14.67±8.96	21.92±3.63	39.34±3.93	57.12±3.44
	Ethyl acetate	29.22±8.21	51.11±9.73	63.66±3.22	76.32±3.43	27.99±4.83	51.33±6.96	59.22±4.29	74.66±2.66
Pseudocalymma alliaceum	Hexane	10.97±2.11	16.54±4.33	23.99±5.33	39.46±3.70	9.15±4.39	13.11±3.88	20.21±2.33	31.17±7.22
	Chloroform	17.11±2.11	26.45±2.92	49.72±2.22	68.33±2.33	21.92±6.94	29.17±4.93	43.32±6.40	63.77±3.44
	Ethylacetate	34.66±5.21	53.74±2.82	68.47±4.22	81.55±4.33	29.12±5.48	36.33±2.99	60.31±4.33	79.44±2.77
Control		0.0±0.0		0.0±0.0		0.0±0.0		0.0±0.0	

Values are mean±standard deviation of 5 replications

the chemosensilla of the larva resulting in feeding deterrence (antifeedant). These findings are in agreement with the earlier reports of Jeyasankar *et al.*[14].

Several authors have reported that plant extracts possess a similar type of antifeedant activity against lepidopteran pests[15]. Jeyasankar *et al.*[16], reported a new crystal compound 2,5-Diacetoxy-2benzyl-4,4,6,6-tetramethyl-1,3-cyclohexanedione that isolated from the leaves of *Syzygium lineare* (*S. lineare*) was effective against *S. litura*. In the present study, hexane, chloroform and ethyl acetate extracts of *B. buxifolia*, *S. pseudocapsicum* and *P. alliaceum* was promising in reducing feeding rate of *S. litura* and *H. armigera.* The rate of feeding significantly varied depending on the concentration of the plant extracts. This indicates that the active principles present in the plants inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence. The present results suggest that leaves extracts sufficiently inhibited the responses of larvae to these specific stimuli. The physical properties of the tested extract probably were not significant in the sense of feeding inhibition, since there were not visible differences between treated and untreated leaves. Therefore, prevention of leaf damage achieved by the application of tested extract could be mainly attributed to their active compounds. These findings are in agreement with the earlier reports of Jeyasankar *et al.*[14,17,18].

CONCLUSION

Pesticidal screening of different plant species against *H. armigera* and *S. litura*. Among the plants screened for antifeedant activity, *Pseudocalymma alliaceum*, *Solanum pseudocapsicum* and *Barleria buxifolia* showed significant antifeedant activity. Further, it may be suggested that the active phytocompounds could be isolated and identified which then can be used for controlling the economically important insect pests.

SIGNIFICANCE STATEMENT

This study discovers the screening of pesticidal plants against economically important insect pests. Twenty six plants screened, *Pseudocalymma alliaceum*, *Solanum pseudocapsicum* and *Barleria buxifolia* showed significant antifeedant activity on *Spodoptera litura* and *Helicoverpa armigera*. Thus, these plants may be used to control the insect pests.

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