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Biological activities of *Solanum pseudocapsicum* (Solanaceae) against cotton bollworm, *Helicoverpa armigera* Hübner and armyworm, *Spodoptera litura* Fabricius (Lepidotera: Noctuidae)

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PEER REVIEW

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Comments
This research holds an important concept in pest control, *i.e.*, controlling the agricultural pests without disturbing the nature and natural enemies in the agricultural field. The results of the present studies are noteworthy and there is a high possibility of developing an ecofriendly phytopesticide.

ABSTRACT

Objective: To evaluate the antifeedant, insecticidal and growth inhibition activities of *Solanum pseudocapsicum* (*S. pseudocapsicum*) seed extracts against *Spodoptera litura* (*S. litura*) and *Helicoverpa armigera* (*H. armigera*). **Methods:** Hexane, diethyl ether, dichloromethane and ethyl acetate seed extracts were prepared and tested for antifeedant, insecticidal and growth inhibitory activities against fourth instar larvae of *S. litura* and *H. armigera*. **Results:** Ethyl acetate extract showed promising antifeedant and insecticidal activities against *S. litura* and *H. armigera*. Percentage of deformed larvae, pupae and adults were maximum in treatment of ethyl acetate extract. Percentage of successful adult emergence was deteriorated by seeds on extract treated larvae. **Conclusions:** Ethyl acetate extracts of *S. pseudocapsicum*, showed higher efficiency of antifeedant, insecticidal and growth inhibition activities. Hence, it can be used to controll agricultural insect pests, *S. litura* and *H. armigera*.

KEYWORDS
Solanum pseudocapsicum, *Helicoverpa armigera*, *Spodoptera litura*, Antifeedant, Insecticidal

1. Introduction

India is basically an agro-based country with more than 80% of Indian population depends on agriculture. Indian economy is largely determined by agricultural productivity. Insect pests are known to cause significant damage to crops and they affect agricultural productivity. In recent years, the use of synthetic organic insecticides in crop pest control programs around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides and lethal effects on non-target organisms[1]. Management of agricultural pests over the half past century has been largely depending on the use of synthetic chemical pesticides for field and post-harvest protection of crops. Potential problems associated with continued long-term use of toxic insecticide include pest resistance

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and negative impact on natural enemies. Among current alternative strategies aiming at decreasing the use of classical insecticides, eco-chemical control based on plant-insect relationships is one of the most promising methods. Plant derived pesticides provide a more natural, "environmentally friendly" approach to pest control than synthetic insecticides. Extensive surveys and reports on plants used as insecticides can be found in the literature[2,3].

The cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (*H. armigera*) is a worldwide polyphagous pest inflicting crop damage in India to the sum of one billion dollars annually and it attacks over 200 crop species belonging to 45 families[4]. In India, this insect occurs as a major pest in many economically important crops including cotton, pigeonpea, chickpea, tomato, okra and blackgram. The tobacco armyworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (*S. litura*) is an economically important polyphagous pest in India, China and Japan, causing considerable economic loss to many vegetable and field crops. This pest attacks more than 112 species of cultivated crops and causes severe loss varies between 10% and 30% for major crops[5,6]. These pests are well justified in their polyphagy on all economically important crops and the hurdles in its management. This necessitates the search for more potent insecticides which are safer to the user and consumer. The present investigation evaluated the biological activities of *Solanum pseudocapsicum* (*S. pseudocapsicum*) against economically important pests.

2. Materials and methods

2.1. Collection and extraction of plant materials

The seeds of *S. pseudocapsicum* were collected from Udhagamandalam, Nilgiris. Plant specimen was identified by Dr. S Premalatha, Assistant Professor, Department of Botany, Government Arts College, Musiri, Trichy District.

The plant materials were thoroughly washed with tap water and shade dried under room temperature (27.0±2.0) °C at Department of Zoology, Government Arts College, Musiri. After complete drying the plant materials were powdered using electric blender and sieved through kitchen strainer. A thousand grams of plant powder was extracted with hexane, diethyl ether and ethyl acetate, sequentially with increasing polarity of solvents and then, filtered through Whatman's No. 1 filter paper. The solvents 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*.

2.2. Rearing of test insects

The larvae of *S. litura* and *H. armigera* were collected from vegetable field at Anaipatti, Musiri taluk, Tamil Nadu, India. Larvae were reared in laboratory condition at the Department of Zoology, Government Arts College, Musiri, Tamil Nadu, India. These laboratory-reared larvae were used for bioassays and the cultures were maintained throughout the study period.

2.3. Antifeedant activity

Antifeedant activity of the crude extracts was studied using leaf disc no choice method[7]. The stock concentration of crude extracts (5%) was adjusted by dissolving in acetone and mixing with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was used as emulsifier[8]. Fresh tomato leaf discs of 2 cm diameter were punched using cork borer and dipped with 0.625 mg/L, 1.25 mg/L, 2.5 mg/L and 5 mg/L concentrations of crude extracts, individually. Leaf discs treated with acetone and without solvent (water) were regarded as control. After air-drying, each leaf disc was placed in Petri dish (1.5 cm ×9 cm) containing wet filter paper to avoid early drying of the leaf disc, and a single 2 h pre-starved the fourth instar larva of *S. litura* and *H. armigera* were introduced. For each concentration five replicates were maintained. Progressive consumption of leaf area by the larva after 24 h was recorded in control and treated discs using graph sheet method. 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*[9].

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

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

2.4. Insecticidal activity

Fresh tomato leaves were treated with different concentrations (as mentioned in antifeedant activity) of crude extracts. Tomato leaves treated with acetone and without solvent were considered as control. Petioles of the tomato leaves were tied with wet cotton plug (to avoid early drying) and placed in round plastic trough (29 cm×8 cm). In each crude extract with different concentrations 10 pre-starved (2 h) the fourth instar larvae of *S. litura* and *H. armigera* were introduced individually and covered with muslin cloth. Five replicates were maintained for all concentrations and the number of dead larvae was recorded after 24 h up to pupation. Percentage of larval mortality was calculated and corrected by Abbott's formula[10].

$$\text{Abbott's percent corrected mortality}=\frac{\%\text{ Mortality in treated}-\%\text{ Mortality in control}}{100\%-\%\text{ Mortality in control}}\times 100$$

2.5. Growth regulation activity

Growth regulation activitiy of crude extracts was studied at four different concentrations (0.625 mg/L, 1.25 mg/L, 2.5 mg/L and 5 mg/L) against the fourth instar larvae of *S. litura* and *H. armigera*. Ten larvae were introduced in a Petri-plate containing tomato leaves treated with different concentrations of crude extracts. Water or acetone treated leaves were considered as control. After 24 h feeding, the larvae were transferred to normal leaves for studying the developmental period. For each concentration five replicates were maintained. During the developmental period, deformed larvae, pupae, adults and successful adults emerged were recorded. In addition, weight gain by the treated and control larvae were also recorded.

2.6. Data analysis

Data analysis was carried out using Microsoft Excel 2007. Two-way ANOVA was performed for all the experimental data, from which least significant difference (P<0.05) was calculated and the significant differences were marked with different alphabets.

3. Results

Crude extracts of *S. pseudocapsicum* were prepared using solvents of hexane, diethyl ether, ethyl acetate and their bioactivities were tested at different concentrations against fourth instar larvae of *S. litura* and *H. armigera*. Data collected from biological activities were subjected to Two -way ANOVA significant difference between the mean was separated using least significant difference (LSD) test.

Antifeedant activity of the crude extracts of the seeds of *S. pseudocapsicum* was studied at four different concentrations and the results are presented in Table 1. Antifeedant activity of the solvent extracts was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. In the present study, irrespective of concentration and solvents used for extraction the antifeedant activity varied

Table 1
Antifeedant index of *S. pseudocapsicum* against the fourth instar larvae of *S. litura* and *H.armigera* at different concentrations (n=5, mean±SD).

Solvent extracts	*S. litura*				*H. armigera*			
	0.625 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L	0.625 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L
Hexane	12.2±6.4^{a}	26.5±4.1^{a}	36.3±3.4^{a}	41.4±6.5^{a}	9.2±5.4^{a}	16.2±5.4^{a}	26.3±3.4^{a}	31.4±5.5^{a}
Diethyl ether	23.7±4.6^{b}	28.2±2.4^{a}	41.9±3.8^{a}	56.7±7.4^{b}	13.7±4.5^{a}	18.1±2.4^{a}	31.9±3.8^{a}	46.6±6.4^{b}
Dichloromethane	26.2±3.4^{b}	38.2±2.1^{b}	48.2±3.4^{b}	66.2±7.3^{c}	16.7±4.2^{b}	22.1±2.7^{a}	29.9±4.8^{a}	50.6±4.4^{b}
Ethyl acetate	31.2±7.3^{c}	45.2±4.8^{c}	57.4±5.2^{c}	80.3±6.6^{d}	31.2±7.4^{c}	35.2±4.8^{b}	47.9±5.2^{b}	82.1±5.6^{c}

Within the column data with different alphabet superscripts are statistically significant (P<0.05 by LSD).

Table 2
Mortality rate of the fourth instar larvae of *S. litura* and *H.armigera* after the treatment of *S. pseudocapsicum* seed extracts at different concentrations (n=5, mean±SD).

Solvent extracts	*S. litura*				*H. armigera*			
	0.625 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L	0.625 mg/L	1.25 mg/L	2.5 mg/L	5 mg/L
Hexane	9.2±5.4^{a}	12.6±7.1^{a}	12.6±6.4^{a}	19.4±5.5^{a}	10.4±3.4^{a}	12.6±7.1^{a}	12.6±6.4^{a}	18.4±6.5^{a}
Diethyl ether	8.9±6.4^{a}	14.4±9.4^{a}	20.4±4.8^{a}	24.8±6.3^{a}	9.9±5.4^{a}	15.4±9.4^{a}	21.4±4.8^{b}	25.8±5.2^{a}
Dichloromethane	9.2±3.4^{a}	20.2±2.4^{b}	39.2±3.1^{b}	46.2±3.3^{b}	12.2±3.2^{a}	20.2±2.0^{b}	32.2±3.1^{c}	42.1±3.3^{b}
Ethyl acetate	11.6±9.4^{a}	24.2±4.6^{b}	46.4±7.8^{c}	75.3±6.2^{c}	21.6±9.2^{b}	24.2±4.1^{c}	46.4±7.8^{d}	66.5±6.6^{c}

Within the column data with different alphabet superscripts are statistically significant (P<0.05 by LSD).

Table 3
Insect growth regulatory activity of *S. pseudocapsicum* seed extracts (5 mg/L) against fourth instar larvae of *S. litura* and *H. armigera* (n=5).

Solvent extracts	*Spodoptera litura*				*Helicoverpa armigera*			
	Larvae	Pupae	Adult	Successful adult emergence	Larvae	Pupae	Adult	Successful adult emergence
Hexane	10.0^{a}	7.5^{a}	9.2^{a}	53.3^{b}	9.0^{a}	7.0^{a}	7.2^{a}	60.3^{c}
Diethyl ether	17.2^{b}	11.8^{a}	14.5^{a}	28.5^{a}	16.2^{b}	11.8^{ab}	15.5^{b}	39.5^{b}
Dichloromethane	16.1^{b}	18.4^{b}	19.4^{b}	26.5^{a}	12.5^{b}	15.4^{ab}	10.4^{a}	26.5^{a}
Ethyl acetate	26.2^{c}	20.8^{bc}	20.5^{b}	16.5^{a}	26.2^{c}	20.8^{c}	21.5^{bc}	21.5^{a}

Within the column data with different alphabet superscripts are statistically significant (P<0.05 by LSD).

significantly. Data pertaining to the above experiment clearly revealed that maximum antifeedant activity was recorded in ethyl acetate extract 80.3% on *S. litura* and 82.1% on *H. armigera* at 5% concentration compared to control. Two–way analysis of variance followed by LSD test showed statistical significance ($P<0.05$).

Insecticidal activity of crude extracts of seeds of *S. pseudocapsicum* was studied at different concentrations and the results are presented in Table 2. Insecticidal activity of solvent extracts was calculated based on larval mortality after treatment. High larval mortality normally indicates potential insecticidal activity of plant extracts. In the present study irrespective of concentration and solvents used for extraction the insecticidal activity varied significantly. Data pertaining to the insecticidal activity clearly revealed that maximum insecticidal activity was recorded in ethyl acetate extract in 75.3% on *S. litura* and followed by 66.5% on *H. armigera*. One–way analysis of variance followed by LSD test showed statistically significant ($P<0.05$). Percentage of deformities due to the treatment of crude extracts from seeds of *S. pseudocapsicum* at 5% concentration is presented in Table 3. Maximum larval, pupal and adult deformities were observed in ethyl acetate extract on both insects. Percentage of successful adult emergence was found to be minimum in ethyl acetate extract in 16.5% and 21.5% on *S. litura* and *H. armigera* respectively. Ethyl acetate extract of seeds was subjected to preliminary phytochemical analysis for the confirmation of major group of compounds. Extracts showed presence of triterpenoids, flovonoids and alkaloids.

4. Discussion

Plants are a rich source of organic chemicals on earth. About ten thousand secondary metabolites have been chemically identified. Many plants have high content of unpalatable substances like phenols, alkaloids, flavanoids, terpenes, quinone, coumarin *etc.*, which play a defensive role against insect pests. These substances possess wide range of biological activities including antifeedant, oviposition deterrent, insecticidal, ovicidal and insect growth regulatory activities. Identifying sources with useful biological activity is only a starting point in the long process of development of a botanical pest management product. Success of botanical in the field depends on numbers of factors such as ongoing availability of the natural resources, adequate biomass to justify extraction, the feasibility of extraction near the harvest site and the stability of the extract in storage after preparation[11]. Antifeedant activity of botanicals against insects has been studied in many countries. Quantification of antifeedant effect of botanicals is of great importance in the field of insect pest management. From an ecological point of view, antifeedants are very important since they never kill the target insects directly and allow them to be available to their natural enemies and thus help in the maintenance of natural balance. The monophagous or oligophagous insects die due to the application of antifeedants on their food plants, due to starvation.

In the present study, ethyl acetate extract of *S. pseudocapsicum* 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. These findings are in agreement with the earlier reports of Jeyasankar and Premalatha[12]. Several authors have reported that plant extracts possess similar type of antifeedant activity against lepidopteran pests[13–17].

Antifeedant chemicals play a major role in the unsuitability of non–host plants as food for insects. Isolation and structure elucidation of these chemicals is important not only for understanding the ecological aspects of insect pest's relationship, but also for their potential in insect pest's control. In the present study, preliminary phytochemical analysis revealed the presence of triterpenoids, flovonoids, alkaloids and quinines in the ethyl acetate extracts indicating higher percentage of antifeedant activity. These findings are in agreement with the earlier reports of Morimoto *et al*[18]. They have reported that quinone, remirol and cyperquinone isolated from the plants of the family Cyperaceae had strong antifeedant activity against *S. litura*.

Screening plant extracts for deleterious effects on insects is one of the approaches used in the searching for novel botanical insecticides. Secondary plant compounds act as insecticides by poisoning *per se* or by production of toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding[19]. In the present study ethyl acetate extract from seeds of *S. pseudocapsicum* exhibited a significant insecticidal activity at a higher concentration. It is possible that the insecticidal property present in the selected plant compound may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died.

In the present study, preliminary phytochemical analysis revealed the presence of alkaloid and quinones in the ethyl acetate extracts indicating higher percentage of insecticidal activity observed in seeds extract of *S. pseudocapsicum*. Similar works have already reported insecticidal activity of many plants and their compounds against different groups of insects[20]. Insect growth regulation properties of plant extracts are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying[21]. In the present study, deformed development of larvae, pupae and adults were noted. Among the solvents extract tested, percentage of deformed larvae, pupae and adults were observed maximum on ethyl acetate extract of *S. pseudocapsicum*.

The morphological deformities at larval, pupal and adult stages are due to toxic effects of subfractions on growth and development processes. Since morphogenetic hormones regulate these processes, it can be suggested that these solvent crude extracts interfere with these hormones of the insects. These results are consistent with the earlier reports on various lepidopteran species[22–27].

In conclusion, ethyl acetate extract of seeds of *S. pseudocapsicum* showed higher antifeedant, insecticidal and growth inhibition activities against agriculture important pests of *S. litura* and *H. armigera*. Hence, it may be suggested that the extract of *S. pseudocapsicum* can be used for the control of economically important insect pests.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

India is an agro–based country in South East Asia. Among the Indian population, nearly 80% of the people depends on agriculture for their food. Besides, after the green revolution, there is a rapid growth in pesticide producing companies. Indiscriminate application of those pesticides over a long period resulted in resurgence of minor pests into major pests. Moreover, our environment also faced several detrimental effects. Thus, there is an urgent need to search for synthetic pesticides towards the control of important polyphagous pests, *H. armigera* and *S. litura*.

Research frontiers

Since the selected two pests are considered as a high devastator of more than 170 agricultural crops, their control in any means, either, deterring them from feeding on agricultural foliage and killing them in the larval stage or arresting their growth, there by indirectly preventing their population explosion are gaining a real momentum at this juncture.

Related reports

In the present study, ethyl acetate extract of *S. pseudocapsicum* showed statistically significant antifeedant activity. This indicates that the extract contain active principles which are responsible for disturbing the feeding behavior in *S. litura* and *H. armigera* by disturbing the chemosensilla. These findings are in line with the earlier findings of Isman *et al.*(1990). Further, morphological deformities observed in the present investigation are in corroborating with the results of Baskar *et al.* (2012).

Innovations and breakthroughs

The data obtained in the present investigation are showing significance in pest control. Furthermore, this study provides first report on the control of selected important agricultural polyphagous pests. Thus, this leads a new path in the research arena to search the diverse actions of selected plant and its active principles in physiological, biochemical and enzymological parameters of pests.

Applications

The ethyl acetate extract of seeds of *S. pseudocapsicum* showed promising antifeedant, insecticidal and growth inhibition activities against the selected pests of *S. litura* and *H. armigera*. Hence, it may be suggested that the extract of *S. pseudocapsicum* can be used for controlling the economically important insect pests as an important component in Integrated Pest Management Programme.

Peer review

This manuscript holds an important concept in pest control, *i.e.*, controlling the agricultural pests without disturbing the nature and natural enemies in the agricultural field. The results of the present studies are noteworthy and there is a high possibility of developing an ecofriendly phytopesticide.

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