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## Bioactivity of *Pseudocalymma alliaceum* (Lam.) Sandwith (Bignoniaceae) against *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)

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### PEER REVIEW

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#### Comments

The present research is an excellent work particularly nowadays. This article will motivate many researchers to conduct their research in this way. This kind of work should be encouraged to save our green world and also, active compounds need to be found out.

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### ABSTRACT

**Objective:** To evaluate the antifeedant, larvicidal and insect growth inhibitory activities of crude extracts of *Pseudocalymma alliaceum* tested against fourth instar larvae of *Spodoptera litura* (*S. litura*) and *Helicoverpa armigera* (*H. armigera*).

**Methods:** Hexane, chloroform and ethyl acetate 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, insecticidal activity against *S. litura* and *H. armigera*. Maximum percentage of deformed larvae, pupae and adults were found on treatment with ethyl acetate extract. Percentage of successful adult emergence was deteriorated by extract treated larvae. Ethyl acetate extracts of *Pseudocalymma alliaceum*, showed higher percentage of antifeedant, insecticidal and growth inhibition activities.

**Conclusions:** This is the first report on *S. litura* and *H. armigera*. Further, the active compounds isolated from the ethyl acetate extracts will be useful for controlling economically important insect pests.

### KEYWORDS

*Pseudocalymma alliaceum*, *Helicoverpa armigera*, *Spodoptera litura*, Antifeedant, Insecticidal

## 1. Introduction

Insect pests play a major role in damaging the agricultural crops and the loss varies between 10% and 30% for major crops[1]. India is basically an agro-based country, and more than 80% of population depends on agriculture and Indian economy is largely determined by agricultural productivity. The intensification of agriculture to fulfill food needs has

increased the number of insect pest species attacking different crops, resulting in the annual production losses of the standing crops. In the past, synthetic pesticides have played a major role in crop protection programmes and have immensely benefited mankind. Nevertheless, the indiscriminate use of synthetic pesticides has resulted in the development of resistance by pests (insects, weeds, etc.), resurgence and outbreak of new pests, toxicity to non-

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target organisms and hazardous effects on the environment endangering the sustainability of ecosystems[2]. Among current alternative strategies aiming at decreasing or minimizing the use of chemical insecticides, eco-chemical control based on plant-insect relationships is one of the most promising methods. Plant derived chemicals offer a more natural and environmentally friendly approach to pest control than synthetic insecticides.

The cotton bollworm *Helicoverpa armigera* (*H. armigera*) (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest worldwide that inflicts crop damage in India to the sum of one billion dollars annually and it attacks over 200 crop species belonging to 45 families[3,4]. In India, this insect occurs as a major pest in many economically important crops including cotton, pigeonpea, chickpea, tomato, okra, and blackgram. The tobacco caterpillar *Spodoptera litura* (*S. litura*) (Fabricius) (Lepidoptera: Noctuidae) is an economically important polyphagous pest in India, China and Japan, causing considerable economic loss to many vegetable and field crops. It causes economic losses of crops from 25.8% to 100% based on crop stage and its infestation level in the field. It has large host range of more than 120 host plants in India including crops, vegetables, weeds and ornamental plants[5,6]. These pests' status is well justified in its 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. Among the control measures, plant derived compounds are more specific and environmentally safer.

*Pseudocalymma alliaceum* (*P. alliaceum*) (Bignoniaceae), commonly called as Garlic vine, is a very common plant remedy for the pain and inflammation of arthritis and rheumatism as well as cold, flu and fever. Generally, leaves are used in the preparation of infusion or decoction. Roots are used in the preparation of cold maceration and tincture and generally taken as a whole body tonic[7–9]. Shrankhla *et al.* reported that larvicidal activity of the hexane extract of *P. alliaceum* exhibited potential larvicidal against *Culex quinquefasciatus* and *Anopheles stephensi*[10,11]. However, this plant has not been reported on biological properties against agricultural insect pests. Hence, in the present study, authors aimed to evaluate the antifeedant, insecticidal and growth inhibitory activities of *P. alliaceum* against economically important pests.

## 2. Materials and methods

### 2.1. Collection and extraction of plant material

The leaves of *P. alliaceum* were collected from Chennai,

Tamil Nadu, India. Plant specimen was identified by Dr. R. Elango Mathavan, Assistant Professor, Department of Biotechnology, PRIST University, Thanjavur, Tamil Nadu, India. The voucher specimen (IPH-25) was deposited at Entomology Lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

The plant materials were thoroughly washed with tap water and shade dried under room temperature [(27.0±2) °C] at Entomology Lab, PG & Research Department of Zoology, A. A. Government Arts College, Musiri. After completing drying, the plant materials were powdered using electric blender and sieved through kitchen strainer. A total of 1000 g of plant powder was extracted with hexane, diethyl ether and ethyl acetate, sequentially with increasing polarity of solvents and filtered through Whatman's No.1 filter paper. The solvents from the crude extract 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 Vadukapatti, Musiri Taluk, Tamil Nadu, India. Larvae were reared under 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 crude extracts was studied using leaf disc no-choice method[12]. 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[13]. Fresh tomato leaf discs of 2 cm diameter were punched using cork borer and dipped with 0.625%, 1.25%, 2.5% and 5% concentrations of crude extracts, individually. Leaf discs treated with acetone and without solvent (water) were considered 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 fourth instar larva of *S. litura* and *H. armigera* was introduced. For each concentration, five replicates were maintained. Progressive consumption of leaf area by the larva after 24 h feeding was recorded in control and treated discs 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*[14].

## 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 concentration, 10 pre-starved (2 h) 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<sup>[15]</sup>.

## 2.5. Growth regulation activity

Growth regulation activities of crude extracts were studied at four different concentrations against fourth instar larvae of *S. litura* and *H. armigera*. Ten larvae were introduced in a Petri plate having 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 of the treated and control larvae were also recorded.

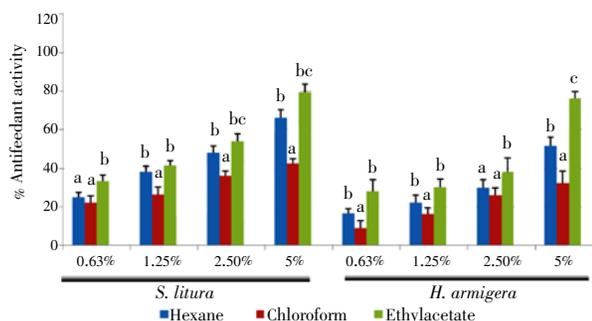
## 2.6. Data analysis

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

## 3. Results

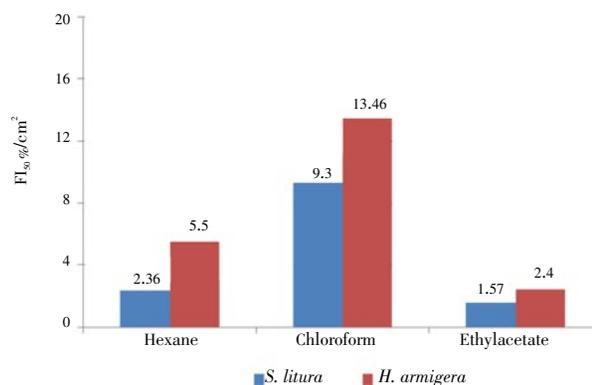
Antifeedant activity of the crude extracts of *P. alliaceum* was studied at four different concentrations and the results are presented in Figures 1 and 2. Antifeedant activity of solvent extracts was assessed based on antifeedant index. Higher antifeedant index usually indicates decreased rate of larval feeding. In the present study, irrespective of concentration and solvents used for extraction, the antifeedant activity varied significantly. Data pertaining to the above experiment clearly indicate that highest antifeedant activity was recorded in ethyl

acetate extract on *S. litura* (79.6%) and on *H. armigera* (76.2%) at 5% concentration compared to control. One-way ANOVA followed by Tukey's multiple range test ( $P \leq 0.05$ ) showed statistical significance ( $P < 0.05$ ).



**Figure 1.** Antifeedant activity of crude extracts of *P. alliaceum* against 4th instars larvae of *S. litura* and *H. armigera*.

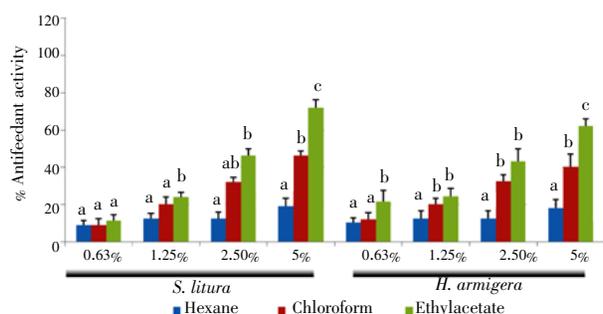
Values are mean of five replications. Within the column similar alphabets are statistically not significant ( $P < 0.05$  by LSD).



**Figure 2.** Antifeedant activity (FI<sub>50</sub>%/cm<sup>2</sup>) of crude extracts of *P. alliaceum* against 4th instars larvae of *S. litura* and *H. armigera*.

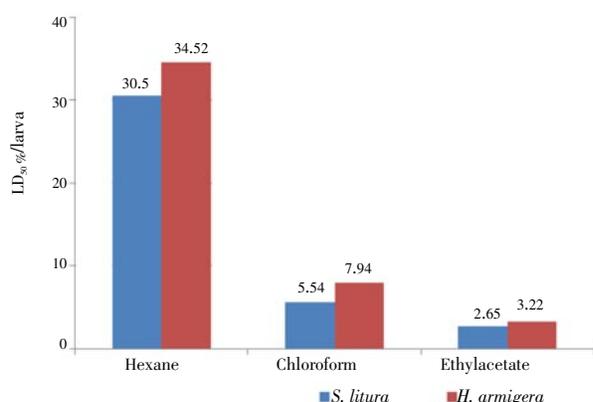
Insecticidal activity of crude extracts of *P. alliaceum* was studied at different concentrations and the results are presented in Figures 3 and 4. Insecticidal activity of solvent extracts was calculated based on larval mortality after treatment. High larval mortality normally indicates potential insecticidal 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 72.1% on *S. litura* and followed by 62.3% on *H. armigera*. One-way ANOVA followed by Tukey's multiple range test ( $P \leq 0.05$ ) showed statistical significance ( $P < 0.05$ ) compared to control. Percentage of deformities due to the treatment of crude extracts of *P. alliaceum* at 5% concentration is presented in Figure 5. Maximum larval, pupal and adult deformities were observed in ethyl acetate extract on both insects. The minimum percentage of successful adult emergence was found in ethyl acetate extract with 14.24% and 15.45% on *S. litura* and *H. armigera* respectively. Significant percent larval, pupal and adult deformities was observed in ethyl acetate extract on both tested insects. Further, ethyl acetate extract was subjected to preliminary phytochemical analysis for the confirmation

of major group of compounds presented in Table 1. Extracts showed positive results for confirmation of terpenoids, flavonoids, phenols and quinines.

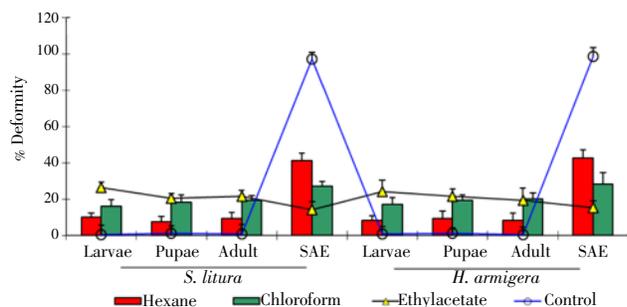


**Figure 3.** Larvicidal activity of *P. alliaceum* against 4th instar larvae of *S. litura* and *H. armigera*.

Values are mean of five replications. Within the column similar alphabets are statistically not significant ( $P < 0.05$  by LSD).



**Figure 4.** Larvicidal activity (LD<sub>50</sub>%/larva) of *P. alliaceum* against 4th instar larvae of *S. litura* and *H. armigera*.



**Figure 5.** Insect growth inhibitory activity of *P. alliaceum* against 4th instar larvae of *S. litura* and *H. armigera*.

Values are mean of five replications. SAE: Successful adult emergence.

**Table 1**

Preliminary phytochemical analysis of different solvent extracts of *P. alliaceum*.

Constituents	Ethyl acetate extract
Alkaloids	–
Anthraquinones	–
Coumarin	–
Catechin	–
Flavonoids	+
Phenols	+
Quinines	+
Saponins	–
Steroids	+
Terpenoids	+
Sugars/glycosides	–

## 4. Discussion

There are numbers of naturally occurring compounds that possess plant protection properties. Already 10000 secondary metabolites have been chemically identified. In nature, many plants have unpalatable substances like high content of 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, insecticidal, and insect growth regulators. Identifying sources with useful biological activity is only the starting point in the long process of development of a botanical pest management product. Success of botanical in the field depends on number 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. 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 for 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 *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 particular solvent extracts inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence (antifeedant). These findings are in agreement with the earlier reports of Jeyasankar *et al*[16]. Several authors have reported that plant extracts possess similar type of antifeedant activity against lepidopteran pests[17–20].

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 our findings, preliminary phytochemical analysis revealed that terpenoids, flavonoids, phenols and quinines were present in the ethyl acetate extracts, indicating higher percentage of antifeedant activity. These findings are in agreement with the earlier reports of Morimoto *et al*[21]. 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 producing toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding<sup>[22]</sup>. In the present study, ethyl acetate extract from of *P. alliaceum* exhibited significant insecticidal activity at higher concentration. It is possible that the insecticidal property present in the crude extract 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 that terpenoids and quinones present in the ethyl acetate extracts indicate that higher percentage of insecticidal activity was observed in seeds extract of *P. alliaceum*. Similar works have already reported insecticidal activity of many plants and their compounds against different groups of insects<sup>[23]</sup>. 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<sup>[24]</sup>. 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 *P. alliaceum*. This is the first report on biological activities against *S. litura* and *H. armigera*.

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<sup>[25–27]</sup>. Ethyl acetate extract of seeds of *P. alliaceum* showed higher antifeedant, insecticidal and growth inhibition activities against agriculture important pests of *S. litura* and *H. armigera* and it is the first report on these insect pests. Hence, it may be suggested that the extract of *P. alliaceum* can be used for controlling the economically important insect pests.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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of Zoology, A. A. Govt. Arts College, Musiri–621 211, Tamil Nadu, India for their support and facilities provided. Authors are also thankful that this work was conducted in the laboratory which is financially supported by UGC, New Delhi, India (Ref No. 42–570/2013 (SR)).

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### Comments

#### Background

Insect pests play a major role in damaging the agricultural crops and the crop loss varies between 10% and 30% for major crops. The indiscriminate use of synthetic pesticides has resulted in the development of resistance by pests. Among current alternative strategies, plant derived chemicals can offer a more natural and environmentally friendly approach to pest control than synthetic insecticides.

#### Research frontiers

This article focuses on the negative impacts of chemical pesticides and also shows the importance of the plant derived pesticides to save the green environment. Thus, there is no doubt, this kind of research must be encouraged and motivated to mitigate the environmental pollution by synthetic pesticides. The plant derived compounds could be possible utilized in the integrated pest management programme in the near future.

#### Related reports

The materials and methods adapted in the present study are reproducible and are universally accepted by several authors. The concentration fixed in the present research are admissible to the non–target organisms like mammals, predators and parasites. The follow up of the methodology are easy even to a farmer.

#### Innovations and breakthroughs

One of the important innovative approach in the present investigation is the first hand report of *P. alliaceum* with special reference to the selected agriculturally important pests. Thus, it paves the way for further exploration of possible utilization of the selected plant against some other field pests which are of economic importance. And also the selected plant is alternative to synthetic pesticide.

#### Applications

This article indicates that the crude extracts showed promising activity against selected insect pests. Further, identification and structural elucidation of the radical compounds which are responsible for the selected biological activity are underway. Outcome of the present research would definitely gain a real momentum in the biological pest control strategies with special reference to phytopesticides, which in

turn, proves as an important component in integrated pest management.

### Peer review

The present research is an excellent work particularly nowadays. This article will motivate many researchers to conduct their research in this way. This kind of work should be encouraged to save our green world and also, active compounds need to be found out.

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