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Research Article
Hypericum hookerianum (Wight and Arn.) as a Potential Bio-Pesticide to Control Cotton Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract

Background and Objective: Cotton is a greatly valued commercial crop which finds heavy losses due to pests. The study aimed to assess the bio-pesticidal activity of *Hypericum hookerianum* (Wight and Arn.) against important field pest *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). **Materials and Methods:** The present study, the methanol extract of *Hypericum hookerianum* was tested for their pesticidal activity against the important agricultural field pest, Cotton bollworm, *Helicoverpa armigera*. The methanolic leaf extract was evaluated at different concentrations against the pest by performing antifeedant(leaf disc choice assay), larvicidal and ovicidal activities. **Results:** The methanol extract showed a 90.0% feed deterrent activity with 96.43% larvicidal activity and 65.77% larval egg mortality. These findings show that the methanol extract of this plant exhibits great potential as a bio-pesticide against the selected field pest. **Conclusion:** This present finding gives a secure alternative to the currently used chemical pesticides by contributing to a sustainable farming method.

Key words: *Hypericum hookerianum*, bio-pesticide, cotton bollworm, *Helicoverpa armigera* methanol extract, *field pest*

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

India is a country where agriculture is the backbone of the economy with nearly 75% of the rural villagers depending on agriculture. The estimated annual growth rate percentage for the food grains and crops to feed the on growing population is affected by the crop loss due to field pests. The quantity of the food produced is deteriorating due to these plant pests and diseases. This results in economic loss due to crop disease, which affects the crop yield. To feed the huge population, one need to double the food production, which will require a threefold increase in the use of fertilizers and much more extensive use of pesticides annually[1]. The most striking fact on the utilization of synthetic pesticides in India is that about 50% of the total pesticides produced are used on cotton crops. It has been estimated that hardly 0.1% of the agrochemical used in crop protection reaches the target pest, while the remaining 99.9% enter the environment and cause undesirable effects on non-target organisms. Ever since plant-based compounds as pesticides have been investigated by the researchers as a dire need.

Several medicinal and aromatic plants have been used as botanical pesticides that do not leave toxic residues in the soil. Many of the plant-derived materials are safer and effective against diseases, nematodes and other organisms in addition to phytophagous insects[2].The components of plants such as alkaloids, flavonoids, plant essential oil, terpenes, coumarins, ketones, fatty acids have been used a biopesticide. Triterpenes, argentatin A and argentatin B from the aerial parts of *P. argentum* was found to show toxicity towards *S. frugiperda* adults with 12.4 and 19.8 ppm respectively as reported by Cespedes[3]. Plant metabolite, sargaquinoic acid from the aerial parts of *R. barba-johannis* was found to be effective against first-instar *S. frugiperda* larvae with[4] a LC_{50} of 5.77 ppm.

Bioefficacy of several plant extracts have been reported against several insect pests. The insecticidal effect of *Mentha pulegium* L. against *H. armigera* (Hübner) has been demonstrated. Antifeedant, contact toxicity and systemic toxicity of *M. pulegium* essential oil were assessed and the results illustrated high level of antifeedant activity on the second instar larva and third instar larvae, i.e., 74.15% and 61.72% respectively[5]. The *M. pulegium* essential oil exhibited a strong antifeedant against other lepidopterans[6-7]. Darabi and Khajehali[8] carried out a similar study and proved that *M. pulegium* exhibited good antifeedant effect of against *Anarta trifolii* (Hufnagel) (Lepidoptera: Noctuidae) Jeyashankar *et al*.[9] have demonstrated that the crude extract of *G. fragrantissima* showed high feeding deterrence activity along with stronger antifeedant activity and Arivoli and Tennyson[10] reported that *Solanum pseudocapsicum* (Solanaceae) showed promising antifeedant activity against larval stages of *H. armigera*.

Helicoverpa armigera (Lepidoptera: Noctuidae) is a major polyphagous agricultural pest, which is also known as American bollworm that infests more than 180 plant species. The adult moth lives for 7-9 days and lays a maximum of 300-500 eggs. The incubation period for the egg is 5-6 days, while 6 larval instar stages are completed within 19 days. The pupa stage is between 7-9 days and the total lifecycle of this worm is completed 35-40 days. This is the serious pest of various economically important crops such as, cotton, groundnut, chilly, tobacco, castor and pulses etc. and also developed resistance against almost all commercially available chemical pesticides.

The present investigation was carried out with the aim to test the bioefficacy of the leaf methanolic extract of *H. hookerianum* (Wight and Arn.) for their antifeedant, ovicidal and larvicidal activity against *H. armigera*.

MATERIALS AND METHODS

Study area: The present study was carried out at Unit of Entomotoxicity, Department of Zoology, Government Arts College for Men (Autonomous), Nandanam, Chennai, Tamil Nadu, India from November 2018-October 2019.

Processing of plant materials: The leaves of *Hypericum hookerianum* (Wight and Arn.) were collected during the season (August-October 2018) from Vattakanal village of Pambar Shola region of Palni Hills, Kodaikanal, Tamil Nadu. The voucher specimen (CABR/15/HH/01)of *H. hookerianum* was prepared and stored in the Department of Botany, Government Arts College for Men (Autonomous), Nandanam, Chennai, Tamil Nadu, India. The leaves were washed thoroughly in running tap water and dried under shade at room temperature (37±2°C and 75±5% Relative Humidity (RH)). After proper drying, the leaves were macerated using a blender and sieved thoroughly. About 50 g of the leaf powder was extracted by the soxhlet extraction method with 500 mL of methanol solvent and filtered through Whatman's No. 1 filter paper. The solvent extract was dried under vacuum at the range of boiling point of methanol solvent using a rotary evaporator. The concentrated methanolic extract was collected and stored in clean vials at 4°C.

Insects rearing: *H. armigera* (Lepidoptera: Noctuidae) Cotton Bollworm was collected from the Bhendi field near Chennai

Fig. 1: Field-collected *Helicoverpa armigera* larva on bhendi fruit

Fig. 2: Field-collected Helicoverpa armigera larva reared on bhendi fruit for stock culture

(Fig. 1) cultured and maintained in the laboratory on Bhendi fruits (Fig. 2.). Rearing conditions were a 12 h photo regime at 28±2°C and 75±5% relative humidity. The insect culture was continuously refreshed with wild moths captured by a light trap in the vicinity of the agricultural farm of Baraniputhur village, Porur, Chennai.

Fig. 3: Experimental design antifeedant assay of crude extracts against *Helicoverpa armigera* larvae

Bioassay

Antifeedant assay: Antifeedant activities of the various solvent extracts of *H. hookerianum* were studied using leaf disc no-choice method[11]. Fresh Bhendi leaf discs of (for *H. armigera*) 3 cm diameter were used for the experiments (Fig. 3). Fresh leaf discs were treated with solvent crude extracts of 50, 100, 200, 400 and 800 ppm concentrations. The leaf disc treated with acetone was considered as the positive control and the negative control was not subjected to any treatment. All the petri dishes(1.5×9 cm) wet filter paper was placed to avoid early drying of the leaf disc single fourth instar larva of *H. armigera* were introduced individually. Five replicates were maintained for each concentration and the progressive consumption of leaf area by the larvae after 24 h was recorded in control and treated discs using leaf area meter (Delta-T Devices, serial No. 15736 F96, UK; Fig. 4):

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

Where:

AFI = Antifeedant Index
C = Area protected in control leaf disc
T = Area protected in treated leaf disc

Ovicidal assay: For ovicidal activity, scales from the egg masses of *H. armigera* were carefully removed using fine camel brush. 500 eggs from both the lepidopterans were separated into five lots each having 100 eggs and dipped in 125, 250, 500 and 1000 ppm concentrations of plant extract and controls as mentioned above. The number of eggs

Fig. 4: Area of leaf consumed by the experimental larvae calculated by leaf area meter

hatched in control and treatments were recorded and the percentage of ovicidal activity was calculated using Abbott's formula[12]:

$$OA\ (\%) = \frac{EHC\ (\%)\text{-}EHT\ (\%)}{EHC\ (\%)} \times 100$$

Where:

OA (%) = Ovicidal activity (%)
EHC (%) = Eggs hatched in control (%)
EHT (%) = Eggs hatched in treatment (%)

Larvicidal assay: For evaluation of larvicidal activity larvae of *H. Armigera* were treated with 50, 100, 200, 400 and 800 ppm of the solvent extract. Petioles of the leaves were tied with wet cotton plug to avoid early drying and placed in a plastic trough (29×8 cm) 20 pre starved (4 h) fourth instar larvae of test organisms were introduced individually and covered with the muslin cloth. Five replicates were maintained and the number of larvae dead after 48 h was recorded and the percentage of larval mortality was calculated using Abbott's formula[12]:

$$Mortality\ (\%) = \frac{MT\ (\%)\text{-}MC\ (\%)}{100\text{-}MC\ (\%)} \times 100$$

Where
MT (%) = Larval mortality in treatment (%)
MC (%) = Larval mortality in control (%)

Statistical analysis: The data were analyzed with SPSS ver 20 by performing a one-way analysis to different means between the treatments. The means which showed significance were marked with the different alphabet (at $p<0.05$ (ANOVA, LSD-Tukey's Test)).

RESULTS

The antifeedant activity of methanol extract of *H. hookerianum* against fourth instar larvae of *H. armigera*: The result of the antifeedant activity of methanol extract of *H. hookerianum* against *H. armigera* is shown in Table 1. These results reveal that the minimum area of leaf protection was recorded in the lowest concentration of extract-treated. At the same time, the maximum feeding deterrence was noticed in the leaves treated with the highest concentration of the extract. It is observed that the feeding deterrence was directly proportional to the increase in the concentration of the leaf extract. In 50 µg cm^{-2} concentration, 21.64% percentage of leaf area was protected by the extract, similarly, 32.36, 47.58, 72.32 and 85.66% leaf protection was recorded from 100, 200, 400 and 800 µg cm^{-2} concentrations respectively against the fourth instar larvae of *H. armigera.* From the data AFI_{50} was calculated using probit analysis and found to be 226.53/cm^2 LCL = 145.18 and UCL = 357.39, Table 1. Furthermore, AFI_{90} was derived from the data and it was found to be 476.40 µg cm^{-2} (LCL = 349.06 and UCL = 859.17).

Ovicidal activity of methanol extract of *H. hookerianum* against freshly laid eggs of *H. armigera*: The ovicidal activity of *H. hookerianum* tested against the freshly laid eggs of *H. armigera* is depicted in Table 2. On treatment with 1000 ppm of the methanolic extract, 79% of the ova were found dead. The percentage of viability decreased with increasing concentration of the extract was 500 ppm, 250 ppm and 125 ppm of the extract yielded 59, 37 and 23% mortality.

Larvicidal activities of methanol extract of *H. hookerianum* against fourth instar larvae of *H. armigera*: Table 3 shows the larvicidal activity of methanol extract of *H. hookerianum* against *H. armigera*. At 50 ppm concentration, larval mortality was found to be 22.35%, whereas, at 100 ppm it was 33.89% in the experimental larvae of *H. armigera*. A mortality

Table 1: Antifeedant activity of methanol extracts of *Hypericum hookerianum* against the fourth instar larvae of *Helicoverpa armigera*

Concentration (µg cm^{-2})	Area of leaf disc (mm^2)	Antifeedant index (%)	AFI_{50} (LCL-UCL)	AFI_{90} (LCL-UCL)	χ^2
0	1249	1.8±0. 64^a (7.71)	226.53	476.40	22.232
50	1249	21.64±1.64^b (26.99)	(145.18-357.39)	(349.06-859.17)	
100	1249	32.36±2.84^c (34.63)			
200	1249	47.58±2.64^d (43.57)			
400	1249	72.32±3.68^e (65.12)			
800	1249	85.66±4.66^f (81.09)			

The value represents the Mean±SE of five replications. AI_{50}: Lethal concentration brings out 50% Mortality and AI_{90}: Lethal Concentration brings out 90% mortality, LCL: Lower confidence limit, UCL: Upper confidence limit, Values in a column with a different superscript alphabet are significantly different at p<0.05 (ANOVA, LSD: Tukey's Test)

Table 2: Ovicidal activity of methanol extract of *Hypericum hookerianum* against the eggs of *Helicoverpa armigera*

	Concentrations tested (ppm)			
Sample	125	250	500	1000
Control	4.55±1.29 (12.25)^a	4.55±1.29 (12.25)^a	4.55±1.29 (12.25)^a	4.55±1.29 (12.25)^a
Methanol	23.56±1.24 (29.00)^b	37.77±2.29 (37.88)^b	59.58±3.82 (56.48)^b	79.33±2.46 (68.44)^b

The value represents Mean±SE of five replications, Values in a column with a different superscript alphabet are significantly different at p<0.05 (ANOVA, LSD-Tukey's Test)

Table 3: Larvicidal activity methanol extract of *Hypericum hookerianum* against freshly molted (0-6 h old) 4th instar larvae of *Helicoverpa armigera*

Concentrations tested	Mortality (%)	LC_{50} (ppm)	LC_{90} (ppm)	χ^2 (df = 4)
Control	1.26±0.32^a (7.21)	227.55	483.10	24.872
50	22.35±1.36^b (28.18)	(139.37-374.09)	(348.21-924.97)	
100	33.89±2.52^c (35.55)			
200	46.64±3.42^d (43.05)			
400	82.16±4.65^e (64.97)			
800	97.33±3.98^f (82.73)			

Values are Mean±SD of five replications, Parentheses hold angular transformation values, LC_{50}: Lethal Concentration brings out 50% Mortality and LC_{90}: Lethal Concentration brings out 90% mortality, LCL: Lower confidence limit, UCL: Upper confidence limit, Values in a column with a different superscript alphabet are significantly different at p<0.05 (ANOVA, LSD: Tukey's test)

percentage of 46.64±3.42, 82.16±4.65 and 97.33±3.98 was observed in the larvae treated with 200, 400 and 800 ppm concentration of methanol extract of *H. hookerianum,* respectively. The results were found statistically significant in comparison with the control larvae, wherein, its mortality was recorded to be statistically less (1.26±0.32; angular transformed value, 7.21). The LC_{50} concentration of the extract was found to be 227.5 ppm with a lower confidence limit of 139.37 ppm and the upper confidence limit of 374.09 ppm. Similarly, The LC_{90} concentration of the extract was found to be 483.10 ppm with the lower confidence limit of 348.21 ppm and the upper confidence limit of 924.97 ppm (df = 4; $\chi^2 = 24.872$).

DISCUSSION

Plants are gifted with plenty of numbers of phytochemicals possessing efficient defense properties against a variety of insect pests and diseases. These natural products are very much studied for their role in the management of insect pests. The antifeedant activity of these bio-based phytocompounds against several insects and pests has been reported by researchers all over the globe. Antifeedant is a compound alone or in combination with any other compound, effectively inhibits the feeding without directly killing the insect pests, i.e., allowing the insect pests to die through starvation[13-14]. Gunderson *et al.*[15] reported the strong effects of plant-based compounds on larval development and adult reproduction of the Lepidoptera *S. frugiperda*. The phytocompounds present in the plants act as insect neurotoxin by inhibiting acetylcholinesterase activity[16] and can cause larval death even in extremely small amounts[17]. This indicates that the active principles present in the plants inhibit larval feeding behavior or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence.

The crude extract of *L. cubeba* Pers. leaves showed significantly high ($p \leq 0.05$) larvicidal and antifeedant activity against the fourth instar larvae of *H. armigera*[18]. Paul and Choudhury[19] studied seven plants of Meghalaya, namely *Pinus kesiya* Royle (Pinaceae), *Lantana camara* Linn.(Verbenaceae), *Litsea cubeba* Lour. (Lauraceae), *Gaultheria fragrantissima* Wall. (Ericaceae), *Mikania micrantha* Kunth. (Asteraceae), *Ambrosia artemisiifolia* Linn. (Asteraceae) and *Eupatorium riparium* Regel (Asteraceae) for their larvicidal and antifeedant activity against fourth instar larvae of the cotton

bollworm, *H. armigera* (Hübner). Out of the seven plants, four plants namely, *L. camara*, *G. fragrantissima*, *L. cubeba* and *P. kesiya* have shown a high level of insecticidal activity against *H. armigera* larvae. The crude extract of *L. cubeba* leaves demonstrated high oral toxicity and feeding deterrence activity.

In the present study, the methanol extract of *H. hookerianum* was promising in reducing the feeding rate of *H. armigera*. The rate of feeding significantly varied depending on the concentration of the extracts of *H. hookerianum*. The results obtained from the antifeedant bioassays in the present study clearly demonstrate that *H. hookerianum* extract effectively reduced the feeding rate of fourth instar larvae of *H. armigera.* The fact that the feeding rate decreased with increasing concentrations of the extract clearly shows that *H. hookerianum* inhibits the larvae's ability to ingest food. These results suggest that *H. hookerianum* could be effectively used to control the insect population by killing its early stages. Therefore, the 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.*[9].

CONCLUSION

The continuous application of chemical pesticides to control the field pests over the past several decades has produced remarkable alterations in the environment and caused the decreasing population of non-target organisms such as neutrals and predators. From the results, it can be concluded that the methanolic extract of the plant has significant antifeedant, ovicidal and larvicidal effects on the pest. To support that, this present investigation has been successful in the assessment of the efficacy of *H. hookerianum* as an alternative green pesticide to control the important agricultural field pest of *H. armigera*.

SIGNIFICANCE STATEMENT

This study reveals the extracts of *Hypericum hookerianum* is a potent bio-pesticidal agent against the Cotton Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). This study has paved the way for future researchers to find a novel biocontrol agent that would improve the crop yield.

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