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Antifeedant and larvicidal activities of *Swertia chirata* Buch-Ham. ex Wall. against *Helicoverpa armigera* Hubner and *Spodoptera litura* Fab.

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ABSTRACT

Hexane, ethyl acetate, methanol and aqueous extracts of *Swertia chirata* (Gentianaceae) were screened against economically important two lepidopteran pests viz, *Helicoverpa armigera* and *Spodoptera litura*. Insects were orally treated in the third instar larval stage by no choice leaf disc method. Among the tested extracts, methanol extract of *S. chirata* highly inhibited the feeding activity and the different developmental stages of *H. armigera* and *S. litura*. Toxicity of *S. chirata* was identified as dosage dependent in both species. In *H. armigera* and *S. litura* larvae, 68 and 56% antifeedant activity was recorded as maximum respectively at 5% concentration of methanol extract. Nearly 80% and 50% larvae of *H. armigera* and *S. litura* were killed respectively by methanol extract (5%) within 96 h duration of treatment. Pupal mortality was recorded after the adult emergence, and it was varied between the treatment concentrations in *H. armigera* and *S. litura*. Due to the toxic effect of methanol extract ultimate adult emergence was gradually reduced with increasing concentration of the treatments. More than 80% of adult emergence was suppressed in both the species.

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Introduction

In a tropical country like India, owing to climatic conditions and its particular environment, agriculture is suffering from severe losses due to pests. The Indian farmers are in need of effective tools to fight against pests [10]. The plant kingdom is the most efficient producer of chemical compounds, synthesizing many products that are used in defence against herbivores. Insect pests are serious limiting factors of food production throughout the world. *Helicoverpa armigera* Hub and *Spodoptera litura* Fab are notorious lepidopteran insects causing severe yield loss to several crops as they are polyphagous feeders. Synthetic pesticides have created many problems in the environment and living systems [14, 5]. The deleterious side effects caused by the synthetic pesticides have forced the scientific community to switch over to biological pesticides especially botanicals. Though the use of botanical pesticides is an age old practice in some countries like India, the actual mechanism of action rendered by botanicals is being explored by the scientists and this practice is revived by the farmers as an ecofriendly pest management strategy [3]. World wide attention has been focused on alternative methods to control the pests. Conventional synthetic organic pesticides are handicapped in the green context by their high toxicity, long-term persistence and propensity of bioaccumulation [11]. Some of them induce malignancy in non-target organisms, while most give rise to development of resistance in insect population or cause resurgence of minor/alternate pests [19]. However, pest suppression can be achieved through judicious use of pesticides, crop rotation, destruction of crop residues, change in sowing dates and other practices [15]. Considering the agro-ecosystems with an increase in population and dwindling land resources there is world wide 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

[15]. Microbial and botanical insecticides offer a more natural, environment friendly approach to pest control than synthetic insecticides [9].

No information is available so far on the antifeedant and larvicidal activities of our experimental *S. chirata*. Therefore the aim of the present work was to study the inhibitory effect of different solvent extracts of *S. chirata* against the two major lepidopteran pests (*H. armigera* and *S. litura*). This is the first report on the antifeedant and larvicidal activity of the solvent extracts of the selected test plant.

Materials and methods

Collection of plant material

Healthy, disease free plants of *S. chirata* (whole plant) were collected from Darjeeling (West Bengal, India). The species was identified and authenticated by Dr. Jeya Jyothi, Taxonomist, Department of Plant Biology and Biotechnology, Loyola College, Chennai and the voucher specimen (LCH-1002) was deposited at the departmental herbarium. Freshly collected plant was washed thoroughly shade dried and powdered using an electric blender.

Preparation of crude extract

The plant powder (2 kg) was soaked sequentially in hexane, ethyl acetate, methanol and water in 1:3 ratios (w/v) for 72 h respectively with intermittent shaking. After 72 h the solution was filtered and the filtrate was concentrated under reduced pressure using rotary vacuum evaporator. The filtrate was air dried to yield 25 g of hexane extract, 42 g of ethyl acetate extract, 37 g of methanol extract and 18 g of aqueous extract and stored at 4°C in air tight containers until further use.

Insect culture

H. armigera and *S. litura* larvae were collected from vegetable agro ecosystem outside Chennai, India. *S. litura* larvae were maintained on the castor (*Ricinus communis* L.) leaves and *H. armigera* larvae were maintained on semi-synthetic diet [16]

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in the laboratory at $28 \pm 1^\circ\text{C}$, 11 ± 1 hr photoperiod and 65-70% relative humidity (RH) in insectary, Entomology Research Institute, Loyola College, Chennai. Adults were released into oviposition chambers for egg laying and provided with 10% honey solution. Fresh cotton (*Gossypium hirsutum* L.) and castor (*Ricinus communis* L.) leaves were kept inside the cages to facilitate the egg laying process of *H. armigera* and *S. litura*. Eggs were collected, kept separately and newly hatched larvae were maintained on the host leaves. The first generation third instar larvae from the laboratory condition were used for antifeedant and larvicidal bioassay.

Antifeedant activity test

Antifeedant activity of crude extracts of the test plant was studied using leaf disc no-choice method [12]. Required concentrations were prepared by dissolving in acetone and mixed with dechlorinated water and tested against third instar larvae of *H. armigera* and *S. litura*. Tween 20 at 0.05% was used as emulsifier [20].

Fresh cotton and castor leaf discs of 4 cm diameter were punched using cork borer and dipped in 0.625%, 1.25%, 2.50% and 5.00% extracts separately and air-dried for 5 minutes. After air drying, treated leaf discs were kept inside the Petri dishes (90 mm diameter) separately 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 (Delta-T Devices, Serial No. 15736 F 96, U.K). Leaf area consumed in plant extract treatment was corrected from the control. The percentage of antifeedant index was calculated using the following formula: $(C-T)/(C) \times 100$, where C is consumed area in control and T is consumed area in treated discs.

Larvicidal activity test

In a separate set of experiments, third instar larvae of *S. litura* and *H. armigera* were orally treated with different concentrations of crude extracts through castor and cotton leaf discs as mentioned above in the antifeedant experiment. A solvent control was also maintained.

Larval mortality was recorded for 96 h. The mortality was adjusted by Abbott's correction factor [1]. Pupal mortality was calculated by subtracting the number of emerging adults from the total number of pupae.

The percent adult emergence and deformities were also recorded. Twenty replicates were maintained for all treatments and controls.

Statistical analysis

The significance of treatments was found out by One Way Analysis of Variance (ANOVA) and effective treatment was separated by Tukey's multiple range tests. Differences between means were considered significant at $P < 0.05$.

Results

Feeding deterrent toxicity

Tables 1 and 2 show the antifeedant activity of hexane, ethyl acetate, methanol and aqueous extracts of *S. chirata* against *H. armigera* and *S. litura* larvae.

Among the tested extracts, methanol extract recorded the highest antifeedant activity compared to other extracts. Antifeedant activity of methanol extract was maximum at all concentrations and was recorded as 37.13, 47.72, 56.64 and 68.53% for *H. armigera* at 0.62, 1.25, 2.5 and 5% concentrations respectively.

In the case of *S. litura* larva, the methanol extract of *S. chirata* showed 28.30, 34.69, 49.62 and 56.58% antifeedant activity at 0.625, 1.25, 2.5 and 5% concentrations respectively. The second effective treatment, which reduced the feeding activity of both lepidopteran larvae was identified as ethyl acetate extract. It gave an antifeedant activity of 43.81 and 37.23% against *H. armigera* and *S. litura* respectively at 5% concentration.

Followed by aqueous and hexane extracts showed antifeedant activities in both test species. In all the treatments, a concentration dependent activity was recorded. Feeding inhibitory effect was higher in *H. armigera* than *S. litura* in all the treatments.

Table 1. Antifeedant activity of crude extracts from *S. chirata* against *Helicoverpa armigera*.

Extract	Antifeedant activity (Mean \pm SD)			
	0.625%	1.25%	2.5%	5%
Hexane	8.69 \pm 2.06 ^b	14.81 \pm 2.49 ^b	24.20 \pm 1.81 ^b	28.72 \pm 4.10 ^b
Ethyl acetate	18.52 \pm 3.07 ^c	24.29 \pm 3.07 ^c	28.98 \pm 5.27 ^b	43.81 \pm 4.89 ^c
Methanol	37.13 \pm 5.59 ^d	47.72 \pm 4.32 ^d	56.64 \pm 5.59 ^c	68.53 \pm 5.43 ^d
Aqueous	17.22 \pm 2.86	21.22 \pm 3.68	26.02 \pm 3.81	33.65 \pm 2.71
Solvent control	3.08 \pm 1.34 ^a			

Values in columns followed by the same alphabets are not significantly different (Tukey's test; $P \leq 0.05$).

Table 2. Antifeedant activity of crude extracts from *S. chirata* against *Spodoptera litura*

Extract	Antifeedant activity (Mean \pm SD)			
	0.625%	1.25%	2.5%	5%
Hexane	1.31 \pm 1.24 ^b	7.92 \pm 4.44 ^b	18.93 \pm 3.25 ^b	20.18 \pm 6.54 ^b
Ethyl acetate	8.21 \pm 2.44 ^c	12.94 \pm 3.84 ^c	27.76 \pm 5.38 ^c	37.23 \pm 5.40 ^c
Methanol	28.30 \pm 3.72 ^d	34.69 \pm 4.30 ^d	49.62 \pm 5.66 ^d	56.58 \pm 5.28 ^d
Aqueous	13.93 \pm 3.80	18.96 \pm 23.91	23.91 \pm 3.19	31.66 \pm 2.95
Solvent control	0.41 \pm 0.18 ^a			

Values in columns followed by the same alphabets are not significantly different (Tukey's test; $P \leq 0.05$).

Insecticidal toxicity

Figures 1 and 2 show the percentage larval mortality of *H. armigera* and *S. litura* recorded at different concentrations of crude extracts of *S. chirata*.

From the data it was clear that crude extract of *S. chirata* killed the larvae of both test species and the toxic effect increased with the increased concentration.

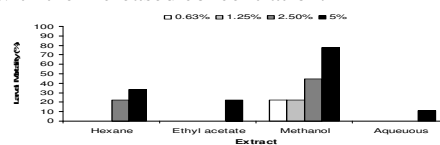


Figure 1. Larvicidal activity of *Swertia chirata* crude extracts against *Helicoverpa armigera*.

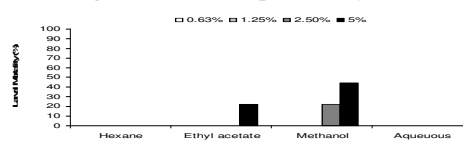


Figure 2. Larvicidal activity of *Swertia chirata* crude extracts against *Spodoptera litura*.

Among the tested crude extracts, methanol extract killed maximum number of larvae. The larvicidal activity of this extract was recorded as 77.7% for *H. armigera* and 46.5% for *S. litura* at 5% concentration. *H. armigera* larvae were more susceptible than *S. litura* larvae. Pupal mortality was varied between the treatments in both the test species.

Maximum pupicidal activity was recorded (33.3%) in methanol extract at 2.5% concentration in *H. armigera* (Figure 3). In *S. litura*, maximum pupicidal activity (33.3%) was recorded in hexane, ethyl acetate and methanol extracts at 5, 5 and 2.5% concentration respectively (Figure 4).

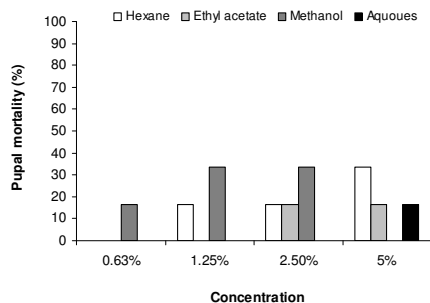


Figure 3. Pupal mortality of *Helicoverpa armigera* by the effect of *Swertia chirata* crude extracts.

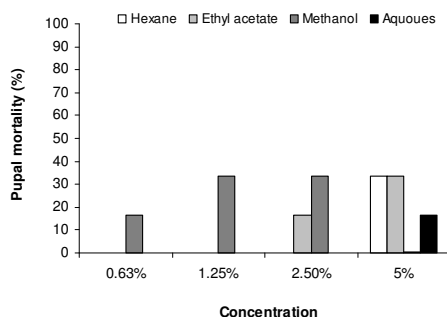


Figure 4. Pupal mortality of *Spodoptera litura* by the effect of *Swertia chirata* crude extracts.

Adult emergence was highly inhibited depending on the concentration in the methanol extract of *S. chirata* followed by hexane, ethyl acetate and aqueous extracts in both insect species (Figure 5 & 6).

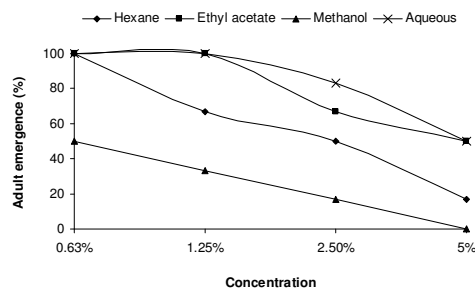


Figure 5. Impact of *Swertia chirata* crude extracts on adult formation of *Helicoverpa armigera*

Discussion

In insect-plant interactions, insects often have unique adaptation to their host plants in locating and selecting the plants by the use of chemical, visual and mechanical cues [21].

According to Mustaparta [18], unsuitable plants are avoided by detection of other chemical cues; such chemical substances may have repellent or toxic properties against insects.

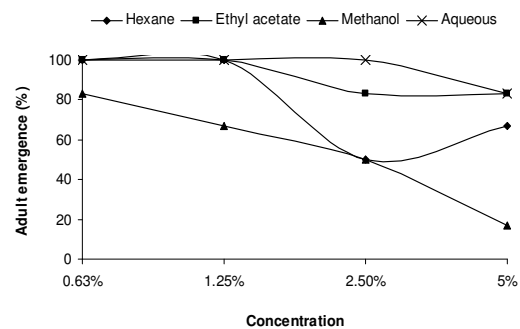


Figure 6. Impact of *Swertia chirata* crude extracts on adult formation of *Spodoptera litura*.

Based on this principle botanical pesticides are invented and utilized for control of insect pests. Accordingly, crude solvent (hexane, ethyl acetate, methanol and aqueous) extracts of *S. chirata* were screened against two phyto-polyphagous lepidopteran pests for knowing the pesticidal potential.

Crude extracts from the leaf, stem, root and seeds of various plant species have been reported to possess antifeedant, insecticidal, and/or growth inhibitory properties [4]. Crude extracts of plants often consist of complex mixtures of active principles [17]. Hummelbruner and Isman [8] reported that synergistic effects of complex mixtures (crude extracts) of phytochemicals are also thought to be important in plant defenses against insect herbivores.

In many countries, plant derived products are being used by the farmers from ancient times and it triggered the scientists to search for ecofriendly insecticides from plant kingdom. Several hundred plants have been reported as insect repellents, antifeedants, attractants, insecticides, ovicides and oviposition deterrents [2,6]. Antifeedants offer first line of crop protection against notorious insects.

According to Isman [13] any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent. In general antifeedants have profound adverse effects on insect feeding behavior [8].

In the present investigation the food consumption of third instar larvae of *H. armigera* and *S. litura* in methanol extract of *S. chirata* treatment was highly reduced followed by the ethyl acetate, hexane and aqueous extracts. This finding indicates that methanol extract of *S. chirata* had higher feeding deterrence, which was identified as concentration dependent against both test species. Because generally methanol extract contains polar chemicals. Frazier [7] signified that alkaloids, phenolics and terpenoids are most probable toxic substances against insects.

Due to the toxic effect of methanol extract of *S. chirata* maximum number of treated larvae was died even though the less consumption of food. Similarly, Leatemia and Isman [17] reported that high concentrations of extracts caused high mortality of larvae even though only very small portions of the leaf discs were consumed.

By following the larval toxicity some insects were died at pupal stage. Further the escaped larvae and pupae were developed into unhealthy adults. From the overall results it was cleared that methanol extract of *S. chirata* acted as promising antifeedant and it led to insect mortality due to the effect of combination of starvation and contact toxicity.

Apart from feeding inhibition and insecticidal activities, larval-pupal intermediates, pupal and malformed insects are formed, and these unhealthy adults were short lived and infertile [22]. Botanical antifeedants and insecticidal agents can play a significant role as part of an Integrated Pest Management [13].

In a conclusion, the present study showed the antifeedant and insecticidal potential of *S. chirata* against *H. armigera* and *S. litura*. However, the active principle present in an effective methanol extract should be analyzed further, which may add new information in the field of botanical pesticides.

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