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Phytopesticidal effects of *Spilanthes acmella* (L.) Murr. leaves on three economically important lepidopteran insect pests

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the pesticide activity of different solvent extracts of *S. acmella* against lepidopteran pests. The pesticidal effect was evaluated with antifeedant and larvicidal activity. DCM extract of *S. acmella* showed promising effect against selected lepidopteran pests.

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ABSTRACT

Objective: To evaluate the antifeedant and larvicidal activities of dichloromethane (DCM), acetone, dimethylsulfoxide (DMSO) and aqueous extracts of *Spilanthes acmella* (*S. acmella*) leaves against *Earias vitella*, *Helicoverpa armigera* and *Spodoptera litura*.

Methods: DCM, acetone, DMSO and aqueous extracts were studied using fruit and leaf discs no-choice method at 0.625%, 1.25%, 2.5% and 5% concentration.

Results: All the extracts showed antifeedant and larvicidal activities against all the tested insects. However, maximum antifeedant activity was detected in DCM extract of *S. acmella* against all tested pest, followed by acetone, DMSO and aqueous extracts at 5% concentration. The leaves extract of *S. acmella* also showed larvicidal activity; maximum larval mortality was observed in DCM extract against *Helicoverpa armigera* (48.88%), *Spodoptera litura* (44.88%) and *Earias vitella* (75.11%) followed by acetone, DMSO and aqueous extracts at 5% concentration.

Conclusions: DCM extract of *S. acmella* could be further purified to develop a formulation for eco-friendly pest control agent.

KEYWORDS

Spilanthes acmella, Antifeedant, Larvicidal, Lepidopteran

1. Introduction

Pest management practice is a vital factor and highly productive inputs are required for increasing crop yields and preventing crop losses before and after harvest[1]. The failure of modern pest control methods has compelled the scientific community to go back to the traditional and indigenous products for tackling the pest problem[2]. The application of chemical pesticides is problematic owing to side effects on the environment, concerns for public health and the rapid development of resistance[3]. Indiscriminate application of chemical pesticides destroys the natural enemies, causes pest outbreaks and results in an ecological imbalance in agro ecosystem[4]. The rapid appearance of resistance to insecticides is also a major concern in pest management. Today, major insect pest species of economic importance are resistant to more than 30 different chemical insecticides[5]. In this context, there is a need for searching eco-friendly compounds especially from plant sources. Several secondary metabolites of plants have been reported

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to be useful in controlling insect pests that are known to affect various physiological and behavioural activities of insects[6]. Globally, there has been a considerable awareness on the needs for evolving more and more non-chemical methods of pest control and avoidance tactics[5].

Botanicals derived from plants are currently recognised as biodegradable, systemic, eco-friendly and non-toxic to mammals and are thus considered as safe alternatives. Their mode of action against pests is diverse[7]. The potential of several secondary chemical compounds in plants disrupting various physiological and behavioural activities of insects could be exploited in the control of insect pests. *Spilanthes acmella* (*S. acmella*) refers to the important medicinal plant distributed in the tropical and subtropical regions around the world with rich source of therapeutic and medicinal constituents. An experiment was conducted to evaluate the antifeedant and toxic effects of *S. acmella* leaves extract against three important lepidopteran pests.

2. Materials and methods

2.1. Plant collection and extraction

The fresh and healthy plant materials of the *S. acmella* leaves were collected during the year 2012 from forest region of Wayanad district, Kerala, India. Plant specimen was identified by the authentic plant taxonomist. The leaves were shade-dried at room temperature and coarsely powdered in a powdering machine. About 500 g powder was taken in an aspirator bottle and soaked with dichloromethane (DCM) (w/v 1:3); this set up was kept for 72 h with occasional shaking at room temperature. After the period of extraction, the content was filtered through filter Whatman No.1 paper and solvent was removed by using the rotary vacuum evaporator at 40 °C. The crude extract was obtained and stored in refrigerator at 4 °C until further use. Remaining residue was sequentially extracted with acetone, dimethylsulfoxide (DMSO) and water.

2.2. Rearing of test insects

Helicoverpa armigera (*H. armigera*) and *Earias vitella* (*E. vitella*) larvae were collected from bhendi fields while *Spodoptera litura* (*S. litura*) larvae were collected from groundnut fields in Kancheepuram district, Tamil Nadu, India. The field-collected insects were reared in the insectaria on their natural diet except *H. armigera* which was reared individually in a plastic vial (20 mL) and fed regularly with bhendi, *Abelmoschus esculentus* L. (Malvaceae) to avoid cannibalism. The pupae were transferred to separate Petri plates and kept inside oviposition chambers. The adult moths that emerged from pupae were provided with a mixture of 10% honey solution and multi-vitamin liquid. Bhendi and groundnut seedlings grown in small paper cups were placed in the cages for oviposition of *H. armigera*, *S. litura* and *E. vitella* respectively. The newly emerged third instar larvae were used for the laboratory experiments.

2.3. Antifeedant activity

Antifeedant activity of different crude extracts of *S. acmella* leaves was evaluated using leaf disc no-choice method. Fresh castor leaf discs of 4 cm diameter were dipped in 0.625%, 1.25%, 2.5% and 5% concentration of crude extracts for about 5 min and were shade dried. One such treated leaf disc was put inside a Petri dish and a single pre-starved (for 2 h) third instar larva of *S. litura* was introduced on the leaf disc. The same procedure was followed for *H. armigera* using cotton leaf discs. Respective solvents were used as negative controls. Five replicates were maintained for each treatment with 10 larvae per replicate (total n=50). The experiment was conducted at laboratory condition [(27±2) °C] with 14:10 light and dark photoperiod and (75±5)% relative humidity. After 24 h, the discs were weighed and the difference between initial and final weights was calculated by formula of Bentley *et al*[8].

The antifeedant activity was assessed using bhendi fruit discs for an *E. vitella*. Bhendi fruit discs (100 mm thick) with seeds were dipped in 0.625%, 1.25%, 2.5% and 5% concentration of crude extracts for about 10 min and were shade dried and fruits were weighed and provided for *E. vitella*. A set containing 10 discs were placed separately in a Petri dish for each treatment and control. Discs of bhendi dipped in respective solvents were used as positive and negative control; without larvae were also maintained to find out the weight loss in the discs due to desiccation at room temperature. After 24 h, the discs were weighed and the difference between initial and final weights was calculated. Real consumption was calculated as follows:

Weight loss due to desiccation (D)=Initial weight-Final weight

Real consumption=Initial weight-(Final weight+D)

2.4. Larvicidal activity

Fresh castor and cotton leaves and bhendi fruit were treated with crude extracts (as mentioned in antifeedant activity). In each treatment, 50 pre-starved (2 h) third instar larvae of *H. armigera*, *S. litura* and *E. vitella* were obtained from laboratory culture and introduced into the respective treatment for 24 h. The larvae were continuously maintained on their non treated natural hosts. Diet was changed every 24 h. Larval mortality was recorded at 96 h. Five replicates were maintained for each treatment with 10 larvae per replicate (total n=50). Percentage of larval mortality was calculated according to Abbott[9].

2.5. Identification of compounds from thin layer chromatography (TLC)

The above prepared plant extracts were applied on pre-coated TLC plates (4×10) with a fluorescent indicator (Merck Silica gel 60 F254; 0.25 mm thick) by using capillary tubes and developed in a TLC chamber using suitable mobile phase chloroform:methanol:H_2O

(7:3:1), ethyl acetate:hexane:chloroform (4:6:1), ethyl acetate:hexane:chloroform (3:4:3), chloroform:methanol (3:7) and sprayed with vanillin-sulfuric acid reagent. The developed TLC plates were air dried and observed under ultra violet light UV TLC viewer 254 nm. They were later sprayed with different spraying reagents and some were placed in hot air oven for 1 min for the development of color in separated bands. The retention factor (R_f) value of the different spots that were observed was calculated.

$$R_f \text{ values} = \frac{\text{Distance travelled by the solute (CM)}}{\text{Distance travelled by the solvent front TLC plates (CM)}}$$

2.6. Statistical analysis

The data related to antifeedant activity was analysed usig One way ANOVA. Significant differences between treatments were determined by using Tukey's HSD multiple range tests ($P \leq 0.05$); concentration dependent larval mortality data were analyzed by linear regression.

3. Results

3.1. Antifeedant activity

The results showed that the DCM extract of *S. acmella* was the most effective treatment against all the tested pests at 5% concentration. DCM extract exhibited antifeedant activity of 53.22% on *H. armigera*, at 5% concentration, followed DMSO, acetone and aqueous extracts (Table 1).

Table 1
Antifeedant activity of different solvent leaf extracts of *S. acmella* against three lepidopteran larvae (means±SD).

Extracts	Concentration (%)			
	0.625	1.25	2.5	5
	H. armigera			
DCM	24.29±1.23^{e}	32.70±2.11^{d}	43.34±2.40^{e}	53.22±1.95^{e}
Acetone	21.42±0.93^{d}	28.92±1.19^{c}	39.07±1.19^{d}	48.25±1.61^{d}
DMSO	18.31±1.41^{c}	26.40±1.62^{c}	35.99±1.21^{c}	45.07±1.30^{c}
Aqueous	15.54±0.83^{b}	21.50±1.68^{b}	31.77±1.08^{b}	41.41±0.73^{b}
Control	2.32±0.62			
	S. litura			
DCM	39.80±2.04^{d}	48.16±3.06^{d}	56.24±1.95^{e}	65.43±2.43^{e}
Acetone	31.51±1.93^{c}	40.62±3.12^{c}	46.72±2.05^{d}	58.63±3.16^{d}
DMSO	29.33±1.68^{c}	37.29±2.65^{c}	40.68±1.71^{c}	52.88±2.04^{c}
Aqueous	23.22±2.52^{b}	31.03±2.23^{b}	35.64±1.50^{b}	41.51±2.26^{b}
Control	2.03±0.70^{a}			
	E. vitella			
DCM	24.72±1.92^{d}	39.95±2.12^{d}	45.39±1.78^{e}	56.72±2.67^{d}
Acetone	19.94±1.39^{c}	32.12±1.74^{c}	39.24±2.69^{d}	46.33±1.67^{c}
DMSO	14.74±3.37^{b}	27.74±2.46^{b}	34.37±1.47^{c}	43.64±2.97^{c}
Aqueous	12.88±2.49^{b}	24.10±2.38^{b}	30.15±2.34^{b}	35.00±4.01^{b}
Control	3.29±0.88^{a}			

Within the columns, followed by the same letter do not differ significantly using Tukey's test, $P \leq 0.05$.

In case of *S. litura*, maximum antifeedant activity of 65.43% was registered in DCM extract of *S. acmella* followed by acetone, DMSO and aqueous extracts at 5% concentration (Table 1). In *E. vitella*, DCM extract of *S. acmella* recorded the highest antifeedant activity (56.72%) followed by acetone, DMSO and aqueous extracts of *S. acmella* leaves at 5% concentration while aqueous extracts of *S. acmella* showed poor antifeedant activity against all the tested insects (Table 1).

3.2. Larvicidal activity

In the present study, crude extracts of *S. acmella* leaves exhibited larvicidal activity against *H. armigera*, *S. litura* and *E. vitella*. DCM extracts of *S. acmella* leaves were the most efficient extract against all the tested lepidopteran larvae at 5% concentration. In *H. armigera*, DCM extract exhibited highest larval mortality of 48.88% followed by acetone, DMSO and aqueous extracts as shown in Figure 1. DCM extract of *S. acmella* was the most promising treatment against *S. litura* which recorded 44.88% larval mortality, followed by acetone, DMSO and aqueous extracts at 5% concentration (Figure 2). In the case of *E. vitella*, DCM extracts of *S. acmella* were superior and recorded (75.11%) larval mortality followed least activity was noticed in aqueous extract of *S. acmella* (Figure 3).

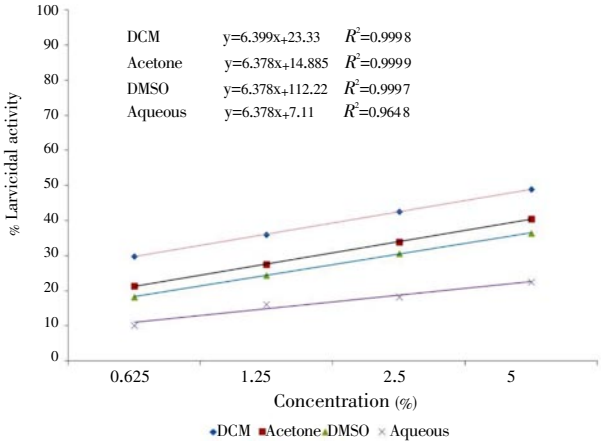

Figure 1. Larvicidal activity of *S. acmella* against *H. armigera*.

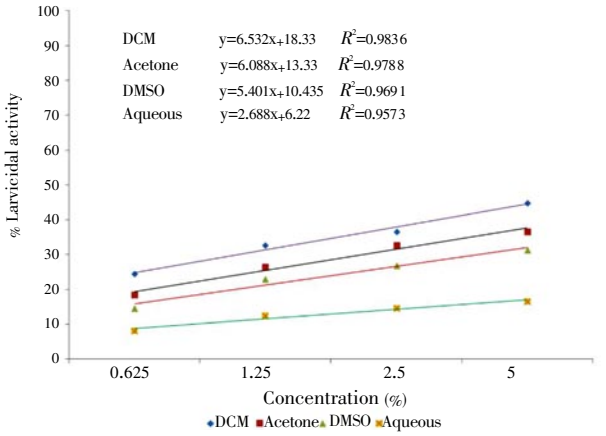

Figure 2. Larvicidal activity of *S. acmella* against *S. litura*.

The linear regression supported that DCM extracts showed superior activity against all the tested pests

(Figures 1-3). Based on linear regression, *E. vitella* (y=11.446x+29.32) is more susceptible than *H. armigera* (y=6.399x+23.33) and *S. litura* (y=6.532x+18.33) by treatment of DCM extract of *S. acmella*. All the extracts exhibited dose dependent activity. R^2 were greater than 0.95 for all the extracts against tested pests.

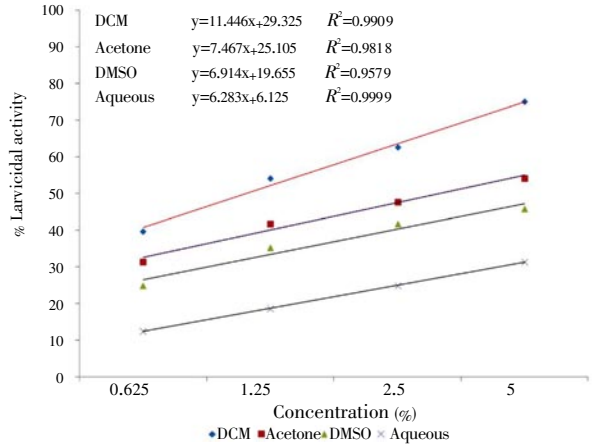

Figure 3. Larvicidal activity of *S. acmella* against *E. vitella*.

The DCM extracts of *S. acmella* exhibited various kinds of abnormalities in larvae, pupae and adults of all the tested pests. It acts as growth regulating activities on the test insects such as incomplete moulting, under developed wings in adults and larval-pupal intermediates were noticed in all the tested pests.

3.3. TLC profiling

TLC profiling of *S. acmella* leaves extracted with DCM, acetone, DMSO and aqueous showed an impressive result by the presence of more phytochemicals (Table 2 and Figure 4). Various phytochemicals gave different R_f values in different solvent systems. This variation in R_f values of the phytochemicals serves as an more important clue in understanding their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. TLC of DCM extract of *S. acmella* revealed the presence of 5 compounds having R_f values of 0.32, 0.67, 0.76, 0.78 and 0.85 respectively while using a solvent phase of chloroform:methanol:H_2O (7:3:1). TLC of acetone extract of *S. acmella* leaves revealed the presence of 7 compounds having R_f values of 0.40, 0.65, 0.70, 0.76, 0.80, 0.81 and 0.85 respectively when a solvent phase of ethyl acetate:hexane:chloroform (4:6:1) was used. The TLC of DMSO extracts of *S. acmella* leaves showed the presence of 7 compounds having the R_f values of 0.27, 0.67, 0.74, 0.79, 0.84, 0.93 and 0.96 respectively while using solvent phase of ethyl acetate:hexane:chloroform (4:6:1). In another solvent phase *i.e.*, chloroform:methanol (3:7), 6 spots were obtained with the R_f values of 0.47, 0.59, 0.71, 0.77, 0.84 and 0.93 in solvent phase of chloroform:methanol of aqueous extract. This information helped in selection of appropriate solvent system for further separation of compound from the plant crude extracts.

Table 2
Major spots in the TLC and R_f values of sequential extracts of *S. acmella* leaves.

Extracts	Solvent phase	No. of spot	R_f values
Dichloromethane	Chloroform: methanol: H_2O (7:3:1)	5	0.32 0.67 0.76 0.78 0.85
Acetone	Ethyl acetate: hexane: chloroform (4:6:1)	7	0.40 0.65 0.70 0.76 0.80 0.81 0.85
DMSO	Ethyl acetate: hexane: chloroform (4:6:1)	7	0.27 0.67 0.74 0.79 0.84 0.93 0.96
Aqueous	Chloroform: methanol (3:7)	6	0.47 0.59 0.71 0.77 0.84 0.93

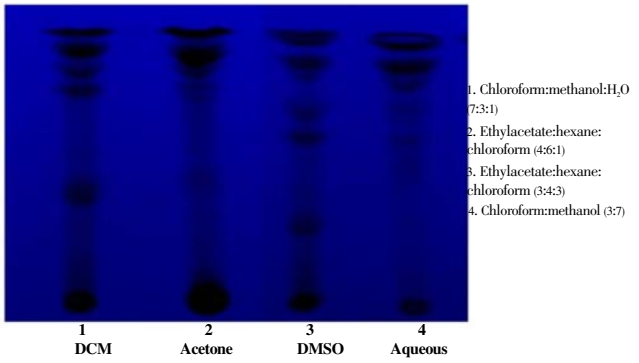

Figure 4. TLC profile of DCM, acetone, DMSO and aqueous leaves extracts *S. acmella* under UV visible light at 254 nm UV TLC viewer.

4. Discussion

4.1. Antifeedant activity

In the present investigation, *S. acmella* leaf extracts were studied under no-choice conditions for antifeedant activity against *H. armigera*, *S. litura* and *E. vitella* larvae. All the extracts showed antifeedant activity against tested insect pests. The maximum antifeedant activity was recorded in DCM extract of *S. acmella*. This result corroborated with the report of Jeyasankar *et al.*[10] who reported that hexane, diethyl ether, DCM and ethyl acetate of *Solanum pseudocapsicum* seed extracts were against *S. litura* and observed that DCM extract recorded

maximum antifeedant activity. DCM leaves extract of *Pterocarpus macrocarpus* Kurz. exhibited antifeedant activity against *S. litura*[11]. Prasad demonstrated that the hexane and DCM extracts of *Andrographis paniculata* leaf inhibited feeding of *S. litura* larvae[12]. In the present study, *S. acmella* derived different solvent extracts exhibited antifeedant activity against *H. armigera*, *S. litura* and *E. vitella*. Similarly, Pavunraj *et al.*[13] stated that solvent extract of *Melochia corchorifolia* exhibited antifeedant activity against *H. armigera*, *S. litura* and *E. vitella*. The crude extract is often attributed to the complex mixture of active compounds. In the present study, the food consumption of third instar larvae of selected lepidopteron pests were highly reduced by the extracts of *S. acmella*, and food consumption declined significantly when compared with control. A similar result has been obtained by Koul *et al.*[14] who reported that DCM extracts of *Melia dubia* leaves were more toxic to *S. litura* and *H. armigera* larvae. *Pedalium murex* Linn. ethanol root extracts affected the food consumption of *S. litura* when compared to control[15]. Ambrosio *et al.*[16] showed that the DCM rinse extracts of the leaves of *Tithonia diversifolia* exhibited significant antifeedant activity against *S. litura*.

4.2. Larvicidal activity of S. acmella

In the present study, DCM extract of *S. acmella* at 5% concentration recorded the larval mortality of 48.96% (*H. armigera*), 43.86% (*S. litura*) and 75.15% (*E. vitella*). This is in accordance with the earlier findings of Sakr *et al.*[17] stated that the DCM extract of *Hyptis brevipes* (aerial parts) exhibited 100% larval mortality against *Spodoptera littoralis*. Hexane extract of *Atalantia monophylla* leaves exhibited larvicidal activity against *H. armigera* and *E. vitella*[18,19]. Baskar *et al.*[20,21] reported that *Hygrophila schulii* (syn. *Hygrophila auriculata*) derived solvent extract showed larvicidal activity against *S. litura* and *H. armigera*. Pavunraj *et al.*[22] stated that ethyl acetate extract of *Hyptis suaveolens* showed larvicidal activity against *H. armigera*, *S. litura* and *E. vitella*. Larvicidal activity of the extracts may be due to the synergistic action of different toxic secondary substances present in the plants that influence different sites of action in physiological biochemicals, resulting in quicker mortality.

In the present study, low polar solvent extract showed maximum antifeedant and larvicidal activity than high polar acetone, DMSO and aqueous extracts of *S. acmella*. In the present study, corroborating with findings of Baskar *et al.*[18,23] and Muthu *et al.*[24] reported that hexane extract of *Atalantia monophylla*, *Couroupita guianensis*, *Fleuggea leucopyrus* exhibited maximum antifeedant and larvicidal activity against *H. armigera* than chloroform and ethyl acetate extracts.

In the present study, DCM extracts exhibited different kinds of abnormalities in larvae, pupae and adults of all the tested insect pests. In this finding, coincide with finding of Baskar and Ignacimuthu[25] ononitol monohydrate isolated from *Cassia tora* and Baskar *et al.*[26] triterpenoid friedelin from *Azima tetracantha* Lam. exhibited different kinds of abnormalities in larvae, pupae and adult of *H. armigera* and *S. litura*.

In conclusion, the DCM extract of *S. acmella* showed higher antifeedant and larvicidal activities against three lepidopteran pests. The regression relationship indicated that *E. vitella* was more susceptible to DCM extract than *H. armigera* and *S. litura*. DCM extract of *S. acmella* leaves could be used to further isolation of active molecules and to develop a new botanical formulation for the management of agricultural pests.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

E. vitella, *H. armigera* and *S. litura* were serious polyphagous pests in tropical countries. To control these pests with chemical pesticides is problematic owing to side effects on the environment. Hence there is a need of an alternate pesticide to control these pests all over the world.

Research frontiers

The present study demonstrates antifeedant and larvicidal activity of *S. acmella* against three lepidopteran pests of *E. vitella*, *H. armigera* and *S. litura*.

Related reports

Synthetic chemical pesticides and insecticides are harmful and produce adverse effects to nontarget organisms and environment. However botanicals are safe to environment and beneficial insects.

Innovations and breakthroughs

S. acmella is commonly knows as toothache plant and paracres. It is a medicinal plant used in various traditional remedy for stammering, toothache, and stomatitis. In the present research work, the authors revealed antifeedant and larvicidal activity of different solvent extracts of *S. acmella* against selected lepidopteran pests.

Applications

From the review of literature it has been found that *S. acmella* is effective against lepipodteran pests and safe to

humans and environment. This research work supports and suggests that *S. acmella* can be used as natural pesticide against phytophagous pests.

Peer review

This is a valuable research work in which authors have demonstrated the pesticide activity of different solvent extracts of *S. acmella* against lepidopteran pests. The pesticidal effect was evaluated with antifeedant and larvicidal activity. DCM extract of *S. acmella* showed promising effect against selected lepidopteran pests.

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