Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2018; 6(3): 1766-1769

Received: 19-04-2018
Accepted: 25-05-2018

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Phytochemical profiling, antifeedant and larvicidal activity of *Gnidia glauca* (Fres.) Gilg. against *Spodoptera litura*

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Abstract
In recent couple of decades biopesticides have been proved to be a potential source of plant protecting agents. An arming health hazards of synthetic pesticides forced farmers to select safer plant protecting agents. *Gnidia glauca* (Fres.) Gilg. is a small shrub. Leaf extracts of *Gnidia glauca* were evaluated for its biological activities against fourth instar larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). Antifeedant and larvicidal activity of acetone, methanol and water leaf extracts of *G. glauca* were estimated in the present study. The antifeedant and larvicidal activity was observed after 24h of exposure to the extracts. All extracts exhibited moderate larvicidal effects. However, highest antifeedant (64%) and larvicidal activity (75%) was observed in methanol leaf extract of *G. glauca* respectively. The results suggest that methanolic leaf extract of *G. glauca* holds a potential to be used as bio-pesticide for the control of destructive polyphagous agricultural pest - *S.litura*. Preliminary Phytochemical screening of the crude extracts was tested. Alkaloids, phenolics, flavnoids and saponins shows positive in all solvents.

Keywords: *Gnidia glauca*, *Spodoptera litura*, antifeedant, larvicidal, biopesticide

1. Introduction
Pest management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of the synthetic pesticide chemicals. Identification of novel effective insecticidal compounds is essential to combat increasing resistance rates. Botanical pesticides have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because botanicals reputedly pose little threat to the environment or to human health. The body of scientific literature documenting bioactivity of plant derivatives to arthropod pests continues to expand, yet only a handful of botanicals are currently used in agriculture in the industrialized world, and there are few prospects for commercial development of new botanical products [1]. Plants are rich source of bioactive compounds including the alkaloids, terpenoids, steroids, carotenoids, flavonoids, glycosides and a range of essential oils. These compounds secondary metabolism synthesized as by products in plants. Neither they do not serve any major physiological functions in plants nor they present in all plant species. Phytochemicals mainly serve a defense function in plants. Their evolution is attributed to selective pressure exerted by plants in a process of self-defense against pests [2].
Gnidia glauca displays a wide range of phytomedicinal properties. It is also utilized in agrochemical applications. *G. glauca* has been reported to contain anti-helminthic, antifungal and anti-diabetic properties [3]. It is also an insecticidal, molluscicidal, piscicidal and homicidal agent [4]. In Kenya, the boiled root is drunk for the treatment of indigestion and the bark is made into arrow poison. It is used in some communities to treat cancer, sore throat, abdominal pain, wounds, burns and snake bites.
Spodoptera litura (Fab.) (Lepidoptera: Noctuidae) is a polyphagous insect pest of cosmopolitan distribution that has about 150 host species and is reported to attack more than 112 different species of cultivated crop plants throughout the world of which 40 species are known in India [5]. *Spodoptera litura* is an economically important polyphagous pest in India, China and Japan causing considerable economic loss to many vegetable and field crops since the larvae of *S. litura* can defoliate many economically important crops [6]. Keeping in view all the facts, the current study was planned to determine the antifeedant and larvicidal effect of the *G. glauca* against *S. litura*

2. Materials and Methods

2.1. Plant collection and extract preparation

The fresh plant material of *G. glauca* was collected from Amboli, Kolhapur, Maharashtra, India during monsoon season. The plant leaves were rinsed with water to remove debris and air dried under shade. Dried plants were chopped finely to a powder. One kilogram of each powder sample was soaked in different solvents for 14 days. Each crude extract was filtered using a vacuum pump, dried by a rotary evaporator to obtain the solidified crude extracts and stored at 4 °C in a refrigerator until further processing**.**

2.2. Rearing of *Spodoptera litura*

Laboratory culture of *S. litura* was maintained at 25 ± 1 ^{0} C, 60 ± 5 % relative humidity and 16:8 h photo: scotophase on artificial diet. An artificial diet with different composition of components is prepared as given in Table 1. Kidney bean (*Phaseolus vulgaris*) seeds procured from market were washed thoroughly and soaked overnight in water. Soaked seeds were ground in an electric grinder thoroughly with the addition of 400 ml double distilled water. Wheat bran, wheat germ, ascorbic acid, casein, yeast powder, methyl parahydroxybenzoate, sorbic acid, cholesterol, streptomycin sulphate, formaldehyde and multivitamin (ABDEC drops) were added to the ground material and mixed thoroughly. Agar was boiled in 200 ml double distilled water with constant stirring till it attained necessary consistency and then ground with rest of the ingredients once again. The whole mixture was poured into plastic trays and covered with thin plastic film. After cooling, the diet was kept in refrigerator and used after 24 h. Neonates, upon hatching from egg, were transferred to glass jars containing fresh thoroughly washed castor leaves. Five-day old larvae were transferred to plastic boxes (30 cm long, 20 cm wide and 7 cm high) containing pieces of diet in groups of two larvae. Boxes were cleaned daily and larvae were fed with fresh diet. When the larvae exhibited gut purge and entered into non feeding wandering stage they were transferred to boxes containing saw dust for pupation. Pupae were collected after 4–5 days and disinfected with 0.02 % sodium hypochlorite. Upon emergence the adults were transferred to oviposition cages. Adults were fed with 20 % honey solution containing vitamin C, E and streptomycin sulphate. Castor leaves with their petiole dipped in water were provided for oviposition inside the cages. All the containers used for rearing were periodically disinfected with Protasan DS® (Qualigens). This enabled to maintain a disease-free and healthy stock culture for further experiments. Larvae for experimental purposes were reared on washed and dried castor leaves in plastic boxes. Care was taken to avoid overcrowding and strict sanitation was maintained to prevent any infection.

2.3. Antifeedant activity

Antifeedant activity of plant extracts was studied using leaf disc no choice method [7]. Fresh castor leaf discs of 4 cm in diameter were punched using cork borer and dipped in 10, 20, 30, 40 and 50 mg/ml concentrations all extracts separately. Leaf discs treated with acetone, methanol and water were considered as control. After air dried, treated leaf discs were kept inside the each petridish (15mm × 90 mm diameter) separately containing wet filter paper to avoid early drying of the leaf disc and a single 2 hrs pre-starved fourth instar larva was introduced into each petridish. A progressive consumption of leaf area by larvae after 24 hr feeding was recorded from control and treated leaf disc using ImageJ Software. Five replicates were maintained for each concentration. The antifeedant activity was calculated using the formula:

Antifeedant activity % = [(C-T) ÷ (C+T)] x 100. Where "C" is the leaf area consumed in control and "T" is the leaf area consumed in treatment.

2.4. Larvicidal activity

Larvicidal activity of crude extracts with different concentrations 10, 20, 30, 40 and 50 mg/ml was determined by topical application method on third instar larvae [8]. A three micro litter extract of above mentioned concentrations were applied separately on the dorsum of the thorax and abdominal regions of third instar larva by using micro-pipette. Larvae were treated with solvents and azadirachtin were considered as negative and positive control respectively. Further larvae were transferred to rearing tubs (8cm ×18cm) lined with wet paper towels and tubs closed with muslin cloth. The treated and control larvae were feed on normal castor leaves. Each concentration treatment contained 20 larvae with three replicates. Larval mortality was observed and results were recorded. Mortality data was corrected by using the Abott's formula [9] and then used for statistical analysis.

2.5. Qualitative test for phytochemicals analysis

All solvent extracts of *G.glauca* were subjected to various chemical tests to identify phytoconstituents using standard methods [10-12]. For qualitative tests of all solvent extract of *G. glauca* diluted to obtained mg/ml concentration and then used for the phytochemical tests.

2.5.1. Test for Phenolics

The extracts were screened for phenols by adding 1 ml of ferric chloride solution to 2 ml of each extract. Formation of blue to the green color indicated the presence of phenolics.

2.5.2. Test for flavonoids

For flavonoids test, aliquots of extracts 0.5 ml mixed with 0.5 ml of respective solvent. Add few drops of 1% of $AlCl_3$. Appearance of yellow color proved the presence of flavonoids.

2.5.3. Test for Tannins

The tannin tests was carried out by adding 0.5 ml of plant extract and 0.5 ml of respective solvent then add few drops of 5% $FeCl_3$. Blackish color shows presence of tannin compounds.

2.5.4. Test for Terpenoids

The presence of terpenoids conformed by mixing 0.5 ml of plant extract and 0.5 ml of solvent. Further add 1 ml chloroform and then add 1 ml H_2SO_4. Reddish brown color confirmed the presence of terpenoids.

2.5.5. Test for Alkaloids

The extracts were tested for alkaloids by first acidifying 5 ml of each extract with 1M HCl. This acidic medium was heated and then treated with Dragendroff's reagent. The formation of an orange or reddish brown precipitate was regarded as positive for the presence of alkaloids

2.5.6. Test for Saponins

About 0.5g of each plant extract was put in a test tube. 3ml of sodium bicarbonate solution was added to the test tube and

shaken vigorously. The mixture is then allowed to stand for about 20 minutes and froth indicates the presence of saponins and no froth indicates the absence of saponins.

3. Results and Discussion

3.1. Antifeedant activity

The percentage antifeedant activity of all solvent extracts of *G. glauca* was evaluated and results pertaining to different concentration were presented in Table 2. The efficiency of all solvent extracts against *S. litura* was assayed by comparing the average leaf area consumed in the treated and control leaf discs. Higher antifeedant percentage indicated the decreased rate of feeding. In the present investigation, antifeedant activity is varied significantly based on the solvents used and concentrations of the solvent extracts. Among the tested solvent extracts, methanol extract was exhibited highest antifeedant activity (64±2.1%) at 50mg/ml concentration followed by acetone extract (32±0.4 %). The least antifeedant activity was noted in water extract (11.00±0.1%) at 10 mg/ml concentration. It is noted that, as the concentration increases the antifeedant activity also increases. The study noted considerable feeding deterrent activity in all tested solvent extracts with special emphasis of methanol extract and acetone extract. In addition, present study noted the dose dependant antifeedant activity in tested solvent extracts against the fourth instar larvae of *S. litura*. Our results are agreed with previous reports on antifeedant activity of various plant extracts against *S. litura* [13, 14].

3.2. Larvicidal activity

The larvicidal activity of different crude leaf extracts of *G. glauca* was tested against fourth instar larvae of an *S. litura*. The perusal of the data clearly revealed that methanol extract at 50 mg/ml concentration showed potential larvicidal effect (75%) (Table.3) followed by acetone extract (48%). Whereas, very poor larvicidal effect (14%) was noted with water extract at 10 mg/ml concentration. The positive control azadirachtin showed 80% mortality which was comparable to methanolic extract of *G. glauca*. Among all tests, methanolic extract showed significant mortality rate as compared to other. As seen in ovicidal activity, as extract concentration increased the larvicidal activity was also increased. The correlative proportion was observed. The results are concurred with efficient larvicidal potential shown by various solvent extract of *E. pedunculatum* at various concentrations. Similarly insecticidal potentiality of *Exacum* spp. is revealed by various studies [15].

3.3. Phytochemical analysis

The results of the phytochemical screening as shown in Table 4. revealed positive response for alkaloids, phenolics, flavonoids and saponins in all solvents in all extracts. Test for tannin and terpenoids shows positive in acetone and methanol and negative in aqueous extract.

Table 1: Composition of artificial nutrient diet for rearing *Spodoptera litura*

Sr. No.	Ingredient	Weight (g) or Volume (ml)
1	Kidney bean	65
2	Wheat germ	65
3	Casein	3
4	Ascorbic acid	4
5	Yeast-powder	25
6	Agar	10
7	Sorbic acid	0.92
8	M-Parabien	0.4
9	Cholestrol	0.25
10	Multi-vitamin	1 Capsule
11	Streptomycin	0.1
12	Formaldehyde	2 ml
13	Sunflower oil	2 drops
14	Distilled water	600 ml

Table 2: Percent antifeedant activity of *Gnidia glauca* leaf extract against *Spodoptera litura*

Crude extract	Concentration mg/ml				
	10 mg/ml	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**50 mg/ml**
Acetone	21±0.4	21±0.4	26±0.2	26±0.0	32±0.4
Methanol	30±1.0	36±1.0	52±0.1	52±0.1	64±2.1
Water	11±0.1	11±0.1	18±0.4	18±0.4	19±0.9
Control	5±0.5				
Azadirachtin (0.1%)	56±1.0				

(Values are the means of three replicates ± standard error)

Table 3: Percent larvicidal activity of different concentrations of G*nidia glauca* leaf extract against *S. litura* .

Crude extract	**Concentration mg/ml**				
	10 mg/ml	**20 mg/ml**	**30 mg/ml**	**40 mg/ml**	**50 mg/ml**
Acetone	34±0.5	36±0.2	36±0.2	42±0.0	48±1.0
Methanol	42±0.5	42±0.5	48±0.1	48±0.1	75±0.5
Water	14±1.0	14±1.0	16±0.0	16±0.0	21±1.5
Azadirachtin (0.1%)	80±1.5				

(Values were the means of three replicates : ± = standard error)

Table 4: Qualitative phytochemical test for *Gnidia glauca* leaf extract in different solvents

Phytochemical constituents	**Acetone**	**Methanol**	**Water**
Phenolics	+	+	+
Flavonoids	+	+	+
Tannins	+	+	-
Terpenoids	+	+	-
Alkaloids	+	+	+
Saponins	+	+	+

(+ = Present ; - = Absent).

4. Conclusion

The methanol extract of *G. glauca* at 50 mg/ml concentration demonstrated highest ovicidal as well as larvicidal activity against *S. litura* followed by acetone, and water. The phytochemical analysis revealed the presence secondary metabolites viz. alkaloids, flavonoids, terpenoids, phenolics and tannins in *G. glauca*. Hence it is inferred that *G. glauca* can be used further for details phytochemical investigation to develop a new botanical formulation for the management of *S. litura*.

5. Acknowledgement

We are thankful to Science Instrumentation Facility Centre (DST-FIST, SR/FST/College-281), Devchand College, Arjunnagar, Kolhapur-591237, Maharashtra, India for providing all necessary facilities.

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