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Antifeedant Activity of Crude Extracts and Fractions Isolated from *Cymodocea serrulate* (R.Br.) Leaf against Tobacco Caterpillar *Spodoptera litura (Fab.)* Lepidoptera: Noctuidae

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Different organic solvent extracts of *Cymodocea serrulata* leaves exhibited antifeedant activity against *Spodoptera litura* larvae. Maximum antifeedant activity (88.95%) was observed in ethyl acetate extract at a 5 percent concentration. Five fractions were isolated from ethyl acetate extract

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by column chromatography and tested individually at four different concentrations, viz., 125, 250, 500, and 1000 ppm, for antifeedant activity. Among the five fractions, fraction I showed promising antifeedant activity (91.12%) at a 1000 ppm concentration. Deformities in larvae, pupae, and adults were also recorded. The preliminary phytochemical analysis showed the presence of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, glycosides, and steroids.

Keywords: Antifeedant activity; Cymodocea serrulata; Spodoptera litura; plant extracts; phytochemical screening.

1. INTRODUCTION

"The application of chemical pesticides plays a significant role in increasing agricultural production by controlling insect pests. However, the chronic effects of chemical pesticides on living organisms and the environment prompt us to restrict the use of many pesticides" [1,2,3,4,5]. "The need for an alternative method of crop protection that is environmentally safe is urgent due to the threats posed by chemical pesticides. The secondary metabolites of several plants have been found to be effective alternatives to synthetic pesticides" [6]. "The mode of action of these secondary metabolites has been studied in detail by many investigators" [7].

"Developing new natural insecticides such as antifeedants, ovipositional deterrents, ovicidals, and insecticidals from some unexplored plant species would definitely be an effective alternative to synthetic chemicals" [8]. "Phyto pesticides can reduce the use of chemical insecticides by avoiding the unnecessary application of synthetic chemicals. Though more than 3000 plants have been reported to have insecticidal properties and to contain biologically active principles with multiracial effects against insect species, only a few plants have been used in pest management programs" [8]. It would be very beneficial to develop novel natural insecticides from some undiscovered plant species, such as insecticidals, ovicidals, antifeedants, and ovipositional deterrents, as an effective substitute for synthetic pesticides. The action of plant-derived compounds on pest insects is exerted in many ways, such as an as an antifeedant [1]; a larvicidal [9]; an ovicidal [10]; oviposition deterrent activity [10]; and a repellent [11].

The marine flora offers an opportunity for the discovery of a number of bioactive compounds [12]. The present study aims to find out the anfifeedant effect of the sea grass *C. serrulata* on *Spoddoptera litura*. So far, there is no detailed report on biological activity, especially the antifeedant activity of *C. serrulate* against insect pests.

2. MATERIALS AND METHODS

The sea grass, *C. serrulata,* was collected from Adirampattinam, Pudukkottai District, Tamil Nadu, India, air-dried, and powdered by a Wiley grinder. The powder (35g) was soaked in 500 ml of hexane for 24 hours with constant shaking. The sample was then filtered through Whtman No. 1 filter paper. The solvent in the filtrate was evaporated under reduced pressure by a vacuum rotary evaporator. The residues were subsequently soaked with ethyl acetate and methanol separately for 24 hours for each solvent, and the extract was prepared as mentioned above. The most effective crude extract (ethyl acetate extract) was subjected to column chromatography over silica gel (100–200 mesh, Merck) using a gradient of hexane–ethyl acetate combinations. The fractions were analyzed by TLC and UV.

2.1 Insect Culture

S. litura larvae were collected from agroecosystems in and around Chennai. They were maintained on castor leaves in the laboratory at 28±1ºC, 11±1 hr photoperiod, and 65-70% R.H. Adults were released into ovipostion chambers for egg laying and provided with a 10% honey solution. Castor leaves were kept inside the cages to facilitate egg-laying. Eggs were collected and kept separately, and newly hatched larvae were maintained on castor leaves. Fourth-instar larvae were used for the experiments.

2.2 Antifeedant Activity Test

"The antifeedant activity of crude extracts and fractions was studied using the leaf disc no-choice method" [13]. "The stock concentration of crude extracts (5%) and fractions (1000 ppm) was prepared by dissolving in acetone and mixing with dechlorinated water. Polysorbate 20 (Tween 20) at 0.05% was used as an emulsifier"

[14,15]. The necessary concentrations were made from the stock and examined against S. litura. A cork borer was used to puncture 3-cm-diameter fresh castor leaf (Ricinus communis) discs, which were then individually dipped in 0.625, 1.25, 2.5, and 5.0% concentrations of crude extracts and 125, 250, 500, and 1000 ppm concentrations of fractions. The discs were then allowed to air dry for five minutes. Following air drying, treated leaf discs were placed individually in 1.5 cm by 9 cm petridishes, and one single *S. litura* fourth instar larvae was placed on top of each leaf disc two hours before it was starved. Acetone-treated leaf discs were used as a negative control. NeemAzal, a company product (reference for comparison), was tested at 125, 250, 500, and 1000 ppm as a negative control. Ten replications were maintained for each concentration and control. Progressive consumption of leaf area by the larvae in a 24-hour period was recorded for control and treatment using a leaf area meter (Delta-T Devices, Serial No. 15736 F 96 UK). The leaf area consumed in the plant extract treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Ben Jannet et al*.* [16]:

$$Antifeedant\ index = \frac{Consumption\ in\ control\ (C) - Consumption\ in\ treatment\ (T)}{C + T} X\ 100$$

2.3 Phytochemical Screening

Phytochemical analysis of *S. acmella* leaf extracts was done using the Harborne [17] methods.

2.4 Data Analysis

The data were subjected to a one-way analysis of variance (ANOVA) to find out the significance among treatments, and the effective treatments were separated by the least significant difference (LSD) (P<0.05).

3. RESULTS

3.1 Preliminary Phytochemical Analysis of *C. serrulata*leafExtracts

Preliminary phytochemical screening of *C. serrulata* leaves revealed the presence of various bioactive compounds. Ethyl acetate extract showed maximum antifeedant activity against *S. litura*, due to the presence of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds, and steroids (Table 1). The present findings coincide with the findings of Das et al. [18], who reported that different phytochemicals in *C. serrulata* showed antimicrobial activity against five potential pathogenic bacteria (*Bacillus subtilis, Klebsiella pneumonia, Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio harveyi).*

3.2 Antifeedant Activity of *C. serrulata*leaf Extracts against *S.litura*

The antifeedant activity of hexane, ethyl acetate, and methanol crude extracts and the fractions of ethyl acetate crude extract from *C. serrulata* were studied at different concentrations, and the results are presented in Tables 2 and 3. A higher antifeedan index normally indicates a decreased rate of feeding. Among the different crude extracts, maximum antifeedant activity was observed at 5% of ethyl acetate extract (8895%), followed by hexane (86.87%) and methanol (83.77%) extracts.

The effect among the different solvent extracts at 5 percent concentration was not statistically significant by LSD at the 5% level. Even at 2.5 percent concentration, ethyl acetate (85.81%) and methanol (80.38%) extracts showed more than 80 p X 100ercent antifeedant activity. NeemAzal showed higher activity (95.59%) than the fractions tested. Among the fractions, the percent antifeedant activity was at its maximum in fraction I (91.12%), followed by fraction III (89.78%) at a 1000 ppm concentration. However, statistical analysis revealed that the effect of NeemAzal and Fraction-I, II, and III was the same at the 5 percent level. At 250 ppm, more than 80 percent antifeedant activity was recorded in fraction-I (84.70%) and fraction-II (80.73%). Deformities in the larval, pupal, and adul stages were also observed.

4. DISCUSSION

Because more people are aware of the need for a safe environment and the damaging effects of chemicals on the environment, the use of plant products and their derivatives in insect pest management is growing daily. The present study indicates that all three solvent extracts of *C. serrulata* exert feeding deterrent activity on *S. litura*.

"The rate of feeding varied significantly depending on the concentration of the plant extract. This suggests that the active principles

Table 1. Preliminary phytochemical analysis of different solvent leaf extracts of *C. serrulate*

S. No.	Test	Test applied	Extracts		
			Hexane	Ethyl acetate	Methanol
1	Alkaloids	Mayer's test	-	+	-
2	Terpenoids	Salkowski test	+	+	-
3	Flavonoids	Alkaline reagent test	-	+	-
4	Tannins	Iron iii trichloride (FeCl3)	-	+	+
5	Phenolic compounds	Gelatin Test	+	+	+
6	Glycosides	Keller Killiani test	+	-	+
7	Steroids	Salkowski test	+	+	+

(Note): (-) Absents; (+) Present

Table 2. Antifeedant activity of different crude extracts of *C. serrulata* against 4th instar larvae of *S. litura*

Extracts	Concentrations (%)			
	0.625	1.25	2.5	5
Hexane	60.34±5.34^{a}	67.07±4.09^{b}	80.38±2.17^{b}	86.87±3.70^{b}
Ethyl acetate	66.72±2.14^{b}	72.40±3.41^{b}	85.81±3.44^{bc}	88.95±4.50^{b}
Methanol	67.91±4.69^{b}	67.71±4.10^{b}	75.59±2.67^{b}	83.77±3.44^{b}
Negative (acetone) control	55.26±6.71^{a}			

Values are represented by the mean ± (n = 10),
In each column, figures marked by the same alphabets do not significantly differ (p = 0.005)

Table 3. Antifeedant activity of different ethyl acetate fractions of *C. serrulata* against 4th instar larvae of *S. litura*

Fractions	Concentrations (ppm)			
	125	250	500	1000
I	69.37±3.26^{b}	84.70±3.83^{c}	87.44±3.14^{b}	91.12±2.85^{b}
II	77.03±2.62^{c}	80.73±3.53^{c}	88.52±5.89^{b}	87.94±3.74^{b}
III	57.14±4.61^{a}	70.45±5.31^{b}	82.60±3.77^{b}	89.78±3.0^{b}
IV	67.59±2.13^{ab}	71.93±3.73^{b}	79.81±1.93^{a}	83.01±2.14^{a}
V	66.08±1.38^{b}	67.88±4.21^{a}	74.45±2.05^{a}	77.66±4.47^{a}
Reference for comparison (NeemAzal)	69.30±4.28^{b}	71.56±1.70^{b}	88.02±3.81^{b}	95.59±4.98^{b}
Negative (acetone) control	55.26±6.71^{a}			

Values are represented by the mean ± (n = 10),
In each column, figures marked by the same alphabets do not significantly differ (p = 0.005)

present in the pant inhibit larval feeding behavior, make the food unpalatable, or that the substances directly act on the chemosensilla of the larva, resulting in feeding deterrence. Several authors have reported that plant extracts possess a similar type of antifeedant activity against *S. litura"* [1,3,4,6,8,9].

Plant products are toxic to field pests and kill nearly 80% of pests in laboratory conditions [4,18,19, 20,21,22]. Antifeedant chemicals play a major role in the defensive mechanisms of non-host plants as food for insects. This is the first report of the sea grass *C. serrulata* having antifeedant activity against *S. litura*.

5. CONCLUSION

The present findings on various solvent extracts prepared from the leaves of *C. serrulata* showed potential antifeedant activity. The ethyl acetate extract of *C. serrulata* exhibited maximum antifeedant activity against *S. litura*. Among the fractions, the percent antifeedant activity was at its maximum in fraction-I due to the presence of active phytochemicals. In further studies, the most effective fraction-I will be purified and a natural herbal formulation prepared for the control of lepidopteran pests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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