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Antifeedant, insecticidal and growth inhibitory activities of selected plant oils on black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

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ABSTRACT

Objective: To evaluate antifeedant, insecticidal and insect growth inhibitory activities of eucalyptus oil (*Eucalyptus globules*) and gaultheria oil (*Gaultheria procumbens* L.) against black cutworm, *Agrotis ipsilon*. **Methods:** Antifeedant, insecticidal and growth inhibitory activities of eucalyptus oil and gaultheria oil were tested against black cutworm, *A. ipsilon*. **Results:** Significant antifeedant activity was found in eucalyptus oil (96.24%) where as the highest insecticidal activity was noticed in gaultheria oil (86.92%). Percentages of deformities were highest on gaultheria oil treated larvae and percentage of adult emergence was deteriorated also by gaultheria oil. **Conclusions:** These plants oil has potential to serve as an alternative eco-friendly control of insect pest.

1. Introduction

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera:Noctuidae) is a worldwide cosmopolitan pest of over 30 important crops, such as, beans, broccoli, cabbage, carrot, spinach, egg plant, lettuce, potato, tomato, turnip, as well as many other plants [1]. However, larvae will feed above ground until about the fourth instar. Larvae can consume over 400 sq cm of foliage during their development, but over 80% occurs during the terminal instar, and about 10% in the instar immediately preceding the last. Thus, little foliage loss occurs during the early stages of development. Once the fourth instar is attained, larvae can do considerable damage by severing young plants, and a larva may cut several plants in a single night. [2,3].

A. ipsilon has developed resistance in recent years to some of the conventional insecticides. Several attempts to combat the pest species on different crops using synthetic chemical pesticides culminated in problems like insecticide resistance, pest resurgence, outbreaks of secondary pests and environmental pollution. Keeping in view the economic importance of the insect pest and the hills crops in Nilgiris, laboratory studies were carried out to asses the effectiveness of the plant oils in controlling the black cutworm on cruciferous vegetables crops. Plant extracts including essential oils must have a great potential for pest management, which we shall review in light of recent literature. This work will complement previous reports on the biological and antimicrobial activities of essential oils as well as plant allelochemicals and their applications [4, 5]. So, a diversified use of essential oils by the development of their use in the pest management sector could be of both economic and ecological benefit.

2. Material and Methods

2.1. Collection of Plant oils

Nilgiris oil, Eucalyptus oil (*Eucalyptus globules*) and wintergreen oil, Gaultheria oil (*Gaultheria procumbens* L.) were purchased from Tamilnadu Government Co-Operative Super Market, Cherring cross, Udhagamandalam, The

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Nilgiris, Tamil Nadu, India and collected oils were used for bioassay against larvae of *Agrotis ipsilon*.

2.2. Rearing of Black cutworm, Agrotis ipsilon

The larvae were collected from cabbage field at Kodappumund, Udhagamandalam, Tamil Nadu, India. Larvae were reared in lab condition at the department of Zoology and Wildlife biology, Government Arts College, Udhagamandalam, Tamil Nadu, India. These laboratory-reared larvae were used for bioassays and the cultures were maintained throughout the study period.

2.3. Antifeedant Activity

Antifeedant activity of plant oils was studied using leaf disc no choice method [6]. The stock concentration of plant oils (2 %) was prepared by mixing with dechlorinated water. Fresh potato leaf discs of 3-cm diameter were punched using cork borer and dipped with 0.25, 0.5, 1.0 and 2.0% concentrations of plant oils individually. Leaf discs treated with water was considered as control. After air-drying, each leaf disc was placed in petridish (1.5 cm X 9 cm) containing wet filter paper to avoid early drying of the leaf disc and single 2hr pre-starved fourth instar larva of *A. ipsilon* was introduced. For each concentration five replicates were maintained. Progressive consumption of leaf area by the larva after 24 hours feeding was recorded in control and treated leaf discs using graph sheet. Leaf area consumed in plant oils treatment was corrected from the control. The percentage of antifeedant index was calculated using the formula of Ben Jannet et al [7].

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

Where C and T represent the amount of leaf eaten by the larva on control and treated discs respectively.

2.4. Insecticidal Activity

Fresh potato leaves were treated with different concentrations (as mentioned in antifeedant activity) of plant oils. Petioles of the potato leaves were tied with wet cotton plug (to avoid early drying) and placed in round plastic trough (29 cm X 8 cm). In each concentration 10 pre-starved (2hours) IV instar larvae of *A. ipsilon* were introduced individually and covered with muslin cloth. Five replicates were maintained for all concentrations and the number of dead larvae was recorded after 24hours up to pupation. Percentage of larval mortality was calculated and corrected by Abbott's formula [8].

Abbott's percent corrected mortality=

$$\frac{\text{\% Mortality in treated} - \text{\% Mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

2.5. Growth Regulation Activity of Plant Oils

Growth regulation activities of plant oils were studied at four different concentrations against IV instar larvae of *A. ipsilon*. Ten larvae were introduced in a petriplate having potato leaves treated with different concentration of plant oils. Water treated leaves were considered as control. After 24hours feeding, the larvae were transferred to normal leaves for studying the developmental periods. For each concentration five replicates were maintained. During the developmental period deformed larvae, pupae, adults and successful adults emerged were recorded. In addition weight gain by the treated and control larvae were also recorded.

3. Results

Plant oils of Gaultheria and Eucalyptus oils and their bioactivities were tested at different concentrations against *Agrotis ipsilon*. The bioactivity data were collected and subjected to one-way analysis of variance (ANOVA). Significant difference between the mean was separated using least significant difference (LSD) test. Antifeedant activity of plant oils was studied at different concentrations and the results are presented in table 1. Antifeedant activity of plant oils was assessed based on antifeedant index. Higher antifeedant index normally indicates decreased rate of feeding. In the present study irrespective of concentration the antifeedant activity varied significantly. Data pertaining to the above experiment clearly revealed that maximum

Table 1

Antifeedant and insecticidal activities of plant oils against fourth instar larvae of *A. ipsilon*

Concentration (%)	Antifeedant		Insecticidal	
	Gaultheria oil	Eucalyptus oil	Gaultheria oil	Eucalyptus oil
0.25	19.79±3.42b	25.74±3.45b	2.70±3.45a	11.74±5.49a
0.5	28.41±2.48b	32.47±8.98b	18.41±2.98a	32.47±6.98a
1.0	54.66±8.41c	62.62±3.41c	53.64±3.41b	48.64±3.62b
2.0	87.21±5.83d	96.24±1.85d	86.92±1.84c	60.24±6.80c
Control	4.22±3.61a	4.22±3.61a	1.20±3.64a	1.20±3.64a

Values are mean of five replications. Within the column similar alphabets are statistically not significant (P>0.05 by LSD).

antifeedant activity was recorded in eucalyptus oil (96.24%) at 2% concentration and followed by gaultheria oil (87.21%) compared to control. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test showed statistical significance (P<0.05) compared to control.

Insecticidal activity of plant oils was studied at different concentrations and the results are presented in table 1. Insecticidal activity of plant oils was calculated based on larval mortality after treatment. High larval mortality normally indicates potential insecticidal activity of plant oils. In the present study irrespective of concentration used for oils the insecticidal activity varied significantly. Data pertaining to the insecticidal activity clearly revealed that maximum statistically significant insecticidal activity was recorded in gaultheria oil (86.92%) at 2% concentration whereas in the case of eucalyptus oil 60.24% was observed. One-way analysis of variance (ANOVA) followed by least significant difference (LSD) test showed statistical significance (P<0.05) compared to control.

Weight gained by larvae was studied at different concentration of plant oils and control. The weight gained by the larvae from the fourth instar to final instar was calculated. Statistically decreased weight gain was observed in gaultheria oil treated larvae (110.12mg) and followed by eucalyptus oil treated (133.52mg) larvae at 2% concentration (Table 2).

Table 2

Effect of plant oils on weight gain of fourth instar larvae of A .ipsilon

Concentration (%)	Name of the Oils	
	Gaultheria oil	Eucalyptus oil
0.25	180.17±7.42b	205.82±8.44b
0.5	162.48±4.48b	180.10±9.68b
1.0	125.63±6.42a	150.64±5.43a
2.0	110.12±5.89a	133.52±6.80a
Control	265.26±8.31c	265.26±8.31c

Values are mean of five replications. Within the column similar alphabets are statistically not significant (P>0.05 by LSD).

Percentage of deformities due to the treatment of plant oils at different concentrations is presented in table 3. Among the two plant oils with four concentrations tested, maximum larval (30%), pupal (23%) and adult (21%) deformities were recorded in gaultheria oil at 2% concentration. and 25.1% in eucalyptus oil at 2% concentration but minimum deformities was recorded in eucalyptus oil. Percentage of successful adult emergence was minimum (26.6%) on gaultheria oil and maximum on eucalyptus oil (35.5%) at 2% concentration. Plant oils were subjected to preliminary phytochemical analysis for the confirmation of major group of compounds. Both plant oils showed positive results for confirmation of alkaloids and triterpenoid.

4. Discussion

In nature many plants have high content of unpalatable substances like phenols, alkaloids, flavanoids, terpenes, quinone, coumarin etc., which play a defensive role against insect pests. These substances possess a wide range of biological activities including antifeedant, oviposition deterrent, insecticidal, ovicidal and insect growth regulators (IGRs). Identifying sources with useful biological activity is only the starting point in the long process of development of a botanical pest management product [9].

Antifeedant is defined as a chemical that inhibits feeding without killing the insect directly, while the insect remains near the treated foliage and dies through starvation. Most potent insect antifeedants are quinoline, indole alkaloids, sesquiterpene lactones, diterpinoids, and triterpinoids [10]. The present study reported that eucalyptus oil was promising in reducing feeding rate of fourth instar larvae of *A .ipsilon*. The rate of feeding significantly varied depending on the concentration of the plant oils. This indicates that the active principles present in the plant oil that inhibit larval feeding behaviour or make the food unpalatable or the substances directly act on the chemosensilla of the larva resulting in feeding deterrence.

Earlier, antifeedant effects of different plant essential oils were reported on various insect species. Krishnappa et al. [11] reported that Tagetes patula essential oil against the fourth instar larvae of S. litura for their antifeedant activity by leaf disc bioassay. Among the compounds tested Terpinolene was the most effective feeding deterrent agent against Spodoptera litura in the laboratory condition. The mean area fed 100 ppm / cm2 and 500 ppm/cm^2. Elumalai *et al*. [12] reported that certain medicinal plant essential oils were tested against the fourth instar larvae of *S. litura* for

Table 3

Percentage of deformed stages of *A. ipsilon* due to the treatment plants oil

Concentration (%)	Gaultheria oil				Eucalyptus oil			
	Larvae	Pupae	Adult	Successful Adult Emergence	Larvae	Pupae	Adult	Successful Adult Emergence
0.25	3.3 a	5.6a	4.3a	86.8b	7.3 a	5.2a	7.0a	80.5b
0.5	10.5 b	11.5ab	4.8 a	73.2b	10.4 a	12.6a	9.2 a	67.8b
1.0	19.4 c	16.6 b	12.5 b	51.5a	15.7 ab	12.9 ab	13.6 b	57.8a
2.0	30.0d	22.5c	20.9c	26.6a	25.1c	18.6b	20.8c	35.5a
Control	1.0a	1.0a	2.0a	96.0c	1.0a	1.5a	1.5a	96.0c

Values are mean of five replications. Within the column similar alphabets are statistically not significant (P<0.05 by LSD).

their antifeedant activity by leaf disc bioassay. All essential oil are showed moderate antifeedant activitiy; however, the highest antifeedant activitiy was observed in the essential oil of Cuminum cyminum, Mentha piperata, Rosmarinus officinalis, Thymus vulgaris and Coriandrum sativum exhibited (100%) complete antifeedant activity at 6mg/cm^2.

Recently, Duraipandiyan et al. [13] they have been reported that antifeedant and larvicidal activities of rhein isolated from Cassia fistula flower against lepidopteron pests S. litura and H. armigera. Significant antifeedant activity was observed against *H. armigera* (76.13%) at 1000 ppm concentration. Rhein exhibited larvicidal activity against H. armigera (67.5), *S. litura* (36.25%) and the LC_{50} values was 606.50 ppm for *H. armigera* and 1192.55 ppm for *S. litura*. The survived larvae produced malformed aduts. In addition, Jeyasankar *et al*.[14] reported that ethyl acetate extract of Solanum psuedocapsicum showed higher antifeedant activity on *A. ipsilon*. Further, Gokulakrishnan et al [15, 16] reported that, 20 plant essential oils have been tested for their antifeedant activity of against three important lepidopteran species such as *S. litura, H. armigera* and Achaea janata . Among the oils tested, most significant antifeedant activity was observed at 1000 ppm concentration on *S. officinalis* (85.56) *S. litura, M. spicata* (82.85) H. armigera and M. spicata (90.55) *A. janata*.

In the present study the preliminary phytochemical analysis revealed that presence of alkaloids and terpinoids in the plant oils. These chemicals may inhibit the feeding of *A. ipsilon*. These results were supported by earlier workers on various insect pests, [17, 18]. Plant oils for deleterious effects on insects are one of the approaches used in the search for novel botanical insecticides. Secondary plant compounds act as insecticides by poisoning per se or by production of toxic molecules after ingestion. These compounds also deter or possibly repel an insect from feeding. In the present study gaultheria oil exhibited significant insecticidal activity at 2% concentration. It is possible that the insecticidal property present in the selected oil compound may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died. Similarly, the larvicidal effect of Basil essential oil tested against A.ipsilon was more effective than its active component (eugenol). The effect was more pronounced at the higher tested concentration. Basil oil at 3% (conc.), only 35 % of the larvae reached the pupal stage with 67.16 % reduction than control and 13 % of the pupae were deformed. Eugenol caused 40 % larval mortality. The reduction in percentage of adult emergence at 3 % and 2 % of basil reached 76.84 and 54.74 %, respectively. The deformities among adult reached 11% and 7 % at 3 and 2 % basil, respectively [19].

Insect growth regulation properties of plant essential oils are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying [20]. In the present study maximum percentage of deformed development of larvae, pupae and adults were noted in gaultheria oil treated larvae. The morphological deformities at larval, pupal and adult stages are due to toxic effects of oils on growth and development processes. Since morphogenetic hormones regulate these processes, it can be suggested that these plant oil interfere with these hormones of the insects. These results are consistent with the recent reports on various lepidopteran species [21-23] who have reported that high larval mortality indicates potential insecticidal properties present in ethyl acetate extracts of Syzygium lineare against *S. litura* and a new crystal compound 2,5-diacetoxy-2- benzyl-4,4,6,6-tetramethyl-1,3-cyclohexanedione was isolated from the leaves of *S. lineare* which was responsible for significant insecticidal properties against larvae of *S. litura*. Its activity was better than the positive control azadirachtin. Furthermore, insecticidal compound was responsible for growth inhibition on *S. litura*. It induced larval, pupal and adult deformities even at low concentration.

In conclusion, gaultheria oil showed greater performance of insecticidal and growth inhibition activities against *A. ipsilon*. Hence, it may be suggested that the gaultheria oil, can be used for controlling the insect pest, *A. ipsilon*, which will replace the chemical pesticides.

Conflict of interests

We declare that we have no conflict of interests.

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