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Antifeedant and Insecticidal Activity of Different Solvent Extracts of *Vitex negundo* (L) against Cotton Leafworm *Spodoptera litura* (Fab.)

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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

Spodoptera litura, commonly knows as cotton leafworm, is a serious polyphagous pest causing damage to more than 150 species of host plants and it is distributed throughout the tropical and sub- tropical regions of the world including India, Japan, China and South East Asia. The chemical pesticides affect the non- target organisms and human beings, directly or indirectly. To find environmentally safe alternative there is need of considering the pesticides of biological origin to replace synthetic pesticides. Anti-insect activity check of plant extract can play important role in ecofriendly control of insect pest. *Spodoptera litura* is a dangerous polyphagous pest. The present work aimed to identify a natural alternative to chemical pesticides for the control of insect pests by examining a variety of factors, including larval weight, duration, antifeedant activity, and mortality. Maximum larval mortality (10.30%) was noted in *Vitex negundo* chloroform extract. The larval duration is much longer in the *Vitex negundo* chloroform and methanol extracts (10.23 days and 10.56 days, respectively) as compared to the control group. The *Vitex negundo* chloroform extract (59.42%) andacetone (37.68%) have the strongest antifeedant effects. In comparison to the control, the larval weight was significantly reduced in the *Vitex negundo* chloroform (0.391 gm), acetone (0.401 gm), and methanol extract (0.420 gm) extracts (0.621 gms).

Keywords: Vitex negundo; anti-insect activity; Spodoptera litura; plant extracts.

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1. INTRODUCTION

Pests are the one of the most increasing and hazardous problems to agricultural cops. Ten to thirty percent of main crop loss in agriculture is attributable to insect pests, which inflict serious damage to crops and directly affect revenue [1]. "The dangerous polyphagous pest *Spodoptera litura*, commonly known as cotton leafworm, present in all tropical and subtropical parts of the world, including India, Japan, China, and South East Asia, damages more than 150 species of host plants" [2,3]. Forty species of the 150 host plants are known to come from India [4]. Most farmers attempt to control the population of *S. litura* by using chemical insecticides. These insecticides have direct or indirect effects on both humans and non-target creatures. Due to a lack of host plant resistance to *S. litura* and inadequate management techniques, it is challenging to handle this pest in the fields [5]. "The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment friendly alternative methods for insect pest control. The use of biopesticides to protect crops from insect pests has become more important in recent years due to rising awareness of the adverse effects of the chemical pesticides' indiscriminate use" (Chari et al. 1990).

"The Sambhaloo (*Vitex negundo* L.), a member of the Verbenaceae family, has thin, gray bark. The herb is abundantly available and has pharmacological effects against a variety of diseases in the conventional medical system. Numerous secondary metabolites, including alkaloids, phenols, flavanoids, glycosidic irridoids, tannis, and terpenes, are present in all plant sections, but particularly in the leaves. Hepatoprotective, anti-inflammatory, anti-tumor, antioxidant, insecticidal, antimicrobial, anti-androgenic, anti-osteoporotic, anti-cataract, and anti-hyperglycemic activity were among the promising bioactivities that the crude extracts and purified components of Vitex negundo displayed" [6].

In the present investigation, an attempt was made to study the effect of extract of *Vitex negundo* leaf against *Spodoptera litura.*

2. MATERIALS AND METHODS

2.1 Collection and Rearing of *Spodoptera litura*

The eggs of *S. litura* were purchased from National Bureau of Agriculture Insect Resources (ICAR), Bangalore. The larvae were fed with castor leaves (*Ricinus communis*. L).

Ten third instar larvae of *S. litura* of uniform size and age were collected from the mass culture maintained in the laboratory were placed in plastic trays (40 X 28 X 10 cm). Only the middle leaves of castot were used.

2.2 Collection and Extraction of Plant Extract

The fresh leaves of *Vitex negundo* were collected from the areas of Nivkane, Diwashi Khurd, Helwak, Rasati from taluka Patan district Satara (MS). The leaves were washed separately with distilled water, shade dried, cut into small pieces and air dried for 14 days in the laboratory before pulverized into fine powders using an industrial electric pulverizing machine at the Department of Zoology, SGM College, Karad. The powders were further sieved to pass through mm2 perforations and kept in an air-tight plastic containers for storage before use at ambient temperature (28 ± 2) °C.

About 300 g of *V. negundo* leaf powders were soaked separately in an extraction bottle containing 500 ml of chloroform, acetone and methanol for 72 hours. The mixture was stirred occasionally with a glass rod and extraction was terminated after three days. Filtration was carried out using a double layer of Whatman No. 1 filter papers and solvent evaporated using a rotary evaporator at 30 to 40 °C with rotary speed of three to six rpm for eight hours. The resulting extracts were air dried in order to remove traces of respective solvents. The extracts were kept in labelled plastic bottles till when needed.

Standard stock solutions were prepared by dissolving 1 g of the crude extracts in 100 ml of solvent.

2.3 Larvicidal Activity

"1% mg/ml concentration of crude extract of *Vitex negundo* was applied using leaf disc method.

Leaf discs of 4cm² diameters were cut and dipped individually in different concentrations of leaf extracts for 5 minutes along with control separately. Third prestarved instar larvae of *Spodoptera litura*, were introduced individually. After 48 hrs, remnants of leaf disc were kept between two transparent sheets [7] and leaf areas consumed by larvae traced on graph paper

[8] and calculated" according to formula of Isman et al. 1990).

The treated leaves were exposed to the 3rd instar larvae. After 24 hrs of treatment, the larvae were continuously maintained on the non- treated castor leaves. Later the larvae were provided with fresh castor leaves every 24hrs. Larval mortality was recorded after 96 hrs of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate.

Percent mortality was calculated by using Abbott's formula [9]. The experiment was conducted at laboratory temperature of 27$\pm$ 2^{o} C with 75 $\pm$ 5 % relative humidity.

$$\% \, Larval \, Mortality \, = \frac{Number \, of \, dead \, larvae}{Number \, of \, larvae \, introduced} \, X \, \frac{100}{1}$$

Percentage larvae mortality was calculated using Abbott corrected mortality formula

$$PT \, = \frac{Po - Pc}{100 - Po} X \frac{100}{1}$$

Where P_T = corrected mortality (%)
P_O = observed mortality (%)
P_C = control mortality (%)

2.4 Antifeedant Activity

"Leaf discs no choice methods [10] were used for bioassay test, after washing with tap water. The crude extracts were dissolved in respective solvents. Fresh castor leaf discs of 4 cm diameter were punched using a borer. The leaf discs were dipped in different solvent extracts. Negative controls were dipped in the representative solvent. Treated leaves were air dried at room temperature and kept in petri plates (9cm diameter). Pre-starved (3 hours) third instar larvae were allowed to feed on the treated leaf disc for 24hours. Five replicates were maintained. For each treatment ten larvae per replicate (total number=50) with one control were maintained. Progressive consumption of the leaf area by the larvae after 24hours were recorded in control and treated discs using the leaf area metre. Area of the leaf eaten by the larvae in plant extract treatment was corrected from the control". Percentage of antifeedant activity was calculated using the formula of Isman et al. (1990).

$$Antifeedant \, activity \, = \frac{Control - Treatment}{Control + Treatment} X \frac{100}{C1}$$

2.5 Larval Duration

The survived larvae were continuously fed with castor leaf. The larval duration was calculated after treated larvae became pupae. Pupal duration was calculated from pupation to the day of emergence of adults.

2.6 Larval Weight

The data on the leaf area consumed was recorded and used for the calculation of the larval weight gain at 3 days after feeding. The leaf discs dipped in acetone, methanol and chloroform were used as negative control since they were used to dissolve the *V. negundo* leaf powder.

2.7 Statistical Analysis

The data obtained in present study was subjected to analysis of variance (ANOVA) significant difference between treatments and control were determined by Tukey's multiple range test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

The *Vitex negundo* leaf extract in 1% chloroform (10.30%) showed the highest level of larval death, followed by the extract in acetone (7.7%). The leaf extract in methanol showed the lowest mortality (3.09%). (Table 1 & Fig. 1). In *P. xylostella*, the aqueous leaf extract of *A. squamosa* at 10% resulted in 66.70% larval death [11]. When used to combat *S. litura, Artemesia nilagrica's* ethyl acetate extract resulted in a 40.24% larval mortality rate [12]. Hence it is inferred that the 1% chloroform extract of *V. negundo* can be used further for the solation of active molecules and to develop a new botanical formulation for the management of *S. litura*.

To evaluate the antifeedant activity of *Vitex negundo* leaf extracts, the amount of leaf area that was consumed by larvae was noted. By comparing the leaf area consumed by the larvae in the treated and untreated groups (13.15%), it is evident that *Vitex negundo's* chloroform leaf extract (59.42%) and acetone leaf extract (37.68%) exhibit significant antifeedant activity, while the methanol leaf extract of *Vitex negundo* exhibited nonsignificant results (Table 2 & Fig. 2). Our findings are consistent with Cassi's earlier observation in 1983 that an aqueous extract of *A. tagala* had antifeedant effect against *S. litura* [13].

Several essential oil constituents contained in the extracts of V. negundo leaves may be responsible for the antifeedant activity against *S. litura*. GC and GC-MS analysis of the essential oils of V. negundo leaves [14] revealed that "this oil contain 65 known compounds, including sabinene p-cymene, betaphellandrene, gamma-terpinene, terpinene-4-ol, beta-caryophyllene, alpha-guaiene, spathulenol, beta-caryophyllene oxide, globulol, viridiflorol, bis [1,1-dimethyl]-methylphenol, abieta-7, 13-diene and several minor unidentified compounds could have the possible antifeedant potential".

The larval duration significantly increased in the *Vitex negundo* chloroform and methanol extracts (10.23 and 10.56 days, respectively) when compared to the control group. Results comparing the control group (9.2 days) with the acetone extract of *Vitex negundo* demonstrate the larval duration (Table 3, Fig. 3).

"Presently, therefore it appears that the ptent antifeedant, larvicidal, larval duration is characteristic at 1.0 mg/ml concentration of *V. negundo*. Antifeedant chemicals play a major role in the unsuitability of non-host plants as food for insects (Jeyasankar & Ignacimuthu, 2010), that inhibits the feeding without killing the insect pests directly, while it remains near the treated foliage and dies through starvation. Higher antifeedant index normally indicate decreased rate of feeding. Leaf extracts of Vitex negundo were found to be effective in reducing the feeding rate of larvae of *Spodoptera litura* with maximum antifeedant activity in methanol and chloroform extracts of Vitex negundo at 1.0 mg/ml extract concentration"[15,16].

Despite eating less food, the majority of treated larvae perished as a result of the harmful effects of plant extracts. Larval death and prolonged larval days may be caused by toxic components in the extract or by an imbalance of the hormones that promote and restrict growth. The findings of the present study strongly imply the need for additional research into the isolation and identification of the active antifeedant qualities, which should be studied further and may lead to the development of an alternate strategy or instrument for the control of *Spodoptera litura*.

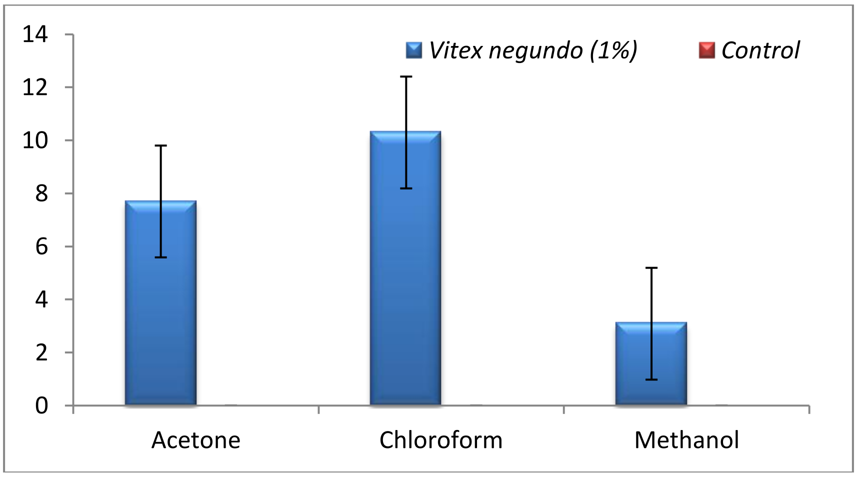

Fig. 1. Histogram showing Percent larval mortality observed after treatment of *S. litura*

Table 1. Percent larval mortality observed after treatment of *S. litura* with different solvent extracts

Treatment	Acetone extract	Chloroform extract	Methanol extract
***Vitex negundo* (1%)**	7.7 ± 0.95 [a]	10.30 ± 0.69 [b]	3.09 ± 0.78 [b]
Control (only solvent)	0	0	0

Within column ± SD followed by the same letter donot differ significantly using Turkey's test, P ≤ 0.05.

a- No significance
b- Significant
c- Highly significant
d- Very highly significant

Table 2. Percent antifeedant activity of plant extracts against *S. litura*

Treatment	Acetone extract	Chloroform extract	Methanol extract
***Vitex negundo* (1.0mg/ml)**	37.68 ± 3.19 [b]	59.42 ± 2.62 [b]	28.81 ± 3.83 [a]
Control	13.15 ± 1.72 [a]		

Within column ± SD followed by the same letter donot differ significantly using Turkey's test, P ≤ 0.05,

a- No significance
b- Significant
c- Highly significant
d- Very highly significant

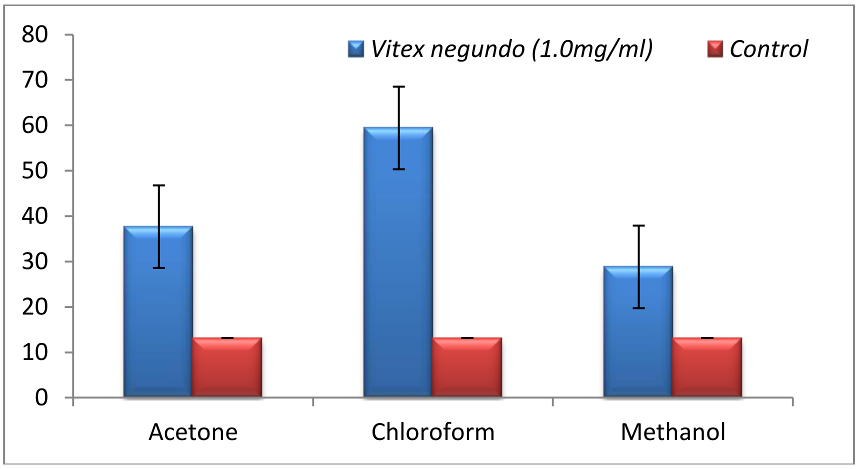

Fig. 2. Histogram showing percent antifeedant activity of plant extracts against *S. litura*

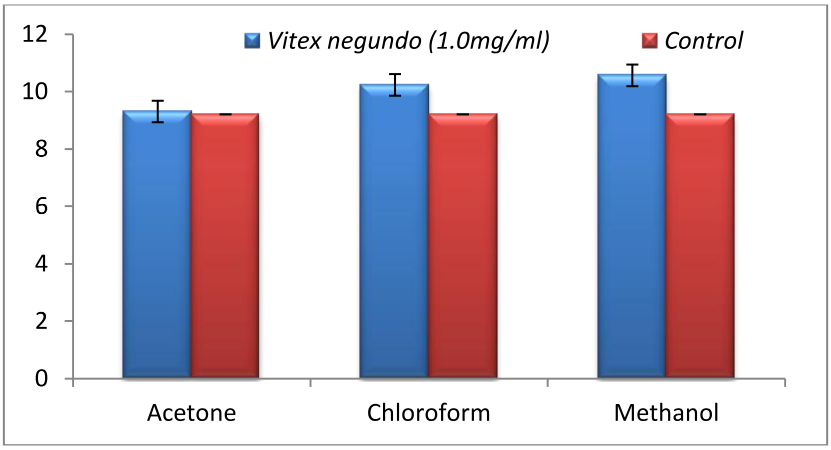

Fig. 3. Total larval duration (days) of *S. litura* after treatment

Table 3. Total larval duration (days) of *S. litura* after treatment

Treatment	Acetone extract	Chloroform extract	Methanol extract
***Vitex negundo* (1.0mg/ml)**	9.3 ± 0.83 [a]	10.23 ± 0.28 [b]	10.56 ± 0.52 [b]
Control	9.2 ± 0.44 [a]		

Within column ± SD followed by the same letter donot differ significantly using Turkey's test, P ≤ 0.05.

a- No significance
b- Significant
c- Highly significant
d- Very highly significant

Table 4. Effect of plant extracts on weight of larva (g)

Treatment	Acetone extract	Chloroform extract	Methanol extract
***Vitex negundo* (1.0mg/ml)**	0.401+ 0.52 [b]	0.391+ 0.81 [b]	0.420 + 0.27 [b]
Control	0.621 + 63 [a]		

Within column + SD followed by the same letter donot differ significantly using Turkey's test, P ≤ 0.05.

a- No significance
b- Significant
c- Highly significant
d- Very highly significant

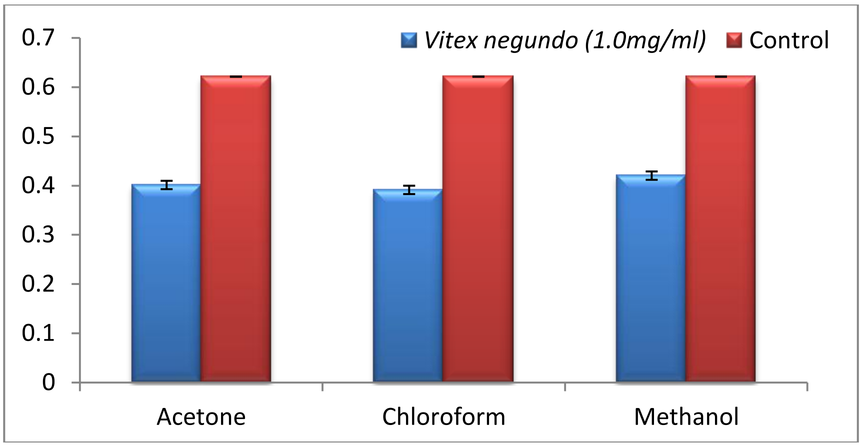

Fig. 4. Effect of plant extracts on weight of larva (g)

4. CONCLUSION

For the management of insects, pest control practitioners are increasingly turning to pesticides generated from natural sources like botanicals as an alternative to traditional chemical pesticides. The *Vitex negundo* (L) leaf extract showed potent antifeedant activity. According to the findings of the current experiment, *Vitex negundo* leaf solvent extracts with 1.0 mg/ml concentrations of effective antifeedant properties. While studying all aspects of *Spodoptera litura's* life cycle, the chloroform extract of *Vitex negundo* leaf shown the greatest impact. The leaf extract of *Vitex negundo* in various solvents will be the most effective, according to the findings of the current investigation.

Therefore the leaf extract of *Vitex negundo* in various solvents can be considered as alternative substitute for chemical pesticides against *Spodoptera litura* due to its various effect on the larval development of *S. litura.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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