Original Research Article

Antifeedantand Insecticidal activity of different solvent extracts of *Vitexnegundo*(L) against cotton leafworm*Spodopteralitura* (Fab.)

ABSTRACT

Spodopteralitura, 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 oftheworld 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.*Spodopteralitura*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 *Vitexnegundo* chloroform extract. The larval duration is much longer in the *Vitexnegundo* chloroform and methanol extracts (10.23 days and 10.56 days, respectively) as compared to the control group.The *Vitexnegundo* 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 *Vitexnegundo* chloroform (0.391 gm), acetone (0.401 gm), and methanol extract (0.420 gm) extracts (0.621 gms).

Key words- *Vitexnegundo*, anti-insect activity, *Spodopteralitura* ,plant extracts

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 affectrevenue (Ferry et al., 2004). The dangerous polyphagous pest *Spodopteralitura*, 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 (Rao, et. al. 1993, &Murgesanand Dhingara, 1995). Forty species of the 150 host plants are known to come from India (Mallikarjuna, et .al., 2004).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. 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(*Vitexnegundo* 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, glycosidicirridoids, 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 Vitexnegundo displayed (Zheng et al., 2015).

In the present investigation,an attempt was made to study the effect of extract of *Vitexnegundo* leaf against *Spodopteralitura*.

Material and Methods

Collection and Rearing of *Spodopteralitura* –

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

Ten third instar larvae of *S. litura*of uniform size andage were collected from the mass culture maintained inthe laboratory were placed in plastic trays (40 X 28 X 10 cm).Only the middleleaves of castotwere used.

Collection and Extraction of plant extract

The fresh leaves of *Vitexnegundo*were collected from the areas of Nivkane, DiwashiKhurd, Helwak, Rasati from talukaPatan 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.

Larvicidal activity-

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

Leaf discs of 4cm² diameterswere cut and dipped individually in different concentrations of leaf extracts for 5 minutesalong with control separately. Third prestarved instar larvae of *Spodopteralitura*, were introduced individually.After 48 hrs, remnants of leaf disc were kept between two transparent sheets (Singh and Singh, 1993) and leafareas consumed by larvae traced on graph paper (Sarma and Kalita, 2001) and calculated according to formulaof 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. (Abbott, 1925). The experiment was conducted at laboratory temperature of 27$\pm$ 2^{o} C with 75 $\pm$ 5 % relative humidity.

$$\frac{\text{Number of dead larvae}}{} \quad \frac{100}{}$$

$$\% \text{ Larval Mortality} = \frac{\text{Number of larvae introduced}}{} \text{ X } \frac{}{1}$$

Percentage larvae mortality was calculated using Abbott corrected mortality formula

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

Where P_T = corrected mortality (%)

P_O = observed mortality (%)

P_C = control mortality (%)

Antifeedant activity

Leaf discs no choice methods (Shrivastava., et al, 1990) were used for bioassaytest, after washing with tap water. The crude extractswere dissolved in respective solvents. Fresh castorleaf discs of 4 cm diameter were punched using aborer. The leaf discs were dipped in different solvent extracts. Negativecontrols were dipped in the representative solvent.Treated leaves were air dried at room temperature andkept in petri plates (9cm diameter). Pre-starved(3 hours) third instar larvae were allowed to feed onthe treated leaf disc for 24hours. Five replicateswereмaintained. For each treatment ten larvae perreplicate(total number=50) with one control weremaintained. Progressive consumption of the leaf areaby the larvae after 24hours were recorded in controland treated discs using the leaf area metre. Area of theleaf eaten by the larvae in plant extract treatment wascorrected from the control. Percentage of antifeedantactivity was calculated using the formulaof Ismanet. al., (1990).

$$\text{Antifeedant activity} = \frac{\text{Control - Treatment}}{\text{Control + Treatment}} \text{ X } \frac{100}{1}$$

Larval duration-

The survived larvae were continuously fed with castor leaf.The larval duration was calculated after treated larvae becamepupae. Pupal duration was calculated from pupation to theday of emergence of adults.

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 wereused as negative control since they were used to dissolve the *V. negundo* leaf powder.

Statistical analysis

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

Result and Discussions

The *Vitexnegundo* 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 (Chandrashekharaiah et al., 2015). When used to combat *S. litura, Artemesianilagrica's* ethyl acetate extract resulted in a 40.24% larval mortality rate (Raja et al., 2003).Hence it is inferredthat the 1% chloroform extract of *V. negundo* can be used furtherfor the solation of active molecules and to develop a newbotanical formulation for the management of *S. litura*.

To evaluate the antifeedant activity of *Vitexnegundo* 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 *Vitexnegundo's* chloroform leaf extract (59.42%) and acetone leaf extract (37.68%) exhibit significant antifeedant activity, while the methanol leaf extract of *Vitexnegundo* exhibitednonsignificant 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.*

Several essential oil constituents contained in the extracts of V. negundo leaves may be responsible forthe antifeedant activity against *S. litura*. GC and GC-MS analysisof the essential oils of V. negundo leaves (Mallavpura et al. 1994) revealed that this oilcontain 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 andseveral minor unidentified compounds could have the possible antifeedant potential.

The larval duration significantly increased in the *Vitexnegundo* 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 *Vitexnegundo* demonstrate the larval duration (Table 3,Fig. 3).

Presently, therefore it appears that the ptentantifeedant, larvicidal, larval duration is characteristic at 1.0 mg/ml concentration of *V. negundo*. Antifeedant chemicalsplay 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 anddies through starvation. Higher antifeedant index normally indicate decreased rate of feeding. Leaf extracts ofVitexnegundowere found to be effective in reducing the feeding rate of larvae of*Spodopteralitura* with maximum antifeedant activity in methanol and chloroform extracts of Vitexnegundo at 1.0 mg/ml extract concentration.

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 *Spodopteralitura*.

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

Treatment	Acetone	Chloroform	Methanol

	Extract	Extract	Extract
***Vitexnegundo* (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
***Vitexnegundo* (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

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

Treatment	Acetone Extract	Chloroform Extract	Methanol Extract
***Vitexnegundo* (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

***Vitexnegundo* (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, $P \leq 0.05$.

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

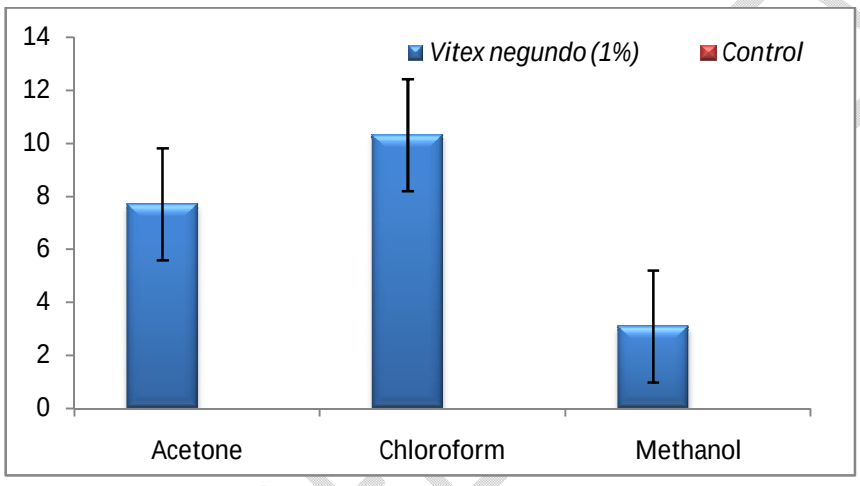

Figure1.Histogram showing Percent larval mortality observed after treatment of *S. litura*

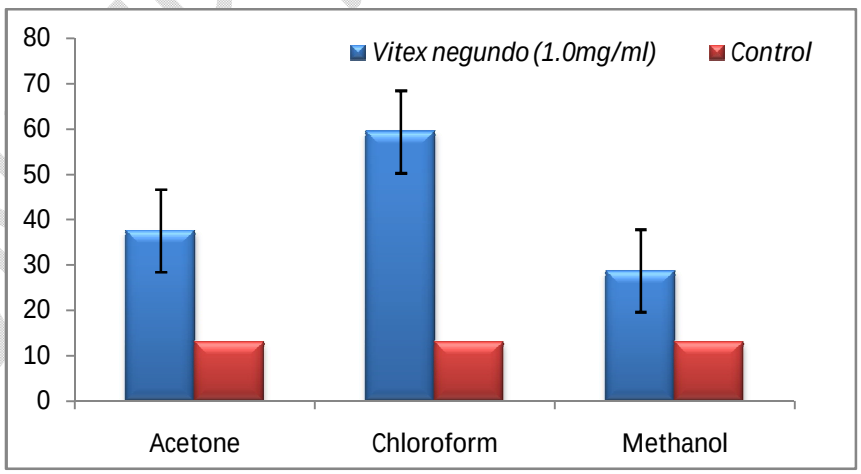

Fig.2.–Histogram showingpercent antifeedant activity of plant extracts against *S. litura*

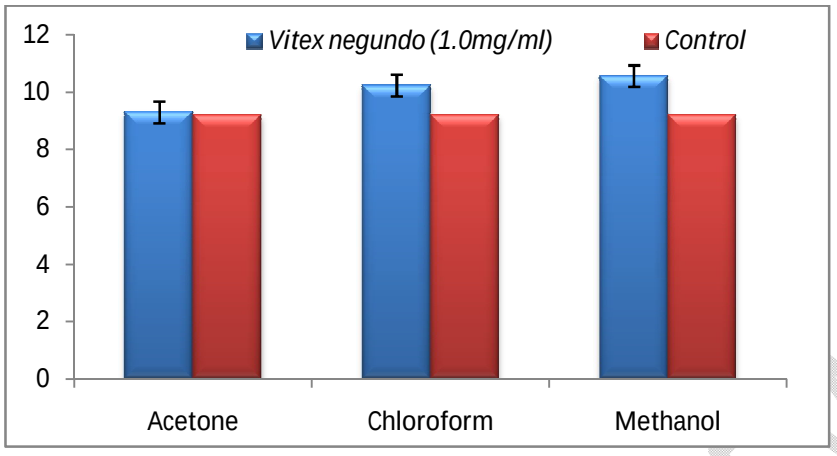

Fig.3. Total larval duration (days) of *S. litura*after treatment

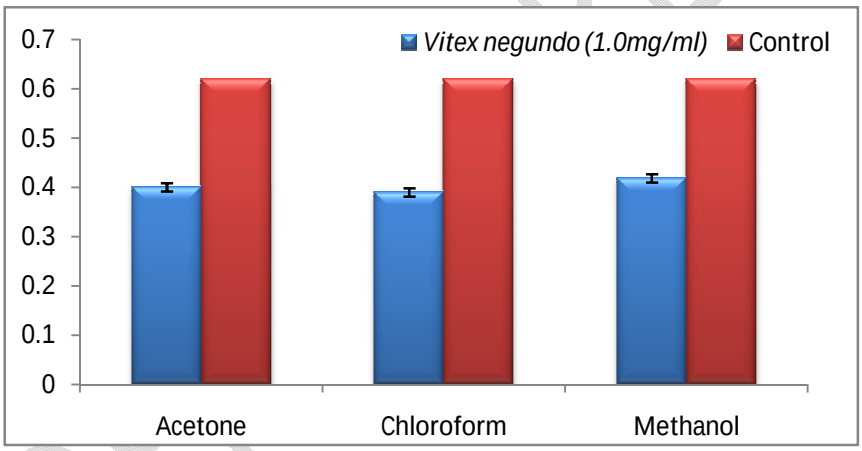

Fig.4. Effect of plant extracts on weight of larva (g)

Conclusion

For the management of insects, pest control practitionersare increasingly turning to pesticides generated from natural sources like botanicalsas an alternative to traditional chemical pesticides. The*Vitexnegundo* (L)leaf extract showed potent antifeedant activity.According to the findings of the current experiment, *Vitexnegundo* leaf solvent extracts with 1.0 mg/ml concentrations of effective antifeedant properties. While studying all aspects of *Spodopteralitura's* life cycle, the

chloroform extract of *Vitexnegundo* leaf shown the greatest impact.The leaf extract of *Vitexnegundo* in various solvents will be the most effective, according to the findings of the current investigation.

Therefore the leaf extract of *Vitexnegundo* in various solvents can be considered as alternative substitutefor chemical pesticides against *Spodopteralitura* due to its various effect on the larval development of *S. litura.*

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