



Insecticidal and growth inhibitory action of *Vitex negundo* Linn. against Asian army worm, *Spodoptera litura* Fab.

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ABSTRACT

Insecticidal and growth inhibitory effects of *Vitex negundo* solvent extracts at different concentrations were evaluated in the laboratory and the results were presented. The different solvent extracts (methanol, hexane, acetone and water) at various concentrations (1,2,4 and 6%) were tested against third instar larvae of *Spodoptera litura*. Insecticidal action of *V. negundo* solvent extracts was determined through topical bioassay. Methanol extract of *V. negundo* was the most toxic and caused maximum mortality (96.3%) recording lowest LD₅₀ of 423 ppm. Aqueous extract recorded lowest mortality of 51.7 per cent. Influence of host materials on the effect of *V. negundo* extract was also tested. Castor, semi-synthetic diet and banana were tested as host materials for rearing *S. litura*. Different solvent extracts of *V. negundo* were prepared at various concentrations (1,2,4 and 6%) and applied to different host materials and allowed to feed by *S. litura* third instar larvae. Observations on larval duration, pupal weight and extent of pupation on different hosts were taken to determine the growth inhibitory action. Among all the hosts, the highest reduction in pupal weight was on semi-synthetic diet reared *S. litura*. Methanol extract caused maximum reduction in pupal weight (45.15 to 72.60%) followed by acetone extract (34.52 to 70 %) and lowest reduction in pupal weight was observed in aqueous extract (52.91 to 56.51 %). Hexane, acetone and methanol extract (6%) caused the highest reduction of 96.51, 96.30 and 92.86 per cent pupation over control while, aqueous extract produced only 52 per cent reduction of pupation of *S. litura* reared on castor. *V. negundo* extracts caused the lowest reduction of pupation (26.93 to 77.78 %) of *S. litura* reared on semi-synthetic diet. In banana, pupation was reduced to the range of 55.56 to 85.72 per cent by aqueous and methanol extracts. A maximum larval duration of 26.33 days with the highest (102.54 %) increase in duration over control was recorded by methanol extract (6%) on semi-synthetic diet. When the larvae were fed with banana, aqueous extract of *V. negundo* (6%) caused longest larval duration of 19 days, when the leaves were treated with acetone extract (6%).

Key words: *Vitex negundo*, Botanical pesticide, *Spodoptera litura*, Crop pest

INTRODUCTION

Spodoptera litura is a polyphagous and most destructive pest and has about 150 host species causing heavy economic loss every year (Rao *et al.*, 1993; Gothama *et al.*; 1995). It is an indigenous pest of a variety of crops in South Asia and was found to cause 26 - 100 per cent yield loss in ground nut (Dhir *et al.*, 1992). In India, this pest was found resistant to several insecticides (Armes *et al.*, 1997; Kranthi *et al.*, 2002). Large scale use of synthetic and broad spectrum insecticides resulted in hazardous effects on environment and human health, resistance development in insect populations, resurgence of pest populations and so forth (Pimental *et al.*, 1992). Most of the research works are being made world wide to find safer, biodegradable substitutes for these synthetic insecticides. The plant kingdom is the most efficient chemical factory. Botanical insecticides are natural toxins

extracted from plants. When compared with chemical pesticides, botanicals are cheaper with less biohazard and mammalian toxicity. Plant derived insecticides breakdown quickly in the environment, resulting in little risk of residues on food crops. The secondary metabolites produced by plants have a wide range of mode of actions. These compounds are deleterious to insects and other herbivores in multiple ways, such as through acute toxicity, affecting insect behaviour, disrupting growth and development of insects, acting as repellents, oviposition deterrents, ovicidal compounds, enzyme inhibitors, and interfering with the consumption and (or) utilization of food (Wheeler *et al.*, 2001; Nathan 2006).

Among the botanicals, only neem (*Azadirachta indica* Juss.) has been successfully commercialized. However, there are many promising leads from numerous plant species, which contain insecticidal compounds (Isman,

1995; Jacobson, 1989; Schmutterer, 1992). *Vitex negundo* L. commonly known as Indian privet is one of the commonly available botanical materials in Kerala all throughout the seasons and well adapted to different agro-ecological situations. *V. negundo* contains a variety of bioactive principles which are to be explored for their insect growth suppressing effects and can be effectively utilized in the pest management programmes. In the present study we investigated the toxic and growth inhibitory activity of different solvent extracts of *V. negundo* against the Asian worm *S. litura*.

MATERIALS AND METHODS

Rearing of *S. litura*

Larvae of *S. litura* were reared separately on castor and banana leaves kept inside separate plastic jars (1.5 litre capacity) closed with muslin cloth. Fresh leaves were given till the pupation of the larvae. Pupae were collected and sorted out for their sexes. The male and the female pupae were differentiated based on the distance between the genital and anal pores. This distance was more than double in female than that in male. Another distinguishing character of the female pupa, was that on either side of the genital pore, a "V" shaped depression or fold extending upto the tenth segment was visible. Male and female pupae were kept in folds of tissue paper in a petridish placed inside a plastic bucket of two litre capacity @ eight pairs per bucket. Mouth of the bucket was then covered with a muslin cloth. Adult moths emerged within a period of 9-10 days. Cotton pieces soaked in honey solution fortified with a few drops of vitamin E were placed on the sides of the bucket for the emerging adults. Folded paper sheets were kept inside the bucket as substrate for egg laying. Egg masses laid on the folded papers were collected and surface sterilized with 0.05 per cent Sodium hypochloride solution for five minutes, thoroughly washed with distilled water to remove traces of chemical and then dried. The egg masses were then kept inside plastic jars containing fresh castor or banana leaves and the rearing was continued for getting a steady supply of larvae for different experiments. Larvae were also reared on semi-synthetic diet as per the method of Ballal (2004). Second instar larvae collected from natural food were released @ two larvae per beaker containing semi-synthetic diet and closed with muslin cloth. The diet was changed at an interval of two days upto third instar stage and daily for later instars. The larvae were allowed to pupate and the pupae were collected from the diet, cleaned well and continued rearing.

Preparation of extracts

Fresh leaves of *V. negundo* were collected from medicinal

plant garden, College of Horticulture, Vellanikkara. Extracts from leaves were prepared by using four different solvents viz., acetone, hexane, methanol and water. Only the middle leaves were used for extraction and were washed and chopped into small pieces before maceration. Fifty gram of the plant material was macerated separately with 100 ml each of acetone, hexane, methanol and water separately in an electric blender. The macerated slurry was first strained through a muslin cloth and then filtered through a Whatman No.1 filter paper kept in a funnel into a volumetric flask. The volume was made upto 100 ml with respective solvents to form the primary stock solution of the extract.

Toxicity bioassay

Solvent extracts of *V. negundo* were prepared at 1, 2, 4 and 6 per cent concentrations. Teepol 0.1 per cent was added to the extracts as a wetting agent. Two ml of the treatments were topically applied on the thoracic tergal plate of the freshly moulted third instar larvae of *S. litura* using Hamilton micro applicator. Ten, third instar larvae were maintained per treatment and each treatment was replicated three times. The treated larvae were then reared on semi-synthetic diet and larval mortality was recorded after 24 hours and the observations continued upto a period of 120 hours. LD₅₀ was calculated by Probit analysis using SPSS programme.

Insecticidal activity

Freshly emerged third instar larvae of uniform size and age were collected from the mass culture maintained in the laboratory. Solvent extracts of *V. negundo* (acetone, hexane, water and methanol) were prepared at different concentrations (1, 2, 4 and 6%). Teepol 0.1 per cent was added to the extracts as a wetting agent. Ten insects formed one treatment and three replications were maintained for each treatment. The different concentrations of the solvent extracts were directly sprayed using an atomizer on the test insects released in clean petridishes. Larvae sprayed with distilled water containing teepol 0.1 per cent served as control. After air drying, the larvae were transferred to beakers (50 ml capacity) containing semi-synthetic diet. Mortality counts were taken at daily intervals and the observations continued upto a period of 120 hours.

Growth inhibitory effect

The effect of *V. negundo* leaf extracts prepared with acetone, methanol, hexane and water on the growth and development of *S. litura* larvae (third instar) was studied by feeding the larvae on treated castor, banana and artificial

diet. Four concentrations, 1, 2, 4 and 6 percentage for each solvent extracts were prepared and applied on fresh leaves of castor and banana and also on artificial diet. Ten larvae (third instar) were released in each treatment and allowed to feed on the treated food (castor, banana and artificial diet) for a period of 24 hours. The larvae were then transferred to respective untreated food and maintained till their pupation. There were three replications for each treatment an untreated control was also maintained. Observations on percentage pupation, weight of pupae and the number of days taken by the third instar larvae for pupation in different treatments fed on castor, banana and artificial diet were taken.

RESULTS

Median Lethal dose

Lowest LD₅₀ of 423 ppm was recorded by methanol extract and was followed by acetone extract with LD₅₀ of 517 ppm. Highest LD₅₀ was recorded by water extract (590 ppm). Hexane extract also recorded higher LD₅₀ of 562 ppm. Among all the four solvent extracts selected, methanol extract was the most effective and toxic one (Table 1).

INSECTICIDALACTIVITY

The results of the study proved that methanol extracts at six per cent level was the most toxic one when compared to other solvent extracts and it recorded highest mortality of 96.4 per cent. Acetone extracts at six per cent level also recorded higher mortality of 60.7 per cent. (Table 2).

Pupal weight, pupation and larval duration of *S. litura*

Pupal weight and pupation showed a decreasing trend with concentrations while larval duration showed an increasing trend with concentrations. When the larvae

were reared on castor, maximum pupal weight reduction was observed in treatment with acetone extract (6%). Similar trend was observed in diet fed larvae also, but when compared with control larvae, maximum weight loss (72.60%) was observed in methanol extract (6%). Methanol extract also proved effective resulting in lowest pupal weight (0.07g) in larvae fed with banana. Hexane and acetone extracts (6%) recorded lowest pupation (3.33% each) when the larvae were fed with treated castor leaves. Methanol extract (6%) also resulted in lower pupation with more than 90 per cent reduction in pupation. Diet fed larvae recorded maximum reduction in pupation on treatment with methanol extract (6%). Similar trend was also observed when the larvae were fed with treated banana leaves with only 13.33 per cent pupation. All the *V. negundo* extracts at higher concentrations resulted in prolonged larval durations. Castor reared larvae recorded maximum larval duration of 19 days when treated with acetone extract (6%). Maximum larval prolongation of 26.33 days was recorded diet fed larvae treated with methanol extract (6%). Larval duration was not much affected in case of banana fed larvae. Aqueous extract (6%) recorded maximum duration of 17.67 days with 23.31 per cent increase over control (Figures 1 - 3).

DISCUSSION

Against *S. litura*, methanol extract was more toxic and efficient in causing mortality and it recorded lowest LD₅₀ of 423 ppm. Highest LD₅₀ was recorded by aqueous extract with a value of 590 ppm. Hexane extract also recorded relatively higher LD₅₀ of 562 ppm while acetone extracts recorded LD₅₀ of 517 ppm (Table 1).

Insecticidal activity of *V. negundo*

Among all the four solvent extracts, methanol extract at

Table 1. LD₅₀ value of different solvent extracts of *Vitex negundo* against *Spodoptera litura*

Treatments (solvent extracts)	Heterogeneity χ^2	Regression equation	LD ₅₀ (ppm)	95 % Fiducial limits	
				Upper	Lower
Hexane	8.006	Y= -1.441+ 25.632 X	562	466	790
Acetone	4.534	Y= - 1.638+ 31.647 X	517	477	570
Water	9.698	Y= - 2.088 + 35.361 X	590	504	783
Methanol	53.049	Y= - 2. 544 + 60.067 X	423	302	633

Table 2. Insecticidal action of different solvent extracts of *V. negundo* against *S. litura*

Treatment(%)	*Mortality (%)			
	Hexane extract	Acetone extract	Water extract	Methanol extract
1	17.9 ^{def}	10.7 ^{ef}	3.5 ^f	0 ^f
2	10.7 ^{ef}	17.9 ^{def}	13.8 ^{def}	21.4 ^{def}
4	35.7 ^{cde}	28.6 ^{cde}	27.6 ^{cde}	21.4 ^{def}
6	53.6 ^{bcd}	60.7 ^{abc}	51.7 ^{bcd}	96.4 ^a

six per cent level recorded significantly highest mortality in *S. litura* (96.4%). This result indicates that the existence of toxic principles in *V. negundo* is more in methanol extracts as reflected by maximum mortality. Insecticidal property of 10% *V. negundo* extracts were reported earlier by Sahayaraj (1998). Experiments conducted by Singh *et al.* (2005) also revealed insecticidal properties of methanol extracts of ginger in which they got 78.9 per cent mortality when the larvae of *Earias vittella* were treated with 10 per cent concentration of methanol extract of ginger. In a field experiment conducted to test the efficiency of different plant extracts, alcoholic extracts of *V. negundo* at two per cent concentration resulted in higher mortality of 74.26 per cent against third instar larvae of *Spilosoma obliqua* (Dubey *et al.*, 2004). Similar were the findings of Gautham *et al.* (2003) in which they had reported maximum insecticidal action of methanolic extracts of *Saussurea heteromala* (a member from Asteraceae family) against *S. obliqua* with a mean per cent mortality of 65.3.

Acetone extract at six per cent level also recorded higher mortality of 60.7 per cent. This is in consonance with the findings of Saradamma (1989), in which she had reported that acetone extracts of *V. negundo* resulted in cent per cent mortality of *S. litura*. Similar findings were also made by Kalavathy *et al.* (1991) where the insecticidal activity of acetone extracts of *V. negundo* extracts against *E. vitella* was reported. Dayrit *et al.* (1995) had recorded that topical application of volatile oils of *V. negundo* caused 91 per cent mortality in third instar larvae of *S. litura*. Devanand and Rani (2008) reported that crude acetone extracts of *Tectona grandis*, *Mangifera indica* and *Momordica charantia* produced higher mortality (> 80%) to *S. litura* and *A. janata*. Hexane and water extracts recorded lowest mortalities of 53.6 and 51.7 per cent respectively at six per cent level.

Pupal weight of *S. litura*

When *V. negundo* solvent extracts treated food were fed to *S. litura* at different concentrations (1, 2, 4 and 6%), effects on development were noted. There was a significant variation in the pupal weight of the test insect in different treatments. Reduction of pupal weight with the increase in concentrations of different solvent extracts was observed in *S. litura* reared on different hosts. *Spodoptera litura* reared on castor revealed that in all the treatments, the pupal weights were lower when compared to control. Increase in concentrations of the extracts caused a decrease in growth and weight of pupae. All solvent extracts of *V. negundo* caused significant reduction in pupal weight of *S. litura* reared on castor as compared to

that in solvents alone. Pupal weight was found to decrease with increase in concentration. Acetone extract of *V. negundo* caused highest reduction (55.25%) in weight of *S. litura* pupae while hexane, water and methanol extracts showed 45 to 49 per cent reduction in pupal weight (Fig.1).

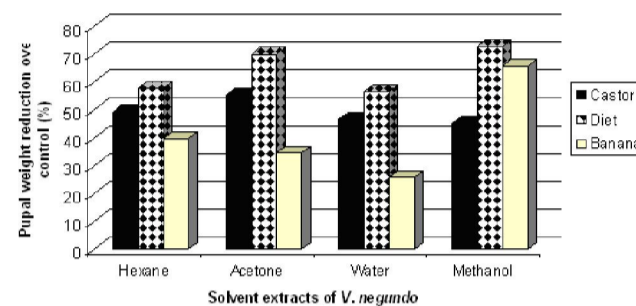


Figure 1. Recuction of pupal weight by solvent extraracts of *V. negundo* against *S. litura*

In case of pupae of *S. litura* reared on semi-synthetic diet, methanol extract caused highest reduction in pupal weight (72.60%) over control while water extract brought about a lowest reduction (56.51%) of pupal weight. Acetone extract also resulted in higher reduction (70%) of pupal weight over control. When the larvae were reared on banana also, methanol extract resulted in highest reduction in pupal weight (65.62%) followed by hexane extract (39.9%). Water extract again showed lowest (52.91%) pupal weight reduction.

It is thus indicated that out of three hosts, *V. negundo* caused the highest reduction (56.5 to 72.60%) of pupal weight in *S. litura* reared on semi-synthetic diet and among the extracts, methanol extract caused the highest reduction (45.15 to 72.60%) and aqueous extract resulted in the lowest reduction (25.91 to 56.51%) of pupal weight of *S. litura* reared on three hosts. The present finding on the reduction of pupal weight in *S. litura* due to *V. negundo* extracts is in conformity with Govindachary *et al.* (1996) who reported decreased pupal weight in *S. litura* due to neem.

Vitex negundo extracts produced significant reduction in the pupation of *S. litura* reared on all hosts *viz.*, castor, semi-synthetic diet and banana. Pupation was reduced with increase in concentration extracts showing the lowest pupation with the highest concentration of six per cent in all solvent extracts. *V. negundo* extracts caused the lowest pupation (3.33 to 53.33%) on castor and highest (20 to 96.67%) on semi-synthetic diet. Among the four solvent extracts, hexane and acetone extracts caused the lowest

pupation (3.33%) on castor, but acetone extract (6%) resulted in the highest pupation (70%) when *S. litura* was reared on semi-synthetic diet indicating its lower influence on pupation. Aqueous extracts caused 40 to 66.67 per cent pupation.

All the solvent extracts of *V. negundo* except water resulted in more than 90 per cent reduction in pupation of *S. litura* reared on castor (Fig.2). Influence of *V. negundo* solvent extracts was comparatively lower in larvae reared on semi-synthetic diet. Here also methanol extract recorded the highest reduction in pupation (77.77 %). All the solvent extracts recorded more than 50 per cent reduction in pupation of *S. litura* reared on banana with the highest reduction in pupation by methanol extract (85.72 %). Aqueous extract caused 31.03 to 55.56 per cent reduction in pupation while 77.78 to 92.86 per cent reduction was caused by methanol extract thus indicating the significant effect of methanol extract in reducing the pupation of *S. litura*. Pupation rate was significantly reduced when *Pieris rapae* L. larvae were treated with the botanical rhodojaponin III (Zhong *et al.*, 2001). Huang *et al.* (2004) reported that azadirachtin at 1 ppm concentration reduced pupation of *S. litura* to 60 per cent.

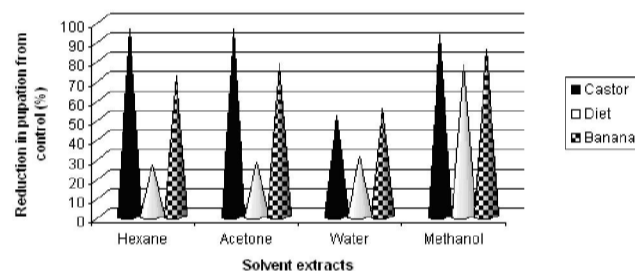


Figure 2. Reduction in pupation by solvent extracts of *V. negundo* against *S. litura*

Results of the present study are in accordance with the findings of Sing *et al.* (2005). They had reported that pupation percentage of *E. vitella* showed a reducing trend with increase in concentration of different solvent extracts of ginger. Benzene fractions of the extracts at two per cent level recorded minimum pupation of only 14.4 per cent.

Larval duration of *S. litura*

When the larvae were reared on castor, acetone extract at six per cent resulted in maximum larval duration (62.81%) and was followed by aqueous extract with 85.87 per cent increase in larval duration over control.

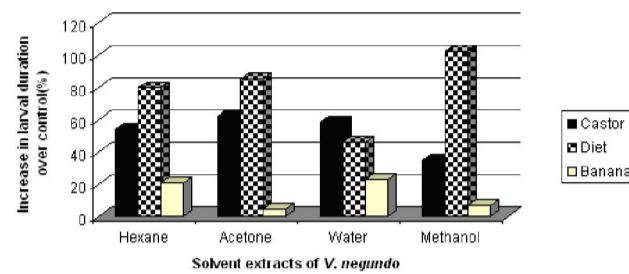


Figure 3. Increase in larval duration by solvent extracts of *V. negundo* against *S. litura*

Solvent extracts of *V. negundo* caused significant increase in larval period of *S. litura*. Larval duration was increased with increase in extract concentration. Larval period showed variation when *S. litura* were reared on different hosts treated with six per cent *V. negundo* extracts. Larvae on semi-synthetic diet had taken the longest period (10.67 to 26.33 days) while it was the shortest on banana from 12.67 to 17.67 days. Among the four solvent extracts, methanol extract caused the longest larval period (15 to 26.33 days) and the shortest with water (17.70 to 18.0 days) (Fig. 3). This is in consonance with the findings of Singh *et al.* (2005). According to them, mean larval period of *E. vitella* prolonged in all fractions of methanol extracts of ginger. The longest duration of 17.3 days (in control, only 9.7 days) was observed in the acetone fraction.

Acetone extracts at two per cent concentration also recorded longer larval duration of 21.67 days. Hexane extracts at four per cent level and the aqueous extract at six per cent level also recorded higher larval duration of 19 days and were on a par with each other. Control larvae reared on banana took an average of 14 to 14.66 days for pupation. Here the insects treated with water extracts at six per cent level took more days for pupation. It was immediately followed by hexane extracts at the same level and acetone extracts at four per cent level. The highest duration observed by the methanol extract treatment was 17.67 days. The lowest larval duration of 12.67 days was observed by hexane at one per cent level. Results of this study are in confirmation with the findings of Martinez and van Emden (2001). According to them, *S. littoralis* third instar larvae when exposed to different concentrations of azadirachtin took longer time to reach the pre-pupa in comparison with the control insects. Similar results relating to the whole larval stage of *S. frugiperda* were obtained by Redfern *et al.* (1981) and *S. litura* by Behera and Satapathy (1997). Larvae kept on treated diet had their pupal ecdysis delayed in comparison

with the control insects. Essential oil of *Vitex trifolia* and *Vitex aganus-castus* were evaluated against fifth instar larvae of *Spilosoma obliqua*. This treatment caused extended larval and pupal period (Tandon *et al.*, 2008). Prolonged larval duration with reduced weight was observed when larvae of rice leaf folder were treated with pure triterpenes isolated from the plant *Dysoxylum malabaricum* Bedd. (Nathan *et al.*, 2009).

All the solvent extracts tested were found to be insecticidal and growth inhibitory in nature. Among the different solvent extracts tested, methanol extract was proved to be superior in causing maximum mortality and growth inhibitory action. *S. litura* is a polyphagous pest with more than 150 species of plants and can be effectively controlled by *V. negundo* solvent extracts. Actual field trials are to be conducted to prove the efficacy of the extracts at field level.

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