

## **Chemical composition and insecticidal efficacy of *Vitex negundo* L. essential oil against fall armyworm, *Spodoptera frugiperda* (J. E. Smith)**

**Rohit S. Parchande., Anuradha K. Shinde and Yankanchi, S. R.\***

### **ABSTRACT**

The fall armyworm, *Spodoptera frugiperda*, is a key pest of maize crops in tropical and subtropical regions and has developed resistance against synthetic pesticides and *Bacillus thuringiensis* toxins. Thus, the experiments were designed to evaluate the chemical analysis and insecticidal efficacy of *Vitex negundo* against *S. frugiperda*. Chemical analysis of *V. negundo* essential oil revealed a total of 36 compounds, and the oil contained more monoterpenes (68.16%) than sesquiterpenes (22.21%) and diterpenes (13.56%). For the ovicidal and insecticidal activities, the essential oil was found to be most effective, with a median lethal concentration (LC<sub>50</sub>) value of 51.31 µL/mL and 0.29 L/larvae, respectively. Larval antifeedant and growth inhibitory activity of essential oil were positively correlated (R<sup>2</sup> = 0.9645) and (R<sup>2</sup> = 0.9727) with oil concentrations, respectively. Therefore, the *V. negundo* essential oil can be used for the development of bioinsecticides to control *S. frugiperda*, a highly invasive and polyphagous pest, which will replace chemical pesticides.

**Keywords:** Bioinsecticide, Essential oil, GC-MS, *S. frugiperda*, *V. negundo*

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### **INTRODUCTION**

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a polyphagous pest in tropical and subtropical regions that causes severe damage to the vegetative stage of maize crops in India (Sharanabasappa *et al.*, 2018). Besides, it feeds on a wide range of host plants from various families and genera (Devappa *et al.*, 2012). To maintain the population of this insect below the threshold level, it is necessary to frequently apply pyrethroid and organophosphate insecticides (Storer *et al.*, 2012). It has been reported that, *S. frugiperda* insects developed resistance against synthetic insecticides such as lambda-cyhalothrin, chlorpyrifos, lufenuron, and *Bacillus thuringiensis* toxins (Carvalho *et al.*, 2013; Nascimento *et al.*, 2016; Burtet *et al.*, 2017). Hence, there is a need to

search for an alternative means for the control of *S. frugiperda* insects.

The efficacy of plant extracts and essential oils to manage insect pests has been studied for many years because they are safe for the environment, public health and active against insect pests (Isman, 2014). The essential oils have been found abundantly in aromatic plants and are by-products of plant metabolism (Rice and Coats, 1994). Essential oils are known to interfere with physiology, behavioural functions, and inhibition of growth in insects, as well as inhibit the oviposition in stored grain pests (Tripathi *et al.*, 2003).

The Indian privet, *Vitex negundo* L. (family: Verbenaceae), is an aromatic medicinal plant commonly available near water bodies, mixed grasslands, and open forests in India. The

*V. negundo* plant contains a number of bioactive compounds that are known for their biological activities. The essential oil of *V. negundo* has shown antioxidant, antibacterial, and larvicidal properties (Balasubramani *et al.*, 2017), and insecticidal activities against stored grain, medicinal, and agricultural insect pests (Sahayaraj, 1998; Sahaf *et al.*, 2008; Karunamoorthy *et al.*, 2008; Deepthy *et al.*, 2010). Thus, the present investigation aims to evaluate the chemical composition and efficacy activities, such as ovicidal, insecticidal, antifeedant, and growth inhibitory activities, of the essential oil extracted from *Vitex negundo* against the fall armyworm *S. frugiperda*, a serious pest of maize and sorghum in India.

## MATERIALS AND METHODS

### Insect collection and maintenance

The fall armyworm larvae, *S. frugiperda*, were collected from infested maize fields around the Kolhapur vicinity. Collected larvae were reared on maize tender leaves in laboratory conditions at 26±2°C, temperature, 75±5 RH, and a natural photoperiod (Sharanabasappa *et al.*, 2018). Sterilised soil was provided for pupation, and then pupae were collected and kept in the insect rearing cage. The emerging adults were provided with a 10% honey solution soaked on cotton swabs offered in small Petri dishes inside the rearing cages and replaced daily. The host plant sapling was provided for oviposition. The laboratory conditions, freshly laid eggs, and early third instar larvae were used in all experiments because early-instar larvae show a high response to slow-acting bioinsecticides.

### Plants collection and isolation of essential oil

The aerial part of *Vitex negundo* L. (family Verbenaceae) was collected from June to September 2022 at the Western Ghats foothills of Kolhapur district. The plant was identified and authenticated by a plant taxonomist, and a voucher specimen (MM2415) was deposited in the Department of Botany, Shivaji University Kolhapur. One kg of mature leaves was washed with tap water and then used for oil extraction with the help of a modified Clevenger type apparatus

with n-hexene for 4 hrs, as described by Li *et al.*, (2014). The yield of EO was determined by the volume of EO/weight of plant material × 100 (v/w). The obtained essential oil was dried over anhydrous sodium sulphate and then stored in airtight brown vials (10 mL) kept in a refrigerator at 4°C for further use.

### Chemical analysis of essential oil

The chemical constituents of essential oil were analyzed by using gas chromatography and mass spectrometry (GC-MS) on a Shimadzu TQ-8050 plus with HS 20. For that, the SH-Rxi-5SIL MS was used in analysis, along with a fused silica column (30 m × 0.25 mm × 0.25 µm) film thickness, and the components were separated using helium as a carrier gas at a constant flow rate of 1 ml/min. The following temperatures were used to inject the 1 µL of EO into the oven; initial oven temperature of 60°C to 250°C at 1°C/min and programmed to 250°C at 5°C/min. Injection mode was split less, with an injection temperature of 250°C; transfer ion source temperature of 200°C and interface temperature of 275°C; slit ratio of 1: 70. The following are the mass detectors operating conditions with an electron impact in the ionization mode at 70 eV; mass can range from 45-500 amu. In the GC-MS NIST 2014 library, the peak compound spectrum was compared to a database of known compound spectra.

### Ovicidal activity

The ovicidal activity of *V. negundo* essential oil was determined by the dipping method as described by Javaregowda and Naik (2007). Different concentrations (10, 20, 40, 80, and 160 µl/ml) of essential oil were prepared in acetone. Scales were removed by a fine brush from the freshly laid egg mass, and 25 eggs were glued to a strip. Egg strips were dipped in each concentration of essential oil, and control eggs were dipped in acetone for a minute. Treated and control eggs were placed in Petri plates containing wet filter paper for hatching. The number of larvae hatched in control and treatment groups was recorded after 72 hrs.

### Insecticidal activity

The topical application method (Pavela, 2005) evaluated the contact insecticidal activity of plant essential oil. Freshly moulted third instar larvae (8–10 mg) were placed in Petri dishes lined with filter paper. A 2 µL acetone solution containing 0.1, 0.2, 0.3, 0.4, and 0.5 µL of essential oil was applied to the dorsal surface of each larva with a micropipette. Control larvae were treated with the same volume of acetone. Treated and control larvae were transferred into a separate Petri dish lined with filter paper and a maize leaf for feeding. Three replicates were maintained for each concentration. Larval mortality was observed after 24 hours of treatment for two days, and mortality results were expressed as µL/ larva.

### Antifeedant activity

The antifeedant activity of essential oil was determined by the no choice method (Isman *et al.*, 1990). Newly moulted third instar larvae were starved for 3–4 hrs prior to each experiment. Leaf discs (1.5 cm dia., 1.76 cm<sup>2</sup> area) were cut from maize leaf using a cork borer. A 10 µL of acetone containing 0.2, 0.4, 0.8, 1.6 and 3.2 µL of essential oil was applied to both sides of the leaf disc, and the same volume of acetone was applied to the control disc. After evaporation of the solvent, the leaf disc was placed in a Petri dish lined with wet filter paper to avoid early drying. A starved larva was released into the centre using a fine brush and allowed to feed. For each concentration, fifteen replicates were maintained. After 3–4 hrs (approximately 50% consumed in the control disc), the larva was removed from the Petri dishes, and then the area consumed in the treated and control discs was measured using Image-J software. A feeding deterrence index (FDI) was calculated using the formula:

$$FDI = [(C-T) / (C+T)] \times 100$$

where C and T represented the leaf area consumed by the larva on control and treated discs, respectively (Akhtar *et al.*, 2010).

### Growth inhibitory activity

Insect growth inhibitory activity of essential oil was determined as described by Reddy *et al.* (2016). Tested essential oil concentrations were

prepared as 30, 60, 90, 120, and 150 µL/ mL of acetone. Maize leaf discs were applied with each concentration of test solution, and control discs were treated with acetone. The treated and control leaf discs were kept in a Petri plate lined with wet filter paper, and then a pre-weighed third instar larva was released. Each concentration was repeated fifteen times, including the control. The larvae from each concentration and control were weighed after 48 hrs. Then, the insect growth inhibitory rate was determined by using the formula (Guo *et al.*, 2014).

$$\text{Growth inhibitory rate} = [(\text{Larva weight in control} - \text{Larva weight in treatment}) / \text{Larva weight in control}] \times 100.$$

### Statistical analysis

The statistical analysis of the results was performed using SPSS version 21.0 software. The mean and SE were calculated using descriptive statistics. A one-way ANOVA was used to compare the significant difference, and means were separated by using the LSD test. The LC<sub>50</sub> and other associated values were calculated using probit analysis (Finney, 1971).

### RESULTS

The aerial part of the essential oil obtained from *Vitex negundo* had a yellowish colour, and the yield was 0.23% (v/w). The colour of the *V. negundo* essential oil was similar, but the yield varied from 0.1-1.6% (v/w) in the previous reports (Padalia, 2016; Balasubramani *et al.*, 2017). The variation in oil yield was dependent on plant polymorphism, seasons, soil type, and variations in precipitation, temperature, light frequency, and duration of the day (Pavela and Benelli, 2016). A total of 36 compounds were identified by GC-MS in the *V. negundo* essential oil (Table 1). The essential oil of *V. negundo* was dominated by terpenes, accounting for 81.93% of the total oil. Of which, 24.84% monoterpene hydrocarbons, 21.32% oxygenated monoterpenes, 14.92% sesquiterpene hydrocarbons, 7.29% oxygenated sesquiterpenes, 1.49% diterpene hydrocarbons, 9.59% oxygenated diterpenes, and 2.48% oxygenated triterpenes were the main compounds in the *V. negundo* essential oil.

Table 1. Chemical constituents of the essential oil of *Vitex negundo*

Constituents	Retention time	% Composition
(+)-4- Carene	7.605	6.06
D-Limonene	7.917	6.53
$\gamma$ -Terpinene	8.671	8.03
(+)-4-Carene	9.386	4.22
Linalool	9.906	5.54
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	10.555	1.29
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	12.288	8.07
$\alpha$ -Terpineo	12.662	1.23
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2	15.336	0.84
1-Oxaspiro [4.5]dec-6-ene, 2,6,10,10-tetrameth	15.498	2.17
1-Oxaspiro [4.5]dec-6-ene, 2,6,10,10-tetrameth	15.937	2.18
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis	18.014	3.24
cis- $\beta$ -Farnesene	19.713	3.01
1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl	19.886	1.75
Alloaromadendrene	20.033	2.15
1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7a,7b-o	20.724	1.77
$\alpha$ -Farnesene	20.929	0.46
Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimet	21.367	0.90
E-11(12-Cyclopropyl) dodecen-1-ol	21.868	1.43
2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octah	24.538	1.24
$\tau$ -Cadinol acetate	24.747	3.56
$\tau$ - Muurolol	25.062	2.49
13-Methyl-E, E-9,11-tetradecadien-1-ol acetate	26.998	2.26
Cyclononasiloxane, octadecamethyl-	27.337	0.93
Neoclovene-(II), dihydro	28.078	1.64
Kolaveloo	31.403	6.50
1-Naphthalenepropanol, alpha.-ethenyldecahy	32.355	3.09
Kolavenol acetate	32.695	5.64
Kaur-15-ene, (5.alpha.,9.alpha.,10.beta.)-	32.925	1.49
beta.-iso-Methyl ionone	33.145	0.79
Cyclooctasiloxane, hexadecamethyl-	33.277	0.75
Lupeol	34.827	2.48
beta-iso-Methyl ionone	36.178	1.06
Oct-5-en-2-ol, 8-(1,4,4a,5,6,7,8,8a-octahydro	36.622	0.92
Oct-5-en-2-ol, 8-(1,4,4a,5,6,7,8,8a-octahydro-	36.983	1.12
4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-bu	37.852	2.53
Total		99.39
<b>Terpenes</b>		
Monoterpene hydrocarbons		24.84
Oxygenated monoterpene		21.32
Sesquiterpene hydrocarbons		14.92
Oxygenated sesquiterpene		7.29
Diterpene hydrocarbons		1.49
Oxygenated diterpene		9.59
Oxygenated triterpene		2.48
<b>Total terpenes</b>		<b>81.93</b>
Other		18.07
<b>Total</b>		<b>100</b>

Table 2. Ovicidal and insecticidal activity of *V. negundo* essential oil against *S. frugiperda*

Activity	LC <sub>25</sub> (95% FL)	LC <sub>50</sub> (95% FL)	LC <sub>90</sub> (95% FL)	Chi-square*	df
Ovicidal	21.60 (16.56-26.56)	51.31(42.83- 62.31)	265.54 (188.25-435.65)	11.31	13
Insecticidal	0.18 (0.14-0.22)	0.29 (0.25-0.34)	0.67 (0.53-1.03)	6.78	13

\*  $p \leq 0.05$ , level of significance of chi-square values

Leaves of *V. negundo* were collected in the spring season from 23 different locations in Uttarakhand state, India, and GC-MS analysis of essential oils revealed 89.2-97.3% of total terpenes (Padalia *et al.*, 2016). Ovicidal and insecticidal results of essential oil extracted from *V. negundo* against *S. frugiperda* eggs and larvae were directly proportional to the concentration of essential oil, and no ovicidal or insecticidal activity was observed in control. *V. negundo* essential oil was toxic to the developing embryo in the egg as well as the early instar larvae of *S. frugiperda*. Both ovicidal and insecticidal LC<sub>50</sub> values with fiducial limits are given in Table 1, and chi-square values are significantly different at  $P \leq 0.05$ . These results are substantiated by the earlier finding of toxic effects of *V. negundo* extract and essential oil on insects, for instance, the essential oil extracted from *V. negundo* was found toxic to diamondback moths, *Plutella xylostella* eggs, and third instar larvae (Dayrit *et al.*, 1991). Methanol extract of *V. negundo* leaves resulted in the highest toxicity to Asian armyworm, *Spodoptera litura* (Deepthy *et al.*, 2010; Sahayaraj *et al.*, 2011), spotted bollworm, *Earias vittella* (Kalavathy *et al.*, 1991), oil seed pest of castor semilooper, *Achaea janata* (Devarshi *et al.*, 2017), and stored grain pest of pulse beetle, *Callosobruchus chinensis* (Yankanchi *et al.*, 2009).

Antifeedant and larval growth inhibitory activities of *V. negundo* essential oil tested through the diet incorporation method, and percent antifeedant and growth inhibition results are given in Figs. 1 and 2. In antifeedant and growth inhibition assays, the essential oil exhibited a strong positive correlation ( $R^2 = 0.9645$ ) and ( $R^2 = 0.9727$ ) with oil concentrations, respectively. At the highest concentration of 3.2  $\mu\text{L}/\text{cm}^2$  essential oil showed

78.18% antifeedant efficacy (Fig.1). There was no feeding activity observed in the *Spodoptera litura* larvae provided with *V. negundo* essential oil treated leaf discs at lower concentrations (Sharma *et al.*, 2008). The highest larval growth inhibition (51.16%) was observed at a 150  $\mu\text{L}/\text{mL}$  concentration of essential oil (Fig. 2) and similar observations were made in the *S. litura* larvae fed with a *V. negundo* essential oil treated diet (Sharma *et al.*, 2008).

The growth inhibitory effect of *V. negundo* was confirmed in a previous study by Deepthy *et al.* (2010), in which they observed more than 70% *S. litura* larval growth inhibition. Yankanchi *et al.* (2014) reported that *V. negundo* extract treated dengue vector mosquitoes; *Aedes aegypti* larvae significantly reduced the growth as compared to the control. Plant essential oils contain maximum terpenes that are lipophilic in nature and have a toxic potency for interfering with basic biochemical processes, causing physiological imbalance. Essential oils are fast acting, broad spectrum insecticides with a neurotoxic mode of action that interfere with octopamine neuromodulators and are controlled by calcium channels. Several studies confirmed that the toxicity actions of plant essential oils are involved in the inhibition of acetylcholinesterase activities (Isman, 2006; Pavela, 2012).

Insecticidal activities are related to essential oil lipophilicity, and the higher the lipophilicity, the better the penetration of oil molecules into the insect cuticle. The *V. negundo* essential oil revealed higher permeability in the larval integument due to its low polarity and hydrophobic nature. Thus, the essential oil involved in insect cuticle permeability leads to toxicity, and these factors might have contributed to the toxicity of *V. negundo* oil in *S. frugiperda*.

Fig. 1 Antifeedant activity of *V. negundo* essential oil against *S. frugiperda*. Means  $\pm$  SE followed by the same letter on bars are not significantly different at  $p < 0.05$

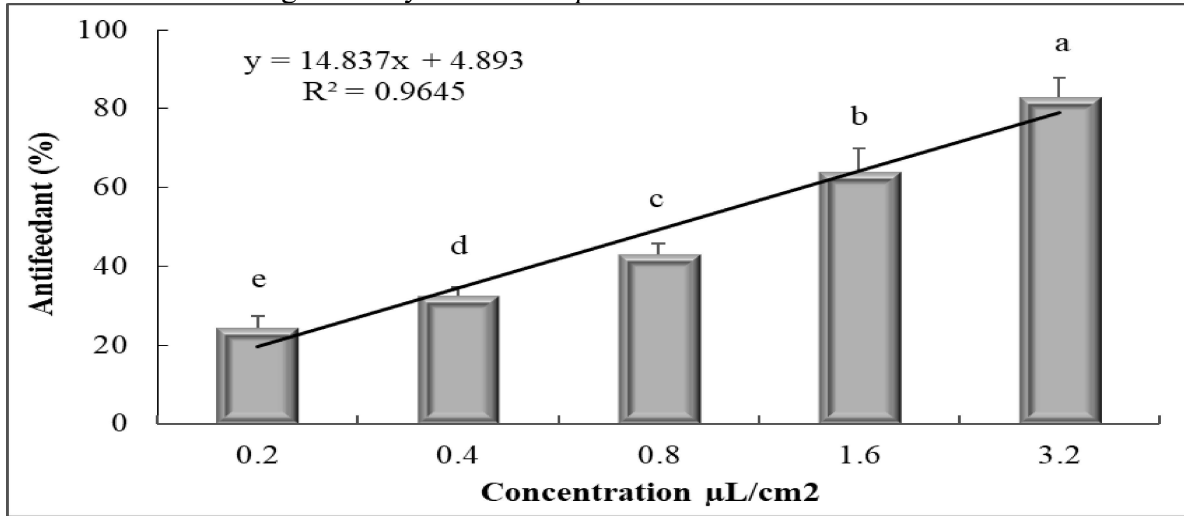
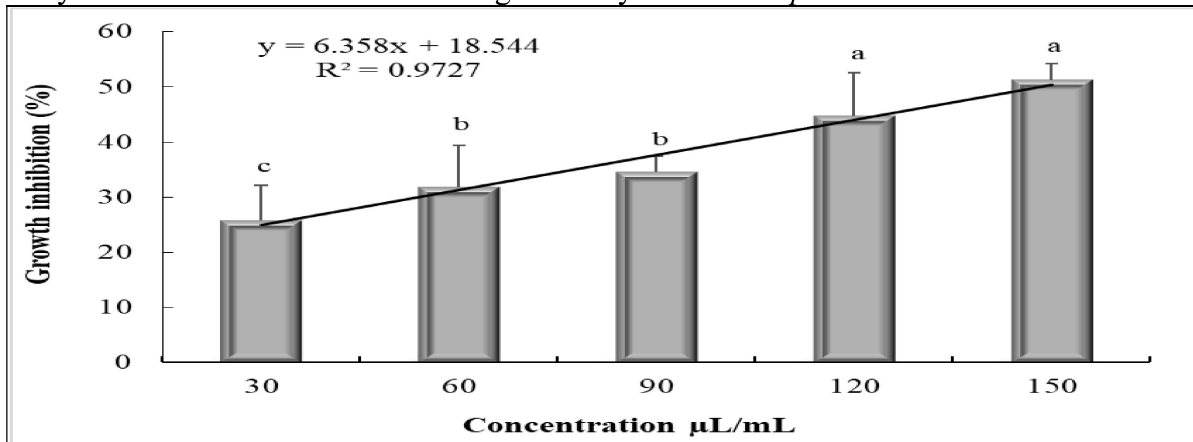


Fig. 2. Larval growth inhibitory activity of *V. negundo* essential oil against *S. frugiperda*. Means  $\pm$  SE followed by the same letter on bars are not significantly different at  $p < 0.05$



Further observation of this study refers to the oil composition, which has the highest content of terpenes, which are already reported in *V. negundo* essential oil (Padalia *et al.*, 2016; Balasubramani *et al.*, 2017) and reported for their insecticidal activities against other insects (Sahaf *et al.*, 2008; Tangadi *et al.*, 2021).

In conclusion, the *V. negundo* essential oil contained a maximum of terpenoids that have shown significant ovicidal, insecticidal, antifeedant, and growth inhibitory activities against *S. frugiperda*. It can be used for the development of bioinsecticides to control this

highly invasive and polyphagous pest, which will replace chemical pesticides.

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