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# Natural mosquito control: *Vitex negundo* and *Argemone mexicana* as larvicidal agents against *Anopheles subpictus*

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### Abstract

Mosquitoes are notorious vectors of severe diseases, posing significant threats to human health. Although pesticides are commonly used for mosquito control, their indiscriminate application can harm the environment and non-target organisms. Consequently, alternative strategies, such as plant-based mosquitocides, are gaining interest. This study evaluated the larvicidal efficacy of methanolic leaf extracts from *Vitex negundo* and *Argemone mexicana* against the fourth instar larvae of *Anopheles subpictus*. The extracts were analyzed for phytochemical compositions. Larvicidal bioassay showed concentration-dependent activity of both extracts against *An. subpictus*. *V. negundo* extract exhibited 86.36% mortality at 400 ppm, while *A. mexicana* extract showed 83.33% mortality at the same concentration. Probit analysis determined LC<sub>50</sub> and LC<sub>90</sub> values for *V. negundo* as 217.06 ppm and 404.32 ppm, respectively, and for *A. mexicana* as 224.45 ppm and 420.34 ppm, respectively. These findings suggest the potential of *V. negundo* and *A. mexicana* as natural larvicidal agents against *An. subpictus*.

**Keywords:** Mosquito control, larvicidal activity, *Vitex negundo*, *Argemone mexicana*, *Anopheles subpictus*, plant extracts, phytochemical analysis, Probit analysis

### Introduction

Mosquitoes play a vital role in transmitting severe diseases to humans and animals <sup>[1]</sup>. Malaria remains a significant global health concern, causing a substantial number of cases and deaths each year. As per a report <sup>[2]</sup> published by the World Health Organisation (WHO), there were 627,000 malaria-related fatalities and 241 million cases of malaria worldwide in 2020. In addition to malaria, Dengue poses a considerable risk to public health, with around 3.9 billion people living in over 129 countries at risk of contracting the disease. The number of Dengue-related deaths is estimated to be around 40,000 annually <sup>[3]</sup>. Over the years, people have been utilizing natural remedies like neem leaves and flowers of *Chrysanthemum cinerariaefolium* to combat arthropod pests effectively <sup>[4]</sup>. While pesticides are essential tools in controlling insect pests that affect both agriculture and public health, their indiscriminate use can lead to adverse consequences <sup>[5]</sup>. The use of synthetic insecticides for mosquito control has drawbacks such as resistance, high costs, and harm to non-target organisms. However, the lack of effective prevention and treatment methods for diseases like Zika, dengue, and Japanese encephalitis necessitates the exploration of alternative strategies to control mosquito-borne illnesses <sup>[1]</sup>. Consequently, there is increasing interest in plant-based mosquitocides as alternatives <sup>[6]</sup>. It is essential to strike a balance between controlling pests effectively and minimizing the negative impacts of pesticide use on the environment and human health. Through responsible and targeted pesticide application, we can manage insect pests while safeguarding our ecosystems and well-being. Proper regulation and adherence to sustainable practices are crucial in achieving this goal. To achieve this, various researchers are searching for natural plant extracts that have pesticidal properties. <sup>[1]</sup>, investigated the larvicidal efficacy of essential oil derived from *Amomum subulatum* in combating important mosquito species, including *Anopheles subpictus*, *Aedes albopictus*, and *Culex tritaeniorhynchus*. Govindarajan, *et al.* <sup>[6]</sup> assessed the larvicidal activity of *Origanum vulgare* EO and its major chemical components

against malaria vectors - *Anopheles stephensi* and *An. Subpictus*. Rajan, *et al.* [7] analyzed the larvicidal efficacy of the methanolic and aqueous extracts of four plants from the Lamiaceae family: *Ocimum sanctum*, *Ocimum basilicum*, *Leucas aspera*, and *Coleus amboinicus* against *Anopheles subpictus*. More recent studies [8-10] confirmed the efficiency of Thyme, Camphor, and Eucalyptus Essential Oils and aqueous leaf extracts of *Murraya paniculata* plants as larvicides against *Aedes aegypti* and *Aedes vittatus* mosquitoes.

*Vitex negundo* and *Argemone mexicana* plants are well-known for their medicinal properties. Leaf extracts of the *V. negundo* plant were reported to possess anti-inflammatory effects [11]. Methanolic leaf extracts of *V. negundo* were believed to contribute to the anti-inflammatory activity [12]. *A. mexicana* was reported to exhibit inhibitory effects on the growth of *Mucor indicus*, *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium notatum* and anticancer activity [13]. Stem and leaf extracts of *A. mexicana* exhibited inhibitory effects on the growth of *Trichomonas vaginalis*, a causative agent of sexually transmitted infections [14]. Aqueous leaf extracts of *Argemone mexicana* were reported to possess efficient larvicidal effects on *Aedes aegypti* [10].

This study aimed to assess the larvicidal efficacy of methanolic leaf extracts of *V. negundo* and *A. mexicana* at various concentrations (50, 100, 150, 200, and 250 ppm) against the 4th instar larvae of *An. Subpictus*.

## Materials & Methods

### Plant Material Collection

Fresh leaves of *V. negundo* and *A. Mexicana* were collected from the Narayankhed area, Telangana State, India. They underwent thorough washing and were subsequently dried for a period of 15 days. After drying, the leaves were powdered using a grinder and stored in a sealed bag until they were used.

### Extract Preparation

To prepare the extracts, 100 grams of the prepared powders were soaked in 250 mL of Methanol separately with frequent shakings for four days. On the fifth day, the solutions were filtered using Whatman filter paper no.1 and allowed to evaporate under a rotating fan for one day. After one day a semi-solid extract was obtained and preserved in the refrigerator at 4 °C until usage. Prior to the larvicidal bioassays, a stock solution of 1000 ppm was prepared by mixing 1 gram of the extract with 10 mL of methanol and later with 990 ml of distilled water. From this stock solution, various test solutions with concentrations of 100, 200, 300, and 400 ppm were prepared through a dilution method. Control stock solution was made with the same solvents in the same ratios excluding the extracts.

### Phytochemical Analysis

Various tests were conducted to identify secondary metabolites in the prepared extracts. The Mayor's Test was employed to identify alkaloids, characterized by the formation of a cream-colored precipitate upon the addition of the Mayor's reagent. The Alkaline Reagent Test was used to detect flavonoids, which exhibited a yellow color upon the addition of NaOH. The presence of terpenoids was determined through the Salkowski Test, where the addition of concentrated H<sub>2</sub>SO<sub>4</sub> resulted in a red or orange color. Saponins were identified using the Froth Test, where the formation of foam was observed upon vigorous shaking. The

Keller-Killiani Test was conducted to detect glycosides, and it involved the addition of HCl and FeCl<sub>3</sub>, resulting in a red or violet color. Lastly, the NaOH Test was performed to identify polyphenols, characterized by the development of a yellow color upon the addition of NaOH.

### Culturing Mosquito Larvae

Larvae of *An. subpictus* were obtained from stagnant water bodies from Sangareddy and Zaheerabad towns, Telangana State, India. These larvae were reared on dog biscuits and dry yeast powder. Fourth instar larvae from the reared population were selected for the larvicidal bioassay.

### Larvicidal Bioassay

The larvicidal efficacy of the crude methanolic extracts of *V. negundo* and *A. mexicana* against the 4<sup>th</sup> instar larvae of *An. Subpictus* was evaluated duly following the WHO guidelines [15]. For each test concentration, 5 larvae of same size were taken separately in 100 mL of the test solutions prepared in 250 mL test cups. After 6, 12, and 24 hours of exposure, the number of dead larvae was counted. The percentage mortality was calculated by taking the average of five replicates and using the formula:

$$\% \text{ PM} = (\text{Number of dead larvae} / \text{Total larvae population}) \times 100.$$

To obtain corrected mortalities, Abbott's formula [16] was applied.

### Statistical Analysis

The statistical analysis of the results was performed using Microsoft Excel software. Regression and Probit analysis were carried out to calculate the concentrations of LC<sub>50</sub> and LC<sub>90</sub>, with a predetermined level of significance set at p<0.05.

## Results

**Phytochemical Analysis:** The phytochemical analysis results of the two extracts (Table 1), revealed the presence of alkaloids, flavonoids, polyphenols, and terpenoids in high quantities in both extracts. Saponins were present in slight quantities in the two tested extracts.

**Larvicidal Bioassay:** The larvicidal bioassay results of the present study (Tables 2 and 3; Figures 1) indicated that both the plant extracts exhibited larvicidal activity against *An. subpictus* in a concentration-dependent manner. Crude methanolic leaf extracts of *V. negundo* exhibited the highest larval mortality percentage of 86.36±0.28 at 400 ppm concentration. At 300 and 200 ppm concentrations the observed mortality percentages were 68.18±0.44 and 50.00±0.50, respectively. The lowest mortality was 27.27±0.49 at 100 ppm concentration. Probit analysis results followed by Regression analysis (Figure 2) indicated that LC<sub>50</sub> and LC<sub>90</sub> values are 217.06 and 404.32 ppm, respectively.

*A. vera* methanolic leaf extracts also showed the same trend in the present study. The highest mortality observed was 83.33±0.37 at 400 ppm. 66.67±0.48 and 45.83±0.51 percentages of mortalities were observed at 300 and 200 ppm concentrations, respectively. The lowest mortality obtained was 29.17±0.48 at 100 ppm concentration. Probit analysis results followed by Regression analysis (Figure 3) indicated that LC<sub>50</sub> and LC<sub>90</sub> values are 224.45 and 420.34 ppm, respectively.

**Table 1:** Identified secondary metabolites in the selected plants' methanolic leaf extracts. Absent: -; Slightly Present: +; moderately present: ++; Heavily present: +++.

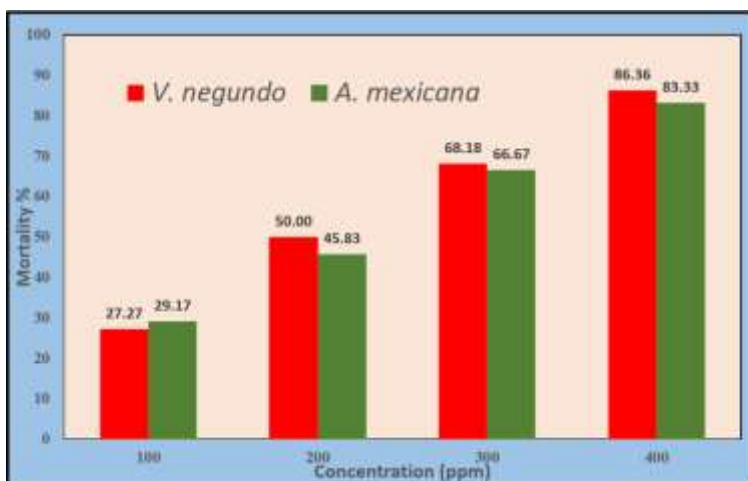
Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Polyphenols
<i>V. negundo</i>	+++	++	+	++	++
<i>A. mexicana</i>	++	+++	+	+	++

**Table 2:** *V. negundo* Methanolic leaf extracts larval mortality percentages ± Standard Deviations against the 4th Instar larvae of *An. subpictus*. LC<sub>50</sub> – 50% Lethal Concentration; LC<sub>90</sub> – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

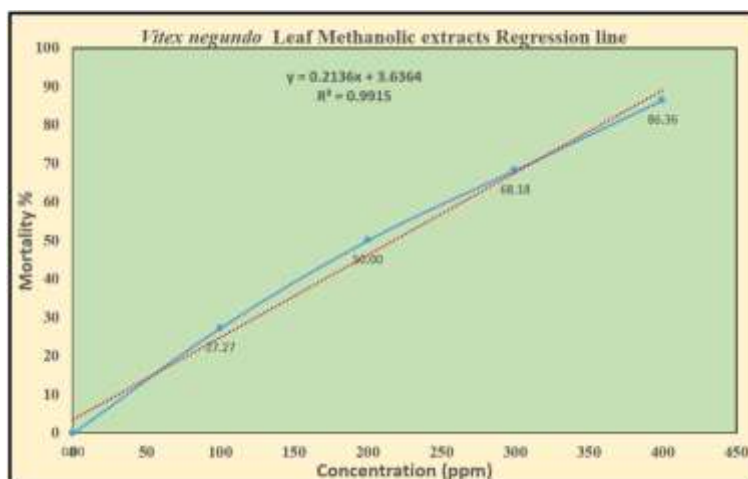
Conc. In ppm	C. limon	LC <sub>50</sub> 95% CL (LCL – UCL)	LC <sub>90</sub> 95% CL (LCL – UCL)
0	0±0.34		
100	27.27±0.49	217.71	404.32
200	50.00±0.50	(216.88	(404.15
300	68.18±0.44	-	-
400	86.36±0.28	217.31)	404.57)

**Table 3:** *A. mexicana* Methanolic leaf extracts larval mortality percentages ± Standard Deviations against the 4th Instar larvae of *An. subpictus*. LC<sub>50</sub> – 50% Lethal Concentration; LC<sub>90</sub> – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

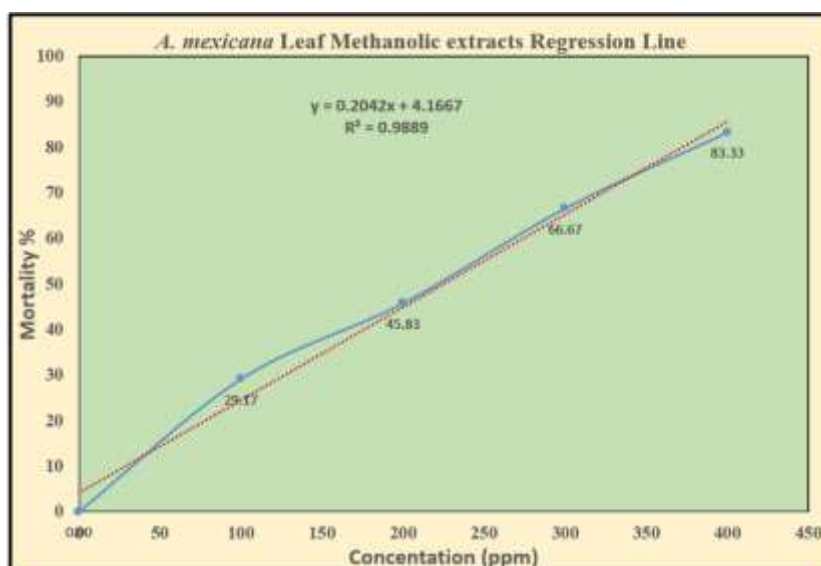
Conc. In ppm	<i>A. mexicana</i>	LC <sub>50</sub> 95% CL (LCL – UCL)	LC <sub>90</sub> 95% CL (LCL – UCL)
0	0±0.20		
100	29.17±0.48	224.45	420.34
200	45.83±0.51	(224.29	(420.17
300	66.67±0.48	-	-
400	83.33±0.37	224.70)	420.58)



**Fig 1:** Larvicidal efficacy of crude methanolic leaf extracts of *V. negundo* and *A. mexicana* against the 4<sup>th</sup> instar larvae of *An. Subpictus*



**Fig 2:** Regression analysis of the larvicidal bioassay of crude methanolic leaf extracts of *V. negundo* against the 4<sup>th</sup> instar larvae of *An. Subpictus*



**Fig 3:** Regression analysis of the larvicidal bioassay of crude methanolic leaf extracts of *A. mexicana* against the 4<sup>th</sup> instar larvae of *An. Subpictus*

### Discussion

The findings of this study demonstrate the potential effectiveness of methanolic leaf extracts of *V. negundo* and *A. mexicana* in controlling the fourth instar larvae of *An. Subpictus*. Phytochemical analysis of the *V. negundo* and *A. mexicana* methanolic leaf extracts revealed the presence of Alkaloids, terpenoids, flavonoids, saponins, and phenols which are proven to possess pesticidal properties. These results are consistent with previous research in this area. For instance, Govindarajan *et al.* [1] conducted a study that showed the toxic effects of *Amomum subulatum* essential oil on third-instar larvae of *An. subpictus*, with LC<sub>50</sub> and LC<sub>90</sub> values of 41.25 µg/ml and 80.29 µg/ml, respectively. Similarly, Rajan, *et al.* [7] reported a significant mortality rate of 92% in *An. subpictus* larvae were exposed to the methanol extract of *Ocimum sanctum* after 24 hours.

Extracts of *V. negundo* and *A. mexicana* were reported to possess various bioactive secondary metabolites in the previous studies. Murugesan, *et al.* [11] conducted gas chromatography-mass spectrometry analysis of the methanolic leaf extract of *V. negundo*, revealing the presence of phenolic compounds. These compounds are known for their potential anti-inflammatory activity. Additionally, Kandasamy, *et al.* [12] conducted phytochemical analysis of the aqueous leaf extract of *V. negundo* and identified alkaloids, saponins, tannins, proteins, and carbohydrates as the key constituents. Regarding *A. mexicana*, Elizondo-Luevano, *et al.* [14] performed phytochemical analysis on crude extracts, which revealed the presence of unsaturated compounds, quinones, sterols, triterpenes, saponins, flavonoids, carbohydrates, and alkaloids.

### Conclusion

This study focused on the larvicidal efficacy of methanolic leaf extracts of *V. negundo* and *A. mexicana* against the *An. subpictus*. Phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, saponins, and terpenoids in both extracts. The bioassay results demonstrated concentration-dependent larvicidal activity, with *V. negundo* showing the highest mortality at 400 ppm and *A. mexicana* exhibiting a similar trend. These findings support previous

research on the pesticidal properties of these plant extracts. By responsibly using targeted pesticides and exploring natural plant extracts, we can effectively control mosquitoes while minimizing the negative impacts on the environment and human health. Further research can focus on identifying the phytochemicals responsible for larvicidal activity and evaluating the efficacy and safety of these plant extracts against other mosquito species.

**Conflict of Interest:** We declare there are no conflicts of interest.

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