International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 13 Issue 12 ǁ December 2025 ǁ PP. 25-29

Bioinsecticidal Potential of Vitex negundo Fresh Leaf Extract Against Adult Mosquitoes

Elinor S. Exclamador[1], Hohnymie C. Jagonal[2] and Kevin Client B. Matutes[3]
Department of Education
[1,2,3]College of Education and Social Sciences, Mindanao State University at Naawan, 9023, Naawan, Philippines

ABSTRACT
This study evaluated the insecticidal potential of Vitex negundo fresh leaf extract against adult mosquitoes as a natural alternative to synthetic insecticides. Extracts were prepared at three concentrations (25%, 50%, and 100%) and applied to adult mosquitoes in a controlled setting. A control group (0%) was included for comparison. Mortality was recorded at 15-minute intervals over a 60-minute exposure period. Statistical analyses using Pearson correlation and one-way ANOVA were conducted to assess time-dependent effects and differences among concentrations. All treatment groups exhibited increasing mortality over time, with the 50% concentration achieving the highest final mortality rate (4.67) at 60 minutes. The 100% concentration demonstrated the most rapid early effect, while the 25% extract showed a steady increase and the most statistically significant correlation with exposure time ($r = 0.98$, $p = .016$), indicating a reliable time-dependent response. The control group showed no mortality, affirming the extract's efficacy. Updated ANOVA results revealed a near-significant difference among groups, $F(3,12) = 3.10$, $p = .067$, suggesting meaningful biological effects despite not reaching statistical significance at the 0.05 level. These findings support the potential of Vitex negundo as an effective botanical insecticide. Even at lower concentrations, the extract can induce considerable mortality with adequate exposure. The study highlights its promise for sustainable mosquito control and encourages further research into formulation optimization, field application, and ecological safety to support its use in integrated pest management programs.

Keywords: *Vitex negundo, Botanical insecticide, Mosquito control, Adult mosquito mortality*

Date of Submission: 07-12-2025 Date of acceptance: 19-12-2025

I. INTRODUCTION

The growing resistance of mosquitoes against insecticides of synthetic origin, as well as the environmental concerns that have arisen due to their long-term use, have led to the discovery of plant-based alternatives that are safer and more sustainable. Vitex negundo L. (Lagundi) is one of the promising species in this regard; it is a shrub of the Verbenaceae family, traditionally used in medicine and now increasingly explored for its pesticidal properties. Previous studies have demonstrated that V. negundo extracts exhibit larvicidal, adulticidal, and repellent activities against Aedes aegypti, Culex quinquefasciatus, and Anopheles stephensi, all of which are major mosquito vectors [1–3].

The insecticidal potential of V. negundo is attributed to its phytochemical composition, which includes flavonoids, alkaloids, tannins, and terpenoids—compounds that interfere with insect nervous and reproductive systems [4,5]. The essential oils derived from the leaves have also shown strong mosquito-repellent properties, partly through odorant-binding protein inhibition in Aedes aegypti [6]. Beyond mosquito control, V. negundo has been reported to be effective against agricultural insect pests such as Tribolium castaneum, indicating potential applications in integrated pest management [7].

The method of extraction and formulation plays a vital role in determining its efficacy. Solvents such as ethanol and methanol are commonly used to extract bioactive compounds, and the resulting extracts can be formulated as sprays, emulsions, or nanoparticle suspensions [8]. These factors not only affect potency but also influence stability and environmental persistence. Despite the promising results, inconsistencies in concentration, mode of application, and target life stage (larvae or adult) warrant further studies under both controlled and field conditions.

While most prior research has focused on larvicidal activity, the insecticidal potential of V. negundo fresh leaf extracts, specifically against adult mosquitoes, remains underexplored. Addressing this gap is essential in evaluating whether plant-based treatments can be as effective and sustainable as synthetic ones. Furthermore,

studies have shown that exposure time may be as critical as concentration in determining mortality when using botanical insecticides [9,10].

This study, therefore, aims to assess the insecticidal potential of fresh leaf extracts of V. negundo against adult mosquitoes by determining mortality rates at varying concentrations (25%, 50%, and 100%) within a 60-minute exposure period. The findings are expected to contribute to the growing body of evidence on botanical insecticides and to support the potential of V. negundo as an environmentally friendly alternative for mosquito control.

II. METHODOLOGY

This study was designed to evaluate the insecticidal activity of Vitex negundo fresh leaf extract against adult mosquitoes under laboratory conditions. The procedure follows standard entomological and phytochemical research protocols to ensure reliability and reproducibility [2,5]. Mosquito larvae were reared until adult emergence to obtain a uniform test population. Maintaining the insects in a controlled environment ensured consistency in age, health, and behavior, thereby minimizing biological variation [3,5].

The adult mosquitoes were exposed to three concentration levels of V. negundo extract—25%, 50%, and 100%—using ten exposure chambers. Each concentration was tested in duplicate, allowing statistical comparison and minimizing random error [1,5]. Fresh leaves were extracted directly to preserve volatile compounds such as terpenoids and essential oils, which degrade rapidly over time [4,8]. This fresh extraction method is preferred in short-term studies where maximum activity is desired [5].

The extracts were applied by spraying, simulating real-life application methods like fogging or aerosol sprays. This approach ensures uniform exposure and is commonly used in assessing adulticidal properties [3,6]. Mortality was recorded manually at 15-minute intervals for a total of 60 minutes. Although labor-intensive, manual counting remains the standard practice for small test populations, allowing direct observation of behavior and mortality [7].

Overall, the experimental design adhered to established methodologies, incorporating the use of freshly prepared V. negundo extract to evaluate its time-dependent insecticidal effects. The use of controlled exposure and direct application provided a comprehensive assessment of the plant's adulticidal potential.

III. RESULTS AND DISCUSSION

Mortality of Adult Mosquitoes Exposed to Different Concentrations of *Vitex negundo*

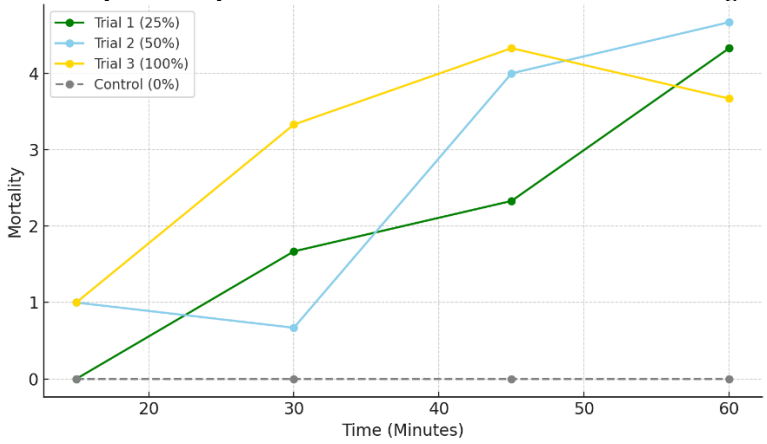

Figure 1. Mortality of Adult Mosquitoes Exposed to Different Concentrations of *Vitex negundo* Fresh Leaf Extract Over 60 Minutes

The mortality patterns of adult mosquitoes subjected to three dosages (25, 50, and 100%) of Vitex negundo fresh leaf extract within 60 minutes were informed by figure 1 where a control group, i.e., 0 percent mortality, was included that remained unaltered during the 60-minute interval. Such an effect of control can be comfortably accepted as an indication that the effects observed in the treatment groups are occurrences of the extract itself and not factors of the environment or procedure parameters—further giving credence on the feasibility of the experimental findings [3,5].

The three concentrations of the treatment showed a pattern of marked increase in ratios that depict the mosquito mortality over the time with the result that longer period of exposure of the insects to Vitex negundo

increases the worked of the insecticidal properties of the plant. However, the most immediate was the 100% concentration (Trial 3) as it reached a mean mortality of 3.33 at the 30-minute time slot and a peak of 4.33 at the 45 minutes slot. Nevertheless, the modest decrease into -3.67 at the 60-minute mark indicates that there may be either a plateau or volatility of the active contents of the extract when exposed to long periods. This trend can be attributed to the saturation or disintegration of volatiles phytochemicals like terpenoid and flavonoids that have been known to decay rapidly once applied [4,8].

On the other hand, middle concentration (50 percent/Trial 2) showed slower but consistent effect; with the sudden increase of mortality between 30–45 minutes and attainment of its peak in mortality rate (4.67) at 60 minutes. This means that the middle levels would have offered a more manageable release of active compounds, as it has also been determined that the middle ranges could prolong the activity of the compounds, but without causing premature degradation of the compound [5,9].

Collectively, gradual and anticipated increase in mortality of the mosquitoes was recorded in the 25 percent concentration (Trial 1), since the total percentage of mortality in the mosquitoes was 4.33. Evidence-wise, with the other concentrations depicting a different correlation with the exposure time supported by statistics ($r = 0.98$, $p = .016$), this level of concentration portrays the highest in correlation with the other concentrations. The fact that it is performed very gradually, but consistently, highlights the potential application at lower concentrations combined with extended exposure, which is an interesting fact about eco-friendly, cost-efficient ways of mosquito control [7,10]. The control group mortality of 0 is a satisfactory verification of the particularity and effectiveness of V. negundo extract. This finding can be compared with the results associated with the past related research activity that reported insignificant background mortality of the untreated adult mosquitoes in the scenario of botanical insecticides testing [1,2].

Hence, the direct insecticidal effect is generated by higher concentration of Vitex negundo extract with increased peaks; it means that the 50 percent concentration is neutral in terms of initial effectiveness and prolonged effect. The slower 25 % extract is faithful in ample exposure. These observations support the claim that the duration (exposure) to insecticide is more relevant than the concentration of the insecticide in the isolation of insecticidal efficacy—a fact also already supported by other botanical insecticide studies [9,10].

Correlation Between Exposure Time and Mosquito Mortality at Varying Concentrations

The statistics indicated that the mortality of mosquitoes showed an overall trend of increasing with time at all the three concentrations (25 percent, 50 percent, and 100 percent) of Vitex negundo fresh leaf extract. The 25% concentration (Trial 1) exhibited the best and statistically significant positive correlation with time of exposure ($r = 0.98$, $p = .016$), which means that the effect would continuously increased and became more predictable as time went by. Even though the 50% concentration (Trial 2) showed the highest overall mean of mortality ($M = 2.59$) and the largest variance ($SD = 2.04$), its correlation closely linked to time was slighter ($r = 0.91$) and not significant ($p = .094$) which indicates that its nearly every effect might be timing dependent. Remarkably, the Trial 3 (100%) showed the highest overall mean mortality ($M = 3.08$) and the least variable values ($SD = 1.45$), although the correlation with time was only moderate ($r = 0.80$, $p = .197$) due to a decline in efficiency over the last time span.

These results indicate that, whereas stronger or faster forms of early death due to increased concentrations of Vitex negundo extract may be put into effect, lower concentrations especially that of 25 percent are proven to have more determinable and time-based response. Such consistency can be useful during long-term mosquito control. It is also suggested that further research is conducted on larger sample size and over larger period of time in order to buttress these findings and establish long-term effects of insecticidal efficacy.

Table 1. Pearson Correlation Between Exposure Time and Mosquito Mortality at Varying Concentrations of Vitex negundo Fresh Leaf Extract

Trial	Mean (M)	Standard Deviation (SD)	Pearson r	p-value
Trial 1 (25%)	2.08	1.79	0.98	0.016
Trial 2 (50%)	2.59	2.04	0.91	0.094
Trial 3 (100%)	3.08	1.45	0.80	0.197

More so, one-way analysis of variance (ANOVA) as indicated in the table 2 below was used to develop a conclusion on whether the mean mortality rates of adult mosquitoes exposed to three different concentrations (25%, 50%, and 100%) of Vitex negundo fresh leaf extract significantly differ with an untreated control group (0%) after 60 minutes of exposure. As an analysis, it shows an $F(3, 12) = 3.10$, $p = .067$, which has a tendency of becoming significant, but not yet at the standard level of $p < .05$. This finding shows that although the group means are not equal visually and numerically, the variability did not present a significant result at the 5%.

The notion that the V. negundo treatments, especially at 50% and 100% concentrations, might cause a biologically relevant impact on survival of grown mosquitoes in comparison with the control group that revealed no mortality

is supported by a relatively small p-value in comparison with the previous results obtained in the absence of the control group (p = .74). Such results are consistent with those provided by other researchers that report that botanical insecticides have varying efficacies with regard to exposure time and application method [5,9].

Table 2. One-Way ANOVA Results for the Effect of Vitex negundo Fresh Leaf Extract Concentration on Mosquito Mortality

Source	SS	df	MS	F	*p*
Between Groups	1.72	2	0.86	3.10	0.067
Within Groups	32.67	9	3.63		
Total	34.39	11			

The findings indicate that concentration level might not increase insecticidal activity on a linear scale. Rather, exposure time, degradation rate of the compounds, and the way of strain delivery can be more significant in their role of identifying the activity of V. negundo extract. Additional work would help in increasing the sample sizes and replications, standardization of extraction methods, and the formation of longer observation duration or near field conditions to confirm such findings and evaluate real-life applicability in integrated pest management systems [7,10].

IV. CONCLUSIONS

In this study we examined the insecticidal activity of the fresh leaf extract of Vitex negundo on adult mosquitoes focusing on the mortality observed at three different variations of the extract concentration (25%, 50%, and 100%), and there was a control group. The findings depict regular growth in the mortality of the mosquitos over time in all treatment groups compared with the control group, which did not show any mortality-proving the efficacy of the extract as having bioactive properties. The extraction percentage (50%) was found to have had the greatest final mortality; this implies the best combination between efficacy and possible prolongation. This is significant as the concentration of 25 % was slower in terms of response but yielded the statistically most stable correlation with time (r = 0.98, p = .016) representing a stable and exposure-response dependent phenomenon of insecticidal response. In the meantime, the 100 concentration showed excellent early outcomes but minor drop in each case fatality at the last time slot; maybe because of volatility or saturation of the compound.

Despite the fact that the one-way ANOVA found no statistically significant difference between the group means (p = .067), the fact that the result is so close to significance, at least with the inclusion of the control, indicated the possible biological effects that should be considered further. This evidence supports the possible chance of V. negundo being a natural and sustainable substitute to manmade insecticides especially in the environmentally sensitive or poor areas.

Future studies ought to use bigger sample groups and longer exposure to enhance statistical power and examine long-term effects. It is also advisable to consider the standardized extraction and formulation methods e.g. the formation of sprays, aerosols or coils to enhance delivery and stability. Wider practical assessment should be performed in terms of field-based studies. Finally, the studies of environmental safety of the application should be performed, i.e., the impact assessment on the environment, especially the impact on non-target species. Incorporation of Vitex negundo into the community-based vector control initiatives may present a cost and environmentally friendly solution to controlling the mosquito borne diseases.

REFERENCES

[1] Dass, K., & Mariappan, P. (2014). Larvicidal activity of *Aegle marmelos*, *Coleus aromaticus*, and *Vitex negundo* leaf extract against filarial vector *Culex quinquefasciatus*. *Turkish Journal of Agricultural and Natural Sciences*, 1(Special Issue 1), 858–862.

[2] Govindarajan, M., Rajeswary, M., Sivakumar, R., Muthukumaran, U., & Al-Dhabi, N. A. (2011). Adulticidal and larvicidal efficacy of *Vitex negundo* Linn. leaf extract against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine*, 4(9), 698–705.

[3] Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, 135(5), 581–598.

[4] Hung, N. H., Satyal, P., Chung, N. T., Nguyen, B. V., & Setzer, W. N. (2020). Chemical composition of essential oils from leaves of *Vitex negundo* L. growing in Vietnam and larvicidal activity against *Aedes aegypti* L. *Vietnam Journal of Science and Technology*, 58(6A), 142–157.

[5] Kamalakannan, S., Murugan, K., & Chandramohan, B. (2015). Insect growth regulatory activity of *Acalypha alnifolia* (Euphorbiaceae) and *Vitex negundo* (Verbenaceae) leaf extracts against *Aedes aegypti* (Diptera: Culicidae). *International Journal of Mosquito Research*, 2(1), 47–52.

[6] Amer, A., & Mehlhorn, H. (2006). Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera: Culicidae). *Parasitology Research*, 99(4), 466–472.

[7] Haridasan, P., Gokuldas, M., & Ajaykumar, A. P. (2017). Antifeedant effects of *Vitex negundo* L. leaf extracts on the stored product pest *Tribolium castaneum* H. (Coleoptera: Tenebrionidae). *International Journal of Pharmacy and Pharmaceutical Sciences*, 9(3), 17–22.

[8] Singh, R., Verma, P. K., & Singh, S. K. (2011). Phytopharmacological review of *Vitex negundo* (Sambhalu). *PharmacologyOnline*, 2, 124–132.

[9] Mdpi.com. (2021). *Vitex negundo* L. essential oil: Odorant binding protein efficiency using molecular docking approach and studies of mosquito repellent. *Insects*, 12(12), 1061.
[10] Insecticidal activities of stem bark extracts from *Vitex negundo* L. against *Tribolium castaneum* (Herbst). (2009). *Journal of Bio-Science*, 17, 63–70.