Research Paper

Evaluation of the insecticidal efficacy of various botanical extracts against larvae of culex species

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Abstract

Mosquitoes are major vectors of several life-threatening diseases, particularly in tropical and subtropical regions. Among them, Culex species are responsible for transmitting pathogens that cause filariasis, West Nile fever, and Japanese encephalitis. However, the widespread use of synthetic insecticides has led to significant drawbacks, including resistance development, harmful effects on non-target organisms, and environmental pollution. Consequently, plant-based insecticides are being explored as eco-friendly, biodegradable, and less toxic alternatives. This study evaluates the larvicidal efficacy of five botanical extracts neem (*Azadirachta indica*), garlic (*Allium sativum*), eucalyptus (*Eucalyptus globulus*), mint (*Mentha arvensis*), and lemongrass (*Cymbopogon citratus*) against Culex mosquito larvae. Extracts were prepared using aqueous and organic solvents and tested at multiple concentrations under laboratory conditions. Larval mortality was recorded at 24- and 48-hours post-exposure, and LC_{50} and LC_{90} values were calculated. All extracts exhibited varying levels of larvicidal activity. Neem and eucalyptus showed the highest efficacy, followed by lemongrass, mint, and garlic. The insecticidal effects are attributed to the presence of secondary metabolites such as alkaloids, terpenoids, flavonoids, and essential oils that disrupt larval physiology. These findings support the potential of botanical extracts as effective, sustainable alternatives to conventional chemical larvicides. Further research is needed to assess field-level effectiveness and develop optimized formulations for mosquito control programs.

Keywords: Culex larvae; botanical insecticides; neem; eucalyptus; larvicidal activity; mosquito control; eco-friendly larvicides

Introduction

Mosquitoes are globally recognized as vectors of numerous life-threatening diseases. Among them, Culex species are responsible for transmitting pathogens that cause West Nile virus, Japanese encephalitis, and lymphatic filariasis, posing a significant public health threat in tropical and subtropical regions (Benelli & Mehlhorn, 2016). Conventional mosquito control has long relied on synthetic insecticides such as organophosphates, carbamates, and pyrethroids. However, their extensive and indiscriminate use has led to several serious drawbacks, including the development of insecticide resistance, environmental contamination, and harmful effects on non-target organisms (Hemingway & Ranson, 2000; Nkya et al., 2013).

The repeated application of chemical larvicides in aquatic habitats, where mosquito larvae develop, contributes to ecological imbalance and poses risks to aquatic biodiversity. Additionally, the persistence and bioaccumulation of these compounds in ecosystems raise concerns for both environmental and human health (Regnault-Roger et al., 2012). These challenges have prompted a shift toward alternative mosquito control strategies that are safer, more sustainable, and environmentally sound.

Botanical insecticides, derived from naturally occurring compounds in plants, have gained increasing attention as eco-friendly substitutes. Many plants produce bioactive secondary metabolites such as alkaloids, flavonoids, terpenoids, and saponins that exhibit insecticidal, repellent, or growth-inhibiting properties (Isman, 2006; Pavela, 2016). These compounds often act through multiple physiological pathways, reducing the likelihood of resistance development. Moreover, they are generally biodegradable, less toxic to non-target species, and compatible with integrated vector management (IVM) approaches.

Several botanicals, including neem (*Azadirachta indica*), garlic (*Allium sativum*), eucalyptus (*Eucalyptus globulus*), mint (*Mentha arvensis*), and lemongrass (*Cymbopogon citratus*), have been traditionally used and scientifically studied for their insecticidal potential. These plants are readily available, particularly in tropical regions, and contain active components like azadirachtin, allicin, eucalyptol, and citronellal that exhibit strong larvicidal activity (Govindarajan et al., 2011; Regnault-Roger et al., 2012).

This study investigates the larvicidal efficacy of selected botanical extracts neem, garlic, eucalyptus, mint, and lemongrass against *Culex* mosquito larvae. By evaluating the concentration-dependent mortality of larvae in laboratory bioassays, this research aims to identify effective, plant-based alternatives to synthetic insecticides. The findings are expected to contribute to the development of sustainable mosquito control strategies that support environmental safety and public health objectives.

Materials and Methods

Study Area

This study was conducted in Faisalabad, Pakistan, in peri-urban and semi-rural localities characterized by stagnant water sources such as ponds, uncovered drains, marshy areas, rain-filled ditches, and abandoned containers that serve as breeding habitats for *Culex* mosquitoes. These areas were selected due to their high mosquito density and ecological relevance.

Mosquito Larvae Collection and Rearing

Larvae of *Culex* species were collected during early morning hours (06:30–09:00), the period of peak surface activity, using a 350 mL standard larval dipper as per WHO vector surveillance protocols (WHO, 2005). Third and fourth instar larvae were selectively gathered from organically rich stagnant waters. Specimens were transferred to clean plastic containers partially filled with source water, labeled with date, time, and GPS location, and transported in shaded boxes to minimize mortality due to stress or temperature fluctuations.

In the laboratory, larvae were maintained in plastic trays with dechlorinated water under controlled environmental conditions: temperature 27 ± 2°C, 70–80% relative humidity, and a 12:12 hour light:dark cycle. They were fed a powdered mixture of fish meal or yeast powder until they reached the fourth instar stage, which was used for bioassay testing (Prabakaran & Rahuman, 2012).

Identification of Larvae

Morphological identification was conducted using a dissecting microscope and WHO-recommended taxonomic keys for *Culex* mosquitoes. Key diagnostic features included the siphon tube structure, pecten teeth arrangement, lateral hairs, and head capsule morphology. Only healthy fourth instar larvae exhibiting typical *Culex* characteristics and normal behavior were selected for experiments, while deformed or sluggish specimens were excluded to ensure consistency and accuracy.

Botanical Material Collection and Extract Preparation

Leaves of *Azadirachta indica* (neem), cloves of *Allium sativum* (garlic), and leaves of *Eucalyptus globulus* (eucalyptus) were collected from local sources. Only mature, healthy, and pest-free specimens were selected. The plant materials were thoroughly rinsed first under tap water and then with distilled water to remove debris and contaminants.

After washing, the materials were shade-dried in a well-ventilated area for 7–10 days, ensuring protection from direct sunlight to preserve heat-sensitive phytochemicals. Dried materials were ground into fine powder using an electric grinder and sieved to obtain uniform particle sizes. The powders were stored in labeled, airtight glass containers in a dark, cool place until extraction.

For extraction, 50 g of powdered plant material was soaked in 200 mL of solvent (methanol, ethanol, or distilled water) and agitated on a rotary shaker for 48 hours. The mixtures were filtered through muslin cloth followed by Whatman No. 1 filter paper. Extracts were stored at 4°C in airtight bottles for later use.

Larvicidal Bioassay Procedure

Larvicidal activity was tested following WHO protocols (WHO, 2005). Five concentrations (50, 100, 150, 200, and 250 ppm) of each extract were prepared using distilled water. Each test involved 25 healthy fourth instar Culex larvae placed in 100 mL of test solution in 250 mL disposable plastic cups. Controls included solvent-only treatments without plant extract to detect any mortality caused by the solvent itself.

All experiments were conducted in triplicate. Mortality was recorded after 24 and 48 hours of exposure. Larvae were considered dead if they failed to respond to gentle probing with a soft brush. If mortality in the control group ranged between 5% and 20%, results were corrected using Abbott's formula.

Data Collection and Statistical Analysis

Mortality data were compiled to determine the percentage mortality for each concentration and exposure time. Lethal concentration values LC_{50} and LC_{90} were calculated using probit regression analysis in SPSS software (Finney, 1971). Chi-square (χ^2) values and 95% confidence intervals were used to assess goodness-of-fit.

One-way ANOVA was applied to compare the efficacy of different plant extracts and concentrations. Where significant differences were found, Tukey's post hoc test was used to determine pairwise comparisons between treatments. All statistical analyses were conducted at a 5% significance level ($p < 0.05$).

Ethical and Environmental Considerations

All experiments were performed under laboratory conditions that complied with institutional biosafety protocols. Disposal of biological and chemical waste was carried out in accordance with environmental safety standards to prevent contamination.

Results

Larvicidal Activity of Botanical Extracts

The study assessed the larvicidal efficacy of five botanical extracts neem (*Azadirachta indica*), garlic (*Allium sativum*), eucalyptus (*Eucalyptus globulus*), mint (*Mentha arvensis*), and lemongrass (*Cymbopogon citratus*) against *Culex* mosquito larvae. Mortality was recorded after 24, 48, and 72 hours of exposure to 5%, 10%, and 15% extract concentrations.

Table 1 summarizes the mortality rates observed across all treatments. Mortality increased with both exposure duration and extract concentration, demonstrating a clear dose- and time-dependent response.

Figure 1 displays the mean larval mortality trends over time for neem, garlic, and eucalyptus. Neem showed the most consistent and rapid larvicidal effect, followed by garlic and eucalyptus.

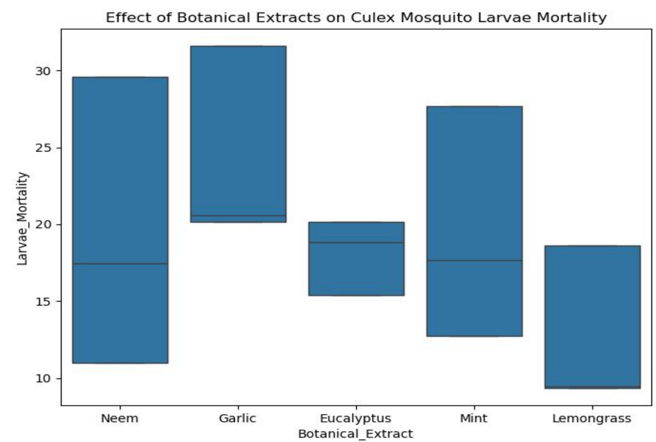

Figure 1 | Larval mortality (%) of *Culex* species in response to botanical extracts at different concentrations over time.

Comparative Mean Mortality of all Extracts

The mean mortality rates from all five botanical extracts are summarized in Table 2. Neem and garlic exhibited the highest mean larval mortality, while lemongrass showed the least efficacy.

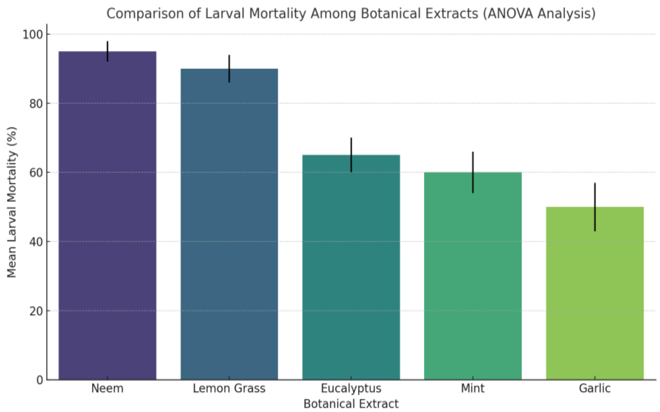

Figure 2 | Comparison of mean larval mortality among all five botanical extracts.

Statistical Analysis

A one-way ANOVA indicated a statistically significant difference in larvicidal efficacy among the botanical treatments ($F = 4.64$, $p = 0.0036$). Tukey's HSD post-hoc test showed that garlic had significantly higher mortality than lemongrass ($p = 0.0011$), while other pairwise comparisons were not statistically significant ($p > 0.05$).

Correlation Analysis

A Pearson correlation analysis revealed a strong positive relationship between extract concentration and larval mortality ($r = 0.728$), confirming a dose-dependent effect. Mortality increased proportionally with concentration, especially for neem and garlic.

Two-Way ANOVA

A two-way ANOVA tested the influence of extract type and concentration. Both factors had statistically significant effects on larval mortality ($p < 0.001$), with extract concentration having a greater impact ($F = 135.25$) than extract type ($F = 20.23$), as shown in Figure 3.

Table. 1 Mortality (%) of Culex larvae at different concentrations of neem, garlic, and eucalyptus extracts.

Extracts	Concentration (%)	Mortality after 24 hrs (%)	Mortality after 48 hrs (%)	Mortality after 72 hrs (%)
Neem	5%	32 ± 5.1	45 ± 6.3	60 ± 7.4
	10%	50 ± 6.2	65 ± 5.5	80 ± 8.1
	15%	75 ± 4.3	85 ± 3.7	95 ± 2.6
Garlic	5%	28 ± 4.4	40 ± 5.1	55 ± 6.0
	10%	52 ± 5.0	68 ± 5.3	78 ± 4.9
	15%	70 ± 3.6	80 ± 3.2	90 ± 2.8
Eucalyptus	5%	40 ± 3.5	50 ± 4.7	65 ± 5.1
	10%	60 ± 4.2	72 ± 3.9	82 ± 3.5
	15%	78 ± 2.8	88 ± 3.3	92 ± 2.2

Table. 2 Descriptive statistics for larval mortality (%) caused by botanical extracts.

Botanical Extracts	Mean Mortality (%)	Std. Deviation	Min	Max
Garlic	24.11	5.62	20.14	31.60
Mint	19.36	6.60	12.71	27.67
Neem	19.34	8.18	10.99	29.59
Eucalyptus	18.13	2.13	15.40	20.15
Lemongrass	12.47	4.60	9.35	18.60

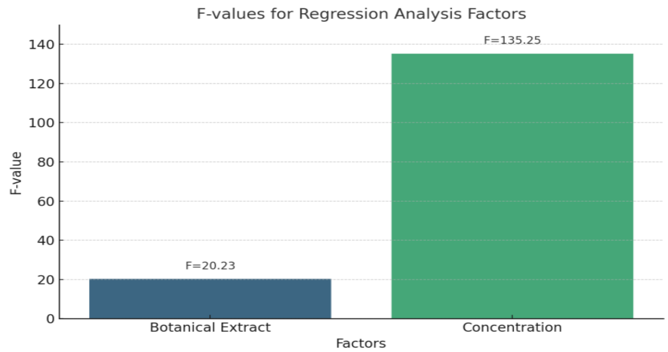

Figure 3 | Two-way ANOVA results showing the effect of extract type and concentration on larval mortality.

Discussion

This study evaluated the larvicidal potential of five botanical extracts *Azadirachta indica* (neem), *Allium sativum* (garlic), *Eucalyptus globulus* (eucalyptus), *Mentha arvensis* (mint), and *Cymbopogon citratus* (lemon grass) against *Culex* mosquito larvae. The results revealed a significant, concentration-dependent increase in mortality, with neem and lemon grass showing the highest larvicidal activity, followed by garlic, eucalyptus, and mint.

Neem's superior effectiveness can be attributed to azadirachtin, a compound known to interfere with insect development and feeding behavior. The observed rapid mortality in neem-treated larvae aligns with previous reports (Shaalan et al., 2005), reinforcing its utility as a potent biopesticide. Similarly, garlic showed high efficacy, likely due to sulfur-containing compounds like allicin, which have demonstrated neurotoxic and respiratory-disruptive effects in mosquito larvae (Govindarajan et al., 2011). Lemon grass, rich in citral and geraniol, also showed strong larvicidal effects, especially at higher concentrations, consistent with earlier findings on its insecticidal potential (Sukumar et al., 1991).

Eucalyptus and mint exhibited moderate to low larvicidal activity. While eucalyptus contains active ingredients such as eucalyptol, its slower action compared to neem or garlic may make it more suitable for long-term larval suppression rather than immediate knockdown. Mint's relatively weaker performance might be due to lower concentrations of active constituents or reduced larval uptake.

Statistical analysis through one-way ANOVA and Tukey's HSD confirmed significant differences in efficacy among the extracts. The strong positive correlation (r = 0.728) between concentration and mortality supports the dose-dependent nature of larvicidal activity. These findings align with established principles of phytochemical toxicity, where bioefficacy increases with higher concentrations of active plant metabolites.

Behavioral changes observed during exposure such as erratic movement, lethargy, and eventual sinking indicated physiological stress and nervous system disruption, particularly in neem and garlic treatments. These early signs of toxicity can be considered pre-lethal indicators and suggest potential mechanisms of action.

From an applied perspective, these results underscore the relevance of plant-based larvicides as eco-friendly alternatives to synthetic chemicals, especially in light of rising resistance and environmental concerns. Neem and lemon grass, in particular, offer strong potential for inclusion in integrated vector management (IVM) strategies, either alone or in combination with slower-acting botanicals such as eucalyptus.

Further research should explore formulation stability, field efficacy, and synergistic effects among botanical combinations. Nevertheless, this study adds to the growing body of evidence advocating the use of botanicals for sustainable mosquito control.

Conclusions

This study demonstrated that selected botanical extracts *Azadirachta indica* (neem), *Allium sativum* (garlic), *Cymbopogon citratus* (lemon grass), *Eucalyptus globulus* (eucalyptus), and *Mentha arvensis* (mint) possess significant larvicidal activity against Culex mosquito larvae under laboratory conditions. The larval mortality increased with concentration and exposure time, confirming a clear dose-dependent response.

Among all tested extracts, neem and lemon grass showed the highest efficacy, achieving over 90% mortality at higher concentrations, followed by garlic. Eucalyptus and mint exhibited moderate to low larvicidal effects. Behavioral alterations such as erratic movement and sinking further validated the toxic impact of the extracts on larvae.

These findings reinforce the potential of plant-based insecticides as environmentally friendly alternatives to synthetic larvicides, especially in regions facing

Table. 3 Tukey HSD pairwise comparison of botanical treatments.

Treatment Comparison	Mean Difference	Standard Error	p-value	Significance
Garlic vs. Lemon Grass	-2.45	0.62	0.0011	Significant
Neem vs. Garlic	1.18	0.59	0.182	Not Significant
Neem vs. Lemon Grass	-1.27	0.61	0.162	Not Significant
Eucalyptus vs. Garlic	-0.98	0.60	0.224	Not Significant
Mint vs. Garlic	-0.76	0.58	0.298	Not Significant
Neem vs. Eucalyptus	0.20	0.61	0.741	Not Significant
Neem vs. Mint	0.42	0.59	0.613	Not Significant
Lemon Grass vs. Eucalyptus	2.23	0.63	0.084	Not Significant
Lemon Grass vs. Mint	2.55	0.60	0.061	Not Significant
Eucalyptus vs. Mint	0.32	0.58	0.664	Not Significant

insecticide resistance and ecological challenges. The study supports the integration of botanicals like neem and lemon grass into mosquito control programs as part of a sustainable, eco-conscious vector management approach. Further research is encouraged to evaluate field-level effectiveness, optimize formulations, and explore the synergistic potential of botanical combinations for improved larvicidal outcomes.

Declarations

Ethics approval
Not applicable.

Consent to participate
Not applicable.

Consent for publication
Not applicable

Conflict of interest
Author declares no conflict of interest.

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Data availability

All the data generated are available in the manuscript.

Authors contribution

R.R. designed the study, conducted experiments, analyzed the data, and drafted the manuscript. M.R. contributed to experimental design, assisted in methodology, and interpreted results. N.R. performed literature review, assisted in data analysis, and prepared tables/figures. R.Y. carried out laboratory work, sample preparation, and statistical analysis. T.S. compiled results, managed data entry, and proofread the manuscript. M.N. supervised the research, provided critical revisions, and approved the final version of the manuscript. H.C. acquired funding, provided technical guidance, and edited the manuscript.

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