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Greenshield: Development And Preliminary Evaluation Of Plant Based Aqueous Extracts For Sustainable Mosquito Larval Management

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Abstract
Mosquito borne diseases continue to create a need for complementary vector-management approaches, particularly those that can be evaluated from locally available botanical resources. The present study, designated GREENSHIELD, was undertaken as a preliminary laboratory screening of aqueous leaf preparations of neem (Azadirachta indica), tulsi (Ocimum tenuiflorum), and eucalyptus (Eucalyptus spp.) against mosquito larvae. Dried leaf powder (10 g) of each plant was separately extracted in either 100 mL or 50 mL of distilled water, producing six preparations coded NE-100, NE-50, TU-100, TU-50, EU-100, and EU-50, respectively. Twenty larvae were exposed to each treatment in the preliminary screening. Observed mortality was 70% for NE-100, 100% for NE-50, 75% for TU-100, 90% for TU-50, 90% for EU-100, and 100% for EU-50. The highest observed mortality occurred with the more concentrated-volume preparations NE-50 and EU-50, whereas TU-50 and EU-100 produced 90% mortality. These preliminary observations indicate that all six aqueous preparations exhibited measurable larvicidal activity under the tested conditions. However, the results are intended as an initial screening and should not be interpreted as LC_{50} or LC_{90} estimates because standardized concentration-response experiments, untreated controls, replicated assays, and non-target safety evaluations are required for quantitative validation. The findings support further investigation of plant-derived aqueous preparations as candidate materials for mosquito larval management.

Keywords: *Mosquito Larvae, Botanical Larvicide, Azadirachta Indica, Ocimum Tenuiflorum, Eucalyptus, Aqueous Extract, Larval Management*

Introduction

Mosquitoes are important vectors of several human diseases, and control of immature stages is an important component of integrated vector management. The World Health Organization recognizes larval source management as a group of interventions that includes habitat modification, habitat manipulation, larviciding, and biological control, with implementation depending on local vector ecology and operational feasibility (WHO, 2026). The continued search for additional larval control materials is also influenced by concerns surrounding insecticide resistance, environmental persistence, and non-target effects associated with some conventional insecticides. Plant-derived products have received considerable scientific attention because plants contain diverse secondary metabolites that may affect mosquito development and survival. Neem (*Azadirachta indica*) is one of the most extensively investigated botanical resources for mosquito control. Reviews and experimental studies have reported ovicidal, larvicidal, pupicidal, and oviposition-related effects of neem-derived preparations, while also emphasizing that activity depends on the plant material, formulation, active constituents, and application conditions (Dua *et al*., 2009; Okumu *et al*., 2007; Isman *et al*., 2023).

Ocimum species have likewise demonstrated mosquito-control potential. Recent work on Ocimum tenuiflorum essential oil and hydrosol preparations has reported larvicidal activity against several disease-vector mosquito species and identified biologically active volatile constituents, although essential oils and aqueous leaf extracts are not equivalent preparations (Kamaraj *et al*., 2024; Raina *et al*., 2024). Eucalyptus-derived products have also shown larvicidal activity in laboratory studies, with activity varying substantially according to species, formulation, and concentration (Kaura *et al*., 2019). Recent reviews indicate that the effectiveness of plant-based larvicides can vary markedly with extraction method, plant part, formulation, mosquito species, and dose. Consequently, preliminary screening should be followed by standardized concentration-response experiments, replication, appropriate controls, and assessment of non-target effects before practical recommendations are made (Noorazlan *et al*., 2024; Wardaya *et al*., 2025). Against this background, the present GREENSHIELD study

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evaluates simple aqueous preparations of neem, tulsi, and eucalyptus leaves as an initial screening approach for mosquito larval management.

Materials And Methods

Fresh leaves of *Azadirachta indica*, *Ocimum tenuiflorum*, and *Eucalyptus* spp. were selected as the botanical materials for the preparation of aqueous plant extracts. The leaves were collected in clean condition and visually inspected to remove damaged or contaminated material. The collected leaves were thoroughly washed with clean water to remove adhering dust and other visible impurities and were subsequently shade-dried under laboratory conditions to minimize direct exposure to sunlight. The dried leaves were then powdered to obtain relatively uniform plant material for extraction. For each plant species, 10 g of dried leaf powder was weighed separately using a laboratory balance. Two aqueous extraction preparations were made independently for each plant. In the first preparation, 10 g of dried leaf powder was mixed with 100 mL of distilled water, whereas in the second preparation, 10 g of the same plant material was mixed with 50 mL of distilled water. Thus, the starting plant-material-to-water ratios were 1:10 (10 g/100 mL) and 1:5 (10 g/50 mL), respectively. Each preparation was maintained separately throughout the experimental procedure, and extracts from different plant species or different extraction volumes were not mixed.

The plant material was allowed to remain in contact with distilled water under laboratory conditions to facilitate the extraction of water-soluble phytochemical constituents. Following extraction, each preparation was passed through a suitable filtration setup to separate the plant residue from the aqueous filtrate. The filtrates were collected separately in clean, labelled containers and were used for the subsequent preliminary mosquito larval bioassay. The photographs documenting the preparation and filtration of the neem aqueous extract are presented in Figure 1. Based on the plant source and extraction volume, six experimental treatment codes were assigned: NE-100 and NE-50 for *A. indica*, TU-100 and TU-50 for *O. tenuiflorum*, and EU-100 and EU-50 for *Eucalyptus* spp. The numerical suffix represents the volume of distilled water used during extraction. Because the same quantity of plant powder (10 g) was extracted in half the volume of water, the 50 ml preparations had approximately twice the starting plant-material-to-water ratio of their corresponding 100 ml preparations. This difference was used to obtain an initial comparison of larvicidal activity between the two aqueous preparation strengths.



(A) (B) (C)



(D)

Figure 1. Preparation of aqueous neem (*Azadirachta indica*) (*Ocimum tenuiflorum*) (*Eucalyptus spp.)* leaf extract: (A) aqueous extraction, (B) filtration setup, (C) filtration of the extract and (D) collection of the filtered extract

Results And Discussion

All six aqueous plant preparations produced observable mortality among the 20 larvae exposed to each treatment. Neem NE-100 produced 14 deaths (70%), whereas NE-50 produced 20 deaths (100%). Tulsi TU-100 produced 15 deaths (75%) and TU-50 produced 18 deaths (90%). Eucalyptus EU-100 produced 18 deaths (90%) and EU-50 produced 20 deaths (100%). Thus, the highest observed mortality was recorded for NE-50 and EU-50, while the lowest observed mortality was recorded for NE-100. The overall pattern showed higher observed mortality in the 50 ml preparations than in the corresponding 100 ml preparations for *neem* and *tulsi*. For *eucalyptus*, both preparations showed high mortality, with EU-50 reaching 100%. These observations provide an initial basis for subsequent replicated dose-response experiments

Treatment	Plant	Extract volume	Dead larvae / 20	Mortality (%)
NE-100	*Azadirachta indica*	100 mL	14 / 20	70
NE-50	*Azadirachta indica*	50 mL	20 / 20	100
TU-100	*Ocimum tenuiflorum*	100 mL	15 / 20	75
TU-50	*Ocimum tenuiflorum*	50 mL	18 / 20	90
EU-100	*Eucalyptus spp.*	100 mL	18 / 20	90
EU-50	*Eucalyptus spp.*	50 mL	20 / 20	100

Table 1. Preliminary mortality of mosquito larvae exposed to the six GREENSHIELD aqueous plant preparations

The preliminary findings demonstrate that aqueous preparations derived from neem, tulsi, and eucalyptus leaves produced measurable mortality in the tested mosquito larvae. The 100% mortality observed with NE-50 and EU-50 suggests that reducing the extraction-water volume while maintaining 10 g of starting plant material may increase the amount of extractable material delivered per unit volume. However, this interpretation should be treated as a concentration-related hypothesis rather than a definitive dose-response conclusion because the actual concentration of active constituents in each preparation was not chemically standardized.

The neem observations are consistent with the broader literature documenting mosquitocidal effects of neem-derived products. Experimental studies have demonstrated larvicidal effects of neem formulations, while reviews emphasize the importance of azadirachtin and other phytochemicals and the influence of formulation and environmental conditions on efficacy (*Dua et al.*, 2009; Okumu *et al*., 2007; Isman *et al*., 2023). The present study used an aqueous leaf preparation rather than neem oil; therefore, direct numerical comparison with neem oil LC_{50} or LC_{90} values reported in other studies would not be scientifically appropriate. The activity observed with tulsi is also biologically plausible because Ocimum-derived preparations contain diverse secondary metabolites. Recent investigations of O. tenuiflorum essential oils and hydrosols have reported larvicidal effects against multiple mosquito species, but extraction chemistry differs between essential oils, hydrosols, and simple aqueous leaf preparations (Raina *et al*., 2024). Similarly, previous work with Eucalyptus and neem oils against Aedes larvae demonstrated concentration-dependent larvicidal activity, supporting the general feasibility of botanical mosquito-control research while also illustrating the importance of standardized concentrations and formulations (Kaura *et al.*, 2019).

A major strength of the GREENSHIELD concept is its simple preparation approach using readily obtainable plant material and water. Nevertheless, the present results should be regarded as preliminary. Recent reviews have emphasized that plant-based larvicidal efficacy is strongly influenced by extraction method, formulation, plant chemistry, mosquito species, and dose, and that promising laboratory observations require validation through standardized and replicated experiments (Noorazlan *et al.*, 2024; Wardaya *et al*., 2025). Future experiments should therefore include an untreated control, multiple standardized concentrations, biological replicates, a defined exposure period, identified mosquito species and larval instar, and appropriate statistical analysis. Non-target aquatic organisms and persistence should also be evaluated before any field application is considered.

Conclusion

The GREENSHIELD preliminary screening demonstrated larval mortality following exposure to aqueous preparations of Azadirachta indica, Ocimum tenuiflorum, and Eucalyptus spp. Among the tested treatments, NE-50 and EU-50 produced the highest observed mortality of 100%, followed by TU-50 and EU-100 at 90%, TU-100 at 75%, and NE-100 at 70%. These findings indicate that the selected plant materials warrant further investigation as candidate botanical larvicidal resources. The present results are preliminary and should be validated through replicated, standardized concentration-response assays, appropriate controls, chemical characterization, and non-target safety testing before practical mosquito-control recommendations are made.

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