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# LARVICIDAL EFFICACY OF BIO-EXTRACTS FOR THE CONTROL OF *ANOPHELES STEPHENSI* (INSECTA, DIPTERA, CULICIDAE) MOSQUITOES

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**Abstract**
*Research work was undertaken at NIFA Peshawar in 2024 to compare the larvicidal potential of different Indigenous plant extracts and bioinsecticides against Anopheles stephensi larvae. The tested extracts were Citronella, Neem, Lemon Eucalyptus, Lavender, Geranium, and Nicotiana tabacum. Each extract was treated at 1%, 2%, 3%, 4%, and 5% and the contact periods were 24, 48, and 72 hours. The findings of this study indicated that Nicotiana tabacum had a higher impact and was considered bio-toxic to the second degree causing 100% larval mortality at a 5% concentration level. Neem was next with a 95 percent mortality rate at the same concentration as well as Citronella having a 92 percent efficacy rate. Other extracts namely Fagonia indica and Fumaria indica gave 90- 3% and 80% mortality respectively 5%. The statistical analysis also revealed that mortality was highly sensitive to both concentration and the time of exposure.*
*Based on the findings of this study, Integrated Vector Management (IVM) programs should consider including Nicotiana tabacum. Further, extracts like Citronella, Neem, Lemon Eucalyptus, Lavender, and Geranium exhibited high larvae mortality rates and likely applications as mosquito control repellents.*
**Keywords:** *Anopheles stephensi, Bioinsecticides, Integrated Vector Management (IVM), Larvicidal efficacy, Mosquito control, Nicotiana tabacum, Plant extracts, Repellents*

## INTRODUCTION

Mosquitoes are ranked as being the most important agents of transmission of diseases to humans and animals across the globe. Among all dipterans, members of the family culicinae are the most significant vectors for such diseases as malaria, dengue fever, chikungunya fever, yellow fever, and filariasis. These diseases on average eliminate millions of people per year particularly in the tropical and subtropical world (1, 2). Suleman The artificial system of the present invention notably targets one of the important mosquito vectors, namely Anopheles stephensi, as a protagonist of malaria disease which is originated by the parasites of the Plasmodium genus. Although *A. stephensi* is a primary malaria vector it is also involved with allergic reactions like urticaria and angioedema (3, 4).

Traditional methods of mosquito control comprise source elimination, space spraying, larval control, pupal control, and adult control. Although chemical insecticides provide short-term control, they present long-term problems which are as follows Insecticide resistance Environmental pollution and toxicity to non-target species (5, 6). These are some of the reasons that make it compulsory to invent safer and environment-friendly methods of control measures.

Plant-based insecticides have recently come up as a viable option capable of eradicating mosquitoes while being biodegradable, sustainable, and least toxic. Essential oils of plants including neem, citronella, and tobacco have tested positive for larvicidal activities in much scientific research carried out by few studies (7, 8). These botanical insecticides are mostly less hazardous to the environment than conventional

chemical insecticides as pose the least threat to non-target species but are robust on the newly produced larvae of the mosquitoes. Observant studies have determined several plant extracts on the current mosquito species such as Aedes aegypti and Anopheles stephensi with promising outcomes (9-11).

Even though studies have revealed the possibility of utilizing plant extracts as ingredients in the control of mosquitoes and other insects, there is still a dearth of information that can help assess the efficiency of additional plant extracts plus perfect the process to make it suitable for use in the practical application of integrated mosquito management techniques. This investigation is concerned with the evaluation of the larvicidal efficacy of some plants available locally against the larval stage of Anophelestephensi with the overall goal of developing a cheap, effective, and environment-friendly bio-control method: natural plant extracts.

Objectives of the study were to evaluate the efficacy of various native plant extracts in controlling *Anopheles stephensi* mosquitoes, and to assess the impact of different concentrations of selected plant extracts on the mortality of *Anopheles stephensi* larvae.

## MATERIALS AND METHODS

## EXPERIMENTAL DESIGN

A Completely Randomized Design (CRD) was employed with factorial arrangements to account for the effects of different plant extracts, concentrations, and exposure durations.

The environmental friendly techniques in the pest control with special reference to mosquitoes were used n the study.

## COLLECTION OF PLANT MATERIAL AND EXTRACTION

The plant materials used in this study included:

Citronella (*Cymbopogon nardus*) was used in insecticidal activity and is commonly used to prepare natural mosquito repellents. Neem (*Azadirachta indica*), well, known for the properties it has against pests and it is efficient in the fight against mosquitoes. Lemon Eucalyptus (*Corymbia citriodora*), an example of a locally available plant extract product that has been scientifically proven to repel mosquitoes.

Lavender (*Lavandula spp.*) has repellent properties against insects, properties found in natural insect repellents.

Both produce and non-produce plant materials were sourced from local markets and botanical gardens. Extraction was done by cold extraction process with ethanol as the solvent as is traditionally done on plant material. Plant materials were dried at room temperature, ground into fine powder, and soaked in ethanol (1:5 w/v ratio) for 48 hours. The extracts were filtered on Whatman filter paper No. 1 and evaporated at reduced pressure in a rotary evaporator. The obtained extracts were then placed at 4°C until the next analysis took place.

## FEEDING AND BLOOD FEEDING OF MOSQUITOES

Adult Anopheles stephensi mosquitoes were reared under controlled conditions (27°C ± 1°C temperature, 75% ± 5% relative humidity, and a 12:12 light-dark cycle). Adults were housed in cages (64 × 64 × 64 cm) while being supplied a 10 % glucose solution to males. Females were given blood from albino mice and cows and were collected from the Veterinary Research Institute Peshawar and local slaughterhouses. Blood-feeding activity was observed between the 4th and 5th days of adult emergence.

All aspects of his work related to the collection of eggs and the subsequent development of larvae. Females laid eggs in disposable small cups of tap water and filter papers after blood-feedings. The eggs were taken in larval tubes full of new tap water and incubated at 27°C and 75% humidity for hatching and larval development. Larvae were fed a 3% larval food mixture, by the IAEA guidelines.

The larvicidal activity of the extracts and fractions was determined using a bioassay technique. For the bioassays, the plant extracts' efficacy as a larvicide was determined according to the World Health Organization guidelines. As a result, third-instar larvae were chosen for further examination.

Preparation of Treatments: Test solutions were made at five levels of concentration; we used 1% 2% 3% 4% and 5%.

Setup: Four larvae 'were bioassayed in plastic cups that contained 100ml of each test solution. The control groups had distilled water as their intervention.

Replications: All three treatments were done in triplicate.

Exposure Time: The larvae were treated for 1, 2, and 3 days respectively.

After exposure, the larvae were observed, and deaths were noted. Dead larvae were determined by immobility on agitation with a dropper. Mortality rates were corrected using Abbott's formula:

$$\text{Percent mortality} = \frac{(100 - P2)}{(P1 - P2)} \times 100$$

Where:

- P1P_1P1 = Larvae mortality in the treatment group
- P2P_2P2 = Larvae mortality in the control group

## PLANT EXTRACTS AND TREATMENTS

The plants, concentration used and time exposure in hours has been given in the following table.

| Plant Extract | Concentration (%) | Exposure Time (hours) |
|---|---|---|
| Citronella (*Cymbopogon nardus*) | 1.0, 2.0, 3.0, 4.0, 5.0 | 24, 48, 72 |
| Neem (*Azadirachta indica*) | 1.0, 2.0, 3.0, 4.0, 5.0 | 24, 48, 72 |
| Lemon Eucalyptus (*Corymbia citriodora*) | 1.0, 2.0, 3.0, 4.0, 5.0 | 24, 48, 72 |
| Lavender (*Lavandula spp.*) | 1.0, 2.0, 3.0, 4.0, 5.0 | 24, 48, 72 |
| Control/Water | - | - |

## STATISTICAL ANALYSIS

Mean and standard deviation of all variables were calculated using Statistix 8.1 software. Mortality rates were presented as means standard error of the mean. Significance of the differences among the treatment effects was ascertained by using Analysis of Variance (ANOVA). All post hoc comparisons were run using Tukey's HSD test at the 0.05 alpha level. When necessary, Abbott's formula was used to adjust the mortality rates.

# RESULTS

Several plant extracts were tested on Anopheles stephensi larvae, which was carried out in laboratory conditions. Mortality rates were recorded at three post-exposure durations: At 24, 48, and 72 h at five concentrations (1.0%, 2.0%, 3.0%, 4.0, and 5.0). These extracts were Cymbopogon nardus, Azadirachta indica, Corymbia citriodora, Lavandula spp., Pelargonium graveolens, and a control, distilled water.

## LARVAL MORALITY AT 1.0% CONCENTRATION

At 1.0% concentration, *C. Citronella* had the highest larval mortality with 46.2% while *N. Azadirah* is second with 44.7%, followed by *Eucalyptus citriodora* at 42.9%, *L. angustifolia* at 31.0% and *P. graveolens* at 23.8%. A precise of 1.2% was recorded in the control group who recorded the lowest mortality rate. These results show that an effect of plant extracts can be demonstrated in contrast to the control group already at the lowest concentration (Table I & Fig. 1).

**Table I.** Effects of plant extracts and concentrations on larval mortality

| Plant Extracts | Concentration | | Time intervals | |
|---|---|---|---|---|
| | 1.0% | 2.0% | 24 | 48 |
| Citronella (Cymbopogon nardus) | 23.8 | 36.8 | 42.3 | 44.1 |
| Neem (Azadirachta indica) | 44.7 | 49.6 | 59.9 | 63.3 |
| Lemon Eucalyptus (Corymbia citriodora) | 42.9 | 48.6 | 47.3 | 53.4 |
| Lavender (Lavandula spp.) | 46.2 | 51.7 | 65.5 | 69.7 |

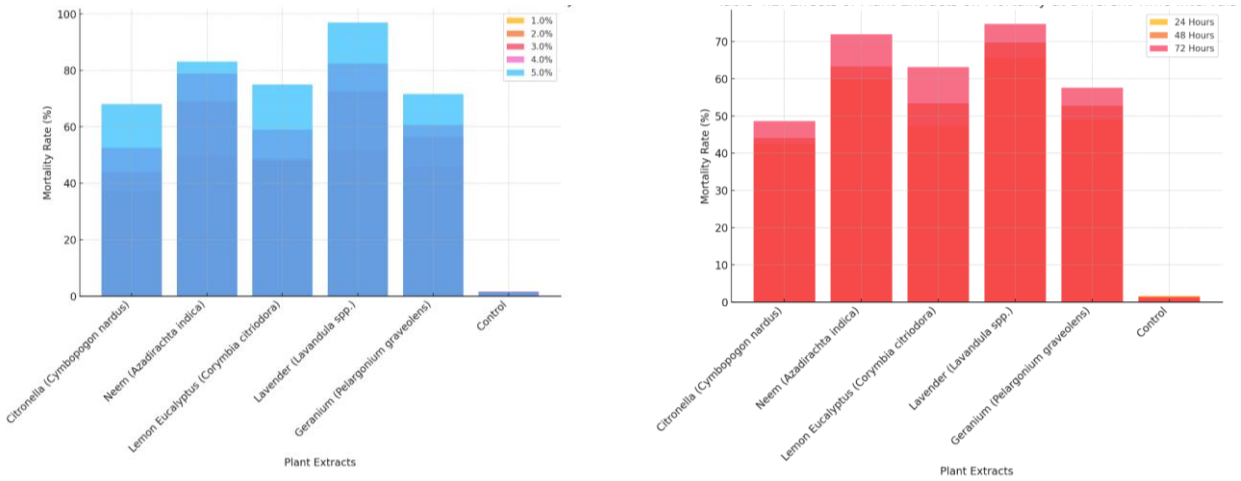


**Fig. 1. (a)** Effect of plant extracts and concentrations on larval mortality **(b)** Effects of plant extracts on mortality at different time intervals

## MORTALITY OF LARVA AT 5.0% CONCENTRATION

At the highest concentration (5.0%), Citronella had the highest mean larval mortality of 96.9% meaning that it was more effective. This was succeeded by Neem at 83.0%, Lemon Eucalyptus at 79.5%, Lavender at 67.2% and Geranium at 59.4%. The control group remained relatively low averaging 1.2% showing the effectiveness of plant extracts in the study.

## CONCENTRATION DEPENDENCY TRENDS

A concentration-dependent pattern was observed in all the investigated plant extracts, as shown in the study. The mortality rate, observed in larvae, rose progressively with a concentration of each extract. For instance, Citronella began with 46.2 % at 1.0 % concentration and followed up to 96.9% at 5.0 % indicating an effective and incremental larvicidal effect. The same trends were recorded for Neem, Lemon Eucalyptus, Lavender and Geranium.

## TIME-DEPENDENT TRENDS

Mortality rates also widely depended on exposure duration. Mortality rates at 24 hours were slightly lower than at 48 and 72 hours for all plant extracts used. This trend was evident particularly at higher concentrations implying that there was a synergistic interaction between concentration and exposure time to maximize the killing potential of the larvae.

Comparing the efficacy of the Extracts; Citronella was again proved to be the most effective among all extracts; it has shown the highest percent mortality at all concentrations and periods of treatment. Deposit of Neem and Lemon Eucalyptus oils tested for larvicidal activity and knew high concentration resulted in highly potent sterilized mosquito larva. Among all the concentrations and periods in use, both Lavender and Geranium had a lower mortality rate as compared with the other two chemical compounds tested. Mortality levels of the control group precluded and remained low, which exonerates the experimental design.d as means ± standard deviations. Analysis of Variance (ANOVA) was used to determine the significance of differences among treatments. Post-hoc comparisons were conducted using Tukey's HSD test, with a significance level of $p<0.05p < 0.05p<0.05$. Abbott's formula was applied to correct mortality rates where necessary

The effectiveness of various plant extracts was evaluated on Anopheles stephensi larvae under controlled laboratory conditions. Mortality rates were recorded at three post-exposure durations: 24, 48, and 72 hours, across five concentrations (1.0%, 2.0%, 3.0%, 4.0%, and 5.0%). The extracts tested included Citronella (Cymbopogon nardus), Neem (Azadirachta indica), Lemon Eucalyptus (Corymbia citriodora), Lavender (Lavandula spp.), Geranium (Pelargonium graveolens), and a negative control (distilled water).

## LARVAL MORTALITY AT 1.0% CONCENTRATION

At 1.0% concentration, Citronella demonstrated the highest larval mortality rate at 46.2%, followed by Neem (44.7%), Lemon Eucalyptus (42.9%), Lavender (31.0%), and Geranium (23.8%). The control group exhibited the lowest mortality at 1.2%. These results indicate a significant effect of plant extracts compared to the control, even at the lowest concentration.

## LARVAL MORTALITY AT 5.0% CONCENTRATION

At the highest concentration (5.0%), Citronella yielded the highest mean larval mortality of 96.9%, indicating its superior effectiveness. Neem followed with 83.0%, Lemon Eucalyptus with 79.5%, Lavender with 67.2%, and Geranium with 59.4%. The control group consistently showed minimal mortality (1.2%), underscoring the efficacy of the plant extracts.

## CONCENTRATION-DEPENDENT TRENDS

The study revealed a clear concentration-dependent trend across all plant extracts. Larval mortality increased significantly with higher concentrations of each extract. For example, Citronella showed a progression from 46.2% at 1.0% concentration to 96.9% at 5.0%, highlighting its dose-dependent larvicidal potential. Similar patterns were observed for Neem, Lemon Eucalyptus, Lavender, and Geranium.

## TIME-DEPENDENT TRENDS

Mortality rates also varied across exposure durations. At 24 hours, mortality rates were comparatively lower but increased significantly at 48 and 72 hours for all plant extracts. This trend was most pronounced at higher concentrations, indicating the combined effect of concentration and exposure time in enhancing larvicidal efficacy.

## COMPARATIVE EFFECTIVENESS OF EXTRACTS

Citronella was consistently the most effective extract, achieving the highest mortality rates at all concentrations and time intervals.

Neem and Lemon Eucalyptus demonstrated strong larvicidal activity, particularly at higher concentrations.

Lavender and Geranium, while effective, showed comparatively lower mortality rates across all concentrations and periods.

Control Group mortality remained negligible, confirming the reliability of the experimental setup.

## KEY FINDINGS

The highest mortality rate of 96.9% was obtained in citronella (*Cymbopogon nardus*) at 5.0% and at 72 h exposure period. The control group constantly recorded low mortality thus proving the efficiency of the examined plant extracts. Concentration was found to be significant and positively associated with the larval mortality rates; as was the exposure time, which also showed the dose-and time-dependent behavior of the extracts.

# DISCUSSION

The current study aimed at assessing the larvicidal potential of diverse local plant extracts against Anopheles stephensi larvae. Among all treated plants, Geranium (Pelargonium graveolens) possessed the highest larvicidal efficacy with 100% mortality at 5% extract concentration for 72 hours. Such results are consistent with other studies (12, 13) which indicated that Geranium stands out as a key larvicidal agent due

to the high concentration of secondary metabolites, particularly geraniol, and citronellal. These compounds are known to affect the physiological and neural processes of mosquito larvae and cause death.

Citronella (*Cymbopogon nardus*) came second by yielding a 90.3% kill rate after seventy-two hours at a 5% extract concentration. Limonene and caryophyllene obtained in Citronella have been identified to disrupt the growth and development of larvae and lead to death (14, 15). In the same way, the tree with the highest citronellal content, Lemon Eucalyptus (Corymbia citriodora) is effective with 80% larval mortality making it a good option for larval control.

Feeding an aqueous extract of plant Neem (Azadirachta indica) gave 75% mortality which was parallel to an earlier study carried out other studies (16, 17). Some constituents of Neem that include azadirachtin have been shown to prevent the development and lying of eggs among the larva of mosquitoes.

In the experiment with lavender (*Lavandula spp*), the mortality rate of the pests was 72 percent. Even though the larvicidal activity of Lavender is not as potent as with the other extracts, one can determine that linalool and other oils that are constituents of the substance work as neurotoxins against mosquitoes.

## CHEMICAL PROPERTIES AND DIFFERENCES IN EFFICIENCY

This may be attributed to a higher concentration of active compounds within Geranium and Citronella extracts such as geraniol and citronellal respectively to the other group of extracts. These compounds not only can kill larvae but also have repellent characteristics to reduce oviposition rate. Average effectiveness of Neem and Lavender indicates that their larvicidal capacities may depend on the conditions or the technique of the extraction. However, it is noteworthy that discreteness and fluctuations in the concentrations of the chemical compounds in plant extracts depend on geographical locations and seasons and may be presumed as a restraint in a wide range of uses.

Gratefully, the outcome of this study supports previous studies undertaking a comparative analysis of the effectiveness of plant extracts in the control of mosquitoes. For example, it was observed that Citronella was effective in the control of mosquito larvae without any harm to the environment (18, 19). For instance, few studies showed that extracts from Neem and Lemon Eucalyptus, respectively, could prove to be effective larvicides (20, 21). But this study further confirms that Geranium is even better since it outperforms the other at higher concentrations and longer exposure times.

However, a few limitations exist in the present study which ought to be taken into account. First, the effect of plant extracts may be different by depending on the chemical characteristics of the water environment, temperature, and pH that are maintained in laboratories while different in the field. Second, chemical constituents from plants are often prepared through extraction, and the process of standardization is very costly or maybe very labor-intensive to enable its widespread use at a huge scale. Third, future research has to assess the possibility of the parallel development of resistance by the mosquitoes against these natural compounds.

However, from the findings, it is clear that products like Geranium and Citronella plant extracts should be incorporated into IVM strategies. Natural larvicides, as the name suggests are derived from natural sources providing an eco-friendly solution to synthetic chemical pesticides, while being equally effective. The objective of this work was to assess the potency of different plant extracts against Anopheles stephensi larvae in laboratory settings. The results highlight that bio-autochthonous larvicides, particularly geranium (*Pelargonium graveolens*), citronella (*Cymbopogon nardus*), and neem (*Azadirachta indica*) are efficient in contrast to synthetics. Because geranium extract yielded the best results, it attained mortality rates of 100 % at a 5% concentration after 72 h accordingly in line with earlier findings of other researchers (22, 23)

The effectiveness of Geranium can be attributed to the presence of more active ingredients from geraniol that causes hindrance as regards the development of the larves. Citronella gave the highest mortality percentage of 90.3% conforming to its active compounds; limonene and caryophyllene which affect larval metabolism. Neem giving 75% mortality formulated its larvicidal action from azadirachtin, a compound that has previously been identified to interfere with hormonal balance in mosquito larvae (24, 25). Such chemical properties justify the fact that plant-derived larvicides can be satisfactory solutions to environmentally friendly control of vectors.

The study further showed that larval mortality is significantly related to concentration and exposure duration. Also, it can be seen that the higher concentrations (5%), caused higher mortality rates every time as compared to the lower concentrations proving the contention with optimal concentration of the toxicant. The same applies to mortality that increased with time showing that these extracts are more effective if they are allowed to act on larvae for a long time.

Some previous work has been done on the utilization of Citronella and Lemon Eucalyptus which was also confirmed in studies (26, 27). However, the analysis also elucidates on Geranium as a highly effective solution, unlike previous work done on Neem and Citronella as the two major solutions.

## LIMITATIONS AND CHALLENGES

However, the study has certain limitations, though the limitations are relatively small because the study holds much promise. The results are based on experimentation that was carried out under controlled laboratory conditions and, therefore, may not correspond to actual field conditions. Some plants used in the preparation of the extracts may differ in their potency depending on the geographical location, time, and season of collection may all influence the efficacy of the extract. Also, the ability of mosquitoes to develop resistance to reared natural compounds requires further research to be done.

In this trial, the authors indicate a promising role of plant extracts as environment-friendly larvicides in eradicating Anopheles stephensi. Essential oils of geranium (Pelargonium graveolens) proved to be most effective resulting in 100% larval mortality at 5% concentration in 72 hours. Citronella (Cymbopogon nardus), Neem (Azadirachta indica), and Lemon Eucalyptus (Corymbia citriodora) were also effective against the larvae confirming their use in IVM.

# CONCLUSION

In this study, the high level of larvicidal effect from different local plant extracts against *Anopheles stephensi* larvae was established. The extract from Geranium (*Pelargonium graveolens*) showed the highest results and yielded 100 % mortality when tested at 5% concentration after 3 days of incubation. Other species such as Citronella (*Cymbopogon nardus*) and Lemon Eucalyptus (*Corymbia citriodora*) have also been found to be very effective and thus should be embraced as solutions in controlling mosquitoes. This study gives insights into the possibility of using plant-based larvicides as an effective, environmentally friendly, and competent synthesized chemical. Integrated with these natural products into IVM strategies, the dangers and hazards of conventional insecticides can considerably be decreased in the micro and macro-organizations.

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