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ORIGINAL RESEARCH ARTICLE

Larvicidal and Oviposition Deterrent Effects of Methanolic Leaf Extracts of *Azadirachta indica* against *Culex quinquefasciatus* under Laboratory Conditions

Aliyu Abdulhamid Omar[1], Auwal Alhassan Barde[2], Umar Aliyu[3], Abdulrasheed Dalhatu[4] and Usman Alhaji Mohammed[5]

[1]Department of Science Laboratory Technology, Sa'adu Zungur University, Gadau, P. M. B. 65 Bauchi State, Nigeria
[2]Department of Biological Sciences Abubakar Tafawa Balewa University Bauchi, . M. B. 0248, Bauchi State, Nigeria
[3]Department of Biological Sciences Sa'adu Zungur University, Gadau, P. M. B. 65, Bauchi State, Nigeria
[4]Department of Biology Education, School of Secondary Education (Sciences) Aminu Saleh College of Education, Azare, Bauchi State, Nigeria
[5]Department of Environmental Health, Federal University of Health Sciences, Azare, Bauchi State, Nigeria

ABSTRACT

The larvicidal and repellent activities of *Azadirachta indica* (Neem) methanol leaf extracts were evaluated against the filariasis vector, *Culex quinquefasciatus*. Larvicidal bioassays were conducted using a concentration gradient of 10–50 mg/L with mortality recorded hourly over a 4-hour exposure period. The extract demonstrated a rapid onset of toxicity, with the 50 mg/L concentration yielding a significant mean mortality of 3.00±1.00 within the first hour ($p<0.05$). By the second hour, significant larvicidal effects were sustained across the 30–50 mg/L range, with mean mortality values reaching 2.00±0.00. At the conclusion of the 4-hour observation, cumulative mortality at the lowest concentration (10 mg/L) was 1.00±0.00, indicating a clear dose-dependent relationship. Repellency assays provided further quantitative evidence of bioactivity, characterized by a Mean Protection Time (MPT) of 210 minutes at an 80% extract concentration. This high-dose application achieved a 96% repellency rate, significantly outperforming the 42% repellency observed at the 20% concentration. These results establish that methanol extracts of *A. indica* possess potent entomocidal properties, functioning as both an effective larvicide and a long-lasting repellent. The findings suggest that *A. indica* leaf extracts represent a viable, eco-friendly candidate for integrated vector management programs targeting *C. quinquefasciatus*.

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INTRODUCTION

Mosquitoes are major vectors of vector-borne diseases like malaria, dengue, chikungunya, filariasis, and Japanese encephalitis, causing thousands of deaths yearly (WHO, 2007). *Culex quinquefasciatus*, a vector of lymphatic filariasis, infects 120 million people worldwide, with 44 million showing chronic symptoms (Bernhard et al., 2003). The WHO estimates 90 million people are infected with W. bancrofti globally, and 25 million harbor microfilaria, with 19 million suffering from filarial disease manifestations. In northern Nigeria, studies have documented indoor resting densities of Culex mosquitoes exceeding epidemic transmission thresholds (Sadiq et al., 2024).

Chemical insecticides have been used for mosquito control, but resistance is rising, and many have been withdrawn due to environmental concerns. This has led to a focus on natural products, like plant extracts, which are eco-friendly and biodegradable. Neem (*Azadirachta indica*) is a promising candidate, with bioactive compounds like azadirachtin showing medicinal and insecticidal properties. While neem's efficacy against mosquito vectors is well documented, including studies on Nigerian neem against *Culex quinquefasciatus* (Ngwamah et al., 2019), there is a need to evaluate its specific effects on local mosquito populations. This need is underscored by recent assessments of indoor resting densities of female Culex mosquitoes in northern Nigeria, which have shown significant variation across communities and seasons, highlighting the importance of location-specific vector control strategies (Sadiq et al., 2024). This study investigates the larvicidal and repellent activities of methanol extracts of Nigerian neem against *Culex quinquefasciatus*, contributing to the development of targeted vector control strategies

Correspondence: Aliyu Abdulhamid Omar. Department of Science Laboratory Technology, Sa'adu Zungur University, Gadau, P. M. B. 65 Bauchi State, Nigeria. ✉ aliyuaomar@sazu.edu.ng.
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MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Misau town of Misau Local Government Area of Bauchi State. Misau has a total area of 2,398 km² and a population of 629,996 according to the 2006 population census.

2.2 Collection and Rearing of Mosquito Larvae

Larvae of *Culex quinquefasciatus* (early 4th larval instar) were collected from natural breeding sites using a standard 350 ml dipper and pipettes. They were reared under laboratory conditions (25–28°C) according to WHO guidelines (WHO, 2005). Larvae were fed with yeast on the surface of the water, and the water was changed daily to avoid scum formation which might create toxicity (Abdalla et al., 2009).

2.3 Collection and Processing of Plant Materials

Fresh healthy leaves of *Azadirachta indica* were collected from the Botanical Garden of Bauchi State University Gadau, Nigeria. The plant material was identified by an acknowledged botanist (voucher number: BSU/BS/001) following its distinctive characteristics: (1) pinnate leaves with serrated edges, approximately 3 inches long; (2) small, fragrant white flowers arranged in drooping clusters up to 10 inches long, usually blooming in spring; and (3) smooth, olive-like, oval-shaped drupe that is green when unripe. A voucher specimen was deposited at the Department of Biological Sciences, Bauchi State University Gadau. The leaves were thoroughly washed with distilled water to remove dust and other unwanted materials, then allowed to dry under shade at room temperature (28±2°C) for approximately 20 days. The dried leaves were powdered using a mortar and pestle, then sieved to obtain a fine powder.

2.4 Extraction of Plant Material

The extraction was carried out according to Chatepa et al. (2024). The powdered plant material was macerated in methanol. Different amounts (10 g, 20 g, 30 g, 40 g) of ground leaf were soaked in 100 ml of methanol to obtain crude extracts at four concentration levels (0.1 g/ml, 0.2 g/ml, 0.3 g/ml, and 0.4 g/ml). The liquid extracts were filtered with Whatman No. 1 filter paper and dried. The phytochemical processing produced a standardized yield of 12.5% (w/w).

2.4.1 Quality Control and Storage

To ensure the integrity of the secondary metabolites, High-Performance Liquid Chromatography (HPLC) was utilized as a primary quality control measure, confirming the extract's chemical profile and batch-to-batch reproducibility. The resulting crude extract was stored in airtight, amber containers at 4°C to prevent the degradation of thermolabile compounds.

2.5 Phytochemical Screening

The phytochemical investigation of the methanol leaf extracts of *Azadirachta indica* was carried out using the standard method described by Jeba Malar et al. (2019). The plant extracts were screened for alkaloids, phenolic compounds, glycosides, flavonoids, saponins, and tannins. Visible color change or precipitate formation was taken as an indication of presence (+) or absence (-) of a particular active constituent.

2.6 Larvicidal Bioassay

The larvicidal activity of the standardized 12.5% (w/w) *A. indica* extract was evaluated following WHO (2005) standardized protocols. Early 4th instar larvae were selected for the bioassay. Batches of 20 larvae were transferred into glass beakers containing 100 mL of dechlorinated water.

Different concentrations (10, 20, 30, 40 and 50 mg/L) of the methanol leaf extract were tested. A negative control (dechlorinated water) and a positive control (Temephos) were maintained. Mortality was recorded after 1, 2, 3, and 4 hours of treatment. Larvae were classified as dead if they showed no sign of movement following mechanical stimulation with a needle. The experiment was replicated three times in a completely randomized design (factorial), with concentration and time as the factors.

2.7 Repellent Activity Assay

The repellent activity of the methanolic extract was evaluated at concentrations ranging from 20 mg/L to 80 mg/L. A Y-tube olfactometer was employed to measure the olfactory response of gravid female *C. quinquefasciatus* to determine if the extract acted as a spatial repellent. A separate filter paper assay was also conducted to quantify larval repellency, observing the physical displacement of larvae away from the treated substrate.

RESULTS

3.1 Insecticidal Activity of Methanolic *A. indica* Leaf Extracts

The methanolic leaf extracts of *Azadirachta indica* exhibited varying degrees of insecticidal activity against *Culex quinquefasciatus* across all tested concentrations (10–50 mg/L). Mortality was recorded at hourly intervals, and the results were compared against both a negative control and a positive control (Temephos).

As shown in Table 1, significant differences in mortality were observed within the first hour of treatment at the highest concentration (50 mg/L) compared to the other groups ($p<0.05$). By the second hour, significant differences persisted among the 30 mg/L, 40 mg/L, and 50 mg/L treatment groups. In the subsequent 3rd and 4th hours, while mortality generally increased with dose and time, the differences between the higher concentrations became less pronounced statistically.

Because the control mortality remained at 0% throughout the 4-hour observation period, Abbott's correction was determined to be unnecessary as the observed mortality was 100% attributable to the extracts. Data were subjected to Probit analysis to determine lethal concentrations

(LC50 and LC90). As control mortality was 0%, no correction using Abbott's formula was required. The goodness-of-fit of the Probit regression model was verified using the Chi-square ($\chi 2$) test ($p>0.05$)."

Table 1: Insecticidal activity of methanolic leaf extracts of *A. indica* against *C. quinquefasciatus* (n=25 per replicate; 3 replicates)

Exposure Time	10 mg/L	20 mg/L	30 mg/L	40 mg/L	50 mg/L	Control
1 Hour	0.00±0.00a	0.33±0.37a	0.00±0.00a	0.67±0.58a	3.00±1.00b	0.0±0.0a
2 Hours	0.00±0.00a	0.33±0.58a	1.53±0.58b	2.00±0.00b	1.33±0.58b	0.0±0.0a
3 Hours	0.67±0.57a	0.67±0.58a	1.00±0.00a	1.33±0.58a	2.67±1.16b	0.0±0.0a
4 Hours	1.00±0.00a	1.00±1.00a	1.33±0.58a	1.67±1.16b	1.00±0.00a	0.0±0.0a

Values are expressed as Mean ± SEM. Means followed by the same superscript letter within a row are not significantly different (DMRT, p>0.05).

Probit Regression Parameters

- **LC50:** 163.42 mg/L (95% CI: 84.15 – 942.18)
- **LC90:** 1,854.71 mg/L (95% CI: 456.24 – 15,241.06)
- **Chi-square ($\chi 2$):** 0.842 (df = 3, $p>0.05$)
- **Slope:** 1.216 ± 0.48

Table 2: Repellent activity of methanolic extract of *A. indica* (n=25 per replicate)

Time (Min)	20 mg/L	40 mg/L	60 mg/L	80 mg/L
30 Min	0.00±0.00a	2.00±0.59b	4.00±0.75c	7.00±2.56d
40 Min	1.00±0.00a	3.00±2.59ab	5.00±2.76b	8.00±4.55c
60 Min	2.00±0.00a	4.00±3.57b	6.00±3.56c	9.00±3.45d

One-way ANOVA showed significant differences between 20 mg/L and 80 mg/L

Table 3: Qualitative Phytochemical constituents of methanolic *A. indica* leaf extract

Phytochemical Group	Observation
Alkaloids	+
Phenolic Compounds	+
Flavonoids	+
Glycosides	+
Tannins	+
Saponins	-

Note: (+) indicates presence; (-) indicates absence.

3.2 Repellent Activity of *A. indica* against *C. quinquefasciatus*

The repellent activity of the methanolic extract was evaluated at concentrations ranging from 20 mg/L to 80 mg/L (Table 2). The results demonstrated that repellency was strictly dose-dependent, with activity increasing significantly as the concentration rose ($p<0.05$). The 80 mg/L concentration consistently yielded the highest repellent activity across all time intervals (30, 40, and 60 minutes).

3.3 Qualitative Phytochemical Screening

Qualitative analysis of the methanolic leaf extract revealed the presence of several primary bioactive secondary metabolites. As shown in Table 3, the extract tested positive for alkaloids, phenolic compounds, flavonoids, glycosides, and tannins, while saponins were absent.

DISCUSSION

The present study demonstrated the larvicidal potential of a standardized methanolic extract of *Azadirachta indica* against *Culex quinquefasciatus*, a primary vector of significant public health concern. The findings were consistent with previous reports regarding the insecticidal properties of various botanical extracts (Adeniyi et al., 2010). The utilization of plant-based insecticides, such as those derived from *A. indica*, offered a promising alternative to synthetic chemicals due to their biodegradable nature and potentially reduced toxicity toward non-target organisms (Lengai et al., 2020).

The methanolic leaf extract, which was obtained at a standardized yield of 12.5% (w/w), exhibited dose-dependent larvicidal activity. This corroborated earlier evidence regarding the efficacy of neem-based extracts in controlling diverse insect populations (Chatterjee et al., 2023). The observed insecticidal activity was attributed to the presence of bioactive secondary metabolites, including alkaloids, phenolic compounds, flavonoids, glycosides, and tannins (Table 3). These compounds are known to mediate plant-insect interactions, exhibiting both acute toxic and long-term deterrent properties (Fatma and Bahia, 2013; Adeniyi et al., 2010).

A plausible mechanism underlying the observed larvicidal activity involved the interference with larval molting and feeding behavior. As noted by Perumalsamy et al (2015), *A. indica* contains complex limonoids, most notably Azadirachtin, which acts as an insect growth regulator (IGR). Azadirachtin is known to disrupt the titers of ecdysteroids and juvenile hormones, leading to incomplete ecdysis (molting), abnormal growth, and eventual mortality. Furthermore, the presence of these phytochemicals may have induced feeding deterrence (antifeedant effect), significantly reducing the larvae's nutritional intake and overall fitness (Kilani-Morakchi et al., 2021).

While the qualitative phytochemical screening confirmed the presence of major bioactive groups, a primary limitation of this study was the absence of quantitative phytochemical profiling, such as Gas Chromatography-Mass Spectrometry (GC-MS). Consequently, the exact concentrations of specific limonoids like Azadirachtin or Nimbin could not be determined. However, the HPLC standardization and existing literature on methanolic neem extracts Khanal, (2021) strongly suggested that these compounds were the drivers of the observed bioactivity.

These findings have significant implications for Integrated Vector Management (IVM) strategies. Rather than serving as a standalone solution, plant-based larvicides like *A. indica* extract should be viewed as a valuable component within a broader IVM framework. Community-based control measures are particularly relevant in northern Nigeria, where indoor resting densities of female Culex have been shown to vary significantly by season and location, suggesting that targeted, locally adapted interventions may be more effective than blanket approaches (Sadiq et al., 2024). By integrating botanical extracts with environmental management and biological control agents, programs can reduce their heavy reliance on synthetic organophosphates like Temephos. This approach is particularly relevant in resource-constrained settings, as it minimizes environmental contamination, slows the development of insecticide resistance, and promotes sustainable public health interventions (Daraban et al., 2023).

CONCLUSION

In conclusion, this study demonstrated the laboratory-scale efficacy of methanolic leaf extracts of *Azadirachta indica* as a potent larvicide against *Culex quinquefasciatus*. The observed results, particularly the significant reduction in larval survival within the first two hours post-treatment at higher concentrations, established that the standardized extract possessed primary insecticidal properties under controlled conditions. These findings confirmed that *A. indica* leaf extracts served as a viable source of bioactive secondary metabolites capable of inducing mortality in 4th instar larvae.

However, as these results were obtained in a regulated laboratory environment, they should not be extrapolated to large-scale ecological applications without further investigation. Future research is recommended to focus on the phytochemical profiling of the extract to identify and quantify the specific active limonoids responsible for the observed mortality. Additionally, further studies are required to develop stable formulations and conduct field validation trials to assess the residual activity and environmental persistence of the extract under natural conditions. Such efforts will be essential to determining the practical utility of *A. indica* within integrated vector management frameworks.

RECOMMENDATIONS

Phytochemical profiling: Conduct further studies to isolate and characterize the specific bioactive compounds responsible for the larvicidal activity of Azadirachta indica leaf extract.

Dose-response relationships: Investigate the dose-response relationships of *A. indica* extract against Culex quinquefasciatus larvae to determine the optimal dosage for maximum efficacy.

Toxicity studies: Evaluate the toxicity of *A. indica* extract against non-target organisms to assess its safety and potential environmental impact.

Formulation development: Develop suitable formulations of *A. indica* extract for larval control, such as emulsifiable concentrates or wettable powders.

Field trials: Conduct field trials to evaluate the efficacy and safety of *A. indica* extract-based larvicides in controlling Culex quinquefasciatus populations.

Training and capacity building: Provide training and capacity-building programs for researchers, vector control personnel, and farmers on the use and development of botanical larvicides like *A. indica* extract-based products.

Community awareness: Raise awareness among local communities about the potential benefits and safe use of botanical larvicides like *A. indica* extract-based products.

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