Davide *et al*., Pan African Journal of Life Sciences (2026): 10(2): 710-717
10.36108/pajols/6202/01.0230

Publication of Faculty of Basic Medical Sciences and Basic Clinical Sciences , Ladoke Akintola University of Technology, Ogbomoso

www.pajols.org
Online ISSN:2672-5924

RESEARCH **Open Access**

Efficacy and Retreatment Interval of *Azadirachta indica* Ethanolic Extract Impregnated Fabrics Against Mosquitoes

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Received 20th March 2026; Revised 25th August 2026; Accepted 31th August 2026

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ABSTRACT

Background: Malaria remains a significant public health issue in sub-Saharan Africa, particularly in Nigeria, where *Anopheles gambiae* s.l. (Diptera; Culicidae) is the primary vector of *Plasmodium* parasites. The consistent increase in synthetic insecticide resistance among mosquito populations has necessitated the exploration of alternative, environmentally friendly tools for vector control. This research examined the effectiveness of two different mosquito bed net fabrics impregnated with ethanolic extract of *Azadirachta indica* seeds against the *Anopheles gambiae* s.l. population. The research was carried out at the insectary of the Biological Sciences Department at the University of Abuja, Abuja. Larval breeding sites were located at selected Gwagwalada communities.

Method: Harvested larvae were reared to the adult stage following standard procedure. Plant materials were gathered, authenticated, processed, macerated, and filtered with 70% ethanol, then concentrated using a rotary evaporator. Laboratory bioassays utilised a range of (1 ppm to 10,000 ppm) concentrations of neem seed extract. Adult mosquitoes were exposed to treated surfaces of the bed net fabrics embedded (impregnated) with the extracts, with mortality rates recorded at 0-60-minute intervals.

Results: The findings showed a clear increase in mosquito mortality that correlated with both concentration and exposure time. Fabrics impregnated with extracts at elevated concentrations (≥1000 ppm) exhibited significant insecticidal efficacy, achieving mortality rates above 95%, particularly after 60 minutes of exposure. Neem-extract-treated polyester and neem cotton exhibited concentration-dependent efficacy, with lower concentrations (1-100 ppm) requiring re-treatment at 2-3-month and 4-5-month intervals, respectively. Higher concentrations (1,000-10,000 ppm), on the other hand, maintained effectiveness for 4-5 months for polyester and up to 6 months for cotton, permitting less frequent re-treatment. Compared with the control group, the treated fabrics showed a higher mosquito mortality rate. For polyester fabrics, Neem treatment values did not differ significantly among the tested concentrations during April–June ($P > 0.05$). However, significant differences among concentrations were observed from August–September ($P < 0.05$). Similarly, for cotton fabrics, Neem treatment values showed no significant differences among concentrations during April–May ($P > 0.05$), whereas significant differences were observed from July–September ($P < 0.05$).

Conclusion: The results support the incorporation of neem plant-derived insecticides into strategies for managing malaria vectors, especially in areas with significant insecticide resistance.

Keywords: *Azadirachta indica*, *Anopheles gambiae* s.l., extracts, concentrations, fabrics.

1.0 INTRODUCTION

Mosquitoes are among the most medically important insects owing to their hematophagous behaviour; they are vectors of diseases, transmitting many infectious pathogens, including arboviruses, filariae, and protozoans that cause common and emerging diseases, specifically malaria, dengue, Zika, chikungunya, and Japanese encephalitis, amongst humans and other animals [1]. Mosquito-borne diseases occur in almost every country [2], but are most prevalent in tropical and subtropical regions with warm, humid climates [3]. There are 3563 valid species of mosquitoes, belonging to three genera (*Anopheles, Aedes,* and *Culex*) within two subfamilies: Anophelinae and Culicinae [1, 4]. The mosquito vectors of the *Anopheles* genus can transmit malaria and filariasis; the *Aedes* genus can transmit dengue virus, chikungunya virus, Zika virus, and yellow fever virus; while the *Culex* genus can transmit filariasis, Japanese encephalitis virus, West Nile virus and Rift Valley Fever [5, 6]. According to the latest World Malaria Report [7], there were 282 million cases of malaria in 2024, an increase of about 9 million cases (3%) compared with 2023. The estimated number of malaria deaths stood at 610,000 in 2024 compared to 598,000 in 2023. This necessitated the WHO 2021 recommendation for the broad use of malaria vaccine among children living in regions with moderate to high P. falciparum malaria transmission [7]. The vaccine has been shown to reduce malaria cases among young children significantly. Vaccines are now being rolled out in routine childhood immunisation programs across Africa. Even though malaria vaccines in Africa are expected to save tens of thousands of young lives every year, the highest impact will, however, be achieved when the vaccines are introduced alongside a mix of other WHO-recommended malaria interventions; the Integrated vector management programs, employing a variety of tools including insecticide use, in indoor residual spraying (IRS), larval control, insecticide-treated bed nets (ITNs) and chemoprophylaxis [8, 9]. The resistance of mosquito populations to insecticides, however, poses a significant challenge to their efficacy [10]. Resistance mechanisms, including target site mutations and increased metabolic detoxification, have emerged due to widespread insecticide use [11, 12]. In addition, the growing resistance to pyrethroids used in long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) necessitates alternative control strategies. Furthermore, synthetic insecticides often harm the environment and nontarget organisms, underscoring the need to explore alternative pest management strategies [13]. In response to the challenges posed by synthetic insecticide resistance, there is growing interest in plant-based insecticidal products [14]. These herbal insecticides, containing mixtures of bioactive compounds such as alkaloids, terpenoids, and flavonoids, exhibit complex modes of action that slow the development of resistance [15]. Neem seed extract has shown promise as an eco-friendly mosquito-control agent, effective against both larval and adult mosquitoes. The extract's effectiveness is attributed to its bioactive compounds [16]. Studies suggest that neem-based insecticides pose a low risk of resistance and are environmentally friendly [4]. The study by [17] reported a 91% mortality rate in adult Anopheles mosquitoes following 24 h of exposure to papers impregnated with 100% *Azadirachta indica* seed oil extract. Only a very few botanicals have, however, progressed from laboratory study to field use; the purpose of the present study is to investigate the effects of bed net fabrics impregnated with ethanolic seed extracts of *Azadirachta indica* (Neem) against *Anopheles gambiae* s.l.

2.0 METHODOLOGY

2.1 Collection and Preparation of Neem Seeds

mature fruits were collected from A. indica trees in the Gwagwalada Area Council, FCT Abuja. The collected seeds were thoroughly washed under running tap water and then soaked in water in a container for 3 days to remove the outer skin (endocarp). The seeds were air-dried under shade for 3 days. The coats covering the seeds were dehusked by a decorticating process that carefully applied pressure with a mortar and pestle to separate the kernels from the seeds. The seeds were thereafter winnowed and sieved to obtain pure reddish-brown seed kernels Plate 1.



Plate 1. Reddish Brown *Azadirachta indica* Seed Kernels

The seed kernels were allowed to shade-dry and weighed until a constant weight was obtained. These were thereafter pulverised manually using a mortar and pestle [18].

2.2 Preparation of Plant Extracts

The pulverised neem seed kernel of *A. indica* was transferred into a column flask, and 1000ml of 70% ethanol was added to macerate for 48 hours [19]. The resultant filtrate was collected into a conical flask and concentrated using a rotary evaporator (RE-201 D, 2L, 20-280 RPM, ZZKD Machinery) and thereafter kept for bioassay.

2.3 Entomological Field Survey

The mosquito larvae of Anopheles species, identified by their resting position [4], were collected in the early morning before sunrise and at sunset using the dipping method [20]. Also, plastic spoons and, in some instances, plastic pipettes were used to remove larvae from small water bodies where dippers would not be effective; the water, along with the larvae, was then collected and transferred into a basin. In addition, five black, six-litre rubber buckets, half-filled with water, were placed in various shady locations for adult mosquitoes to lay eggs. The containers were observed daily for larvae, which were collected using a small sieve with a mesh size of about 0.55mm that retained the larvae. Sampling was repeated intermittently until the required number of larvae was reached. The collected mosquito larvae were identified based on morphological characteristics using keys described by [21].

2.4 Mosquito Larvae Culture

The harvested mosquito larvae were reared at the Insectary of the Biological Sciences Department, University of Abuja, FCT Abuja. The mosquito larvae were maintained under controlled temperature conditions in the Insectary as prescribed by [22]. Larvae were fed finely ground fish food, yeast, and breadcrumbs. The adult mosquitoes that emerged were kept in plastic mosquito cages with about 20 cm x 20 cm x 20 cm frames and mosquito-net tops. They were provided with cotton pads soaked in 5% glucose/10% sugar solution.

2.5 Preparation of Mosquito Netting Fabrics

The two commonly existing mosquito-netting fabrics (polyethene and cotton) were employed for this study. Impregnations were carried out by dipping the nets in the extract for 30 minutes and allowing them to dry in the shade. Before and after impregnation, each net type was weighed separately, and the average weight was determined [23].

2.6 Preparation of Concentrations from Extracts of *Azadirachta indica*

Serial dilution was performed by preparing a 1% stock solution of the crude extract by weighing 200 mg (0.2 g) of the plant extract into an empty test bottle. A 20 mL aliquot of acetone was added to the test tube, and the mixture was vigorously shaken to ensure uniform dissolution. This was appropriately labelled as a 20 mL stock solution. Serial dilutions at 0.1%, 0.01%, 0.001%, and 0.0001% were subsequently prepared from the stock solution [24]. As a result, five concentrations of the plant extract were obtained: 1%, 0.1%, 0.01%, 0.001%, and 0.0001%. These were converted to ppm using the formula: Per cent = PPM / 10,000.

2.7 Cone Bioassay

The bioassays were performed according to WHOPES guidelines [2]. The set-up was prepared by cutting approximately 25cm x 25cm of net from each net type. Each of which was impregnated with 10 mL of *Azadirachta indica* (A.) Juss. The WHO plastic cone was attached to the piece of net and held together by plastic boards that were clamped together (plate 3). Using an aspirator, five females, non-blood-fed mosquitoes aged 2 to 5 days, were introduced into the cone; this was replicated 5 times for each of the 5 concentrations. Before this exposure, the mosquitoes were transferred into holding tubes and observed for about 20-30 minutes to assess possible adverse handling effects. Mosquitoes were exposed for 60 minutes and subsequently transferred into labelled plastic holding cups containing 10% sugar solution for feeding. Knock-down and mortality were recorded during the exposure and post-exposure period of 60 minutes to 24 hours. Mosquitoes were scored as alive if they were still able to fly; dead or knocked down if immobile or incapable of flying or standing in a coordinated manner [25-29]; and repelled if they were unable to rest on the net. An untreated net of 25cm x 25cm, impregnated in acetone, was used as a negative control for each net type, and Alpha cypermethrin-impregnated nets were used as a positive control.

2.8 Data Analysis

Data on mosquito mortality were analysed using IBM SPSS Statistics version 25.0, and the results were presented in tables. Mortality rates were summarised as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to assess the effects of the extract-impregnated nets on mosquito mortality. Where significant differences were observed, Tukey's Honestly Sig-

nificant Difference (HSD) post hoc test was used to compare treatment means pairwise. Statistical significance was set at $P < 0.05$. Throughout the study period, mortality in the control group remained below 5%; therefore, Abbott's correction was not applied

3.0 RESULTS

Table 1 describes the mortality responses of *Anopheles gambiae* s.l. exposed to neem extract–impregnated polyester material from April to September. Mean values showed no significant differences across all concentrations (P=0.005) in April-June, but significant differences were observed ($P < 0.005$) from August to September.

permitting retreatment after every four to five months. Overall, a sustained 24-hour mortality rate of 80% or higher is considered the threshold for operational effectiveness against *Anopheles gambiae* s.l. [22]. According to the World Health Organisation [23] vector control standards, retreatment of insecticidal materials is recommended when mosquito mortality falls below 80% after 24 hours of exposure.

Table 3 illustrates the monthly efficacy trend of neem-extract-impregnated cotton materials against Anopheles gambiae s.l. across varying concentrations from April to September. Efficacy generally declined over time, with lower concentrations (1–100 ppm) dropping below the [23] recommended threshold of 80% mortality earlier

Table 1. Monthly Mean (±SD) 24-Hour Mortality of *Anopheles gambiae* s.l Exposed to Neem Extract impregnated polyester material (April–September)

Month	Time (hrs)	1 ppm (%)	10 ppm (%)	100 ppm	1,000 ppm	10,000 ppm	Control
April	MT 24	4.00 ± 0.00^{a} (80%)	4.67 ± 0.33^{a} (93%)	4.67 ± 0.33^{a} (93%)	5.00 ± 0.00^{a} (100%)	5.00 ± 0.00^{a} (100%)	0.00 ± 0.00^{d} (0%)
May	MT 24	4.00 ± 0.00^{a} (80%)	4.67 ± 0.33^{a} (93%)	4.67 ± 0.33^{a} (93%)	4.67 ± 0.33^{a} (93%)	4.67 ± 0.33^{a} (93%)	0.00 ± 0.00^{d} (0%)
June	MT 24	3.67 ± 0.33^{ab} (73%)	4.00 ± 0.00^{ab} (80%)	4.33 ± 0.33^{ab} (87%)	4.67 ± 0.33^{a} (93%)	4.67 ± 0.33^{a} (93%)	0.00 ± 0.00^{d} (0%)
July	MT 24	3.33 ± 0.33^{b} (67%)	4.00 ± 0.00^{ab} (80%)	3.67 ± 0.33^{b} (73%)	4.33 ± 0.33^{ab} (87%)	4.17 ± 0.31^{ab} (83%)	0.00 ± 0.00^{d} (0%)
August	MT 24	2.33 ± 0.33^{c} (47%)	3.33 ± 0.33^{b} (67%)	3.33 ± 0.33^{b} (67%)	3.67 ± 0.33^{b} (73%)	4.33 ± 0.33^{ab} (87%)	0.00 ± 0.00^{d} (0%)
September	MT 24	2.67 ± 0.33^{c} (53%)	2.33 ± 0.33^{c} (47%)	2.67 ± 0.33^{c} (53%)	3.00 ± 0.00^{b} (60%)	3.50 ± 0.29^{b} (70%)	0.00 ± 0.00^{d} (0%)

Note: Different letters indicate significant differences between concentrations ($P \leq 0.05$)

Table 2 indicates that lower concentrations of Neem-extract impregnated polyester (1–100 ppm) lose their insecticidal potency more rapidly and therefore require retreatment at intervals of approximately two to three months. In contrast, higher concentrations (1,000–10,000 ppm) maintain their effectiveness for a longer duration, than higher doses did. This pattern supports the recommendation for shorter retreatment intervals at lower concentrations and longer intervals at higher concentrations. It was observed that mean values did not differ significantly across concentrations (P=0.005) during April-May, whereas significant differences were observed ($P < 0.005$) from July to September.

Table 2. Retreatment intervals of the Neem-extract impregnated polyester materials based on the decline in mortality (%) of *Anopheles gambiae* s.l across months

Concentration	April (%)	May (%)	June (%)	July (%)	Aug (%)	Sep (%)	Recommended Retreatment Time
1 ppm	80.0	80.0	73.0	67.0	47.0	53.0	Efficacious within 2 months. Sharp decline below 80% from June indicates rapid loss of potency; retreat every 2 months.
10 ppm	93.0	93.0	80.0	80.0	67.0	47.0	Activity sustained for 3 months before dropping; retreat quarterly.
100 ppm	93.0	93.0	87.0	73.0	67.0	53.0	Effective up to June; retreat before end of 3rd month.
1,000 ppm	100.0	93.0	93.0	87.0	73.0	60.0	Maintained effectiveness ≥87% up to July; retreat after 4 months to sustain full efficacy.
10,000 ppm	100.0	93.0	93.0	83.0	87.0	70.0	High stability; remains ≥87% up to August. Retreatment only needed after 5 months.

Month	1 ppm (%)	10 ppm (%)	100 ppm (%)	1,000 ppm (%)	10,000 ppm (%)	Control (%)
April	4.00 ± 0.00^{a} (80.0%)	4.67 ± 0.33^{a} (93.4%)	5.00 ± 0.00^{a} (100.0%)	5.00 ± 0.00^{a} (100.0%)	5.00 ± 0.00^{a} (100.0%)	0.00 ± 0.00^{c}(0)
May	4.00 ± 0.00^{a} (80.0%)	4.67 ± 0.33^{a} (93.4%)	5.00 ± 0.00^{a} (100.0%)	5.00 ± 0.00^{a} (100.0%)	5.00 ± 0.00^{a} (100.0%)	0.00 ± 0.00^{c}(0)
June	4.00 ± 0.00^{a} (80.0%)	4.00 ± 0.00^{b} (80.0%)	4.67 ± 0.33^{b} (93.4%)	5.00 ± 0.00^{a} (100.0%)	5.00 ± 0.00^{a} (100.0%)	0.00 ± 0.00^{c}(0)
July	3.67 ± 0.33^{b} (73.4%)	4.00 ± 0.00^{b} (80.0%)	3.67 ± 0.33^{c} (73.4%)	4.67 ± 0.33^{b} (93.4%)	4.67 ± 0.33^{b} (93.4%)	0.00 ± 0.00^{c}(0)
August	3.33 ± 0.33^{c} (66.6%)	3.67 ± 0.33^{c} (73.4%)	4.00 ± 0.00^{d} (80.0%)	4.33 ± 0.33^{c} (86.6%)	4.67 ± 0.33^{b} (93.4%)	0.00 ± 0.00^{c}(0)
September	3.00 ± 0.00^{d} (60.0%)	3.33 ± 0.33^{d} (66.6%)	3.67 ± 0.33^{c} (73.4%)	4.00 ± 0.00^{d} (80.0%)	4.33 ± 0.33^{c} (86.6%)	0.00 ± 0.00^{c}(0)

Note: Different letters indicate significant differences between concentrations ($P \leq 0.05$)

Table 4 illustrates the monthly efficacy trend of neem-extract impregnated cotton materials against *Anopheles gambiae* s.l. across varying concentrations from April to September. Efficacy generally declined over time, with Lower concentrations (1-100 ppm) falling below the [22] recommended 80% mortality threshold sooner than higher doses.

4.0 DISCUSSION

Table 4. Retreatment Intervals of Neem-Extract Impregnated Cotton Materials Against *Anopheles gambiae* s.l.

Concentration	April (%)	May (%)	June (%)	July (%)	August (%)	September (%)	Recommendation (Retreatment Interval)
1 ppm	80.0	80.0	80.0	73.4	66.6	60.0	Retreatment every 2 months (efficacy drops <80% by July)
10 ppm	93.4	93.4	80.0	80.0	73.4	66.6	Retreatment every 3 months (decline <80% after June)
100 ppm	100.0	100.0	93.4	73.4	80.0	73.4	Retreatment every 3 months (mortality falls <80% by July)
1,000 ppm	100.0	100.0	100.0	93.4	86.6	80.0	Retreatment every 5 months (falls to borderline 80% in September)
10,000 ppm	100.0	100.0	100.0	93.4	93.4	86.6	Retreatment every 6 months (still >80% up to September)

Neem seed extracts have demonstrated significant promise in controlling adult mosquitoes, particularly *Anopheles gambiae* s. l., a primary malaria vector. The effectiveness observed in the present study corresponds with previous findings that neem limonoids such as azadirachtin, salannin, and nimbolide can disrupt insect neurophysiology by interfering with cholinergic transmission and muscle coordination, ultimately leading to paralysis and death [25, 20]. Various studies have shown that neem seed extracts can significantly reduce adult mosquito populations when applied to breeding habitats. For instance, a study on malaria vector control in the Sahel region of Niger revealed that twice-weekly applications of neem seed powder reduced adult female Anopheles gambiae populations by 49% [21]. The extracts work by targeting larval stages, leading to high mortality rates and reduced adult longevity. Studies indicate neem seed extracts have a low risk of resistance development, positioning them as a potential alternative to synthetic insecticides. In a similar study by [26], the effect of Azadirachta indica (neem) seed extract on the larvae and adult *Anopheles* mosquitoes in Nasarawa Local Government Area of Nasarawa State was determined. Impregnated papers with neem seed oil were used against adult *Anopheles* mosquitoes, and the larvicidal effect was also determined. Adult *Anopheles* mosquitoes exposed to impregnated papers with neem seed extract at concentrations of 20%, 40%, 80%, and 100% showed efficacy of 5%, 29%, 66%, and 91%, respectively. Mortality rate was recorded at 24 h of exposure with LC_{50} (Lethal concentration) of 4.45ml. The larvae were also exposed to different concentrations of neem seed extract (1%, 4%, 8%, and 10%) for 72 h, and the mortality rate was 100%, with an LC_{50} (Lethal concentration) of 4.16ml. The study suggested that adult Anopheles mosquitoes will require more of the extract to achieve 100% mortality than larvae do. Also, research on neem oil-based nanoemulsions demonstrated that higher concentrations (2000 ppm) provided longer-lasting efficacy (up to 12 hours) against *Anopheles stephensi* compared with lower concentrations (500 ppm) [27]. Similarly, in the present study, high concentrations (100–10,000 ppm) consistently produced sig-

nificantly higher mortality than low concentrations (1–10 ppm), with the differences becoming more pronounced as exposure time increased. The results across months and replicates confirmed that mortality variation was not due to chance but directly attributable to treatment levels. These findings validate the hypothesis that extract concentration is critical to efficacy, with higher concentrations yielding significantly improved mosquito control outcomes. The analysis of the results showed significant differences in mean mortality rates across different concentrations of both plant extracts ($p < 0.05$).

In addition, this study demonstrated that mosquito susceptibility varied significantly with the type of fabric used for impregnation with the extract. Cotton fabrics exhibited higher efficacy than polyester, achieving higher and more sustained mortality rates. The results showed that the efficacy of extract-impregnated fabrics declined progressively over time, particularly at lower concentrations. For instance, at 1 ppm, the polyester fabric remained effective for only two months, while at 10,000 ppm, the fabric maintained effective mortality levels (>80%) for five months. This indicates that lower concentrations dropped below effective thresholds within a short period. Mid-range concentrations (100–1,000 ppm) maintained moderate efficacy for approximately three to five months. On the other hand, the efficacy of neem-impregnated cotton fabric persisted for three months at the lowest concentration (1 ppm). In contrast, the fabrics treated with the highest concentration (10,000 ppm) retained efficacy for up to six months. The fabrics exhibited higher efficacy than polyester, achieving higher and more sustained mortality rates. This could be attributed to the porous, absorbent nature of cotton, which enhances retention and the gradual release of active compounds. In contrast, polyester showed reduced effectiveness due to its hydrophobic nature, which limits absorption and accelerates loss of active ingredients. In conclusion, mosquitoes were more susceptible to extracts delivered via cotton fabrics, confirming the importance of material type in vector control applications. Similarly, studies by Lama *et al.* [16] found that silk fabric dyed with the highest concentrated neem extract showed excellent mosquito repellence (66.67%). In comparison, lower concentrations exhibited moderate to average repellences (40-60%), confirming that neem extracts could be an alternative to synthetic anti-mosquito and antibacterial chemicals and could be commercialised in protective textiles. Similar observations have been documented, in which plant-derived insecticides demonstrated greater effectiveness on absorbent natural materials than on synthetic textiles due to improved retention and reduced volatility of bioactive substances [28].

In addition, studies by [29] show that re-treatment intervals for neem-based insecticides are concentration-dependent. For example, in his study on neem oil-based formulations, he found that higher concentrations (10% and 20%) provided longer-lasting efficacy against mosquitoes, with re-treatment intervals ranging from 7-14 days, compared to lower concentrations (1% and 5%), which required more frequent re-treatment [27, 29]. A field trial in Nigeria found that neem-treated bed nets (10% concentration) retained efficacy for up to 6 months, with a recommended re-treatment interval of 3-6 months [30]. These trends indicate that re-treatment intervals are concentration-dependent, with higher concentrations requiring less frequent re-application. The observed decline in efficacy over time highlights the influence of environmental degradation and supports the need for periodic re-treatment to maintain optimal mosquito control.

In conclusion, this study is notable for its use of mosquito bed net fabrics, making it relevant to indoor vector control practices; most existing literature predominantly focuses on sprays, oils, or larvicidal applications of botanicals. This study introduces a practical innovation by showcasing extract-impregnated fabrics, particularly cotton, as viable delivery systems, bridging the gap between laboratory research and real-world applications, and addressing the limitation of theoretical botanical studies. Additionally, the study provides novel comparative evidence that cotton outperforms polyester in retaining and releasing active compounds, an area that has received limited attention in previous research. This provides material-science insights into vector control strategies, a perspective largely overlooked in botanical insecticide research.

Conflicts of Interest

The authors declare that there is no conflict of interests.

Authors' Contributions

PSD conceived and designed the study, contributed to data collection, and manuscript writing. **DYM** contributed to data analysis tools and manuscript writing. **CAO** contributed to analysis of data, data analysis tools and manuscript writing. All authors approved the final copy of the manuscript.

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