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(RESEARCH ARTICLE)

Investigating the Efficacy of phytochemical components of some plants as repellents against *Anopheles gambiae* Species Siblings (Mosquitoes) In Batagarawa Local Government Area, Katsina State, Nigeria

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Abstract

Anopheles gambiae (mosquitoes) are primary vectors of malaria in Africa, transmitting the disease to millions of people annually. The use of synthetic repellents has been effective in preventing mosquito bites, but concerns about environmental pollution, toxicity, and resistance development necessitate the exploration of alternative, eco-friendly solutions. In this study the phytochemical components of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract as potential repellents against *Anopheles Gambia* were investigated. The phytochemical extract of these plants was collected, their chemical composition analyzed using gas chromatography-mass spectrometry (GC-MS) and their repellent activity evaluated through laboratory and field experiments. The results show that specific phytochemicals, such as limonene, beta-pinene, and gamma-terpinene, exhibit significant repellent effects against *Anopheles gambiae* (mosquitoes) with varying degrees of efficacy depending on the plants species and concentration. The ecological implications of using plants-based repellents were also identified, including potential impacts on mosquito behavior and resistance development. Furthermore, we examined the effects of plants extracts on mosquito oviposition, larval development, and adult emergence which shows a significant effective on all the stages. This research highlights the potential of plants-derived phytochemicals as environmentally friendly and sustainable mosquito repellents, contributing to malaria prevention strategies in Africa. The findings of this study have significant implications for the development of novel, plant-based mosquito repellents and provide insights into the ecological dynamics of *Anopheles gambiae* (mosquitoes) in response to natural repellents.

Keywords: Efficacy; Repellent; Phytochemical; *Anopheles gambiae* Mosquitoes; Neem (*Azadirachtolides*); Citronella (*Cymbopogon Nardus*); Lemongrass (*Cymbopogancitratus*)

1. Introduction

Malaria remains a significant public health challenge in Africa, with *Anopheles gambiae* mosquitoes being the primary vectors responsible for transmitting the disease. Malaria is a life-threatening disease transmitted through the bite of infected female Anopheles mosquitoes and caused by parasite species of the genus Plasmodium. In the tropical part of the world, the main malaria parasite is Plasmodium falciparum which is transmitted by the *Anopheles gambiae* complex (Service, 2004). Malaria is the most highly prevalent parasitic infection in sub-Saharan Africa, which results in high morbidity and mortality, economic and social impact, premature death, reduced productivity and huge medical cost (Kayode et al.,2003). In another development, despite the progress in malaria control efforts by different organizations and researchers, the disease continues to claim hundreds of thousands of lives annually. Particularly in sub-Saharan Africa. The World health organization (2022) reported that, about half of the world population is at risk of being infected with malaria. In 2021, there were approximately 247 million cases and 619, 000 deaths due to malaria worldwide. WHO African regions accounted for 95% of the global malaria case and Nigeria accounted for 27% of malaria cases and 31%

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death due to malaria (WHO, 2022). Vector controls are imperative strategies in the reduction of disease transmission. presently, mosquito vector control majorly depends on the use of long-lasting insecticide treated mosquito nets (LLIN/ITNs) and indoor residual spray (IRS) in some rural communities. Although, WHO recommends universal coverage of Insecticide Treated Nets (ITNs) specifically, Long Lasting Insecticidal Treated Nets (LLINs) and Indoor Residual Spray (IRS) as core interventions in malaria-endemic countries to reduce global malaria burden in 2030 by 90% globally (Federal Ministry of Health, 2015). nevertheless, the increase of insecticide resistance is threatening the effectiveness and sustainability of this control method (Keita et al., 2016; Thaiwa et al., 2018).

The widespread use of synthetic insecticides and repellents has contributed to the development of resistance in mosquito populations, compromising the efficacy of these interventions. Furthermore, concerns about environmental pollution and toxicity associated with synthetic chemicals have sparked interest in exploring alternative, eco-friendly solutions for mosquito control. Plant-based repellents have been used for centuries in various cultures, offering a promising avenue for sustainable mosquito management. Citrus plants, in particular, have been recognized for their insect-repellent properties, attributed to their rich phytochemical composition. The peels and essential oils of citrus fruits, such as oranges, lemons, and limes, contain a complex mixture of compounds, including limonene, beta-pinene, and gamma-terpinene, which have shown potential as mosquito repellents.

This study aims to investigate the efficacy of phytochemical components of plants extracts as repellents against *Anopheles gambiae* mosquitoes, with a focus on understanding the ecological implications of using these natural compounds. By exploring the repellent activity of plants-derived phytochemicals, this research seeks to contribute to the development of novel, environmentally friendly, and sustainable mosquito repellents that can be integrated into malaria prevention strategies in Africa.

1.1. Study area

The study was conducted in Bananarama Local Government Area of Katsina State, Northern Nigeria. Katsina State is located between latitudes 11°08′N and 13°22′N and longitudes 6°52′E and 9°20′E. The state covers an area of 23,938 sq km and lies in the Northern Nigerian Sahelian Savannah. It is bordered by Niger Republic to the North, Jigawa and Kano States to the East, Kaduna State to the South and Zamfara State to the West. The state has 34 Local Government areas.

Bananarama Local Government Area in Katsina state covered an area of 433 square kilometers. It shares borders with other LGAs in the state and has a tropical savanna climate with average temperatures of the area covers the range from 31.43°C to 35°C during the seasons.

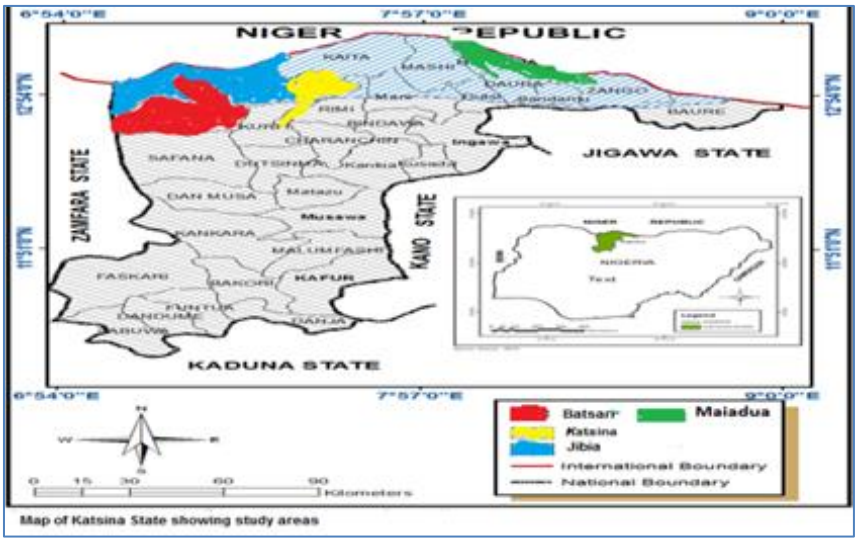

Figure 1 Map of Katsina State, showing the study area

1.2. Population and Demographics

As of 2006, the population of Bananarama LGA was approximately 184,575, with a projected population of around 337,900 in 2022. The area is predominantly inhabited by Hausa people, with a mix of farming and trading activities.

1.2.1. Study Design

This study employed a Completely Randomized Design (CRD) and combined field and laboratory experiments to investigate the efficacy of plant extracts against *Anopheles gambiae* mosquitoes. The research design consisted of the following key components:

- To prepare and evaluate the phytochemical composition of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extracts for potential mosquito control properties.
- To identify and characterize Anopheles mosquito breeding sites in the study area and collect larvae for further study.
- To rear *Anopheles gambiae* mosquito larvae to adulthood in a controlled environment and study their biology.
- To confirm the species identity of *Anopheles gambiae* mosquitoes through morphological identification.
- To assess the susceptibility of Anopheles mosquitoes to commonly used insecticides, including Dieldrin, Lambda cyhalothrin, Bendiocarb, and Malathion.
- Adulticidal efficacy assessment: Investigation of the adulticidal effects of plant extracts against *Anopheles gambiae* species (mosquitoes).

2. Materials and methods

2.1. Sample Collection and Preparation

Selected plants were collected, cleaned and dried under the shed of tree, kept in a container and transferred to the laboratory for extraction and analysis

2.2. Mosquito Larval Collection and Rearing

Anopheles mosquito larvae were collected from natural breeding sites and reared in the laboratory according to standard protocols.

Adult mosquitoes from the study area were collected using the pyrethrum spray catch/ collection (PSC) method as described by (WHO, 2021a).

2.3. Morphological Identification *Anopheles gambiae* Mosquito

All *Anopheles gambiae* mosquito collected indoor and those exposed to insecticides impregnated papers and ITNs were morphologically identified using Afrotropical Anopheline morphological taxonomic keys as updated by Coetzee, (2020a). The morphological features were observed with the aid of X10 Zeiss light stereo microscope and LCD digital microscope (1-1200x, China). The morphological feature includes; absence of lateral abdominal tufts of hair, speckled legs, 3- banded palps and pale interruptions on the 3rd main dark area of vein one of the wings. Individual identified mosquitoes were placed in 1.5 ml Eppendorf tubes containing silica gel plugged with tissue paper and kept at the Federal College of Education Katsina Laboratory.

2.4. Phytochemical Analysis

Preliminary phytochemical screening was conducted to identify the major bioactive compounds present in the plant extracts, utilizing established methods.

2.5. Adulticidal Bioassay

The adulticidal efficacy of crude plant extracts was evaluated using a modified version of the World Health Organization (WHO) protocol (1998)

The phytochemical analysis of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract revealed the presence of various bioactive compounds, including flavonoids, Alkaloids, Saponins, etc. The results, summarized in Table 1, 2 and 3, show that certain chemical constituents were detected, while others were absent, providing insight into the extract's potential adulticidal properties against *Anopheles gambiae* mosquitoes.

3. Result and discussion

Table 1 Qualitative analysis of the phytochemicals content of Neem (Azedarach indica)

Phytochemicals	**Neem (*Azedarach indica*)**
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins	-
Paleobotanies	-
Terpenoids	+
Cardiac glycoside	-
Reducing Sugar	-
Limonoids	+
Sterols	+
Gallic acid	+
Catechins	-
Triterpenoids	+
Vitamin C	+
Phenolic compounds	+
Essential oils -	+

Table 2 Qualitative analysis of the phytochemicals content of Citronella (*Cymbopogon Nardus*)

Phytochemicals	**Citronella (*Cymbopogon Nardus*)**
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins	-
Paleobotanies	-
Terpenoids	+
Cardiac glycoside	-
Reducing Sugar	-
Limonoids	+
Sterols	+
Gallic acid	+
Catechins	-
Triterpenoids	+
Vitamin C	+
Phenolic compounds	+
Essential oils -	+

Table 3 Qualitative analysis of the phytochemicals content of Lemongrass (*Cymbopogancitratus*)

Phytochemicals	**Lemongrass (*Cymbopogancitratus*)**
Alkaloids	+
Flavonoids	+
Saponins	-
Tannins	-
Paleobotanies	-
Terpenoids	+
Cardiac glycoside	-
Reducing Sugar	-
Limonoids	+
Sterols	+
Gallic acid	+
Catechins	-
Triterpenoids	+
Vitamin C	+
Phenolic compounds	+
Essential oils -	+

The phytochemical screening of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract revealed a diverse range of bioactive compounds, including alkaloids, flavonoids, saponins, terpenoids, limonoids, sterols, gallic acid, triterpenoids, vitamin C, phenolic compounds, and essential oils. The presence of these compounds suggests that Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract may possess various biological activities, including antioxidant, anti-inflammatory, and insecticidal properties.

The detection of flavonoids, phenolic compounds, and vitamin C in the extract is consistent with previous studies that have reported the antioxidant activity of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*). These compounds may contribute to the extract's potential health benefits and insecticidal activity.

The presence of terpenoids, limonoids, and essential oils in the extract is also noteworthy, as these compounds have been reported to possess insecticidal and repellent properties. The essential oils, in particular, may play a role in the extract's adulticidal activity against *Anopheles gambiae* mosquitoes.

The detection of alkaloids, saponins, and sterols in the extract suggests that Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) may possess a range of biological activities, including anti-inflammatory and antimicrobial properties. Gallic acid, which was also detected in the extract, has been reported to possess antioxidant and antimicrobial activities.

Overall, the phytochemical profile of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract suggests that it may be a valuable source of bioactive compounds with potential applications in medicine, agriculture, and public health. The results of this study provide a foundation for further research into the biological activities and potential uses of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*).

3.1. Adult Mortality Results

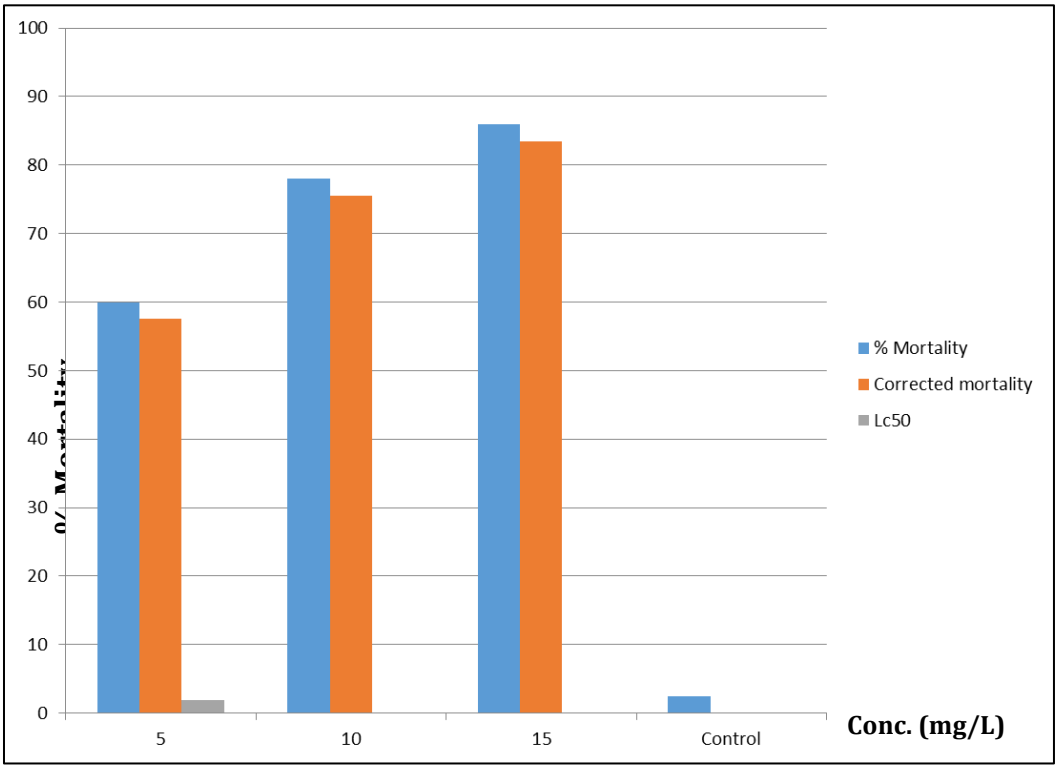

Figure 2 Chart Adult mortality at different concentration of Neem (Azedarach indica) from 24 to 192h at different concentrations (5, 10, and 15 mg/L)

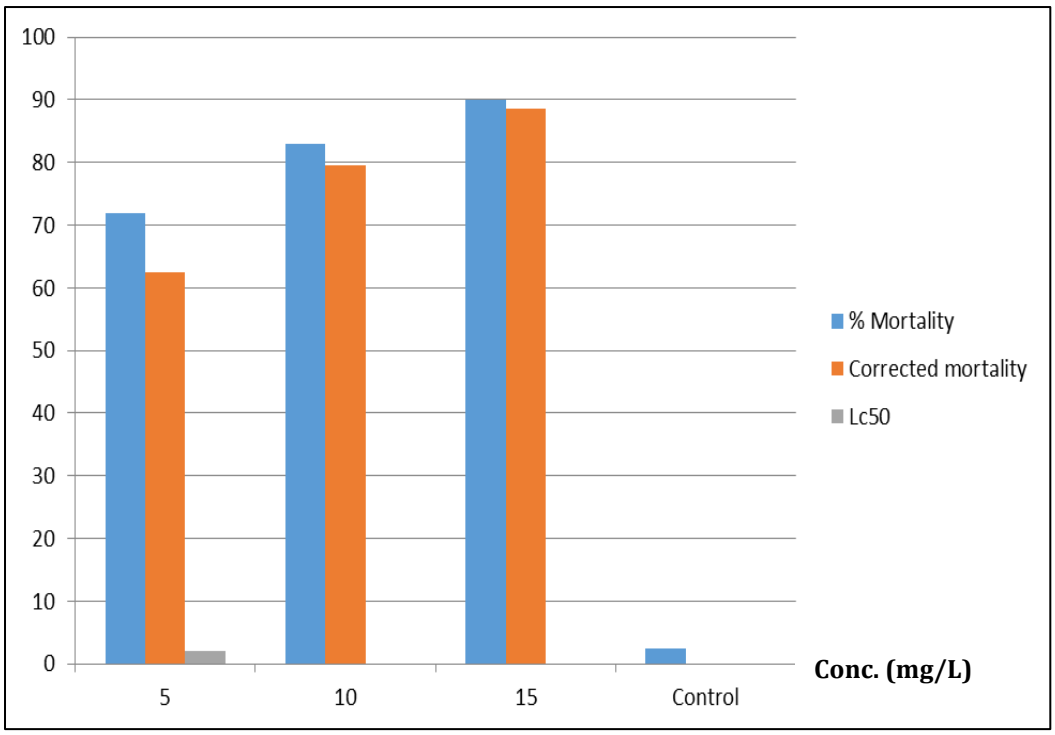

Figure 3 Chart Adult mortality at different concentration of Citronella (*Cymbopogon Nardus*) from 24 to 192h at different concentrations (5, 10, and 15 mg/L)

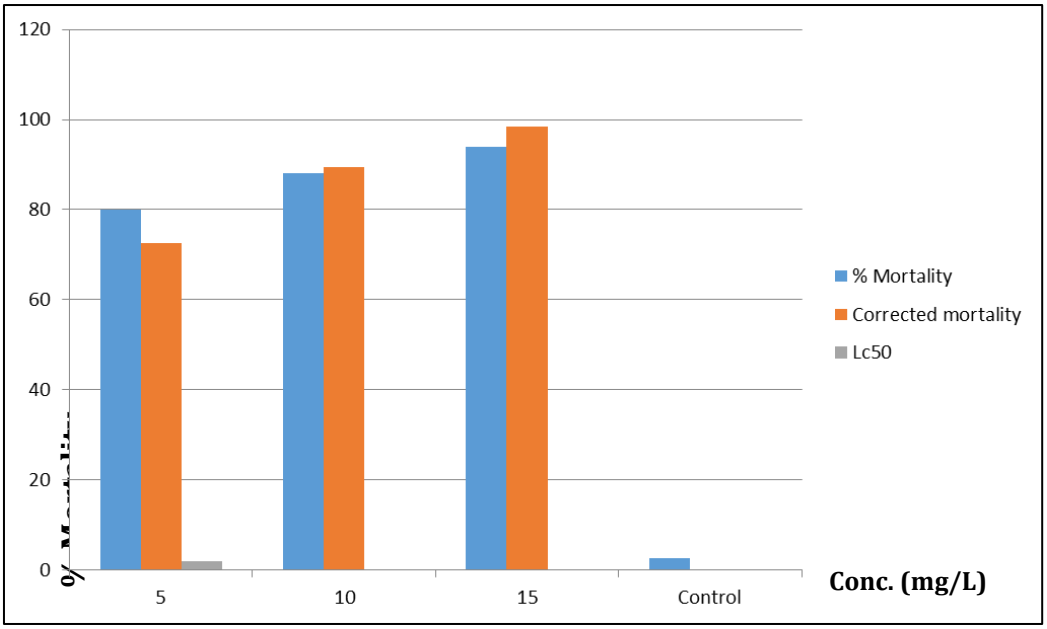

Figure 4 Chart Adult mortality at different concentration of Lemongrass (*Cymbopogancitratus*) from 24 to 192h at different concentrations (5, 10, and 15 mg/L)

The results of the adulticidal activity of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract against *Anopheles gambas* mosquitoes show a clear dose-response relationship. As the concentration of the extract increases, the percentage mortality of the mosquitoes also increases.

3.2. Key Findings

For Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*), at a concentration of 5 mg/L, the extract induced 60%, 72% and 80% mortality in the mosquito population. Increasing the concentration to 10 mg/L resulted in a significant increase in mortality, with 78%, 83% and 88% of the mosquitoes killed. The highest concentration tested, 15 mg/L, resulted in the highest mortality rate, with 86%, 90% and 94% of the mosquitoes killed.

3.3. Implications

The findings suggest that Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract have potent adulticidal activity against *Anopheles gambiae* mosquitoes, which are the primary vectors of malaria in many tropical regions. The dose-dependent increase in mortality rates indicates that the extract's efficacy can be optimized by adjusting the concentration.

3.4. Potential Applications

The results of this study have implications for the development of natural insecticides for vector control. Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract may offer a promising alternative to synthetic insecticides, which can have negative environmental and health impacts.

4. Conclusion

The study demonstrates the potential of Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract as a natural adulticide against *Anopheles gambiae* mosquitoes. The extract showed a dose-dependent increase in mortality rates, with higher concentrations resulting in higher mortality. These findings suggest that Neem (*Azadirachtolides*), Citronella (*Cymbopogon Nardus*) and Lemongrass (*Cymbopogancitratus*) extract could be a promising alternative to synthetic insecticides for vector control.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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