LARVICIDAL EFFICACY OF ACETONE EXTRACT OF ONION (*Allium cepa*) AGAINST ANOPHELES GAMBIAE LARVAE IN ENUGU METROPOLIS, NIGERIA

BY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES, GODFREY OKOYE UNIVERSITY, UGWUOMU NIKE, ENUGU. ENUGU STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF SCIENCE (B.Sc), DEGREE IN BIOTECHNOLOGY

SUPERVISOR: DR AMADI NNAMDI

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JULY, 2026

APPROVAL

This research titled “Larvicidal Efficacy of Acetone Extract of Onion (Allium cepa) against Anopheles Gambiae Larvae in Enugu metropolis, Nigeria ” has been assessed and approved by the Committees of Department of biological sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University Enugu Nigeria.

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CERTIFICATION

This is to certify that this research titled "Larvicidal Efficacy of Acetone Extract of Onion (Allium cepa) against Anopheles Gambiae Larvae in Enugu metropolis, Nigeria " was carried out by Wanogho Ejovwokoghene Charles under the supervision of Dr. Amadi Nnamdi in the Department of Biological Sciences, Godfrey Okoye University, Enugu, Nigeria.

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DEDICATION

This work is dedicated to God Almighty, who has been my ultimate source of strength and guidance. Through every challenge and triumph, His divine hand has sustained me, illuminating the path and making this achievement possible.

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ABSTRACT

Malaria continues to be one of the major health challenges in Nigeria and in most parts of sub-Saharan Africa due to the prevalence of Anopheles gambiae mosquito as the main vector that transmits the disease. The increase in resistance of mosquitoes to artificial insecticides has led to reduced efficacy of conventional vector control strategies, hence the demand for more environmentally friendly and safer measures. In this research, the larvicidal effect of acetone extract of onion (Allium cepa) on larvae of Anopheles gambiae sensu lato collected from Emene and Thinkers Corner in Enugu metropolis, Nigeria, was studied. Mosquito larvae were collected from their natural habitats such as stagnant pools, gutters, ponds, and drainage channels and kept in laboratory conditions. The onions were extracted through a Soxhlet apparatus using acetone. The extracts were used at the following concentrations: 10%, 20%, 30%, 40% and 50%. A total of 100 third and fourth instar larvae were exposed to each of the concentrations in four replicates, with distilled water serving as the control. The rate of larval mortality was observed at various intervals of time and the results subjected to analysis of variance (ANOVA). Mean mortality ranged from 61.67% at 10% concentration to 87.50% at 50%, with complete mortality achieved after 50 minutes of exposure. The overall mean mortality was 81.84 ± 35.42%, and larvae from Thinkers Corner showed slightly higher mortality than those from Emene. Statistical analysis indicated that location, concentration, exposure time, and their interactions significantly influenced larval mortality ($p < 0.05$). The findings suggest that Allium cepa extract is a promising, low-cost botanical larvicide with potential for inclusion in integrated malaria vector management programmes.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Malaria, remains one of the leading causes of death from infectious diseases globally, responsible for an estimated 405,000 fatalities every- year, with over half of the world's population at risk of infection (Haileselassie et al., 2022). Despite several intervention programs and control measures implemented over the years, there has been little noticeable reduction in malaria cases over the past two decades. Reports also indicate that, globally, the number of malaria cases has shown no substantial reduction within the last five years (WHO, 2020). Furthermore, the disruption caused by the COVID-19 pandemic has negatively affected ongoing malaria control programs, making it unlikely that the goals described in the World Health Organization's Global Technical Strategy for Malaria 2016–2030 will be fully realized (Feachem et al., 2019; World Health Organization, 2020; World Health Organization, 2020).

Efficacious preventive medicines and vaccines for the prevention and control of various vector transmitted diseases are not accessible or are inadequate in many regions of the world. Thus, chemical based vector control tactics remain an important tool in disease prevention and management (Bhatt et al., 2015; World Health Organization, 2015).

In 2018, the United States President's Malaria Initiative funded Indoor Residual Spraying (IRS) in more than 5.7 million households as part of worldwide malaria intervention efforts (Haileselassie, 2022). In addition, about 1.8 billion pyrethroid-treated bed nets were delivered worldwide from 2010 to early 2020, with almost 1.6 billion of those going to nations in sub-Saharan Africa (Nonè, 2025).

However, the extensive use of pesticides has exerted a tremendous selective pressure on mosquito populations that favor the formation and spread of insecticide resistance. Now resistance to most pesticides certified by the World Health Organization for public health use has been reported in various parts of the globe (Moyes et al., 2021; Ranson et al., 2011).

Resistance among malaria vectors, particularly among Anopheles mosquitoes, is becoming a serious public health problem. According to reports, mosquitoes from several malaria-endemic locations have developed resistance to one or more pesticide classes. The World Health Organization (2010) reported that resistance had been documented in approximately 90% of malaria-endemic countries, with 32% reporting resistance to all four main classes of insecticides recommended for vector control up to 2016 (pyrethroids, carbamates, organophosphates and organochlorines).

More recently resistance to clothianidin, one of the newest active components used in indoor residual spraying (IRS) products and which is regarded particularly efficient for insecticide-resistance management, has also been detected in portions of Central Africa (Makoni, 2020). Evidence further suggests a growing trend of rapid development of insecticide resistance following repeated exposure. This situation is worrisome because it limits the available chemical options for vector control and threatens the effectiveness of existing and future interventions aimed at controlling disease vectors

(Wilson et al., 2020).

Malaria is still a great cause of sickness and death in many underdeveloped nations, notably in sub-Saharan Africa. The major carriers of the disease are Anopheles mosquitoes. The increasing resistance of these vectors to current synthetic pesticides has created an urgent need for new, environmentally acceptable and sustainable vector control solutions. In recent years, plant-derived bioactive chemicals have attracted interest owing to their insecticidal, larvicidal and repellent activities against mosquito vectors.

Onion (Allium cepa) is an extensively grown vegetable which has a wide range of naturally existing phytochemicals such as flavonoids, phenols, and organosulfurs. These bioactive compounds have gained the attention of many scientists owing to their insecticidal and biological properties. Previous research works have established that extracts from various plant species have the capability to inhibit the survival, feeding, and reproductive abilities of mosquitoes. Onion extract is among those natural products whose ability to control mosquitoes has been studied lately as more and more people are looking for safe natural products as an alternative to artificial insecticides. However, not much information exists on the effectiveness of onion extract in controlling Anopheles mosquitoes. Understanding the genetic mechanisms responsible for resistance in mosquito populations is essential for effective vector management. Resistance genes in Anopheles mosquitoes may influence the effectiveness of both synthetic insecticides and plant-based control agents. Therefore, identifying and characterizing resistance genes associated with exposure to onion extract will provide important information for developing alternative malaria vector control approaches.

Improved vector control tactics and constant monitoring of pesticide resistance patterns in mosquito populations are needed to properly combat this threat. Monitoring the susceptibility status of vectors remains essential for understanding the contribution of resistance to the recent decline in malaria control and eradication efforts (Wilson et al., 2020).

Heavy usage of insecticides for agricultural, livestock production and public health programs has contributed significantly to the increasing difficulty of insecticide resistance in malaria control efforts (Rivero et al., 2010). Mosquitoes, which serve as vectors of several human diseases, are responsible for transmitting infections like malaria, yellow fever, dengue fever, hemorrhagic fever, filariasis and encephalitis (Okorie et al., 2011).

These vector-borne diseases continue to pose serious public health problems and have contributed to increased morbidity and mortality, especially among vulnerable populations. In Nigeria alone, the economic burden associated with malaria treatment and prevention has been estimated at about 132 billion naira (Jimoh et al., 2007; FMOH/NMCP, 2009).

To reduce the effect of malaria, public health authorities in Nigeria have introduced several malaria control and eradication programs (Jimoh et al., 2007). Control of malaria in Nigeria is based on vector control measures such as use of Long Lasting Insecticide Nets (LLINs) and Indoor Residual Spraying (IRS). Other methods include intermittent preventive therapy (IPT), which involve elimination of mosquito breeding sites through environmental management (Bloland et al., 2002).

While these interventions have been part of the process that has helped reduce the prevalence of malaria in many of the affected regions, this condition continues to remain one of the most important public health problems. In spite of measures being implemented to tackle the problem, the ongoing transmission of malaria, as well as the development of resistant mosquito populations to the available insecticides, among others, has hampered the success of the initiatives for controlling malaria. Consequently, malaria still remains an endemic problem in many places across the world, especially sub-Saharan Africa (WHO, 2015).

1.2 Statement of the Problem

Effective management of malaria requires minimizing the interaction between humans and mosquito vectors through effective vector control. Chemical insecticides have for a long time been used as the main strategy in such programs. The emergence and spread of insecticide resistance in Anopheles mosquitoes in Africa have proved to be a serious challenge in the efforts to maintain the progress made in the fight against malaria. In Nigeria, vector control programs are still focused on the widespread use of LLINs and IRS. Although this has led to the decline in the incidence of malaria, there are inadequate data regarding the status, distribution, and genetics of insecticide resistance in mosquito population in many parts of the country.

Furthermore, there is a growing need to investigate environmentally sustainable approaches that can complement existing vector control methods. The use of synthetic pesticides has led to the development of resistance in mosquito populations and also prompted concerns on environmental contamination, toxicity to non-target creatures and human health risks. This situation highlights the need to explore safer and more sustainable mosquito control options, including the use of plant-derived extracts with insecticidal properties.

Onion (Allium cepa) extract has been reported to contain bioactive compounds that may possess insecticidal and repellent activities against mosquitoes. However, there is insufficient scientific information regarding its efficacy and the probable resistance reaction of Anopheles mosquitoes to onion extract under local conditions. Therefore, this work is necessary to access the effectiveness of onion extract against Anopheles mosquitoes and to determine its potential role as an alternative strategy for malaria vector control in Nigeria.

1.3 Aim

To evaluate the larvicidal efficacy of acetone extract of onion (Allium cepa) against Anopheles gambiae s.l. larvae in Emene and Thinkers Corner, Enugu Metropolis, Nigeria.

1.4 Objectives

The objectives were to:

1. collect and identify Anopheles gambiae s.l. larvae from Emene and Thinkers Corner in Enugu Metropolis.
2. determine the mortality rate of Anopheles gambiae s.l. larvae exposed to

different concentrations of acetone onion extract.

3. compare larval mortality across concentrations, exposure periods, and locations

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Malaria and Malaria Vector Control

Malaria remains one of the most serious public health challenges, particularly in low- and middle-income countries. The disease is caused by protozoan parasites of the genus Plasmodium, which are carried from person to person by the bite of an infected female Anopheles mosquito. Although several interventions have been employed over time, malaria remains endemic in tropical and subtropical areas. Sub-Saharan Africa is one of the regions with a high prevalence of malaria infection due to its favorable weather, which is conducive to mosquito breeding. It remains one of the major causes of morbidity and mortality in many African nations (World Health Organization, 2024). Even though interventions have been going on for some time now, the disease malaria remains an enormous health burden across the world. In 2023 alone, the number of malaria cases was estimated to be around 263 million with the number of deaths estimated to be around 597,000 deaths, out of which 95% were from Africa. Some of the countries that have been heavily affected include Nigeria, owing to poor environmental sanitation, limited quality healthcare services and insecticide resistance in mosquitoes.

The vector control method plays an essential role in the prevention of malaria disease and may be attained using various measures like long-lasting insecticide-treated bednets, indoor residual spray, environmental management, and elimination of mosquito breeding sites. While these methods have played an important role in the reduction of transmission of the malaria infection in many parts of the world, their sustainability is now at stake due to the emergence of insecticide resistance among Anopheles mosquitoes (Hemingway et al., 2016).

2.2 Insecticide Resistance in Anopheles Mosquitoes

Insecticide resistance has been identified as one of the biggest challenges in the fight against malaria vectors. According to the World Health Organization (2010), it refers to the capability of mosquitoes to withstand exposure to insecticides meant to kill them. The report indicated that in more than 90% of the malaria-endemic countries, resistance to at least one type of insecticide used in the vector control has been observed.

There are different factors that contribute to the development of insecticide resistance in mosquitoes. The constant and repetitive use of synthetic insecticides in agriculture and public health settings creates high selection pressure on insects, only those with the trait of being resistant survive. Over time, these mosquitoes reproduce and transfer the trait to their progeny. As mentioned by Riveron et al. (2019), the evolution of insecticide resistance has tremendously limited the efficacy of the most common groups of insecticides including pyrethroids, carbamates, organophosphates, and organochlorines in malaria control.

From the research studies conducted in Nigeria and across the African continent, it is evident that the resistance in Anopheles gambiae population is a prevalent phenomenon. For example, Awolola et al. (2007) have found pyrethroid resistance in mosquito population in the southwestern region of Nigeria. Oduola et al. (2010) have also found the same resistance pattern in Lagos State, Nigeria.

There are different strategies used by mosquitoes to counteract the effects of insecticides. These strategies can be classified as metabolic resistance, target-site resistance, behavioral resistance, and cuticular resistance. Metabolic resistance refers to the enhanced function of enzymes which metabolize insecticides before they act at target sites within the body of the mosquito.

Behavioral resistance entails modification of behavior of the mosquito which interferes with its contact with the insecticide.

Cuticular resistance is due to change of the mosquito cuticle, which results in a reduction of insecticide penetration. However, target-site resistance remains one of the most studied resistance types. This resistance is often accompanied with changes in the voltage-gated sodium channel (VGSC) gene, known as knockdown resistance (kdr) mutations that decrease the sensitivity of mosquitoes to pyrethroids and DDT. Since the discovery of kdr mutations in Anopheles gambiae, genetic markers have shown to be invaluable tools to monitor the prevalence and strength of pesticide resistance in malaria vector populations. Recent studies have shown the increasing prevalence of resistance-associated mutations across Africa, underscoring the necessity for continued surveillance and development of alternate vector control measures to sustain malaria control efforts (WHO, 2023; Cleanclay et al., 2026).

2.3 Environmental and Health Concerns Associated with Synthetic Insecticides

The utilization of synthetic pesticides has been a very important factor in the elimination of malaria, but there are various environmental and health concerns due to the long-term and unselective utilization of these pesticides. Synthetically produced insecticides have a very long shelf life in the environment and thus result in water pollution as well as harmful effects on other living things like birds and mammals. Mossa (2016) reported that many synthetic insecticides are toxic to humans, and repeated exposure may contribute to respiratory complications, neurological disorders, and disruptions in the endocrine system. In addition, the continuous accumulation of pesticide residues in the environment can reduce biodiversity and destabilize ecosystem functions.

The increasing problem of insecticide resistance has further compelled public health authorities to either raise application doses or frequently alternate between different classes of insecticides. These adjustments often result in higher operational and program costs. As a result, there is a growing focus on exploring alternative vector control strategies that are environmentally friendly, biodegradable, affordable, and sustainable in the long term.

2.4 Plant-Based Insecticides as Alternative Vector Control Agents

Plant-derived insecticides are increasingly being recognized worldwide as viable alternatives to conventional synthetic chemicals. Botanical extracts contain naturally occurring bioactive compounds that can interfere with mosquito survival, feeding patterns, development, and reproduction. In contrast to synthetic insecticides, many plant-based products are biodegradable and generally pose lower risks to human health and the environment.

Isman (2020) highlighted that botanical insecticides play an important role in integrated vector management due to their wide range of phytochemicals that can disrupt insect physiological processes. In a similar view, Pavela and Benelli (2016) reported that plant extracts may help reduce the likelihood of resistance development because they contain multiple active compounds that act on different biological targets in insects.

A number of medicinal plants have demonstrated both larvicidal and adulticidal activities against mosquito vectors. Govindarajan et al. (2011) found that various plant extracts significantly reduce mosquito larval survival rates. Likewise, Shaalan et al. (2005) reviewed several plant-derived compounds with mosquitocidal effects and concluded that botanical products represent promising eco-friendly alternatives for inclusion in vector control strategies.

2.5 Onion (Allium cepa) and Its Bioactive Properties

Onion (Allium cepa) is a widely cultivated plant belonging to the family Amaryllidaceae and is valued globally for both nutritional and medicinal uses. It contains a variety of biologically active constituents such as flavonoids, phenolic compounds, sulfur-containing substances, and antioxidants, which are known to exhibit antimicrobial, antifungal, insecticidal, and repellent activities.

Block (2010) described onion as a significant source of organosulfur compounds that produce a range of biological effects against both microorganisms and insect pests. In a similar perspective, Mgbeahuruike et al. (2019) noted that the bioactive constituents found in Allium species are responsible for their medicinal and pesticidal properties. Although onion extracts have demonstrated insecticidal potential in some experimental studies, there is still limited evidence regarding their efficacy against Anopheles gambiae as well as the potential for resistance development after prolonged exposure. This limitation underscores the need for further research to explore how exposure to onion extracts may influence resistance mechanisms and gene expression in mosquito populations.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Study Area

The study was conducted within Enugu Metropolis, located in southeastern Nigeria. Enugu State covers approximately 7,161 km² and serves as one of the major urban centres in the region. According to the 2006 national population census, the state had a population of over 700,000 residents. It shares boundaries with Abia State and Imo State to the south, Ebonyi State to the east, Benue State to the northeast, Kogi State to the northwest, and Anambra State to the west. The accompanying map shows the location of Enugu State within Nigeria.

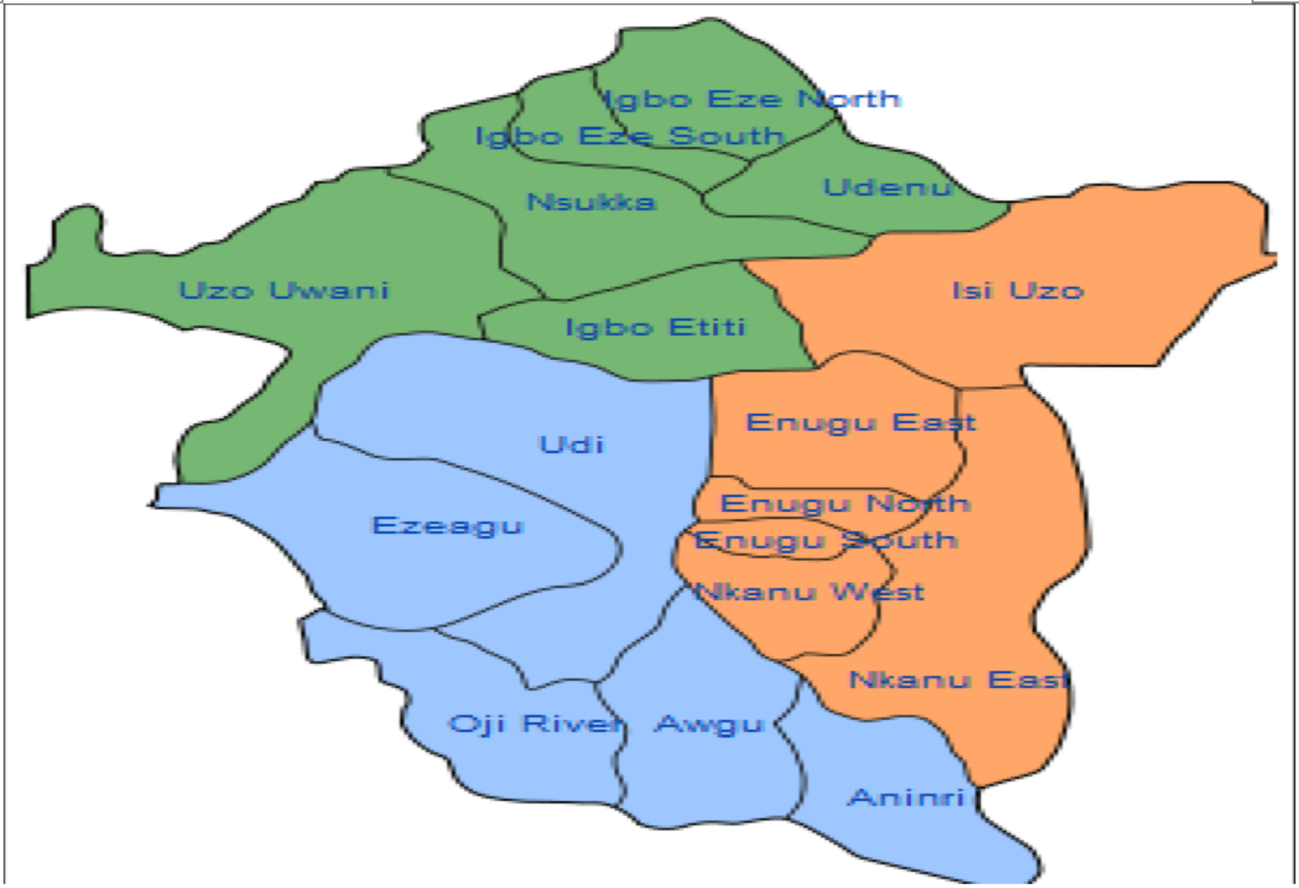

Figure 2.1 Map of Enugu State indicating the location of the 17 local government areas
Source: (Ukabia, 2010).

3.2 Study Design

The field and laboratory experimental designs were used in this study between March to May (2026).

3.3 Sampling Technique

Standard Operating Procedure for Anopheles larval and pupal survey.

This SOP specifies materials and methods for conducting larval surveys of anopheline mosquitoes.

Description: Larval sampling involves collecting juvenile mosquitoes from aquatic areas (Silver, J.B. 2008).

Target species and physiological states: Traps larvae and pupae of anopheline mosquitoes (Silver, J.B. 2008).

Entomological surveillance indicators : Larval sampling is performed for all immature vector indices, and larvae are reared and utilized as specimens for pesticide resistance bioassays (Silver, J.B. 2008).

Larvae may record early or late instars (4 developmental periods or instars).

3.4 Materials

350 ml dipper with handle.

5L plastic container (gallon).

3 ml plastic pipette.

Pencil /markers.

White tray.

Digital device and data collection form (Collins et al., 2018).

3.5 Description of Anopheles larval habitats

1. Mosquito larvae were detected at the water surface, usually near vegetation or floating trash. They generally occur on the margins of larger pools and ponds and not in open deep water (Collins et al., 2018).
2. Anopheline larvae were commonly recovered from a range of environments including transient pools, ground pools, pig wallows, wheel tracks, brackish swamps, freshwater swamps, streams, drains and ditches. Aquatic environment preferences of several mosquito larval species (Collins et al., 2018).
3. Searches for suitable aquatic habitats were performed over the entire village area to assess availability and occupancy. (Collins et al., 2018).

3.6 Sampling procedure (Collins et al., 2018).

- We gathered all materials required.
- We identified a potential habitat:
    - We moved slowly, step gently, so as not to shake the water or disturb the vegetation more than is required.
    - The water was approached against the sun so that no shadow should be cast on the water.
    - Dipping was done in severe winds on the windward side of the habitat where larvae and pupae are expected to be concentrated most heavily.
    - We looked for larvae and pupae before we started dipping.

Larval Collection Procedure

4. Larvae were collected using a standard mosquito dipper.

- For effective sampling of Anopheles larvae, the dipper was held at an angle of approximately 45° and gently lowered about 3 cm below the water surface before being moved steadily toward visible larvae.
- Sampling effort was standardized across all breeding sites by maintaining a consistent number about 5-10 of dips at each location.
- A waiting period of about two minutes was observed between successive dips to allow larvae that had submerged to return to the water surface.

4. Water was removed from the aquatic habitat to minimize future disturbance to the habitat.

5. The collected samples were poured into the plastic container with the help of the funnel.

6. Some of the water from the environment was saved for the transported larvæ.

7. Collected samples were clearly labelled (Collins et al., 2018).

3.7 Mosquitoes Larvae Sample Collection-

Larva collections in all the 2 locations (Emene) Thinkers Corner and Abakpa were carried out utilizing the procedures outlined above and Entomological collection techniques utilized as conventional mosquito collection techniques. Mosquito larvae and pupae were sampled at random from selected breeding habitats within the study locations. Collections were carried out weekly between 8:00 a.m. and 10:00 a.m., with sampling schedules adjusted according to prevailing rainfall conditions. The breeding habitats surveyed included drainage channels, ponds, temporary stagnant pools, muddy water bodies, and water accumulations resulting from household and agricultural runoff.

Anopheles larvae were identified in the field by their characteristic habit of resting parallel to the water surface, whereas larvae of other mosquito species typically remained suspended at an angle. The immature stages were carefully collected using a 350 mL dipper and transferred into 500 mL plastic containers with loosely fitted covers to ensure adequate air circulation during transportation.

The collected specimens, together with water from their breeding habitats, were transported to the laboratory and insectary facilities of the Department of Microbiology, Godfrey Okoye University, where they were maintained under controlled conditions and reared to the adult stage for further investigation.

3.8 Study inclusion

Mosquitoes (females Anopheles) larval that are causative agents of malaria.

3.9 Exclusion criteria

Anopheles mosquitoes was picked.

3.10 Laboratory Rearing of Mosquitoes

Larval development was examined routinely and all pupated specimens were collected with Pasteur pipettes into shallow beakers and stored in adequately labeled cages for adult emergence (Imam et al., 2014). The larvae were fed on Oxford Cabin biscuit and yeast and the adults were fed 10% sugar solution (Imam et al., 2014).

3.11 Laboratory Analysis for Determining the Larvicidal Activity of Onion (Allium cepa) Extract

Fresh bulbs of onion (Allium cepa) were obtained at a local market in Enugu Metropolis. The bulbs were cleansed carefully in distilled water to eliminate dirt and other impurities. These were chopped up into little pieces and air-dried at room temperature for two weeks to consistent weight. The dried onion samples were pulverized to fine powder using an electric blender.

The powdered onion sample was extracted using a Soxhlet extraction apparatus. The powdered onion (about 200 g) was put into a thimble and extracted with the solvent, acetone, for 6–8 h. The extraction was carried out until the solvent in the siphon tube ran clear, indicating that the extraction was complete. The acetone extract obtained was concentrated by rotary evaporator and dried in water bath at 40°C to eliminate residual solvent. Crude extract was stored in sealed containers at 4°C until use.

The extraction was prepared at different concentrations as follows:

- ❖ 10% concentration: 10 mL extract + 90 mL distilled water
- ❖ 20% concentration: 20 mL extract + 80 mL distilled water
- ❖ 30% concentration: 30 mL extract + 70 mL distilled water
- ❖ 40% concentration: 40 mL extract + 60 mL distilled water
- ❖ 50% concentration: 50 mL extract + 50 mL distilled water

For each concentration, 25 larvae in four replicates were prepared instar Anopheles gambiae s.l. larvae were introduced into containers containing 100 mL of each prepared concentration. A control experiment containing only distilled water was also set up using 25 larvae each experiment.

After being exposed to the extract, the larvae were monitored at 10, 20, 30, 40, and 50 minutes. Larvae that showed no movement after gentle probing with a glass rod were considered dead.

The following formula was used to determine the percentage mortality:

The formula for percentage mortality is (number of dead larvae × total number of larvae exposed) × 100.

Where:

- Number of Dead Larvae = Total number of larvae that died after exposure.
- Total Number of Larvae Exposed = Total number of larvae introduced into the test solution.

For example, if 20 larvae died out of 25 larvae exposed:

Percentage Mortality (%) =

$(20 \div 25) \times 100$

$= 80\%$

The mortality rates obtained at different concentrations and exposure periods were recorded and compared to determine the larvicidal efficacy of the onion extract against Anopheles gambiae s.l. larvae.

3.13 Microscopy

The mosquito's morphological identification was done using the Leica identification microscope to be sure that vectors tested were anopheles' species.

Data analysis.

Excel descriptive and Three-way ANOVA were used to analyze the data. The WHO's 1998 recommendations were followed for evaluating the pesticide susceptibility bioassay results.

CHAPTER FOUR

RESULTS

4.1

Susceptibility test results of the i dentified mosquitoes

The results of susceptibility tests showed that resistance, intermediate and susceptible among the mosquitoes tested indicate resistance at less than 90% mortality, intermediate for those between 90%- 97% mortality and susceptible for those greater than or equal 98% mortality (98%-100%).

4.2 Morphological identification

Under a Leica ide microscope, morphological identification was carried out utilizing the Gilles and Coetzee (1978) characteristic. All Anopheles mosquitoes have a dark patch (wing spots) on the upper edges of their fore wings. The palps are long and divided into three sections, with a pale mark on the second dark section. Based on the aforementioned physical traits, the mosquitoes from the three study locations were determined to be Anopheles species (Gilles and Coetzee, 1978).


Figure 4.1: Anopheles mosquitoes viewed under the microscope

Interpretation

The overall mean mortality of Anopheles gambiae s.l. larvae exposed to acetone onion extract was 81.84 ± 35.42% . Mortality was slightly higher in Thinkers Corner (82.43 ± 35.12%) than in Emene (81.22 ± 35.84%). However, the difference between the two locations was small, with only about 1.21% higher mortality in Thinkers Corner.

Implication

This suggests that acetone onion extract produced high larval mortality in both locations. The small difference between Emene and Thinkers Corner may indicate that larvae from both sites responded similarly to the extract. However, this is only a descriptive observation; the ANOVA result is needed to confirm whether the location effect is statistically significant.

Table 4. 1. Mean mortality of Anopheles gambiae s.l. larvae exposed to acetone onion extract by location

Location	N	Mean mortality (%) ± SD	Minimum (%)	Maximum (%)
Emene	144	81.22 ± 35.84	0	100
Thinkers Corner	148	82.43 ± 35.12	0	100
Overall	292	81.84 ± 35.42	0	100

Interpretation

No mortality was recorded at 0 min, confirming that mortality occurred only after exposure to the extract. Mortality increased markedly after exposure, reaching 98.25 ± 3.26% at 3 min, 99.00 ± 2.20% at 5 min, and 100.00 ± 0.00% at 50 min. The lower mean mortality at 10 min (77.50 ± 29.25%) suggests that this time point included lower concentrations where larval death was slower.

Implication

The result indicates that exposure time strongly influenced larval mortality. The extract showed rapid larvicidal action, especially at time points linked with higher concentrations. However, because different concentrations contributed differently to each time point, the time effect should not be interpreted alone. The final conclusion should come from the three-way ANOVA testing Location × Concentration × Time.

Table 4.2: Mean mortality of Anopheles gambiae s.l. larvae exposed to acetone onion extract by exposure time

Exposure time (min)	N	Mean mortality (%) ± SD	Minimum (%)	Maximum (%)
0	40	0.00 ± 0.00	0	0
3	16	98.25 ± 3.26	88	100
5	20	99.00 ± 2.20	92	100
10	32	77.50 ± 29.25	20	100
15	24	97.83 ± 3.54	88	100
20	40	90.80 ± 18.37	44	100
30	40	97.70 ± 5.27	80	100
40	40	99.40 ± 2.13	88	100
50	40	100.00 ± 0.00	100	100
Overall	292	81.84 ± 35.42	0	100

Interpretation

Location, concentration, and exposure duration all significantly affected larval mortality, according to the three-way ANOVA, with p-values less than 0.05. With a partial eta squared of 0.997 for exposure time and 0.943 for concentration, the largest effect was found. Although location had a reduced effect size (partial eta squared of 0.076), it was still significant.

The interactions were also significant. This means the effect of concentration depended on exposure time, and the mortality pattern differed across locations; Emene and Thinkers Corner.

Implication

The result confirms that acetone onion extract caused larval mortality in a dose-dependent and time-dependent manner. Higher concentrations killed larvae faster, while lower concentrations required longer exposure. The significant location effect suggests that larvae from Emene and Thinkers Corner may not have responded exactly the same way, although the difference between both locations was small. The significant three-way interaction means that the combined effect of concentration and exposure time varied between the two locations.

Table 4.3 Three-way ANOVA showing the effects of location, concentration and exposure time on larval mortality

Source of variation	df	Mean square	F-value	p-value	Partial eta squared	Interpretation
Location	1	77.592	18.001	<0.001	0.076	Significant
Concentration	4	3898.684	904.462	<0.001	0.943	Significant
Time	8	39275.212	9111.516	<0.001	0.997	Significant
Location Concentration	x 4	12.949	3.004	0.019	0.052	Significant
Location × Time	8	15.220	3.531	<0.001	0.114	Significant
Concentration Time	x 36	894.334	207.478	<0.001	0.961	Significant
Location Concentration Time	x 21 x 32	7.826	1.815	0.019	0.148	Significant
Error	219	4.311	—	—	—	—

Model summary: $R^2 = 0.997$; Adjusted $R^2 = 0.997$.

Interpretation

Larval mortality increased with increasing concentration of acetone onion extract. The lowest mortality was recorded at 10% concentration with 61.67 ± 0.30%, while the highest mortality was recorded at 50% concentration with 87.50 ± 0.26%. Tukey HSD showed that 10% differed significantly from all other concentrations. Mortality at 30% and 40% did not differ significantly, while 40% and 50% also showed no significant difference.

Implication

This indicates a concentration-dependent larvicidal effect. However, the response began to level off from 30% upward, suggesting that very high concentrations may not produce much additional mortality once the extract has already reached near-maximum larvicidal effect.

Table 4.4 Mean larval mortality across concentrations of acetone onion extract

Concentration (%)	N	Mean mortality (%) ± SE
10	48	61.67 ± 0.30[a]
20	56	82.14 ± 0.28[b]
30	60	86.07 ± 0.27[c]

40	64	87.06 ± 0.26^{cd}
50	64	87.50 ± 0.26^{d}

Means with different superscript letters are significantly different at $p < 0.05$ based on Tukey HSD. Means sharing at least one superscript letter are not significantly different.

Interpretation

No larval mortality was observed at 0 minutes of exposure. However, mortality increased markedly following exposure to the acetone onion extract. The lowest recorded mortality after exposure was at 10 minutes (77.50 ± 0.37%), while full mortality (100.00 ± 0.33%) was achieved at 50 minutes.

The Tukey post-hoc grouping indicated a clear separation between the early exposure times (0, 10, and 20 minutes) and the later exposure periods. From 30 minutes onward, mortality levels were consistently high and gradually reached near or complete lethality. These results suggest that exposure duration has a strong effect on larval mortality. Generally, longer exposure resulted in increased mortality; however, once mortality approached 98–100%, differences between the later time intervals became minimal. Overall, the findings indicate that acetone onion extract exhibits strong and rapid larvicidal activity, particularly at extended exposure times.

Table 4.5 Mean larval mortality across exposure periods

Exposure time (min)	N	Mean mortality (%) ± SE
0	40	0.00 ± 0.33^{a}
10	32	77.50 ± 0.37^{b}
20	40	90.80 ± 0.33^{c}
30	40	97.70 ± 0.33^{d}
15	24	97.83 ± 0.42^{d}
3	16	98.25 ± 0.52^{d}
5	20	99.00 ± 0.46^{de}
40	40	99.40 ± 0.33^{de}
50	40	100.00 ± 0.33^{e}

Superscript letters are based on Tukey HSD homogeneous subsets. Means sharing at least one superscript letter are not significantly different at $p < 0.05$.

Interpretation

Mean mortality was significantly higher in Thinkers Corner (82.43 ± 0.17%) than in Emene (81.22 ± 0.17%), $p < 0.001$.

Although the location difference was statistically significant, the actual difference was small, about 1.21%. This suggests that acetone onion extract was effective in both locations, with only slight variation in larval response between Emene and Thinkers Corner.

Table 4.6 Mean larval mortality by location

Location	N	Mean mortality (%) ± SE
Emene	144	81.22 ± 0.17^{a}
Thinkers Corner	148	82.43 ± 0.17^{b}

Means with different superscript letters differ significantly at $p < 0.05$ based on Bonferroni pairwise comparison.

Interpretation

The overall mean mortality of Anopheles gambiae s.l. larvae exposed to acetone onion extract was 81.84 ± 35.42% . Mortality was slightly higher in Thinkers Corner (82.43 ± 35.12%) than in Emene (81.22 ± 35.84%). However, the difference between the two locations was small, with only about 1.21% higher mortality in Thinkers Corner.

Implication

This suggests that acetone onion extract produced high larval mortality in both locations. The small difference between Emene and Thinkers Corner may indicate that larvae from both sites responded similarly to the extract. However, this is only a descriptive observation; the ANOVA result is needed to confirm whether the location effect is statistically significant.

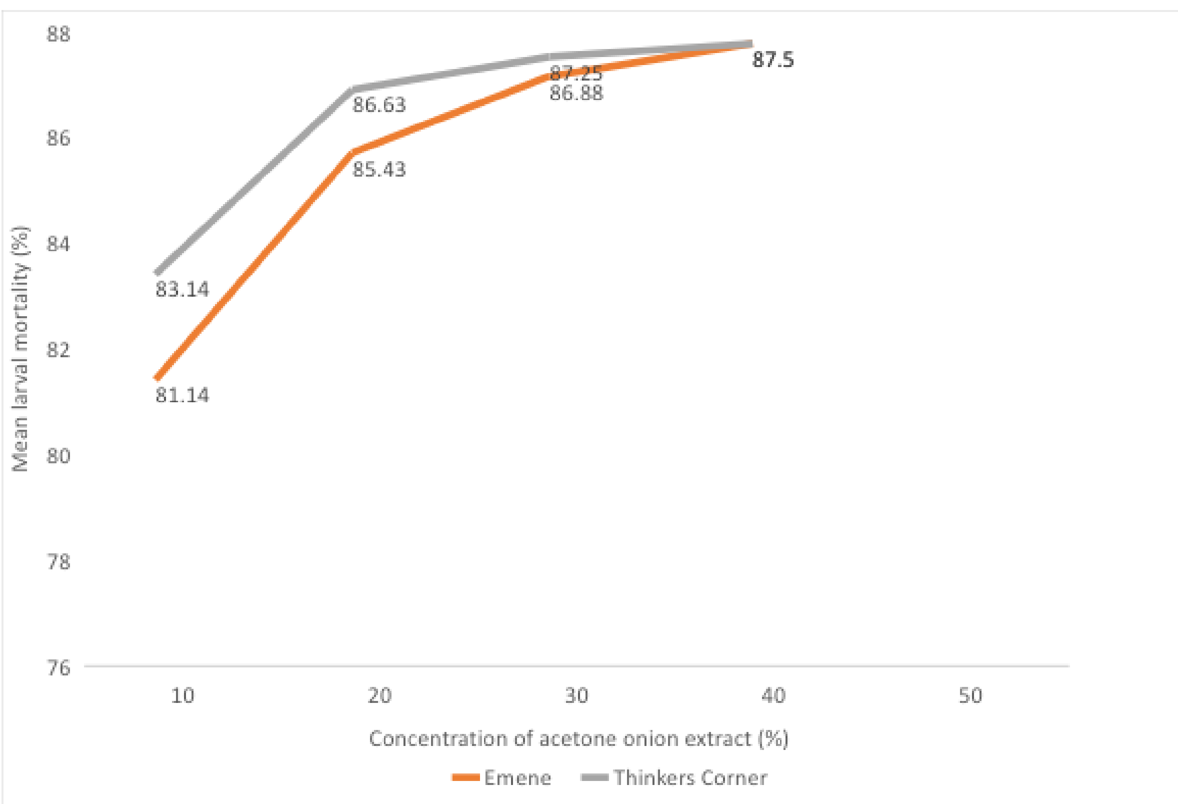

Fig 4.2: A graph showing the interaction between concentration and location on larval mortality

CHAPTER FIVE

DISCUSSION, RECOMMENDATION AND CONCLUSION

5.1 Discussion

This work evaluated the larvicidal activity of acetone extract of onion (Allium cepa) against Anopheles gambiae sensu lato larvae collected from Emene and Thinkers Corner in Enugu Metropolis. The results indicate that onion extract demonstrated strong larvicidal activity against mosquito larvae, with mortality increasing as the concentration of the extract increased. This suggests that the bioactive constituents in onion have toxic effects on mosquito larvae and could potentially serve as an alternative to conventional synthetic larvicides.

The average larval mortality recorded in Emene was 81.22 ± 35.84%, while Thinkers Corner showed a slightly higher mean value of 82.43 ± 35.12%. Overall, the combined mean mortality for both locations was 81.84 ± 35.42%. The close similarity in mortality between the two sites suggests that the response of Anopheles gambiae s.l. larvae to onion extract was fairly uniform across the study area, indicating that local environmental conditions may not have significantly influenced the effectiveness of the extract.

A clear concentration-dependent pattern was observed, where larval mortality increased steadily with higher concentrations of acetone onion extract. At 10% concentration, mortality was about 81.14% in Emene and 83.14% in Thinkers Corner, rising to 87.50% at the highest concentration tested. This trend shows that the larvicidal effect of onion extract is dose-related. The higher concentrations likely contain more active compounds, which may interfere with essential biological functions in the larvae, ultimately leading to increased death rates.

The present study demonstrated that exposure time had a significant influence on the larvicidal activity of acetone extract of Allium cepa against Anopheles gambiae s.l. larvae. Larval mortality increased progressively with increasing exposure duration, reaching complete mortality (100%) after 50 minutes of exposure. This finding suggests that prolonged contact between the larvae and the bioactive compounds in the onion extract enhances toxic action, resulting in irreversible physiological damage and eventual death. Similar observations have been reported by Govindarajan et al. (2011), who found that botanical extracts generally exhibit time-dependent larvicidal activity because the active phytochemicals require sufficient contact time to penetrate the larval cuticle and disrupt vital metabolic processes. Likewise, Shaalan et al. (2005) reported that many plant-derived insecticides become increasingly effective with longer exposure periods, leading to higher mortality rates.

Concentration-Dependent Mortality

The increase in larval mortality with increasing concentration observed in this study confirms the concentration-dependent nature of the insecticidal activity of onion extract. Higher extract concentrations likely contained greater amounts of biologically active compounds such as flavonoids, phenolic compounds, and organosulfur compounds, which have been reported to interfere with respiration, enzyme activities, and nervous system functions in insects. The findings agree with Isman (2020), who explained that botanical insecticides exhibit enhanced insecticidal activity as the concentration of active phytochemicals increases. Similar dose-dependent larvicidal responses have also been reported by Pavela and Benelli (2016) in studies involving several medicinal plant extracts against mosquito larvae.

Differences between Study Locations

Although larval mortality was slightly higher in Thinkers Corner than in Emene, the difference between both locations was relatively small despite being statistically significant. This observation suggests that larvae collected from both environments responded similarly to acetone onion extract, indicating comparable susceptibility among the mosquito populations. The slight variation may be associated with local ecological factors such as water quality, breeding habitat characteristics, previous insecticide exposure, or differences in larval physiology. Similar geographical variations in mosquito susceptibility have been reported by Awolola et al. (2007), who noted that environmental conditions and insecticide selection pressure can influence susceptibility patterns among Anopheles populations in different locations.

Three-Way ANOVA Results

The three-way ANOVA demonstrated that location, concentration, exposure time, and their interactions significantly influenced larval mortality. Among these factors, exposure time produced the greatest effect, followed by extract concentration, indicating that these variables are the primary determinants of larvicidal efficacy. The significant interaction between concentration and exposure time suggests that increasing concentration becomes more effective when larvae remain exposed for longer periods. This finding supports the concept that botanical insecticides act through cumulative physiological disruption rather than immediate toxicity alone. Similar interaction effects have been documented in botanical larvicidal studies where both dosage and exposure duration jointly determine mosquito mortality (Govindarajan et al., 2011).

Public Health Significance

The findings of this study have important implications for malaria vector control in Nigeria. The increasing resistance of Anopheles mosquitoes to conventional synthetic insecticides has created an urgent need for environmentally friendly alternatives. The high larval mortality achieved with acetone onion extract indicates that this readily available plant could serve as a potential botanical larvicide within integrated vector management programmes. Because onion is inexpensive, biodegradable, and widely cultivated in Nigeria, its utilization may reduce dependence on synthetic insecticides while minimizing environmental contamination and slowing the development of insecticide resistance. These observations support recommendations by the World Health Organization that integrated vector management should include sustainable and environmentally compatible control measures where appropriate.

5.2 Conclusion

This study showed that acetone extract of onion (Allium cepa) has notable larvicidal effects against Anopheles gambiae s.l. larvae collected from Emene and Thinkers Corner in Enugu Metropolis. Mortality was observed to increase as the concentration of the extract increased, indicating a clear concentration-dependent response. Overall, the results suggest that onion extract has potential as a simple, environmentally friendly, and cost-effective option for controlling malaria mosquito larvae.

REFERENCES

Awolola, T. S., Oduola, A. O., Oyewole, I. O., Obansa, J. B., Amajoh, C. N., Koekemoer, L. L., and Coetzee, M. (2007). Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of Anopheles gambiae s.s. in southwestern Nigeria. Journal of Vector Borne Diseases, 44(3), 181–188.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K. E., Moyes, C. L., Henry, A., Eckhoff, P. A., Wenger, E. A., Briët, O., Penny, M. A., Smith, T. A., Bennett, A., Yukich, J., Eisele, T. P., Griffin, J. T., Fergus, C. A., and Gething, P. W. (2015). The Effect of Malaria Control on Plasmodium Falciparum in Africa between 2000 and 2015. Nature, 526(7572), 207–211.

Bloland, P. B., Williams, H. A., Population, N. R. C. U. C., and Program on Forced Migration and Health at the Mailman School of Public Health, C. U. (2002). Preventive Interventions. In Www.ncbi.nlm.nih.gov. National Academies Press (US).

Cleanclay, W. D., Akanni, M. H., Bajepade, T. I., Ajeoge, J. K., and Zakari, S. (2026). Mechanisms of carbamate resistance in the malaria vector: Anopheles gambiae [Review of Mechanisms of carbamate resistance in the malaria vector: Anopheles gambiae]. Parasite Epidemiology and Control, 33, e00486.

Collins, C. M., Bonds, J. A. S., Quinlan, M. M., and Mumford, J. D. (2018). Effects of the removal or reduction in density of the malaria mosquito, Anopheles gambiae s.l., on interacting predators and competitors in local ecosystems. Medical and Veterinary Entomology, 33(1), 1–15.

Feachem, R. G. A., Chen, I., Akbari, O., Bertozzi-Villa, A., Bhatt, S., Binka, F., Boni, M. F., Buckee, C., Dieleman, J., Dondorp, A., Eapen, A., Sekhri Feachem, N., Filler, S., Gething, P., Gosling, R., Haakenstad, A., Harvard, K., Hatefi, A., Jamison, D., and Mpanju-Shumbusho, W. (2019). Malaria eradication within a generation: Ambitious, achievable, and necessary. Lancet (London, England), 394(10203), 1056–1112.

Govindarajan, M., Mathivanan, T., Elumalai, K., Krishnappa, K., and Anandan, A. (2011). Mosquito larvicidal, ovicidal, and repellent properties of botanical extracts against Anopheles stephensi, Aedes aegypti, and Culex quinquefasciatus (Diptera: Culicidae). Parasitology Research, 109(2), 353–367.

Haileselassie, W., Parker, D. M., Taye, B., David, R. E., Zemene, E., Lee, M.-C., Zhong, D., Zhou, G., Alemu, T., Tadele, G., Kazura, J. W., Koepfli, C., Deressa, W., Yewhalaw, D., and Yan, G. (2022). Burden of malaria, impact of interventions and climate variability in Western Ethiopia: An area with large irrigation based farming. BMC Public Health, 22(1), 196.

Hemingway, J., Ranson, H., Magill, A., Kolaczinski, J., Fornadel, C., Gimnig, J., Coetzee, M., Simard, F., Roch, D. K., Hinzoumbe, C. K., Pickett, J., Schellenberg, D., Gething, P., Hoppé, M., and Hamon, N. (2016). Averting a Malaria disaster: Will Insecticide Resistance Derail Malaria control? The Lancet, 387(10029), 1785–1788.

Imam, H., Zarnigar, Sofi, G., and Seikh, A. (2014). The basic rules and methods of mosquito rearing (Aedes aegypti). Tropical Parasitology, 4(1), 53–55.

Insani, E. M., Cavagnaro, P. F., Salomón, V. M., Langman, L., Sance, M., Pazos, A. A., O. Carrari, F., Filippini, O., Vignera, L., and Galmarini, C. R. (2016a). Variation for Health-Enhancing Compounds and Traits in Onion (Allium cepa L.) Germplasm. Food and Nutrition Sciences, 07(07), 577–591.

Insani, E. M., Cavagnaro, P. F., Salomón, V. M., Langman, L., Sance, M., Pazos, A. A., O. Carrari, F., Filippini, O., Vignera, L., and Galmarini, C. R. (2016b). Variation for Health-Enhancing Compounds and Traits in Onion (Allium cepa L.) Germplasm. Food and Nutrition Sciences, 07(07), 577–591.

Isman, M. B. (2020). Botanical Insecticides in the Twenty-First Century—Fulfilling Their Promise? Annual Review of Entomology, 65(1), 233–249.

Jimoh, A., Sofola, O., Petu, A., and Okorosobo, T. (2007). Quantifying the economic burden of malaria in Nigeria using the willingness to pay approach. Cost Effectiveness and Resource Allocation, 5(6), 6.

Mgbeahuruike, E. E., Holm, Y., Vuorela, H., Amandikwa, C., and Fyhrquist, P. (2019) An ethnobotanical survey and antifungal activity of Piper guineense used for the treatment of fungal infections in West-African traditional medicine [Review of An ethnobotanical survey and antifungal activity of Piper guineense used for the treatment of fungal infections in West-African traditional medicine]. Journal of Ethnopharmacology, 229, 157–166.

Mossa, A.-T. H. (2016). Green Pesticides: Essential Oils as Biopesticides in Insect-pest Management. Journal of Environmental Science and Technology, 9(5), 354–378.

Moyes, C. L., Vontas, J., Martins, A. J., Ng, L. C., Koou, S. Y., Dusfour, I., Raghavendra, K., Pinto, J., Corbel, V., David, J.-P., and Weetman, D. (2021). Correction: Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLOS Neglected Tropical Diseases, 15(1), e0009084.

None Laxmi, Tamer Oraby, Tyshenko, M. G., Danquah, I., and Bhattacharyya, S. (2025). Insecticide-treated net use and elimination of malaria in Sub-Saharan African countries: Assessing the Global Technical Strategy using an evolutionary game approach. BMC Infectious Diseases, 25(1), 1707–1707.

Oduola, T., Bello, I., Adeosun, G., Ademosun, A.-W., Raheem, G., and Avwioro, G. (2010). Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. North American Journal of Medical Sciences, 2(5), 230–233.

Okorie, P. N., McKenzie, F. E., Ademowo, O. G., Bockarie, M., and Kelly-Hope, L. (2011). Nigeria Anopheles Vector Database: An Overview of 100 Years" Research. PLoS ONE, 6(12), e28347.

Oxborough, R. M. (2016). Trends in US President's Malaria Initiative-funded indoor residual spray coverage and insecticide choice in sub-Saharan Africa (2008–2015): Urgent need for affordable, long-lasting insecticides. Malaria Journal, 15(1).

Pavela, R., and Benelli, G. (2016). Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. Trends in Plant Science, 21(12), 1000–1007.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., and Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? Trends in Parasitology, 27(2), 91–98.

Rivero, A., Vézilier, J., Weill, M., Read, A. F., and Gandon, S. (2010). Insecticide Control of Vector-Borne Diseases: When Is Insecticide Resistance a Problem? PLoS Pathogens, 6(8), e1001000.

Riveron, J. M., Huijben, S., Tchapga, W., Tchouakui, M., Wondji, M. J., Tchoupo, M., Irving, H., Cuamba, N., Maquina, M., Paaijmans, K., and Wondji, C. S. (2019). Escalation of Pyrethroid Resistance in the Malaria Vector Anopheles funestus Induces a Loss of Efficacy of Piperonyl Butoxide–Based Insecticide-Treated Nets in Mozambique. The Journal of Infectious Diseases, 220(3), 467–475.

Shaalan, E. A.-S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., and Mansour, A.-H. (2005). A review of botanical phytochemicals with mosquitocidal potential. Environment International, 31(8), 1149–1166.

WHO, (2015) World malaria report 2015 [Review of World malaria report 2015]. Www.Who.Int. https://www.who.int/publications/i/item/9789241565158

WHO, 2024 Results Report 2023 Shows Notable Health Achievements and Calls for

Concerted Drive toward Sustainable Development Goals Www.Who.Int. https://www.who.int/news/ item/07-05-2024-who-results-report-2023-shows-notable-health-achievements-and-calls-for-concerted-drive-toward-sustainable-development-goals

Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., and Lindsay, S. W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. PLOS Neglected Tropical Diseases, 14(1), e0007831.

World Health Organisation. (2020). World Malaria Report 2020. Www.Who.Int. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020