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# Evaluation of Aqueous extracts of *Hyptis suaveolens, Curcuma longa* and *Ixora coccinea* against 3rd instar larva of *Culex quinquefasciatus*

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**Abstract**
Mosquitos have been implicated to be vectors of many debilitating diseases that affect both humans and livestock. Out of over 3500 mosquito species that have been identified, *Culex quinquefasciatus* stands out because they are hardy and can survive in different environmental conditions compared to *Aedes* species and *Anopheles* species. *Culex quinquefasciatus* are carriers of different pathogens ranging from bacteria, fungi, protozoans and helminths. This research aims to assess the larvicidal potential of aqueous solvent extract of the leaves of *Hyptis suaveolens*, *Curcuma longa* and *Ixora coccinea* against 3rd instar larva of *Culex quinquefasciatus.* Adult *Culex quinquefasciatus* were collected blood fed, reared in an entomological cage. The resulting 3rd instar of F1 generation were assayed using serial concentrations of 10 % stock solutions of the leaves extracts while feeding *ad libitum*. The result obtained revealed that aqueous extracts of the three plants at different concentrations (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) was found to cause varied mortality in C. *quinquefasciatus* third (3rd) instar larvae after exposure. Extract of *Hyptis suaveolens* recorded significantly higher mortality (27.33 ± 0.57) at 50 mg/L concentration than *H. suaveolens* (14.67 ± 0.57) and *C. longa* (14.33 ± 0.57) when larvae were exposed to the same concentration. Lethal dosage studies required to yield 50 % and 90 % mortality for larvae were found to be smaller (LD 50 = 27.9 mg/L and LD 90 = 49.4 mg/L) when *Ixora coccinea* was tested compared to that of others tested. Out of the leaves extracts tested, *Ixora coccinea* was observed to be the most potent laicide. By way of recommendation, other plants should be tested on different mosquito species in a bid to obtain more plants that can control the vector.

**Keywords**: Toxicology, Mosquito physiology, *Ixora coccinea*, Larva control, *Culex quinquefasciatus.*

## Introduction

Mosquito-borne diseases have been implicated to pose continuously significant public health challenges worldwide. Among the various mosquito species, *Culex quinquefasciatus* stands out as a major vector responsible for transmitting pathogens that result in diseases in different climatic areas, like St. Louis encephalitis, West Nile virus, Ross River Virus, Alfuy, Corriparta, Almpiwar, Dengue, Japanese Encephalitis Virus, Sindbis, *Brugia timori, Brugia malayi* and lymphatic filariasis (Bellone and Anna-Bella, 2020). It is also the main vector of *Wuchereria bancrofti*, a filarial parasite that is responsible for human lymphatic filariasis, leading to morbidity that is acute and chronic, affecting all ages and sexes in tropical and sub-tropical areas worldwide (Dayvion et al., 2021). Conventional methods of mosquito control, including chemical insecticides, have often been associated with environmental risks, development of resistance, and concerns over their impact on non-target organisms. As a result, there is a growing interest in exploring alternative, eco-friendly strategies to combat mosquito populations and the diseases they transmit. (Peniche *et al.*, 2022)

*Culex quinquefasciatus* is distributed worldwide. Significant global morbidity and mortality are due to mosquito-borne diseases. Diseases that are transmitted by mosquito species pose a serious public health concern, causing a threat to a larger number of about 40% of the entire world's population. In spite of the many efforts of entomology and parasitology researchers, the vector is still implicated in major global illnesses and mortality. *Culex* species are the most abundant and widely distributed mosquito species in the World due to their ability to withstand harsh conditions compared to *Aedes* species and *Anopheles* species. The abundance of *Culex quinquefasciatus* is due to their ability to breed in a wide range of water bodies, especially the heavily polluted. (Omotayo et al., 2022)

The *Culex* genus is known to be vectors of human diseases that include Elephantiasis, Dengue fever and West Nile fever in sub-Saharan Africa (Turell, 2012). They possess high biting ability, mostly at night, that has been reported to exceed on the average, 10 bites per person per night, thereby creating a high level of discomfort and nuisance to humans and other animals alike. Out of all the *Culex* species, *Culex pipiens* predominates sub-Saharan Africa and *Cx. quinquefasciatus* remains the abundant sibling species of the complex in both tropical and sub-tropical regions of the world where the transmission of lymphatic filariasis is high. (Samy et al., 2016). Lymphatic filariasis is the second most common cause of permanent disability worldwide, and it is largely transmitted by both *Anopheles* mosquitoes in Nigeria, with *Culex* mosquitoes contributing to its transmission to an extent. (Omotayo et al., 2022).

Botanical extracts have garnered attention as potential candidates for mosquito larvicidal agents due to their inherent bioactive compounds with insecticidal properties. Plant species have a history of traditional use and are known to possess diverse bioactive compounds with insecticidal potential. (Yagoo et al., 2023). The utilisation of plant-derived products for pest control has a long-standing history, with many plant extracts demonstrating bioactivity against a range of insect pests. These extracts often contain secondary metabolites such as alkaloids, terpenoids, flavonoids, and phenolic compounds that exhibit various levels of insecticidal activity by disrupting vital physiological and biochemical processes in the insects. (Lim et al., 2023). *Hyptis suaveolens* (L.) is commonly called bush tea, bush mint, pignut in the English language, Hausa calls it daddoya-ta-daji, *efiri* in Yoruba and in Igbo it is called nchuanwu (Umedum et al., 2014). It is associated with the family Lamiaceae, with bioactive essential oils against insects. The plant has its major constituents, include B-caryophyllene, sabinene, terpinolene and 4-terpineol. (Peniche et al., 2022). Composition and quantity of known constituents in *H. suaveolens* can vary due to different chemotypes (Azevedo et al., 2001). Extracts of *H. suaveolens* have been observed to show larvicidal and repellent activity on the Culicidae family, revealing its potential as an insecticide. The intrinsically limited water solubility of constituents of essential oils has affected viable larvicide development. (Azevedo, et al., 2001)

*Ixora coccinea* L (Rubiaceae), from Portuguese origin, has been differently called West Indian Jasmine, Jarum-jarum, Techi, Rangan, Kheme tanea, Chann Santan, Pan, Jungle Geranium and Jungle flame (Okhale et al., 2018). The leaves are leathery, oblong and glossy. In length, the leaves are about 3-6 inches, there are different colours of flowers in existence, including yellow, red, purple, white, orange, and pink, blossoming all year round (Lambert and Sofia, 2021). The plant has a lot of medicinal uses due to its antibacterial, anti-oxidative, gastroprotective, antidiarrheal, antinociceptive, hepatoprotective, antimutagenic, chemopreventive and antineoplastic properties. Its potential in controlling the aquatic stage of mosquitoes has been demonstrated using leaf and flower against the 4th instar of larvae of *Anopheles* and *Aedes aegypti*. All parts of the plant are useful especially in the Indian traditional system of medicine (Okhale et al., 2018). *Curcuma longa* (L.), Turmeric is also called atale pupa in the Yoruba language, in the Hausa language it is called gangamau (Amadi et al., 2018). It is traditional to the Chinese and belongs to the family Zingiberaceae. The plant has fragrance and perennial rhizomatous herb, and the leaves are dotted, large and pointed (Lim et al., 2023). Turmeric can grow between 1 to 2 m in height, and flowers are funnel-shaped. The leaf, rhizome, and flowers of *C. longa* have been studied and reported to possess anti-cancer, antimicrobial, larvicidal, insecticidal, and antioxidant activities. (Das et al., 2015).


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*Curcuma* species extracted leaf oil has been observed to possess fumigant toxicity against insects like beetles that are generally present in stored products, the plant is also confirmed to possess tetrahydrocurcumin which is odorless and heat-resistant antioxidant compound, there are also several polyphenolic compounds such as eugenol, curcumin, cinnamic acid, linalool, limonene, vanillic acid and turmerone (Nataya et al., 2019).

*Hyptis suaveolens*, *Ixora coccinea*, and *Curcuma longa* are chosen for their unique chemical profiles and documented insecticidal potential. *Hyptis suaveolens* is known for its essential oils rich in monoterpenes and sesquiterpenes (Peniche et al., 2022), while leaves of *Ixora coccinea* have been reported to contain Ixora tannin A-2 (a trimeric A-type proanthocyanidin), procyanidin A2, cinnamtannin B-1 (Ambreen et al., 2013). Turmeric contains curcuminoids and essential oils that have demonstrated larvicidal effects against other mosquito species (Lim et al., 2023).

The control of *Culex quinquefasciatus* can be difficult because there might be genetically different populations of these culicines within a single country (Wike et al., 2014). Reducing the proliferation of this species requires either biological or chemical control, in which synthetic pyrethroid, carbamate, or organophosphate insecticides are diluted and applied to breeding sites (Ministerio et al., 2011). Some mosquito species have developed resistance to insect growth regulators (IGRs). Hence, there is a need to identify and evaluate newer IGR compounds to be used in vector control programs (Sadanandane et. al., 2012).

Resistance has been induced within a few generations of *Culex quinquefasciatus* (Mehlhorn, 2011). For the avoidance of resistance, products with a different array of endotoxins should be alternated (Schuster et. al., 2006). Mosquitoes are known to be vectors of various diseases worldwide. There is therefore a need to identify ways in which these debilitating and life-threatening agents can be controlled or the diseases eliminated from the vectors. (Wilson et al., 2020).

Despite the burgeoning interest in botanical extracts as potential alternatives to synthetic insecticides for mosquito control, there remains a conspicuous dearth of comprehensive investigations into the comparative effects of aqueous leaf extract of *Hyptis suaveolens*, *Ixora coccinea*, and *Curcuma longa* on the developmental stages of *Culex quinquefasciatus* larvae. While individual studies have elucidated the larvicidal potential of these plant species against various mosquito vectors, the nuanced interplay between solvent extraction methods and larval developmental dynamics in the context of *Culex quinquefasciatus* remains markedly underexplored.

The research gap delineated herein underscores the exigency for comprehensive studies that not only elucidate the influence of solvent extraction methodologies on phytochemical profiles and larvicidal potency but also delve into the nuanced effects of these botanical extracts across diverse developmental stages of *Culex quinquefasciatus* larvae. Addressing this research lacuna holds profound implications for advancing our understanding of eco-friendly mosquito control strategies and catalysing the development of novel botanical-based interventions with enhanced efficacy and sustainability.

The proposed study holds substantial significance as it seeks to address the limitations of conventional mosquito control methods by exploring sustainable and environmentally friendly alternatives. The findings of this research can contribute to the development of effective and safe larvicidal agents, potentially leading to the reduction of mosquito-borne disease transmission and minimising the adverse effects of chemical insecticides on ecosystems. Botanical extracts have the potential to affect the population dynamics of several insect pests and disease vectors and could also potentially lead to their eventual eradication when used appropriately (Martins and Silva, 2004). Various simulation models that predict the effects of plant extracts against vectors of certain diseases and their populations can help in making management decisions.

This study aims to determine the effects of aqueous leaf extracts of *Hyptis suaveolens*, *Ixora coccinea* and *Curcuma longa* on 3rd instar larvae of *Culex quinquefasciatus*. The objective of the study is to assess mortality through bioassays and determine lethal concentration.


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## Materials and Methods

### Study Area

The research was carried out at the Nigerian Defence Academy, Kaduna State. Kaduna State is one of the North-Western states of Nigeria. The state lies between latitude 11° 15′N to 11°3′N and longitude 7° 30′E to 7°45′E. Its peculiar vegetation is characterised by guinea savannah type, grassland ecosystem with trees being widely spaced so that the canopy does not close. The open canopy allows sufficient light to reach the ground to support an unbroken herbaceous layer consisting primarily of grasses. There are distinct seasons which are wet and dry annually, a dry season that lasts between four to seven months and a rainy season that lasts between four to five months. The annual rainfall ranges between 1016 mm and 1524 mm, with a relative humidity ranging between 60% and 80%. Kaduna state shares borders with several states, which include Katsina, Kano, and Zamfara in the northern direction, Bauchi and Plateau States to the eastern direction, Federal Capital Territory and Nasarawa States to the southern direction and Niger State located in the western part. Kaduna State has a land mass of about 46.53 square kilometres with a population that is over 5 million. (Micheal, 2014)

### Collection, Identification, Authentication and Preparation of Plant Leaves

The leaves of *Hyptis suaveolens, Ixora coccinea,* and *Curcuma longa* were collected within Kaduna metropolis. The leaves were identified and authenticated at the herbarium of the Department of Biological Sciences; a voucher number was received for each identified plant at the Herbarium. The leaves were air-dried under shade, pulverised with pestle and mortar, and taken to the laboratory for extraction.

### Preparation of Aqueous Extract

The pulverised leaves of *Hyptis suaveolens, Ixora coccinea* and *Curcuma longa*, weighing 200g each, were separately placed in three different conical flasks labelled for each leaf. A litre (1000 ml) of distilled water (aqueous solvent) was poured into the different flasks and allowed to stand for 72 hours.

The whole content in each flask was filtered using muslin cloth, and the filtrates were allowed for 24 hours after which another filtration process was carried out using cotton wool to enable the extracts from the different conical flasks to drain gently into different evaporating dishes. The evaporating dishes containing the extracts were placed in water baths between 40˚C and - 65˚C. The dishes were left in the water baths until the solvents in the extracts were completely evaporated. (Mohammed & Elourfi, 2005)

### Preparation of test solutions of leaf extracts

### Preparation of stock solution

Stock solution (10 %) of solvent extracts of *Hyptis suaveolens, Ixora coccinea* and *Curcuma longa* were prepared from 250 mg of the solid residue dissolved in 25 mL of borehole water (WHO, 2005).

### Serial dilution of stock extracts

Serial dilutions of the stock solutions were obtained with a medical syringe to obtain test concentrations, 10 mg/L, 20 mg/L, 30 mg/L and 40 mg/L (Adebote and Adeyemi, 2011). Different 5 mL syringes were used to take and apply 0.1 mL to 0.5 mL from the stock solution onto 100 mL of water. Control concentration, devoid of the plant extract, consisted of 100ml of borehole water (Adebote and Adeyemi, 2011).

### Collection of field species of mosquito larvae

Blood-fed adult female *Culex quinquefasciatus* were collected from a poultry farm, APIA Peace Farm, Kamazou, Kaduna. The collection was carried out by using a test tube to trap mosquitoes while resting on the wall (Belkin, 1965) from indoors. The trapped mosquito was transferred into a collecting bottle (Agada *et al.,* 2020). This process was repeated until a good number is collected (about twenty (20) blood fed mosquito adults).

### Breeding collected mosquitoes

The blood-fed mosquitoes were transferred into two (2) entomological cages that measure 30 cm × 30 cm × 30 cm protected with a net with a mesh size of 1 mm (Adebote and Adeyemi, 2011), which had a petri-dish containing borehole water with relative humidity (Barik et al., 2016). The adult mosquitoes in the cage were fed with sugar solution soaked in cotton wool (Porter et al., 1961), which is aimed at supplying the adults with energy for physiological functions. *Culex quinquefasciatus* mosquitoes in the entomological cage oviposited in the water


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obtained from a bore-hole within 2 to 6 days after collection from the field (Gerberg et al., 1969). Within 3 to 4 days after oviposition, the eggs hatched and first instar larvae were obtained (Adebote and Adeyemi, 2011). The larvae that emerged from oviposited eggs were fed and allowed to grow through the various developmental stages until they became adult. Adults were allowed to mate and subsequently blood fed from quail birds after which female *Culex quinquefasciatus* oviposited. Egg rafts were allowed to hatch, from which first instar larvae were obtained. These larvae were fed and allowed to develop to third instar larvae for the mortality assay (WHO, 2005).

**Identification of adult mosquitoes**

The blood-fed adult female mosquitoes that oviposited, were isolated and preserved for identification using of keys for mosquito species identification described by the service (2015). The adult female mosquitoes were labeled with appropriate labels including dates and locations of collection after oviposition in plastic petri-dishes.

**Bioassay of mosquito larvae using solvent leaf extracts of *Hyptis suaveolens, Ixora coccinea,* and *Curcuma longa***

Constant measure (0.02 mg) (Eugene, 1970) of ground digestive biscuit was added to the petri-dish, which served as feed for F1 generation third instar larvae for each bioassay. The larvae were allowed to feed *ad libitum* while the experimental process continued until they metamorphosed into the pupae stage.

Similarly, pupae of the F1 generation that have not been exposed to the extracts were tested for mortality just like third instar larvae. Also, adults that have not been exposed to extracts were tested by soaking 10 % sugar solution and serial concentrations in cotton wool after starving the adult mosquito species overnight following standard procedure of WHO (2005).

The leaf extracts of *Hyptis suaveolens, Ixora coccinea*, and *Curcuma longa* were investigated based on concentration. Growth inhibition, varying developmental stages, mortality efficacy and morphological deformation were screened.

Screening for mortality of larvae, pupae and adults involved separating each stage in batches of twenty (20). Each batch was transferred to separate petri dishes containing 100 mL of distilled water in case of larvae and pupae, while adults were tested using an appropriate concentration and sugar solution soaked in cotton wool. Each bioassay had three replicates, and a replicate for control was carried out concurrently at comparable conditions (room temperature). Replicates of the controls were exposed without solvent extracts.

The dead and moribund larvae were recorded after an exposure period of twenty-four (24) hours. Larvae and pupae were touched gently with a glass rod and were considered dead in the absence of any sign of movement. The larvae were considered moribund if they only moved a little without any sign of swimming or vigorous movement. The moribund larvae could never revive and thus were counted as dead larvae (WHO, 2005). Several concentrations (10 mg/L, 20 mg/L, 30 mg/L and 40 mg/L) were used to determine the mortality effect of the leaf extract of *Hyptis suaveolens, Ixora coccinea*, and *Curcuma longa* on the larvae (Adebote and Adeyemi, 2011).

**Data Analysis**

Mortality data of exposed test specimens of *Culex quinquefasciatus* in the various test concentrations used were subjected to probit analysis (Finney, 1971). Analysis of variance (ANOVA) was used to test for significant differences in larval mortality of the leaf extracts of *Hyptis suaveolens, Ixora coccinea*, and *Curcuma longa*. Duncan's multiple range test was employed in separating differing means (Adebote and Adeyemi, 2011).

## Results

**Effects of Aqueous extracts of *Hyptis suaveolens, Curcuma longa* and *Ixora coccinea* on *Culex quinquefasciatus third instar larvae***

The mortality of *C. quinquefasciatus* third (3rd) instar larvae after exposure to aqueous extract of the leaves of *Hyptis suaveolens, Curcuma longa* and *Ixora coccinea* and monitored for 24hrs is presented in table 1.1 Aqueous extract of the three plants at different concentrations (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) was found to cause varied mortality in C. *quinquefasciatus* third (3rd) instar larvae after exposure. Aqueous extract of *Hyptis suaveolens* recorded significantly higher mortality (27.33 ± 0.57) at 50 mg/L concentration than *H. suaveolens* (14.67 ± 0.57) and *C. longa* (14.33 ± 0.57) when larvae were exposed to the same concentration. Significantly lower mortality was


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recorded for aqueous extracts of the three plants when larvae were exposed to lower concentrations of 10mg/L (*H. suaveolens*: 7.67 ± 0.57; *C. longa*: 0.33 ± 0.57; *I. coccinea*: 10.33 ± 0.57) and control (*H. suaveolens*: 0.33 ± 0.57; *C. longa*: 0.33 ± 0.57; *I. coccinea*: 0.33 ± 0.57) ($p<0.05$).

**Table 1. 1. Larval mortality of *Culex quinquefasciatus* due to solvent extracts**

| Concentration (mg/L) | --------------Mean Mortality (Mean + SD)---- -------- Aqueous Extracts ---------- | | |
|---|---|---|---|
| | ***H. suaveolens*** | ***C. longa*** | ***I. coccinea*** |
| **0.0** | $0.33±0.57^{a}$ | $0.33±0.57^{a}$ | $0.33±0.57^{a}$ |
| **10** | $7.67±0.57^{b}$ | $4.00±0.00^{c}$ | $1303±0.57^{a}$ |
| **20** | $9.33±0.57^{b}$ | $5.33±0.57^{c}$ | $12.67±0.57^{a}$ |
| **30** | $13.33±0.57^{b}$ | $6.67±0.57^{c}$ | $17.33±0.57^{a}$ |
| **40** | $11.67±0.57^{b}$ | $8.33±0.57^{c}$ | $24.33±0.57^{a}$ |
| **50** | $14.67±0.57^{b}$ | $14.33±0.57^{b}$ | $27.33±0.57^{a}$ |

SD = Standard Deviation; Different superscript indicates significant difference at $P \leq 0.05$

**Table 1.2. Lethal dose determination of leaves tested**

| **Statistics** | ***H. suaveolens*** | ***C. longa*** | ***I. coccinea*** |
|---|---|---|---|
| F Value | 212.400 | 238.440 | 874.967 |
| P Value | 0.000 | 0.000 | 0.000 |
| LD 50 | 47.8 | 53.94 | 24.26 |
| LD 90 | 98.5 | 95.55 | 47.75 |

However, the evaluation study to determine the lethal concentration required to yield 50 % and 90 % mortality, relatively smaller (LD 50 = 24.26 and LD 90 = 47.75 ) when 3rd instar larvae of *Cx quinquefasciatus* were exposed to aqueous extract of *Ixora coccinea* than when exposed to extracts of other leaves tested. Similarly, LD 50 and LD 90 for extracts of *Curcuma longa* were higher (LD 50 = 68.2 and LD 90 = 81.2) to result in 50 % and 90 % larval mortality, respectively (Table 1.2).

## Discussion

The high mortality of larvae recorded with aqueous extract of *Ixora coccinea* are compared to aqueous extracts of *Hyptis suaveolens* and *Curcuma longa* could be associated with the presence of one of the phytochemicals absent in the two plants but present in *I. coccinea* or as a result of the synergistic effect of some phytochemicals in *I. coccinea* such alkaloids, phenols, terpenoids, and flavonoids which were reported by Ahmed *et al.* (2016), Iqbal et al. (2021)


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and Ahmed et al. (2021) to possess larvicidal effects on larvae of mosquito species. These phytochemicals find their ways into mosquito larvae causing a decrease in the insect's growth rate, with consequential mortality of the larvae. Mortality may also result from failure of the larvae to respond to food requirements as a result of the presence of allelochemical compounds such as terpenoids, which are responsible for feeding inhibition (Ninkuu, 2021). Similarly, the larvicidal properties recorded with aqueous extract of the leaves sampled are consistent with the studies of Murugan et al. (2012) and Ikram and Ali (2017), who reported that aqueous extracts of different plants, such as *Citrus sinensis* orange peel and *Cymbopogon nardus* whole plant, respectively, have larvicidal properties which result in increased mortality with increased concentrations. It also corroborates the findings of Adeniyi et al. (2024), who presented the efficacy of aqueous leaf extract of *H. suaveolens* as larvicidal against *Cx. quinquefasciatus*.

## Conclusion

The leaves of *Hyptis suaveolens, Curcuma longa* and *Ixora coccinea* were tested on the 3rd instar larva of *Culex quinquefasciatus. Ixora coccinea* was found to be potent as a larvicide among other extracts tested. Also, it was determined that a lower dosage of similar plant extract is needed to obtain a total kill of *Culex quinquefasciatus* larvae. We recommend that while other plant extracts should be assessed for their larvicidal potential, *Ixora coccinea* should be formulated and made available for mosquito control.

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