Larvicidal Effects of *Citrus* Peels Extracts against *Culex Pipiens* Mosquitoes

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Abstract

Background: Mosquitoes transmit severe diseases such as malaria, lymphatic filariasis, dengue fever, and yellow fever. These diseases result in significant morbidity and mortality in humans around the world. This study aimed to provide information on the larvicidal potential of different *Citrus* species peels against *Culex* mosquito larvae.

Methods: Ethanol extracts of the peels of four *Citrus* species, such as *Citrus sinensis* (sweet orange), *Citrus reticulata* (tangerine), *Citrus latifolia* (lime), and *Citrus paradisi* (grape) fruits were tested for larvicidal properties against *Culex pipiens* at different concentration (3 mg, 5 mg, 7 mg and 8 mg in 100 mL) on 25 larva per concentration. Percentage mortality was recorded for each of the concentrations used.

Results: Only *Culex latifolia* induced the death of all the mosquito larvae (100%) at 5 mg/mL concentration of the extract, meanwhile *Culex reticulata* resulted in the lowest mortality rate (80%). However, a hundred percent (100%) mortality of the larvae, was observed at 8 mg/mL of all the types of extracts evaluated.

Conclusion: The result indicates that the peels of these *Citrus* species, especially *Culex latifolia*, hold potential for control of *Culex pipiens* mosquito larvae.

Keywords: *Culex pipiens*, *Citrus* peels, ethanol extracts

Introduction

Mosquitoes constitute a nuisance and are of great public health concern globally. *Culex pipiens* mosquitoes are considered vectors of several diseases such as lymphatic filariasis (LF), West Nile Virus, Japanese Encephalitis, Saint Louis Encephalitis, Dengue and Rift Valley Fever with some being fatal in the absence of treatment and others causing lifelong disabilities and impairment.[1] *Culex* mosquito species are also responsible for a serious nuisance problem with high biting rates exceeding 100 bites/person/night.[2] The *Culex pipiens quinquefasciatus* is a prominent vector species that feeds on both humans and animals,[3] thereby increasing its implication in pathogen transmission to both host groups. Factors such as low or inefficient drug distribution in Nigeria,[4] mass drug administration (MDA) failure in Ghana and Burkina Faso[5] and the ability of the culicine mosquitoes to transmit low levels of microfilariae[6] have further led to a review of the World Health Organization (WHO) filariasis elimination strategy to include vector control.[7] In Nigeria, *Culex* and *Aedes* species are responsible for transmitting LF, dengue and yellow fever.[8] Larval source management through larviciding and or environmental modification represents a key supplementary means recommended for mosquito vector control in Nigeria.[4] Efforts towards a search for effective natural mosquito larvicide will contribute to the drive for the incorporation of mosquito larval control into the overall integrated mosquito vector management plan.

Citrus fruit is grown and consumed worldwide and in many cases, processed into juice to preserve them from spoilage. Waste materials from *Citrus* could be processed into flavonoids, dietary fibre, methane, essential oils, carotenoids, and other essentials

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materials.[9,10] These processed materials could serve as antimicrobials and antioxidants against different organisms.[11] Due to the chemical composition of *Citrus* peels, leaves, flowers, and other parts of citrus, their effectiveness as biological control agents differ from one organism to another. This study was designed to provide information on the larvicidal potential of the peels of different *Citrus* species against *Culex* mosquito larvae. This information will serve as a basis for determining the possible use of *Citrus* peels to control *culex pipiens* mosquito larvae, the vectors of such deadly diseases such as lymphatic filariasis.

Methods

Four *Citrus* species, such as *Citrus sinensis* (sweet oranges), *Citrus reticulata* (tangerine), *Citrus paradisii* (grape), and *Citrus latifolia* (lime) were purchased from a local market in Ilorin and identified by a plant taxonomist at Kwara State University, Malete, Nigeria (Figure 1).

The *Citrus* species peels were removed and air dried at room temperature for some days. The dried peels were pulverized with a blender in the laboratory into powder and were weighed using the weighing balance, yields were 140.9 g, 86.5 g, 46.5 g, and 50.0 g for sweet orange, tangerine, grape, and lime respectively. The powdered sieved peels were dissolved in a volume of ethanol (mL) which is five times (5x) their weight (mg). Fifty grams of lime peel were dissolved in 250 ml of ethanol and kept for 24 hours with periodic shaking using a rocker at 125 rpm, the same process was repeated for each *Citrus* species. The filtered and the filtrate were collected. The procedure was repeated three times with a new volume of ethanol. The filtrates were pooled and placed in a water bath for the ethanol to evaporate.

Culex pipiens larvae were collected from natural breeding sites around the Kwara State University campus, Malete, Kwara State, Nigeria using a dipper (Figure 2). Mosquito larvicidal assay was conducted at different test concentrations (3 mg, 5 mg, 7 mg, and 8 mg)

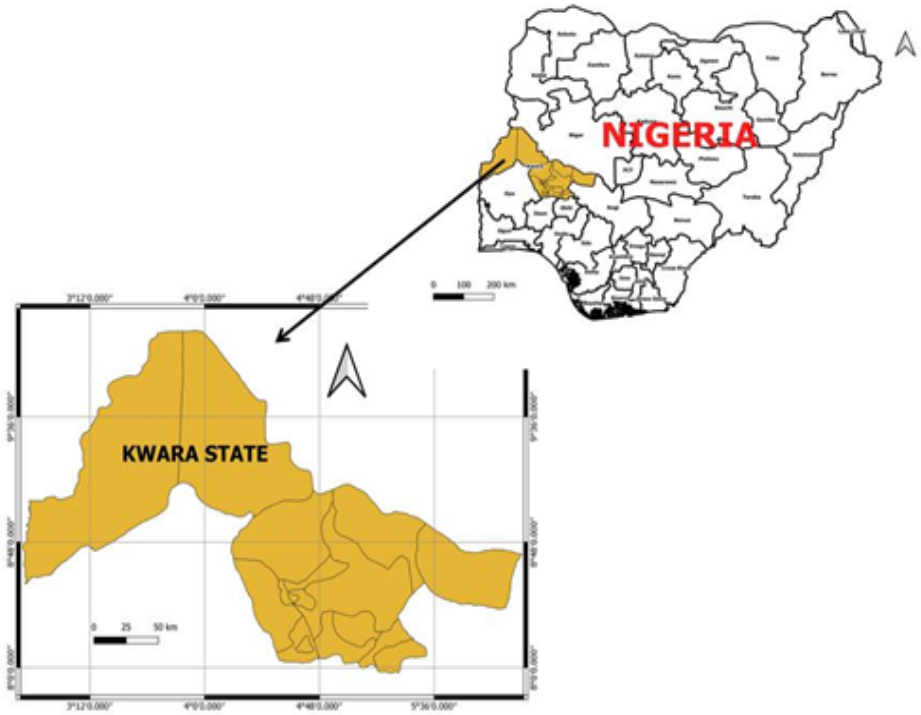

Figure 1 Map of Nigeria and Kwara State as the Study Area

Figure 2 Larval Collection from the Study Area

of *Citrus* peels extract. One hundred milliliters of distilled water were taken in a series of 250 mL glass beakers. The measured extracts were dissolved in 2 mL of the solvent (ethanol) used to prepare the extract. The dissolved *Citrus* peel extract was added to a beaker's 100 mL of water. A control was also maintained by adding 2 ml of solvent ethanol to 100 mL water, 25 larvae per concentration were introduced into different beakers. The temperature (28±2ºc) and humidity (78±5%) were recorded during the experiment in the laboratory using a thermohygrometer. The mosquito larva mortality rates were recorded at the end of 24 hours and the experiment replicated thrice.[12] Controls were exposed to the solvent (ethanol alone). During the treatment period, the larvae were not fed.

The percentage mortality of *Culex* larvae induced by each *Citrus* peel extract was calculated for each concentration. Ethical approval for this study was obtained from the University of Ilorin Ethical Review Committee no. UERC/ASN/195.

Results

The result of mortality rates induced by the different *Citrus* peel extracts against *Culex pipiens* mosquito larvae was presented in Table. *Citrus latifolia* showed the highest larvicidal activity against the larvae compared

Table Mortality Rates of Mosquito Larvae Exposed to Different *Citrus* Peel Extracts

Citrus species	Observed Percentage Mortality (%+SD)			
	3 mg/mL	5 mg/mL	7 mg/mL	8 mg/mL
Citrus sinensis	80±1.15	88±2.08	92±1.00	100
Citrus reticulata	80±.0.00	80±0.57	92±1.15	100
Citrus latifolia	80±0.57	100	100	100
Citrus paradisi	76±0.57	96±0.57	100	100

to lowest activity induced by *Citrus paradisi.* At the lowest concentration of 3 mg/mL, *Citrus sinensis*, *Citrus reticulata*, and *Citrus latifolia* had the same percentage of mortality (80%), whereas *Citrus paradisi* induced 76% *Culex pipiens* mosquito larva mortality. At 5 mg/mL, only *Citrus latifolia* brought about the death of all the mosquito larvae (100%), meanwhile *Citrus reticulata* showed the lowest mortality rate (80%). At 7 mg/mL, larvicidal activities of *Citrus sinensis* and *Citrus reticulata* were the same (92%), meanwhile *Citrus latifolia* and *Citrus paradisi* elicited 100% mortality of the larvae. At the highest concentration (8 mg/mL), all the *Citrus* peel extracts induced 100% mortality against the larvae. *Citrus latifolia* peel extract showed a consistently higher mortality rate at the different concentrations.

Discussion

This study identified the larvicidal activities of different *Citrus* peel extracts against *Culex pipiens* mosquitoes. The result has shown that the effectiveness of the *Citrus* peel extract is high. The high effective concentration was found to be 8 mg/mL, although the lowest concentration of 3 mg/mL also demonstrated some effectiveness in controlling *Culex* larvae. For all the different species of *Citrus* used in the study, the higher the concentration of the extract, the higher the mortality rate of the *Culex* larvae. This is in tandem with a similar study conducted in India.[13] However, it is in deviance with another study elsewhere, where the absolute mortality was observed at a lower concentration.[14] This could be due to some factors, such as the handling of the *Culex* species used or other effects of experimental errors. Minor or significant stress could have occurred on the *Culex* species used during transportation or transferring the species in the laboratory during the experimental process. Another factor could be attributed to the lack of a photoperiod of 12 hours light and 12 hours dark as the World Health Organization required during the experimental process.[12] For *Citrus lemon* peel extract, the absolute mortality occurred at 8 mg/mL, this is in disagreement with other studies where absolute mortality occurred at 100 mg/mL.[15,16] This could be due to the differences in the method of extracting the active ingredient for the experimental studies. However, limonene remains the active ingredient found in lemon peels which are found to be active against insects and could be used in obstructing the activities of larvae.[17] *Citrus sinensis* peel extract showed a progressive mortality rate in *Culex* species as the concentration of the extract increased, this was also found in a similar study conducted elsewhere in South-South Nigeria.[18] Moreover, the known active ingredient (saponins) is effective against the life cycle of many insects that are of public health importance to humans and animals.[18–20] For this study, the 7 mg/mL concentration *Culex reticulata* peel extract showed almost complete mortality in *Culex* species, which agrees with previous studies using different vectors. For *Citrus reticulata*, the effective ingredient includes terpenes limonene, sabinene gamma-terpinene, octanal, and capraldehyde; these have been proven to be responsible for mortality in vectors.[21,22] Although grapefruit is used as an anti-obesity and body cleansing promoter,[23–25] it also has antibacterial, antifungal, and larvicidal properties.[26–29] In the same vein, in this study, *Citrus paradisi* (grapefruit) had a full lethal effect on *Culex* species larval at 7 mg/mL concentration. This aligns with other reports where *Citrus paradisi* was used alongside *Allium sativum* to control of *Culex quinquefasciatus* larvae and adults. The effectiveness of *Culex paradisi* peel extract has been linked to diallyl-disulphide, linalool, citronellal, and caryophyllene oxide as the major active ingredient.[30] Overall, the results of this study demonstrate that the different *Citrus* peel extracts, particularly *Culex latifolia*, hold potential for the control of *Culex pipiens* mosquito larvae.

The limitation of the study is that we did not consider fractionation and testing of the bioactive components of the extracts. Therefore, further studies should include these aspects.

In conclusion, the *Citrus* peel extracts of *Culex latifolia* have a potential effect to be used for the biocontrol of *Culex pipiens* mosquito larvae.

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