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Larvicidal Efficacy of *Citrus sinensis* Peel Extract against *Culex quinquefasciatus*: Implications for Sustainable Mosquito Control

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Abstract

Culex quinquefasciatus, a vector of lymphatic filariasis and many otherarboviral infections, remains a major public health concern, especially in regions lacking access to modern vector control tools. Growing resistance to synthetic insecticides has driven interest in eco-friendly alternatives; including plant-based larvicides such as *Citrus sinensis* (orange) peels. This study evaluated the larvicidal activity of ethanolic *C. sinensis* peel extract against *Cx. quinquefasciatus* larvae under laboratory conditions. Air-dried orange peels were pulverized and subjected to cold maceration in ethanol. Extracts were tested at concentration of 100–8,333 ppm on third to early fourth instar larvae, with four replicates per concentration. Mortality was recorded at 24 and 48 hours. LC_{50} and LC_{90} values were determined using linear interpolation. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test at a 5% significance level. High concentrations (1,667–8,333 ppm) caused complete mortality (25.00 ± 0.00) within five minutes, with no significant difference among treatments ($p > 0.05$). Atmoderate concentrations, mortality followed a dose-response trend: 400 ppm resulted in 54 and 57% mortality at 24 and 48 h, while 800 and 1000 ppm achieved 100% mortality at both time points. Estimated LC_{50} and LC_{90} values were 385.2 and 713.0 ppm. No control group mortality was recorded throughout the study. *C. sinensis* peel extract exhibitspotent larvicidal activity against *Cx. quinquefasciatus*, particularly at concentrations above 400 ppm. Its efficacylow cost and availability suggest potential as a natural component in integrated mosquito control strategies, particularly in resource-limited settings.

Keywords: *Citrus sinensis*, larval mortality, mosquito control, botanical insecticide, *Culex quinquefasciatus*

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Introduction

Mosquito-borne diseases remain a major public health concern in many parts of the world, particularly in tropical and subtropical regions where environmental conditions favour mosquito breeding [1, 2]. *Culex quinquefasciatus*, one of the most widely distributed mosquito species, is a prominent vector of lymphatic filariasis and many other arboviral infections, contributing significantly to disease burden and economic losses in affected regions [3-10]. Control of mosquito population has traditionally relied heavily on insecticides application. However, this overreliance has raised concerns due to environmental toxicity, risk to human health and the rapid development of insecticide resistance [4, 11]. Consequently, there is growing interest in sustainable, environmentally friendly alternatives, such as plant-derived larvicides, which are often safer, cost-effective and locally available [12].

One promising plant-derived or botanical source is *Citrus sinensis* (sweet orange), whose peels are rich in bioactive phytochemicals such as essential oils, limonoids, and flavonoids, compounds with documented insecticidal and antimicrobial properties [13]. These natural compounds can disrupt key physiological functions in mosquito larvae, including membrane integrity, respiration and enzyme function [14]. While various *Citrus* species have shown efficacy against mosquito vectors, further research is needed to standardize extraction protocols, determine lethal concentration thresholds and evaluate their effectiveness against locally prevalent species such as *Cx quinquefasciatus* [15].

Exploring the larvicidal potential of *C. sinensis* peels using ethanol as a solvent offers a practical and efficient approach, as ethanol is widely recognized in herbal research for its ability to extract a broad spectrum of phytochemicals [16]. The use of cold maceration further helps preserve heat-sensitive bioactive components, ensuring the extract retains maximum potency [3]. Also, orange peels are a locally available agricultural byproduct that is often discarded, making their application in mosquito control both cost-effective and environmentally responsible [17, 18]. This study, therefore, aims to systematically evaluate the larvicidal activityof ethanolic *C. sinensis* peel extract against *Cx quinquefasciatus* larvae under controlled laboratory conditions. Ultimately, findings from this study should help determine the potential of *C. sinensis* peel extract as a cost-effective, eco-friendly plant-based larvicide for mosquito control initiatives.

Material and Methods

To achieve the study objective, a series of extract concentrations were prepared and larval mortality was evaluated after 24 and 48 h of exposure. Lethal concentration values (LC_{50} and LC_{90}) were estimated to determine the doses required for 50 and 90% mortality, respectively, specifically

Collection and Preparation of *Citrus sinensis* Peels

Fresh peels of *C. sinensis* (L. Osbeck) were obtained from Zuba market, located within the Gwagwalada Area Council, Abuja, Nigeria. The plant material was formally identified and authenticated by a taxonomist at the Department of Biological Sciences, University of Abuja and a voucher specimen was archived for reference, in accordance with the World Health Organization (WHO) recommendations [19].

Following collection, the peels were thoroughly rinsed and air-dried in a shaded, well-ventilated area at an average ambient temperature of 28 ± 2°C for approximately two months. This approach minimized direct sunlight exposure, thereby reducing the risk of phytochemicals degradation [20]. Once a constant weight was achieved, indicating adequate drying, the peels were milled into a fine powder using a mechanical grinder. The powdered material was then stored in tightly sealed amber glass containers at room temperature, in a dark, dry environment, to preserve chemical stability and quality, in line with WHO guidelines for handling herbal materials [20].

Preparation of Ethanolic Extracts

Solvent selection and extraction procedure

Ethanol (95%, analytical grade) was selected as the extraction solvent due to its proven ability to dissolve a broad spectrum of phytochemicals, including both polar and moderately non-polar compounds.This makes it a preferred choice in phytochemical research and is consistent with WHO guidelines for preparing herbal extracts intended for medicinal use [20]. Extraction process was performed using the cold maceration technique, which avoids heat exposure and thus helps preserve thermolabile bioactive compounds, ensuring optimal retention of the extract's phytochemical integrity [21].

Preparation of Various Extract Concentrations

To prepare the extract concentrations, accurately weighed portions of powdered *C. sinensis* peel were macerated in a fixed volume of ethanol (100 mL) at weight-to-volume (w/v) ratios ranging from 1 to 5%. The specific concentrations are provided in Table 1.

After maceration, the mixtures were separately filtered through Whatman No. 1 filter paper. The resulting filtrates were then concentrated under reduced pressure using a rotary evaporator at 40°C to remove ethanol, yielding the crude extracts [5]. To prepare lower concentrations, 500 ml of distilled water was added to 100 ml of each extract, resulting in the diluted lower concentrations described in Table 2.

Table 1: Preparation of ethanolic extracts at varying concentrations

Concentration (% w/v)	Weight of Powder (g)	Volume of Ethanol (mL)	Concentration (ppm)
1	1	100	10,000
2	2	100	20,000
3	3	100	30,000
4	4	100	40,000
5	5	100	50,000

Twenty grams of dried *C. sinensis* peel powder were macerated in 98% ethanol for 24 h at room temperature with intermittent stirring to enhance solute-solvent interaction. This method, consistent with WHO recommended phytochemical extraction practices [20], was selected to preserve the integrity of bioactive compounds. Following maceration, the mixture was filtered to obtain a concentrated ethanolic solution with an estimated concentration of 100,000 ppm, equivalent to 10% w/v (10 g per 100 mL of solvent).

Table 2: Test concentrations of ethanolic extract of *Citrus sinensis*

Extract (%w/v)	Original Concentrations (ppm)	New Concentrations in ppm (resulting from 100 mL of Original Extract + 500 mL of distilled water)
1	10,000	1,667
2	20,000	3,333
3	30,000	5,000
4	40,000	6,667
5	50,000	8,333

To further prepare lower test concentrations, the above procedure was repeated.

Table 3: Preparation of lower test solutions from 1000 ppm intermediate stock

Target Concentration (ppm)	Volume of 1000 ppm Stock (mL)	Final Volume (mL)	Volume of Distilled Water Added (mL)
100	10	100	90
200	20	100	80
400	40	100	60
800	80	100	20

From this stock solution, 1 mL was carefully diluted with distilled water to a final volume of 100 mL in a standard volumetric flask, yielding an intermediate solution of 1000 ppm. Test solution of 100, 200, 400 and 800 ppm were then prepared by serial dilution of the intermediate solution, each adjusted to 100 mL with distilled water. The dilution process is provided in Table 3.

Source and Collection of *Culex* Mosquito Larvae

Larvae of *Cx. quinquefasciatus* were collected from a range of natural and artificial breeding habitats within the Abuja metropolis. These included stagnant watersources such as roadside drainages, polluted water in discarded containers, abandoned vehicle tires, swampy areas, construction pits and uncovered water storage vessels, sites commonly associated with *Cx. quinquefasciatus* larval development.

Larval collections were conducted using the standard WHO dipping method, in which a white plastic dipper was gently lowered onto the water surface at an angle of approximately 45 degrees to minimize disturbance and increase collection efficiency [15, 19]. Larvae and water from each dip were transferred into clean plastic buckets and promptly transported to the laboratory for subsequent bio-larvicide bioassays.

Experimental Design

Larvicidal efficacy was evaluated following the WHO standardbioassay protocol for mosquito larvae [19]. Assays were conducted in clean, transparent plastic containers, each containing 500 mL of *C.sinensis* extract at the desired concentrations. In the first experiment, 25 third- to early fourth-instar *Cx. quinquefasciatus* larvae were introduced into containerswith extract concentrations ranging from 1,667 to 8,333 ppm (Table 2). Each concentration was tested in quadruplicate, alongside a negative control containing only distilled water to evaluate natural mortality. A second set of assays was conducted with lower concentrations (100–200 ppm; Table 3), following the same procedure. Each concentration had four replicates, and distilled water served as the negative control.

Experimental Conditions

The bioassays were conducted under controlled laboratory conditions – Temperature: Maintained between 25–28°C; Photoperiod: A consistent 12:12 h light: dark cycle was observed; Feeding: No food was provided during the assay period, as recommended; Contamination Prevention: Care was taken to avoid contamination; all handling was performed gently to minimize stress or injury to larvae.

Mortality Assessment

Larval mortality was recorded after 24 and 48 h of exposure. Larvae were considered dead if they showed no movement when gently probed with a fine brush. Live and dead larvae counts were recorded separately for each replicate. Corrected mortality rates were calculated using Abbott's formula when control mortality ranged between 5–20% [19].

Statistical Analysis

Mortality data were analyzed using both descriptive and inferential statistics to evaluate the larvicidal activity of *Citrus sinensis* peel extract against *Cx. quinquefasciatus*. Mean values and standard errors were calculated for each treatment group, and comparisons among groups were determined using one-way ANOVA followed by Tukey's post hoc test at a 5% significance level ($p = 0.05$). Treatments sharing the same superscript letters within the same row were not statistically. Since no larval mortality occurred in the control groups throughout the experiment, Abbott's correction formula was deemed unnecessary.

To illustrate the concentration-mortality relationship, a dose-response curve was generated using Python-based computational tools. Data visualization was performed using the Matplotlib library, while numerical processing and array manipulation were handled with NumPy. Lethal concentrations (LC_{50} and LC_{90}) were estimated via linear interpolation using the interp1d function from the SciPy library, providing accurate estimates of the extract concentrations required to achieve 50 and 90% larval mortality, respectively.

Results and Discussion

Exposure of *Cx. quinquefasciatus* larvae to higher concentrations (1,667–8,333 ppm) of *C. sinensis* ethanolic peel extract resulted in complete mortality (25.00 ± 0.00) within 24 h. No mortality was observed in the control group (0.00 ± 0.00). Notably, 100% larval deaths occurred rapidly within the first five minutes of exposure, making the 48-hour observation period unnecessary. Statistical analysis revealed no significant differences among the treated groups ($p > 0.05$), as all concentrations produced identical mortality outcomes, whereas the difference between treated and control groups was highly significant ($p < 0.001$). Since no mortality occurred in the control treatments throughout the study, Abbott's formula was deemed unnecessary.

Table 4 illustrates a concentration-dependent increase in the mortality of *Cx. quinquefasciatus* larvae following exposure to ethanolic extracts of *Citrus sinensis* peels. No larval deaths were recorded at the lower concentrations of 100 and 200 ppm within 24 and 48 h. A notable rise in mortality was observed at 400 ppm, while complete larval mortality occurred at both 800 and 1000 ppm. These findings indicate that the larvicidal potency of the extract becomes significantly effective at concentrations exceeding 400 ppm.

Table 4: Mortality rate (%) of *Cx. quinquefasciatus* larvae exposed to ethanolic extract of *C. sinensis* peels

Concentration (ppm)	% Mortality at 24h	% Mortality at 48 h
100	0	0
200	0	0
400	54	57
800	100	100
1000	100	100

A clear concentration-dependent increase in larval mortality was observed when *Cx. quinquefasciatus* larvae were exposed to *C. sinensis* peel extract for 24 h. Mortality rates rose significantly at concentrations above 400 ppm, reaching complete lethality at 800 and 1000 ppm (Fig. 1). The computed LC_{50} and LC_{90} values were approximately 385.2 and 713.0 ppm, respectively, highlighting the extract's potent larvicidal properties. These outcomes underscore the potential application of *C.sinensis* as a natural alternative for mosquito larval control strategies.

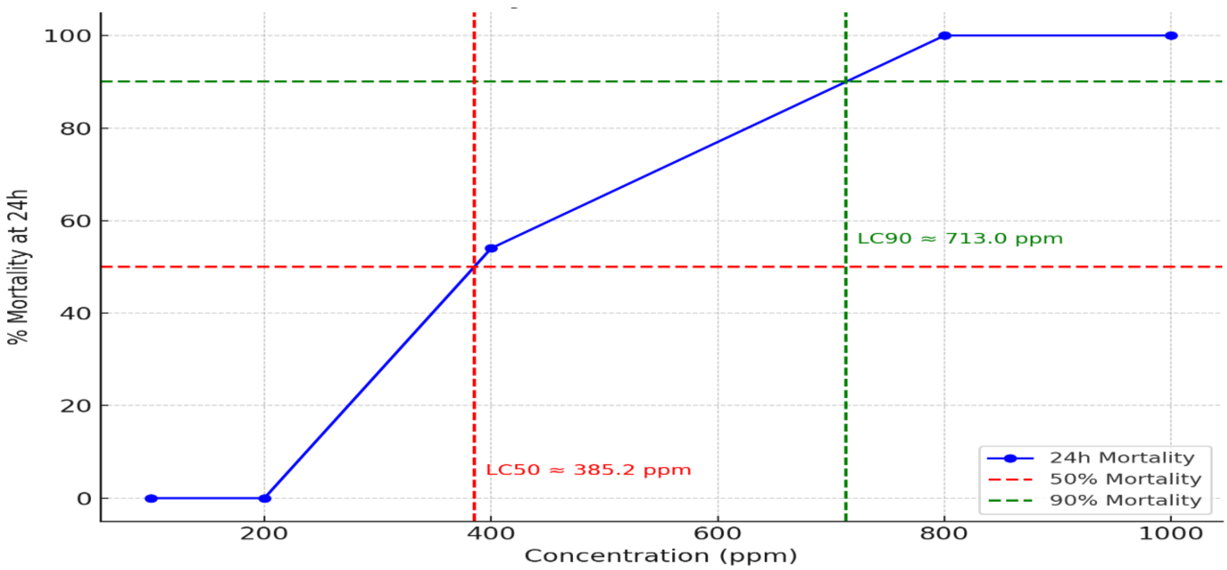

Figure 1: Dose-response relationship between *Citrus sinensis* extract and 24 h mortality of *Cx. quinquefasciatus* larvae

This study demonstrates the clear, concentration-dependent larvicidal activity of *C. sinensis* ethanolic peel extract against *Cx. quinquefasciatus* larvae. Complete mortality occurred at concentrations of 800 ppm and above, with LC_{50} and LC_{90} values of 385.2 and 713.0 ppm, indicatingstring efficacy. These findings align with previous botanical studies reporting the effectiveness of plant-based biopesticides against mosquito vectors, including *Culex* and *Anopheles* species [15, 22]. The rapid onset of mortality, especially at higher doses suggests a fast-acting mechanism, likely mediated by synergy interactions among key phytochemicals such as limonene and linalool [23].

Notably, 100% mortality within five minutes at higher concentrations points to rapid penetration of potent contact toxicity. This observation aligns with earlier reports on the quick insecticidal action of monoterpenes and flavonoid-rich extracts derived from citrus and related plant residues [24]. Supporting evidence from GC-MS analyses in a related study confirms the abundance of bioactives like limonoids and citral in *C. sinensis* peels [25]. These compounds are thought to compromise larval physiology by impairing respiration or damaging cuticle structures, mechanisms also supported by recent histopathological investigations [26].

The use of citrus peel extract offers dual benefits, potent bioactivity against mosquito larva and a sustainable approach to vector control. As an abundant agricultural byproduct, orange peels are often discarded. Their valorization in vector management not only mitigates waste but also addresses vector (mosquito) proliferation in endemic regions [3, 27]. Compared with synthetic insecticides, citrus-based compounds offer greater environmental compatibility, as they tend to degrade more rapidly and pose less risk to non-target organisms [28]. The absence of mortality in control groups throughout the experiment strengthens the reliability of the results and suggests that the observed larvicidal effects were due to the extract itself, aligning well with WHO's criteria for evaluating plant-based larvicides [1].

The negligible mortality observed at lower concentrations (100 and 200 ppm) highlights the existence of a critical threshold, below which the extract is insufficiently potency. This emphasizes the importance of determining and maintaining optimal doses for effective field application [29]. Based on current results, formulations in the 800–1000 ppm range appear more suitable for real-world application, especially in regions with dense breeding sites or prolonged mosquito activity. Other botanicals such as *Azadirachta indica*, *Eucalyptus globulus*, and *Ocimum gratissimum* have also shown similar patterns, where standardized concentrations are essential to prevent the risk of inducing resistance [30].

Conclusion

In conclusion, *C. sinensis* peel extract demonstrates good potential as a cost-effective, natural larvicide against *Cx. quinquefasciatus*. The findings provide a foundation for future studies focused on optimizing extract formulations, evaluating long-term impacts, and conducting large-scale field trials. Additionally, future research could exploresynergistic combinations with other botanical agents to enhance both efficacy and cost-effectiveness [31]. In light of the rising resistance to synthetic insecticides, plant-based alternatives like *C. sinensis* offer a promising path toward safer and sustainable mosquito control strategies.

Conflict of interests: The authors declare no conflict of interest.

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