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EA conceived and supervised the project. EA, GA, MM, and AE wrote the main manuscript text, prepared figures and tables; AA and EJ performed the analysis of the results, and contributed to the writing of the manuscript. All authors have approved the final version of the manuscript for publication.

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The Efficiency of Fresh and Commercial Aqueous Extract of *Ocimum basilicum* Leaves against *Culiseta longiareolata* Mosquito Larvae

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Abstract:
Mosquitoes are carriers of diseases that affect both humans and animals. It is crucial to manage their spread during their early developmental stages using natural, environmentally safe materials. This research aimed to assess the effectiveness of *Ocimum basilicum* leaves-derived aqueous extract (basil plant) as a larvicide against mosquito larvae (*Culiseta longiareolata*). Third and fourth instar larvae (fifteen larvae per replicate, n = 4) were subjected to varying concentrations of the leaves' aqueous extract (200, 400, 800, and 1600 ppm), with mortality rates recorded after 24 and 48 hours. Both fresh and stored aqueous basil leaf extracts demonstrated efficacy in the eradication of mosquito larvae. The results indicated that the fresh basil leaf extract was more effective than the stored extract, and larval mortality increased proportionally with higher concentrations and longer exposure times. Specifically, after 24 and 48 hours, the mortality rates for the fresh aqueous extract were 36.7% and 66.7%, respectively, compared to 23.3% and 43.3% for the stored extract. Statistically, a significant value was noticed in the fresh extract ($p\leq 0.01$). The lethal concentration (LC_{90}) after 48 hours of treatment was determined to be 2425 ppm for the fresh basil leaf extract and 2902 ppm for the stored extract. Furthermore, the LC_{50} values for the fresh and stored aqueous basil extracts after 24 hours were 1920 ppm and 2361 ppm, respectively. These results suggest that the aqueous extract of basil leaves (O*. basilicum*) with high concentrations holds potential as a larvicide for controlling *Cs. Longiareolata* mosquito larvae.
Keywords: *Ocimum basilicum,* aqueous extract, *Culiseta longiareolata*, fresh and stored plant, larvicide, and mosquito larvae.

INTRODUCTION

Mosquitoes belong to the family *Culicidae*. They serve as significant vectors for many diseases affecting both humans and domestic animals (Cui *et al.*, 2006; Lim *et al.*, 2018; Hernandez-Valencia *et al.*, 2023). Some mosquitoes are responsible for the transmission of many important pathogens and parasites, such as viruses, bacteria, protozoans, and nematodes, which cause serious diseases. The most important genera of mosquito-borne disease pathogens are *Aedes, Anopheles, Culex, Mansonia,* and *Culiceta*. They lead to diseases such as malaria, dengue fever, yellow fever, filariasis, Zika, West Nile, and Rift Valley fever (Dilshad *et al.*, 2016; Onen *et al.*, 2023; Zaynab, 2019). Although the text identifies *Culiseta longiareolata* as a virus vector. This role is not confirmed within the given context. Thorough recognition and having a comprehensive understanding of the efficiency of the different mosquito vectors is essential for developing an effective control mechanism (Day, 2016; Hazratian *et al.*, 2019; Mwingira *et al.*, 2020).

The mosquito is recognized for its ability to adapt its egg-laying behavior in response to environmental conditions (Hazratian *et al.*, 2019). Female mosquitoes sting and suck blood to complete egg formation. This avoidance is primarily triggered by chemical signals released by predators, particularly the backswimmer (Hazratian *et al.*, 2019; Kiflawi *et al.*, 2003; Spencer *et al.*, 2002). This effect remains and continues for several days even after the predator has been removed (Mwingira *et al.*, 2020; Blaustein *et al.*, 2004).

A major problem in effective vector control, particularly for vector-borne diseases, is the rapidly increasing resistance of vector populations to commonly used insecticides (Kumar *et al.*, 2024). This mosquito can resist pesticides, which leads to environmental pollution. On a large scale, Pyrethroids are used as insecticides, which have promoted the development of resistance mechanisms within these mosquito populations (Ranson, 2017; Black IV *et al.*, 2021). This resistance is related to many factors, including enhanced detoxification procedures, changes in modifications in cellular signaling pathways, and gene expression (Black IV *et al.*, 2021). Plant-based bioinsecticides are now considered a much safer and less toxic alternative to synthetic compounds (Sattar *et al.*, 2016; Iqbal and Ashraf, 2019; Ishtiaq *et al.*, 2019).

Ocimum basilicum belongs to the family *Labiatae,* which is considered a native annual herb growing mostly in the tropical regions, and has several traditional and medicinal benefits (Makri and Kintzios, 2008; Shahrajabian *et al.*, 2020). It is also considered a significant crop due to the biologically active compounds found in its essential oil. These compounds include linalool, methyl chavicol, 1,8-cineole, and ursolic acid (Nacar and Tansi, 2000; da Costa *et al.*, 2015; Chenni *et al.*, 2016; Shahrajabian *et al.*, 2020; Silva *et al.*, 2008). Moreover, various studies have identified a wide array of active chemical constituents in *O. basilicum* leaf extracts, such as phenolics (e.g., rutin and quercetin), flavonoids (e.g., quercitrin), and phenolic acids (e.g., caffeic, chlorogenic, and gallic acids) (Irondi *et al.*, 2016; Sestili *et al.*, 2018).

In Yemen, biological control of mosquito larvae (*Aedes aegypti*) by *Mentha pipreta* (methanol extract) was studied (Al-Ajmi *et al.*, 2021). Research concerning mosquito vector control utilizing plant extracts in Yemen still limited, particularly regarding its publication and investigation into *Culiseta longiareolata* mosquitoes. Consequently, the current study aimed to assess the larvicidal effect of *O. basilicum* aqueous extract against *Cs. longiareolata* larvae.

MATERIALS AND METHODS

Larval collection

All methodological steps followed the ethical guidelines for handling invertebrate samples; the experimental procedures were implemented in the Faculty of Science. Mosquito eggs and first-instar larvae were collected from the Bait Bos (Al-Sabe'ein) breeding site in Sana'a, Yemen.

Larvae were transferred and reared under laboratory conditions (25°C, 12:12 light-dark cycle) (Carnaghi *et al.*, 2024). When they reached the late third instar and early fourth instar larval stages, larvae were identified using different larval keys (Das *et al.*, 2007; Foster and Walker, 2019; Harbach, 2007).

Plant collection:

In March 2022, fresh leaves of *O. basilicum* were collected from the Al-Sabe'ein region, Sana'a City and identified by Prof.Dr/ Hassan Ibrahim, Faculty of Science, Sana'a University, with the herbarium number (1183). Leaves were air-dried under laboratory conditions for a week, then crushed into a fine powder and stored in airtight bottles. Both *O. basilicum* leaves powder and commercially available samples were studied.

Extraction

Fifty grams of each sample (fresh and commercial) was extracted separately in 500 mL of distilled water for 24 h. Aqueous extracts then filtrated, evaporated and dried in the oven at 40°C for 3 days. Dried extracts were then stored in a dark containers for further use.

Larvicidal activity

Larvicidal activity was evaluated following established protocol with slight modifications (Larvicides, 2005). Stock solutions of *O. basilicum* were prepared by dissolving 0.2, 0.4, 0.8, and 1.6 g of the extract in distilled water to reach 1000 ml of final volume of 200, 400, 800, and 1600 ppm, respectively. Under laboratory conditions, larvicidal activity of the *O. basilicum* against late 3^{rd} and early 4^{th} *Cs. longiarealate* larvae was tested by exposing larvae to 250 ml of each solution to determine the lethal concentrations of the extract, (LC_{50} and LC_{90} values). All concentrations were studied in four replicates of 15 larvae.

The death rate of each replicate in 24 and 48 hours of exposure was calculated using the following formula:

Percentage of mortality

$$= (Number\ of\ dead\ mosquitoes\ /(\text{number of mosquitoes tested}) * 100$$

Statistical analysis

Statistical program Statistical Package for the Social Sciences (SPSS, VERSION 27) was used in this study to determine LC_{50} and LC_{90}.

RESULTS

Larvicidal activity of *O. basilicum* against *Cs. longiareolata* larvae was evaluated. At different concentrations, larvicidal effects against *Cs. longiareolata* larvae showed more efficacy with different degrees of mortality. The efficacy at both 24-hour and 48-hour intervals of fresh *O. basilicum* extract against *Cs. Longiareolata* showed strong larvicidal activity than stored leaf extract. Moreover, the mortality rate of fresh and stored leaf extracts of 10%, 16.7%, 23.33%, and 36.7% at 200, 400, 800, and 1600 ppm against *Cs. longiareolata* mosquito larvae were correlated. In contrast, lower efficacy was noticed in the preserved leaves extract, which produced mortality rate of 3.33, 6.7, 16.7, and 23.33, respectively. The aqueous extract of fresh plant leaves was significantly better than that of the commercial one after 24 hours ($p \leq 0.05$) (Figure 1).

After 48 hours of incubation, fresh and commercial plant extracts exhibited more effect in a concentration depending manner. High mortality rate (66.7%) was observed in the high concentration (1600 ppm) and low mortality rate (16.7%) was observed in the low concentration (200 ppm). Conversely, the highest concentration of the extract of commercial basil extract (1600 ppm) obtained 43.3% mortality rate, and the lowest concentration (200 ppm) produced a 10% mortality rate after 48 hours (Figure 2).

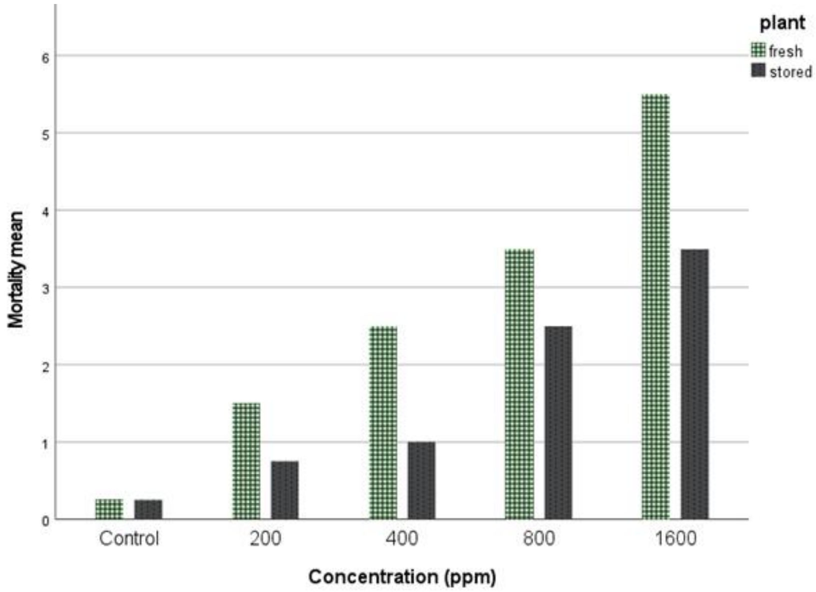

Fig. 1. Larvicidal effect of fresh and commercial aqueous extracts of *Ocimum basilicum* against *Culiseta longiareolata* larvae after 24 h.

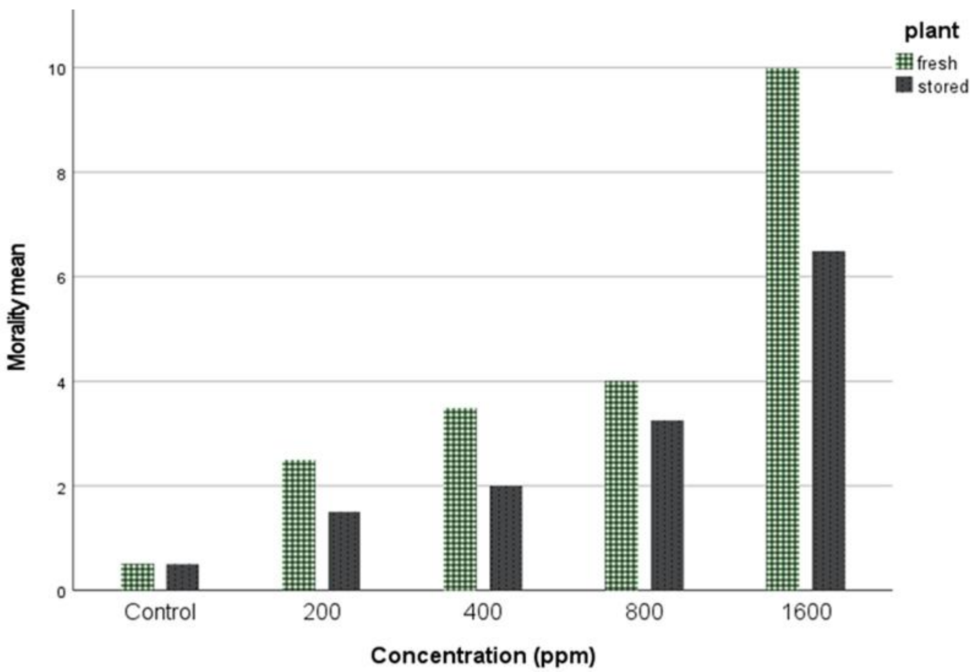

Fig. 2. Larvicidal activity of fresh and commercial aqueous extract of *Ocimum basilicum* against *Culiseta longiareolata* larvae after 48 hours of exposure.

The fresh leaf extract of *Ocimum basilicum* exhibited LC_{50} and LC_{90} at 1920 ppm and 3587 ppm, respectively, at 24 hours and 48 hours of exposure with a standard error of 4.10±0.754 (Table 1).

Table 1. Statistical analysis of the larvicidal efficacy of *O. basilicum* leaves aqueous extract on Culex quinquefasciatus and *Culiseta longiareolata* larvae**.**

***Ocimum basilicum* extract**	**Time of Exposure**	**LC_{50}**	**LC_{90}**	**Mean ± S.E.**
Fresh Leaves	24 hours	1920	3587	2.65±0.437
	48 hours	1259	2445	4.10±0.754
Commercial Leaves	24 hours	2361	4046	1.60±0.294
	48 hours	1601	2902	2.70±0.529

DISCUSSION

The use of chemical agents for mosquito control is associated with environmental contamination, posing risks to both human health and populations. Furthermore, mosquitoes have developed resistance to chemical interventions; therefore, the utilization of plant extracts as larvicides represents a highly effective alternative (Amir *et al.*, 2017; Iqbal and Ashraf, 2019).

Fresh plant aqueous extract of dried basil leaves (*O. basilicum*) demonstrated superior efficacy in inducing mosquito larvae (*Cs. longiareolata*) mortality compared to the aqueous extract obtained from Commercial basil leaves. This enhanced effectiveness is attributed to the a lot existence of chemical constituents, including linalool, methyl eugenol, eugenol, 1,8-cineole, glycosides, anthocyanins, sugars, flavonoids, saponins, tannins, alkaloids, phenols, and terpenoids (Purushothaman *et al.*, 2018). Furthermore, investigations of *O. basilicum* leaf aqueous extracts have consistently identified a diverse array of active phytochemicals, such as flavonoids, phenolic acids, and phenolics (Irondi *et al.*, 2016; Sestili *et al.*, 2018).

The rate of larval mortality was increased, particularly after a prolonged experimental period, which is potentially attributable to the progressive accumulation of active materials within the larval body over 48 hours. In Sudan, the effectiveness of ethanolic extracts of *O. basilicum* leaves and flowers against *Culex quinquefasciatus* and *Anopheles arabiensis* larvae was evaluated (Rudayni *et al.*, 2021). Similarly, another study examined the larvicidal activity and phytochemical profile of sweet basil (*Ocimum basilicum* L.) leaf extract against Asian tiger mosquitoes, subsequently recommending its application as a pesticide (Chan *et al.*, 2022).

The larvicidal efficiency of *Ocimum basilicum* and *Ocimum gratissimum* aqueous leaf extracts against *Culex quinquefasciatus* larvae has been reported. The results showed *that O. gratissimum* leaf extract was more effective than *O. basilicum* leaf extract (Akinnibosun *et al.*, 2023). The essential oil of *O. basilicum* was evaluated against *Aedes aegypti* and *Culex quinquefasciatus*. The nanoemulsion was tested at different concentrations, and the highest effectiveness was observed for *Cx. quinquefasciatus* (de Sousa dos Santos *et al.,* 2024). The methanolic extracts of *Lavandula dentata* and *Nerium oleander* exhibited a higher efficacy against *Cs. longiareolata* (Dris *et al.*, 2024). Nanoemulsion from the essential oil of *Ocimum basilicum* leaves showed larvicidal activity against *Cx. quinquefasciatus* (Sundararajan *et al.*, 2018).

This study tested different concentrations of the lethal concentration (LC_{90}), which was 1105 ppm for fresh leaf extracts and 2210 ppm for commercial extracts, for 48 hours. A similar study used three plants; one of them was *O. basilicum*. (El Zayyat *et al.*, 2017) used acetone extract of *O. basilicum* against *Culex pipiens* adults and larvae. The results were significantly more effective with *O. basilicum* (LC_{50}- 0.064) against *Cx. pipiens* larvae. (Akinnibosun *et al.*, 2023) reported that *Ocimum gratissimum* aqueous leaf extracts was more effective against *Culex quinquefasciatus* larvae (LC_{50} of 920.0 ppm) than *Ocimum basilicum* (LC_{50} of 1000 ppm).

Therefore, the present study suggests that the fresh leaf extract of basil (*Ocimum basilicum*) has larvicidal activity and can be used as a larvicide against *Cs. longiareolata* larvae at high concentration.

CONCLUSION

This study demonstrates that the aqueous extract of *Ocimum basilicum* leaves has larvicidal ativity against *Culiseta longiareolata,* with superiority of fresh extract over commercial one. The larvicidal activity was also concentration- and time-dependent, with the fresh extract achieving markedly lower LC_{50} and LC_{90} values after 48 hours. These findings establish the fresh basil extract as a potent and eco-friendly alternative to chemical insecticides and highlight the critical importance of using fresh, non-degraded plant material for optimal efficacy in mosquito vector control.

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CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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