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Exploring the Effectiveness of *Ocimum basilicum* Extracts in Mosquito Larvae Management

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ABSTRACT

Mosquito control is a key method for managing diseases transmitted by these insects, and targeting their early life stages offers several advantages, such as ease of application and greater susceptibility to environmental and chemical factors. This study aimed to investigate the chemical constituents and the larvicidal effects of ethanolic extracts from the leaves and flowers of *Ocimum basilicum* on mosquito larvae. Fresh *O. basilicum* was harvested, dried for one week in the shade, and then extracted using ethanol. They were placed in containers and subjected to bioassays according to WHO guidelines. Mortality rates were recorded after a 24-hour recovery period, and dead larvae were examined for any morphological changes. Probit analysis was performed using SPSS version 19. Phytochemical analysis of *O. basilicum* leaves revealed the presence of flavonoids, tannins, glycosides, and steroids, while saponins, alkaloids, and terpenoids were absent. For the flowers, tannins, steroids, terpenoids, and flavonoids were found, but saponins, glycosides, and alkaloids were not present. The LC50 values for the leaf and flower extracts were found to be 17.78 ppm, 16.98 ppm, 15.48 ppm, and 15.84 ppm, with LC95 values at 56.23 ppm, 64.56 ppm, 66.06 ppm, and 50.11 ppm, respectively, for *Anopheles arabiensis* and *Culex quinquefasciatus*. Larvae treated with ethanolic extracts showed visible morphological changes, including discoloration and deformities in the digestive tract.

Keywords: Ethanol extract, Larvicidal, Anopheles arabiensis, *Ocimum basilicum*, Culex quinquefasciatus, Phytochemicals

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Introduction

Mosquitoes, such as *Anopheles*, *Culex pipiens*, and *Aedes*, that belong to the Diptera: Culicidae family, are vectors for a variety of serious diseases. The use of chemical insecticides, while effective, is costly and poses significant environmental risks. Furthermore, the growing resistance of many mosquito species to these chemicals has become a major concern [1-3]. In recent years, there has been a growing focus on finding new, naturally occurring compounds from plants to replace chemical insecticides. Plants have proven to be valuable sources of novel

natural insecticides. Many plant-based mosquito repellents and insecticides have shown promising results as effective inhibitors of mosquito populations [4-9].

Ocimum basilicum L., commonly known as basil and belonging to the Lamiaceae family, is widely utilized in traditional medicine worldwide. *Aedes aegypti* has shown a high sensitivity to extracts from *O. basilicum* leaves, especially at concentrations ranging from 0.3% to 1.5%. When ethanol is used to extract basil leaf powder, its toxicity to insects increases, making it a recommended solution for controlling pests like the American cockroach [10-12]. Additionally, basil essential oils are particularly effective against the 3rd instar larvae of *A. aegypti*, yielding favorable LC50 and LC90 values in laboratory tests. These oils have also demonstrated 100% mortality rates while tested against mites. Furthermore, basil leaves have proven effective in controlling agricultural pests in rice, with high mortality rates observed [9-11]. This study aims to analyze the chemical components and evaluate the larvicidal properties of ethanolic extracts from the leaves and flowers of *O. basilicum*, contributing to the exploration of natural alternatives for mosquito control.

Materials and Methods

Plant collection

Fresh *O. basilicum* plants were collected from the garden of the College of Science at Imam Mohammed Iben Saud Islamic University. The leaves were identified and processed by a faculty member. After collection, the plant parts were air-dried in a shaded area for one week. Once dried, they were ground into a fine powder and stored in sealed plastic containers until needed for further analysis and experiments.

Phytochemical analysis of extracts

To analyze the phytochemical composition, selected parts of *O. basilicum* (leaves and flowers) were subjected to preliminary screening using methods described by Mohamed Nour [13]. This screening was conducted to detect the presence of key chemical compounds, including alkaloids, tannins, saponins, flavonoids, steroids, glycosides, and terpenoids.

Preparation of ethanolic extracts

Ethanolic extracts were prepared by soaking the powdered plant parts in ethanol, following the method established by Hamid *et al*. [14]. The ethanol was allowed to extract the compounds for further analysis.

Mosquito larvae

Mosquito larvae, specifically *A. arabiensis*, and *C. quinquefasciatus*, were collected and cultured under controlled laboratory conditions at the Biology Department, Faculty of Science. The larvae were placed in plastic containers and provided with the required nutrients for growth and development.

Larval bioassay

The larvicidal properties of the ethanolic extracts were tested according to the standard procedure approved by the World Health Organization (WHO) [15]. A set of 20 third- and early fourth-instar larvae from *A. arabiensis* and *C. quinquefasciatus* was placed in 250 ml of tap water in individual plastic cups. The extracts were tested at varying concentrations, and each concentration was replicated three times. Control groups, that did not receive any extract, were also included. Mortality was observed after 24 hours, and the dead larvae were analyzed for morphological changes under a microscope.

Statistical analysis

The data collected from the bioassay were calculated using SPSS version 19. Probit analysis was used to determine the LC50 and LC90 values for the larvae of *A. arabiensis* and *C. quinquefasciatus*. Additional statistical parameters, such as the regression coefficient (R^2), slope, and x-coefficient, were calculated to assess the efficacy of the extracts.

Results and Discussion

Phytochemical Composition of *O. basilicum* Leaves and Flowers

The phytochemical analysis of *O. basilicum* leaves and flowers revealed varying amounts of different compounds **(Table 1)**.

Table 1. Phytochemical composition of *O. basilicum* leaves and flowers

Plant Part	Alkaloids	Saponins	Tannins	Flavonoids	Glycosides	Steroids	Terpenoids
O. basilicum Leaves	-	-	+	+	+	+	-
O. basilicum Flowers	-	+	-	+	++	-	+

- "-" indicates the absence of the compound.
- "+" indicates the presence of the compound.
- "++" indicates the compound is present in relatively higher amounts.

Effect of ethanolic extracts of basil leaves and flowers on mosquito larvae (24 Hours)

The findings of the present study demonstrated that the ethanolic extract from basil leaves, when tested at varying concentrations, resulted in a lethal concentration (LC50) of 17.78 ppm for *Anopheles arabiensis* and 16.98 ppm for *Culex quinquefasciatus* larvae. On the other hand, the ethanolic extract from basil flowers showed an LC50 of 15.48 ppm for Anopheles arabiensis and 15.84 ppm for *Culex quinquefasciatus* larvae **(Tables 2 and 3; Figures 1 and 2)**.

The LC50 data revealed that the flower extract exhibited a stronger effect on *Anopheles* larvae than on *Culex* larvae. Moreover, the flower extract demonstrated greater efficacy and biological activity than the leaf extract.

Table 2. The action of ethanol extract of leaves on mosquito larvae (24 hrs)

Conc. (ppm)	Log- Conc	*A. arabiensis*		*C. quinquefasciatus*	
		Mortality (%)	Probit	Mortality (%)	Probit
59.3	1.773	97	6.88	92	6.41
47.44	1.676	82	5.92	87	6.13
35.58	1.551	75	5.07	80	5.84
23.72	1.375	57	5.18	52	5.05
11.86	1.074	47	4.92	32	4.53
R2		0.64		0.97	
slope		2.36		2.79	
x-coefficient		2.07		1.43	
LC_{50}		17.78 ppm		16.98 ppm	
LC_{95}		56.23 ppm		64.56 ppm	

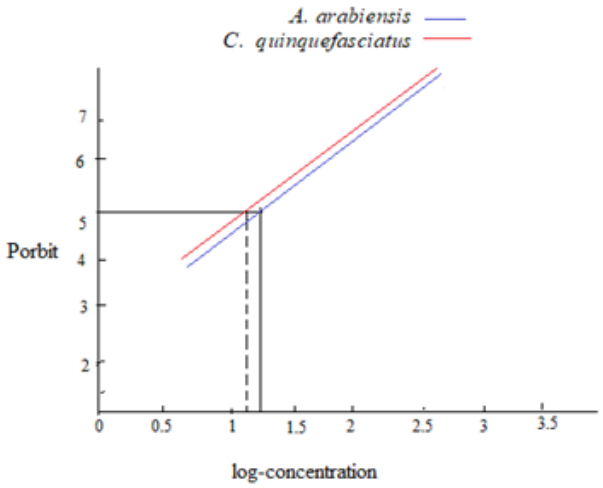


Figure 1. Log- Probit curve of action of ethanol extract of leaves on mosquito larvae (24hrs)

Table 3. The action of ethanol extract of flower on mosquito larvae (24hrs)

Conc. (ppm)	Log- Conc	*A. arabiensis*		*C. quinquefasciatus*	
		Mortality (%)	**Probit**	**Mortality (%)**	**Probit**
58.56	1.76	90	6.28	97	6.88
46.86	1.67	85	6.04	87	6.13
35.15	1.54	72	5.58	77	5.74
23.43	1.36	57	5.18	62	5.31
5.86	0.76	35	4.61	30	4.48
R2		0.912		0.88	
slope		1.60		2.12	
x-coefficient		3.25		2.69	
LC_{50}		15.48 ppm		15.84 ppm	
LC_{95}		66.06 ppm		50.11 ppm	

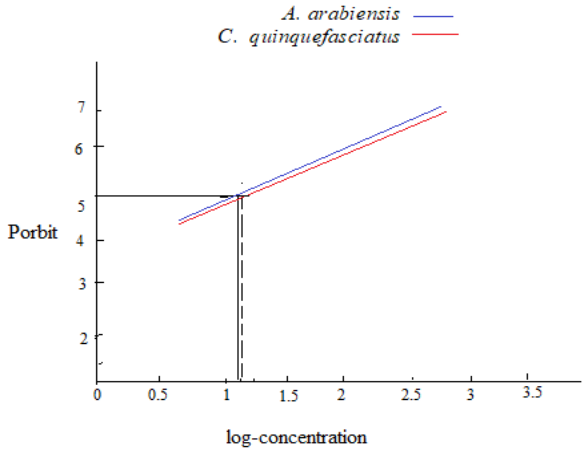


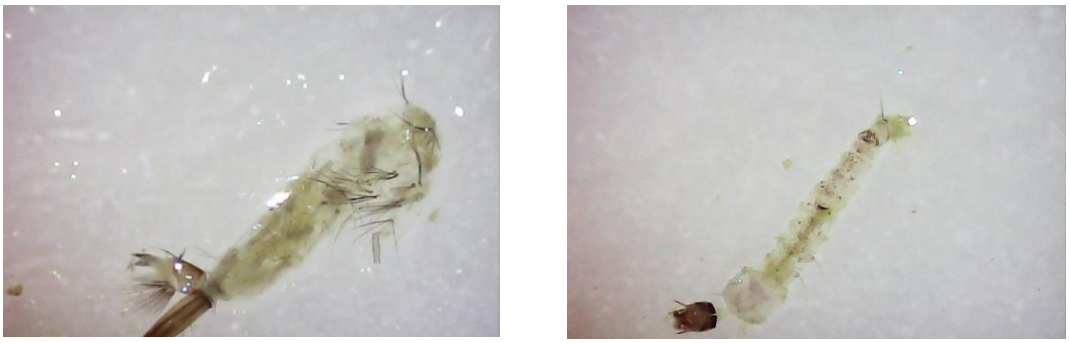
Figure 2. Log- Probit curve of action of ethanol extract of flower on mosquito larvae (24hrs)

Damage to Mosquito Larvae Caused by Basil Plant Extracts

Mosquito larvae exposed to basil plant extracts exhibited several deformities. Notable changes included discoloration (bright coloration), detachment of the alimentary canal, and swelling of the alimentary canal, which was not properly connected to the head **(Figure 3)**.

a)

b)



c) d)

Figure 3. The damage to mosquito larvae caused by basil plant extracts. a) Control larva, b) Bright color larva, c) Larvae swollen alimentary canal that was not attached to the head, d) Larva disconnected the alimentary canal

Mosquitoes, recognized as key vectors for transmitting diseases, present a significant challenge in both public health and entomological research [16]. Recently, there has been growing interest in the use of plant-based products as alternatives for pest control. Unlike synthetic insecticides, plant extracts offer a rich source of bioactive compounds that are generally less toxic to mammals and biodegradable [17, 18]. This shift is crucial due to concerns over the increasing resistance of mosquitoes to synthetic chemicals and the environmental and food safety risks posed by traditional insecticides [19].

Ocimum basilicum, known for its diverse phytochemical content, is rich in compounds such as terpenoids, flavonoids, alkaloids, tannins, and glycosides, which contribute to its pharmacological benefits [20]. In this study, the phytochemical analysis of *O. basilicum* leaves revealed the presence of glycosides, tannins, steroids, and flavonoids, with alkaloids, saponins, and terpenoids absent. In contrast, the flowers contained tannins, steroids, terpenoids, and flavonoids, but lacked alkaloids, glycosides, and saponins.

The study found that the phytochemicals in basil have significant larvicidal effects on mosquito larvae. The findings are in line with the research of Azhari *et al*. [9], which demonstrated the larvicidal and repellent properties of *O. basilicum* against the dengue vector (*A. aegypti*). Basil has also been shown to have insecticidal properties against *Culex quinquefasciatus* larvae under laboratory conditions, with aqueous plant extracts exhibiting effective LC50 values of 5.32% [21]. These results further support the potential of basil as an effective mosquito control agent when used in water extracts and other solvents.

In another study, hexane extracts of basil leaves were tested against *Anopheles arabiensis* larvae, demonstrating excellent repellent activity against adult mosquitoes for up to two hours [22]. Although the current study focused on larval stages, the efficacy of ethanol extracts corroborates previous findings. Ethanol extracts of *O. basilicum* exhibited significant repellency and larvicidal activity against *A. aegypti* and *C. quinquefasciatus*, supporting the consistency of these results with prior studies [23].

Furthermore, basil's essential oils, when used as larvicides, show promising effects against *Anopheles arabiensis* larvae, with an LC50 value of 58 mg/l and LC90 of 143 mg/l, contributing to malaria control efforts [24]. These findings align with the current study's results and emphasize the potential of plant-based bio-agents in mosquito control programs. Many studies have suggested that essential oils of *O. basilicum* could serve as a natural repellent [25-27], with *Anopheles* larvae proving more susceptible than *Culex* species [28]. Basil leaf essential oils have also been recommended as an efficient repellent and a moderate larvicide against *A. aegypti* [29, 30].

The current study also highlighted the morphological changes induced by basil extracts in mosquito larvae, including discoloration, swelling, and detachment of the alimentary canal. These deformities are consistent with observations in other studies, where plants like *L. camara* and *A. indica* caused similar morphological changes in mosquito larvae [14, 31]. These findings confirm the effectiveness of *O. basilicum* as a potential bio-pesticide for mosquito control.

Conclusion

The study found that the ethanol extract of *O. basilicum* demonstrated greater effectiveness against *Anopheles arabiensis* larvae compared to *Culex quinquefasciatus* larvae, with the flower extract showing higher mortality

rates than the leaf extract. Based on these findings, the study recommends *O. basilicum* ethanol extract as an effective, eco-friendly natural larvicide.

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