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The Efficiency of Ethanolic Extract of *Ocimum basilicum* Leaves and Flowers agiainst Mosquito Larvae

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ABSTRACT

Mosquito control remains a powerful method to control mosquito-transmitted diseases. Focusing on early stages is very important for many reasons because they are easy to handle, significantly affected by ambient environmental influences, natura.032.0l, and chemically toxic. The current study was aimed to examine the chemical components and larvicidal effect of Ethanolic extract of *O. basilicum* leaves and flowers on mosquito larvae. Fresh *Ocimum basilicum* was collected, dried in the shade for one week, and extracted with ethanol. The larvae were placed in plastic containers and bioassayed according to the protocol approved by WHO. After 24 hours of the recovery period, mortality percentage was recorded. Dead larvae were examined under a microscope to observe any morphological changes. The statistical analysis using SPSS program version 19 was done to determine Probit data. The phytochemical investigation of *O. basilicum* leaves presented many components such as flavonoids, glycosides, tannins, and steroids, whereas alkaloids, saponins, and terpenoids were absent.
Moreover, *O. basilicum* flowers showed the presence of only tannins, terpenoids, steroids, and flavonoids, but not Saponins, glycosides alkaloids. The action of leaves and flower extracts were given LC_{50} values of 17.78 ppm, 16.98 ppm, 15.48 ppm, and 15,84 and LC_{95} values were 56.23 ppm, 64.56 ppm, 66.06 ppm, and 50.11 ppm against larvae of *Anopheles. Arabiensis* and *Culex. quinquefasciatus*, respectively. Moreover, when treated with the ethanolic extract, mosquito larvae showed some morphological changes, such as decoloration and alimentary canal deformity.

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

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INTRODUCTION

Mosquitoes, including *Culex pipiens*, Anopheles, and aedes which belong to the family of Diptera: Culicidae, are responsible for transmitting many diseases. In addition to their higher cost, chemical insecticides remain one of the main factors that cause damage to the environment. Most mosquitoes are becoming resistant to insecticides [1, 2]. Research in the last years has concentrated on the discovery of new active compounds from plants as natural products [3]. Plants are significant resources for discovering new natural products to replace chemical ones [4]. Many mosquito insecticides derived from plants proved to be inhibitors [5].

Ocimum basilicum L - belongs to the family Lamiaceae - is used globally in folk medicine [6]. The Aedes *aegypti*, showed high sensitivity to *Ocimum basilicum* L leaf extract with concentrations of 0.3%, 0.6%, 0.9%, 1.2%, 1.5% [7]. Basal leaf powder, when extracted by ethanol, becomes more toxic to insects. Therefore, it is recommended to use for controlling American cockroaches [8]. Basil essential oils were more effective against the third instar of *A. aegypti* larvae. It gives the best LC_{50} and LC_{90} values when applied in the laboratory [9, 10]. Oil constituents gave 100% mortality when tested against mites [11]. The basil leaves gave good results when used against agriculture pests in rice and showed a high mortality rate [12]. The current study was aimed to examine the chemical components and larvicidal effect of Ethanolic extract of *O. basilicum* leaves and flowers on mosquito larvae. The study results would likely be aimed to contribute to the search for natural products in mosquito control programs.

MATERIALS AND METHODS

Plant material

Fresh samples of *Ocimum basilicum* were collected randomly from the garden of the College of Science, University of Imam Mohammed Iben Saud Islamic, where plant parts were processed and identified by a department member. Leaves and flowers samples were dried in the shade for one week, then were powdered and kept in a sealed plastic container until using for phytochemical investigation and bioassay experiments.

Phytochemical screening of extracts

The selected parts of *O. basilcum* were screening preliminary phytochemical to identify the chemical constituents (main classes). The methods described by Mohamed Nour (2009) [13]. were used to screen alkaloids, saponins, tannins, flavonoids, glycosides, steroids, and terpenoids.

Preparation of ethanolic extracts

The plant parts (leaves and flowers) were extracted by ethanol, according to Nosiba S *et al.* [14].

Larvae of mosquitoes

Under laboratory conditions, mosquito larvae were collected and reared in the Biology Department laboratory, Faculty of Science. The larvae were placed in plastic containers and provided with all nutrients required.

Larval bioassay

The larvicidal application was performed according to the protocol approved by WHO [15]. The test included 20 larvae (third and early fourth instar stages) of mosquito *A. arabiensis,* and *C. quinquefasciatus* put 250 ml of tap water into container cups. Sequences of concentrations were used. Each attention was of three replicates of extract, in addition to control groups without section. After 24 hours, the mortality percentage was recorded. Dead larvae were counted and examined under a microscope to observe morphological changes.

Data and statistical analysis

Data were analyzed using SPSS program version 19 to determine Probit parameters that included: percentage mortalities of larvae *A. arabiensis and C. quinquefasciatus*, LC_{50} and LC_{90} values at specific P-value, slope, regression (R^2), and x-coefficient.

RESULTS AND DISCUSSION

The phytochemical constituents of O. basilicum leaves and flowers

O. basilicum leaves showed the variant amount of phytochemical components **(Table 1)**.

Table 1. The phytochemical constituents of *O. basilicum* leaves and flowers

Plant part	Alkaloids	Saponins	Tannins	Flavonoids	Glycosides	Steroids	Terpenoids
O. basilcum leaves	-	-	+	+	+	+	-
O. basilcum flowers	-	+	-	+	++	-	+

- means the absence of the main class

+ indicates the presence of the main class

++ implies the presence of the main class in a relatively higher concentration

The action of ethanol extract of leaves and flower of basil plants on mosquito larvae (24hrs)

The results obtained by the current study showed that leave ethanol extracts of basil plant

tested at different concentrations of the lethal concentration (LC_{50}), which was 17.78 ppm for *Anopheles. arabiensis* and 16.98 ppm for *Culex. quinquefasciatus* larvae. Meanwhile, flowers ethanol extract at different concentrations showed lethal concentration (LC_{50}) that was 15.48 ppm for *Anopheles arabiensis* and 15,84 ppm for *Culex. quinquefasciatus* larvae **(Tables 2 and 3; Figures 1 and 2)**.

According to LC_{50} value, the flower presented good action against *Anopheles* larvae compared to *Culex* larvae. Additionally, the flowers extract indicated more potency and biological activity compared to the extract obtained from leaves.

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

Conc. (ppm)	Log- Conc	*A. arabiensis*		*C. quinquefasciatus*	
		Mortality %	Probit	Mortality %	Probit
٥٩,٣	1.773	97	6.88	92	6.41
٤٧,٤٤	1.676	82	5.92	87	6.13
٣٥,٥٨	1.551	75	5.07	80	5.84
٢٣,٧٢	1.375	57	5.18	52	5.05
١١,٨٦	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	

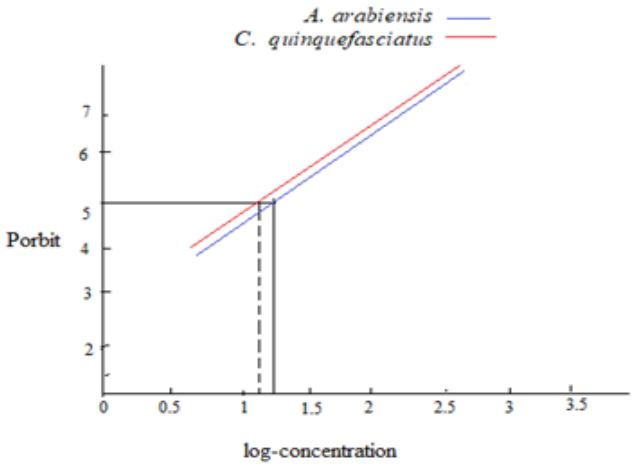

Figure 1. Log- Probit curve of action of ethanol extract of leaves on mosquito larvae (24hrs)

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}		15.48ppm		15.84ppm	
LC_{95}		66.06ppm		50.11ppm	

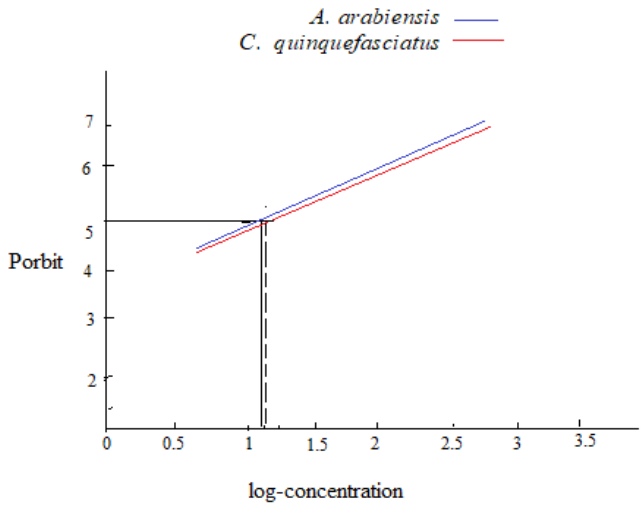

Figure 2. Log- Probit curve of action of ethanol extract of flower on mosquito larvae. (24hrs)

The damage on mosquito larvae caused by basil plant extracts

Mosquitoes larvae treated with plant parts section of basil revealed larvaeal malformation. Discoloration (bright color), disconnected alimentary canal, and swollen alimentary canal that was not attached to the head were the main changes observed **(Figure 3)**.

Control larva

Bright color larva

Larvae swollen alimentary canal that was not attached to head

Larva disconnected the alimentary canal

Figure 3. The Damage on mosquito larvae caused by basil plant extracts

The mosquitoes are a series of vectors transmitted diseases, and control plans remained reflected as a very difficult triangle, both in health and entomological sciences [16]. Recently, the use of plant products received significant attention and proved to be alternative sources as parasitic control agents since - compared to synthetic products - they constitute a rich source of eco-friendly bioactive compounds. The plant extracts are suitable and safe alternatives due to their low toxicity to mammals and easy biodegradability [17, 18].

Identifying novel effective mosquitocidal compounds are essential to overcome the increasing resistance rates of synthetic insecticide concern for the environment and food safety, the unacceptability of many chemicals insecticide [19]. The Ocimum basil proved to have richness in phytochemical components such as terpenoids, alkaloids, phenolics, flavonoids, tannins, saponin, reducing sugars, cardiac glycosides, steroids, and glycosides that cause pharmacological advantages [20]. The current study showed that the phytochemical constituents of *O. basilicum* in leaves were tannins, glycosides, steroids, and flavonoids, while saponins, alkaloids, and terpenoids were absent. On the other hand, *O. basilicum* flowers showed the presence of tannins, terpenoids, steroids, and flavonoids. While, Saponins, glycosides alkaloids were not detected.

The study suggested that the basil content phytochemicals act more toxic to mosquito leave and other stages. In the present study, the larvicidal activity of O. basilicum seemed to be supported by Azhari *et al.* [9]. Further, it has the larvicidal potential and repellent against the dengue vector *Ae. aegypti*. Also, it showed toxicity in experimental animals. The study of the insecticidal potentiality of basil was tested against *Culex quinquefasciatus* (Say) under laboratory conditions. Five concentrations of aqueous plant extracts against the 4th instar larvae for 24 and 48h were used after exposure. The LC_{50} values demonstrated (5.32%) [21]. The previous results support our results and suggest that bioactive basil content can be more effective with water extract and other solvents. *Ocimum basilicum* L leaves extracted by ethanol and hexane and applied against the larvae of *Anopheles arabiensis* Patton. Results showed that basil leaves hexane extracts at the rate of 10% were the best to repel the adult insect for up to 2h [22]. This result is not similar to the result obtained by the current study because the application was in adult mosquitoes. Moreover, when the ethanol extract efficacy was considered, it supports our findings. In the dose-response test of *Ocimum basilicum* (*O. basilicum*), a high repellency against *Ae. Aegypti* is exhibited and a strong, effective dose against *Cx. quinquefasciatus* is obtained. In addition, the two essential oils exhibited moderate repellency against *Ae. aegypti, An. dirus* and *Cx. quinquefasciatus*, at 60, 90, and 78 min with *C. nardus*, and 54, 96, and 72 min with *S. aromaticum*, respectively [23]. These results were consistent with results obtained by the current study. The leaves powder (LP) of *Ocimum basilicum* is extracted by ethanolic and used as a larvicide of *Anopheles arabiensis* 3rd instar larvae. The study accomplished malaria control findings by using basil as a bio-agent (Basil leaves extracts LC50 of 58mg/l and LC90 of 143mg/l) [24]. The previous studies were similar to the results obtained by the current study. Many plants may affect the mosquito that given lethal effect against *Anopheles* and *Culex larvae*, it can be recommended to be used in mosquito control programs [15, 24, 25]. Many researcher suggest that essential oils of Ocimum basilicum could be used as natural repellent [26, 27] *Anopheles larvae* is more susceptible than *Culex* [28]. Kumar *et al.* recommended the efficient use of basil leaf essential oil as an efficient repellent and as a moderate larvicide agent against *Ae. aegypti* [29, 30]. The previous studies indicate that the plant has the ability to inhibit mosquito larvae and control it without using chemical pesticides due to the sensitivity of mosquito stages. Some plants such as L. Camara *A. indica* leaves, and flower leaves give changes in morphology [14, 31]. This finding is similar to the results of the current study. Changes in *O. basilicum* leaves, and flower ethanol extracts in morphological (Bright color, swelled disconnected alimentary canal and head) of *A. arabiensis* and *C. quinquefasciatus* were noted.

CONCLUSION

The study concluded that according to LC's values obtained, the plant extract displays more potentiality against *A. arabiensis* larvae than C. *quinquefasciatus* larvae, and the flowers were better than leaves in mortality rate. Therefore, the study recommended that *O. basilicum* ethanol extract is the best natural larvcial and it is eco-friendly.

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