The Efficiency of Ethanolic Extract of *Ocimum basilicum* Leaves and Flowers against 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, naturally, 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 ppm 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.

**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].

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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 LC₅₀ and LC₉₀ 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. basilicum 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, LC₅₀ and LC₉₀ values at specific P-value, slope, regression (R²), 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).

| Plant part     | Alkaloids | Saponins | Tannins | Flavonoids | Glycosides | Steroids | Terpenoids |
|----------------|-----------|----------|---------|------------|------------|----------|------------|
| O. basilicum leaves | -         | -        | +       | +          | +          | +        | -          |
| O. basilicum 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** |
| 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$_{95}$   |           | 50.11 ppm     |                    | 56.23 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 larvaal 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).

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 basilicum 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 leaf 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 LC50 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 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 larvicde 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 larvicde 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 larvicidal and it is eco-friendly.

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REFERENCES

1. El Ouali Lalami A, El-Akhal F, Ez Zoubi Y, Taghzouti K. Study of Phytochemical Screening and Larvical Efficacy of Ehtanolic Extract of Salvia officinalis (Lamiaceae) from North Center of Morocco against Culex pipiens (Diptera: Culicidae) Vector of Serious Human Diseases. Int J Pharmacog Phytochem Res. 2016;8(10):1663-8. Available online on www.ijppr.com

2. Krishnappa K, Pandiyan J, Elumalai K, Baranitharan M, Jayakumar S, Gokulakrishnan J. GC–MS analysis and mosquitocidal properties of Loranthes pentandrus Linn.(Loranthaceae) against human vector mosquitoes (Diptera: Culicidae). Acad J Med Plants. 2019;7(12):261-8. doi:10.15413/ajmp.2019.0154

3. Lupi E, Hatz C, Schlagenhauf P. The efficacy of repellents against Aedes, Anopheles, Culex aIxodes spp. A literature review. Trav Med Infect Dis. 2013;11(6):374-411.

4. Asadollahi A, Khoobdel M, Zahraei-Ramazani A, Azarmi S, Mosawi SH. Effectiveness of plant-based repellents against different Anopheles species: a systematic review. Malar J. 2019;18(1):1-20. doi:10.1186/s12936-019-3064-8.

5. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc. 1991;7(2):210-37.

6. Miele M, Dondoro R, Ciarallo G, Mazzel M. Methyleugenol in Ocimum basilicum L. Cv. genovese gigante. J Agric Food Chem. 2001;49(1):517-21.

7. Husna I, Setyaningrum E, Handayani TT, Kurnia Y, Palupi EK, Umam R, et al. Utilization of Basil Leaf Extract as Anti-Mosquito Repellent: A Case Study of Total Mosquito Mortality (Aedes aegypti 3rd Instar). J Phys: Conf Ser. 2020;1467(1):012014.

8. Ukoorije BR, Island W, Island W, Island W. The efficacy of Ocimum gratissimum leaf powder and ethanol extract on adult Periplanata americana under laboratory condition. Open Access Libr J. 2018;5(04):1. doi:10.4236/oalib.1104455

9. Azhari H, Abdurahman H, Mashitah M, DO SJ. Bioactive compounds from Basil (Ocimum basilicum) essential oils with larvicidal activity against Aedes aegypti larvae. 3rd Int. Conf Bio, Env Chem, Singapore; 2012;46:21-4.

10. Amer A, Mehlhorn H. Larvical effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). Parasitol Res. 2006;99(4):466-72.

11. Perumalsamy H, Kim JY, Kim JR, Hwang KN, Ahn YJ. Toxicity of basil oil constituents and related compounds and the efficacy of spray formulations to Dermatophagoides farinae (Acari: Pyroglyphidae). J Med Entomol. 2014;51(3):650-7.

12. López MD, Jordán MJ, Pascual-Villalobos MJ. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. J Stored Prod Res 2008;44(3):273-8.

13. Mohamed Nour AA. Chemical component of the essential oil of basil (Ocimum basilicum L.) active against Salmonella typhi [Doctoral dissertation, M. Sc. Thesis]. Sudan: University of Gezira; 2009.

14. Hamid NS, Kehail MA, Ibrahim NA, Abdel-Rahman EH. Larvicidal activity of ethanol extracts of Azadirachta indica (neem) against Anopheles Arabiensis and Culex Quinquefasciatus larvae, Gezira state, Sudan. Int J Entomol Res. 2021;6(1):138-41.

15. World Health Organization. Pesticides and their application: for the control of vectors and pests of public health importance. World Health Organization; 2006. WHO/CDS/WHOPES/GCDPP/2006.1.
16. Yousif HY. Susceptibility of Anopheles arabiensis Patton and Culex quinquefasciatus Say Larvae to Ixora (Ixora coccinea L) Leaves and Flowers Extracts. [dissertation on the internet]. Sudan: University of Gezira; 2013. [cited 2013 May 16]. Available from: http://repo.uofg.edu.sd/handle/123456789/2165

17. Aouati A, Roubhi AH, Boudjahem I, Berchi S. Study of toxicological activity of the essential oil of Ocimum basilicum L. against Culicidae larvae in Algeria. AIP Conf Proc. 2019;2190(1):020073. doi:10.1063/1.5138559

18. Madhumitha G, Rajakumar G, Roopan SM, Rahuman AA, Priya KM, Saral AM, et al. Acaricidal, insecticidal, and larvicidal efficacy of fruit peel aqueous extract of Annona squamosa and its compounds against blood-feeding parasites. Parasitol Res. 2012;111(5):2189-99. doi:10.1007/s00436-011-2671-2

19. Shaalan EA, Canyon D, Younes MW, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. Environ Int. 2005;31(8):1149-66. doi:10.1016/j.envint.2005.03.003.

20. Al-Snafi AE. Chemical constituents and pharmacological effects of Ocimum basilicum-A review. Int J Pharm Res. 2021;13(2):2997-3013.

21. Iqbal J, Ishtiaq F, Alqarni AS, Owayss AA. Evaluation of larvicidal efficacy of indigenous plant extracts against Culex quinquefasciatus (Say) under laboratory conditions. Turk J Agric For. 2018;42(3):207-15. doi:10.3906/tar-1711-69

22. Elsiddig FEI, Khei SEM. Effect of Neem, Cafure, and Rehan Organic Extracts Compared to Other Chemical and Biological Standard Insecticides on Anopheles arabiensis Patton Mosquito. [dissertation on the internet]. Agricultural Studies College of Agricultural Studies: Sudan University of Science and Technology; 2007. [cited 2007 April 1]. Available from: http://repository.sustech.edu/handle/123456789/3504

23. Phasomkusolsil S, Soonwera M. Comparative mosquito repellency of essential oils against Aedes aegypti (Linn.), Anopheles dirus (Peyton and Harrison) and Culex quinquefasciatus (Say). Asian Pac J Trop Biomed. 2011;1(1):S113-8. doi:10.1016/S2221-1691(11)60136-6

24. Ali, HE. Effect of Basil (Ocimum basilicum L) Leave Powder and Ethanolic Extract on the 3rd Larval Instar of Anopheles arabiensis (Patton,1905) Culicidae: Diptera. [dissertation on the internet]. Sudan: University of Gezira; 2013. [cited 2013 January 31]. Available from: http://repo.uofg.edu.sd/handle/123456789/2171

25. Panneerselvam C, Murukan K, Kovendan K, Kumar PM, Subramaniam J. Mosquito larvicidal and pupicidal activity of Euphorbia hirta Linn.(Family: Euphorbiaceae) and Bacillus sphaericus against Anopheles stephensi Liston.(Diptera: Culicidae). Asian Pac J Trop Med. 2013;6(2):102-9. doi:10.1016/S1995-7645(13)60003-6

26. Ileke KD, Adesina JM. Toxicity of Ocimum basilicum and Ocimum gratissimum extracts against main malaria vector, Anopheles gambiae (Diptera: Culicidae) in Nigeria. J Arthropod Borne Dis. 2019;13(4):362.

27. Adam AA, Ahmed SA, Mohamed TA, Azrag RA, Mustfa SE, Hamdi AA. Evaluation of repellent activities of the essential oil of Ocimum basilicum against Anopheles mosquito and formulation of mosquitoes repellent cream. Biomed Res Clin Prac. 2019;4:2-5. doi:10.15761/BRCP.1000184

28. Abdalla AI, Kehail MA, Abdelrahim YM, Ibrahim NA. Phytochemical screening of Calotropis procera ait flower parts and their larvicidal potentialities against Anopheles and Culex Larvae, Gezira State, Sudan. Int J Biol Res. 2017;2(2):88-92.

29. Govindarajan M, Rajeswary M. Ovicidal and adulticidal potential of leaf and seed extract of Albizia lebbeck (L) Benth. (Family: Fabaceae) against Culex quinquefasciatus, Aedes aegypti, and Anopheles stephensi (Diptera: Culicidae). Parasitol Res. 2015;114(5):1949-61. doi:10.1007/s00436-015-4384-4
30. Kumar S, Warikoo R, Mishra M, Samal RR. Impact of Ocimum basilicum Leaf Essential Oil on The Survival and Behaviour of an Indian Strain of Dengue Vector, Aedes aegypti (L.). Vector Biol J. 2017;2:2. doi:10.4172/2473-4810.1000122

31. Alghamdi AA, Basher NS. Efficacy of leaves and flowers ethanol extracts of the invasive species Lantana camara Linn as a Mosquito larvicidal. Int J Mosq Res. 2020;7(5):43-7.