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# Larvicidal potency of selected botanical extracts on *Culex quinquefasciatus* (Diptera: Culicidae)

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## Abstract
The increasing need for environmentally safe and sustainable mosquito control methods has led to growing interest in plant-based larvicides. This study evaluated the larvicidal potential of extracts from *Foeniculum vulgare* (fennel) and *Brassica nigra* (black mustard) against *Culex quinquefasciatus* larvae. Plant materials were extracted using two solvents, benzene and water, to assess the influence of solvent type on larvicidal activity. Larval mortality was recorded after 24, 48, 72, and 96 hours of exposure at concentrations of 50, 150, 250, 350, and 450 ppm. Among the tested extracts, benzene extracts exhibited the highest larvicidal efficacy, resulting in greater larval mortality than aqueous extracts. The findings highlight the important role of solvent polarity in extracting bioactive compounds responsible for larvicidal activity. Overall, the study suggests that locally available plants such as *F. vulgare* and *B. nigra* possess promising bioinsecticidal properties and could serve as eco-friendly alternatives to synthetic chemical insecticides in mosquito vector management programs.

**Keywords:** Plant-based larvicides, *Culex quinquefasciatus* control, *Foeniculum vulgare*, *Brassica nigra* and eco-friendly bioinsecticides

## Introduction
Mosquitoes are among the most important insect vectors, transmitting a variety of infectious diseases that affect millions of people worldwide. They play a significant role in spreading illnesses such as malaria, dengue fever, chikungunya, yellow fever, tularemia, filariasis, and dirofilariasis. Among the different mosquito genera, *Culex* species, commonly referred to as house mosquitoes, are particularly important because they thrive in stagnant water and serve as vectors for several pathogens, including the West Nile virus [1].

Controlling mosquito populations at the larval stage is considered one of the most effective approaches for reducing disease transmission. Since mosquito larvae develop in aquatic habitats, targeting this stage can significantly interrupt their life cycle before they emerge as disease-carrying adults. Conventional mosquito control programs have largely depended on synthetic insecticides such as pyrethroids, organophosphates, and carbamates [2]. Although these chemicals have been widely used due to their effectiveness, their long-term and excessive application has led to several concerns, including environmental contamination, adverse effects on non-target organisms, and the development of insecticide resistance among mosquito populations [3].

Mosquito-borne diseases continue to pose a serious threat to public health, particularly in tropical and subtropical regions where environmental conditions favour mosquito breeding. According to the World Health Organisation (WHO), these diseases contribute to more than one million deaths each year, highlighting the urgent need for safer and more sustainable mosquito control measures [4].

In recent years, increasing attention has been given to plant-based products as environmentally friendly alternatives to synthetic insecticides. Numerous studies have reported that plant extracts, essential oils, and powdered plant materials possess significant larvicidal activity against various mosquito species [5, 6]. The effectiveness of these botanical products is largely attributed to the presence of bioactive compounds such as flavonoids, alkaloids, glycosides, and terpenoids. These phytochemicals can interfere with larval growth, development, and physiological functions through mechanisms that differ from those of conventional insecticides [7]. As a result, plant-derived larvicides are considered promising tools for mosquito management, offering effective control while minimising environmental risks and reducing the likelihood of resistance development [8].

## Materials and Methods
### Experimental Site
The present investigation was conducted in the Entomology Laboratory of Vidyasagar College, Salt Lake Campus, Kolkata, India. All experimental procedures were carried out under controlled laboratory conditions, with an ambient temperature of 30 ± 2°C and relative humidity maintained at 75 ± 5%.

### Collection and Maintenance of Mosquito Larvae
Larvae of *Culex quinquefasciatus* were collected from stagnant water bodies and drainage channels located in and around the Vidyasagar College campus. The collected larvae were transported to the laboratory in clean plastic containers containing water from their natural breeding habitats to minimise handling stress.

Identification of the larvae was performed using standard morphological characteristics described in entomological identification keys. The larvae were subsequently maintained in aquaria containing dechlorinated tap water and reared under suitable laboratory conditions until they were used for larvicidal bioassays.

### Collection of Plant Materials

Fresh plant materials of *Foeniculum vulgare* (fennel) and *Brassica nigra* (black mustard) were collected from local sources in the vicinity of the Vidyasagar College campus. The plants were identified and authenticated based on their morphological features using standard botanical references.

The collected leaves were thoroughly washed with tap water followed by distilled water to remove dust, dirt, and other contaminants before further processing.

### Preparation of Plant Extracts

The collected leaves were shade-dried for one week and then finely ground into powder using an electric blender. The powdered plant material was transferred into clean conical flasks for extraction.

Two solvents, benzene and distilled water, were used separately for the extraction process. The plant powder was mixed with the respective solvents and allowed to stand at room temperature for three days. During this period, the mixtures were stirred gently three times daily to facilitate efficient extraction of bioactive compounds.

After three days, the extract-containing layer was carefully separated and transferred to Petri dishes. The remaining residue was discarded. The collected extracts were then dried in an incubator for 2–3 days until the solvents had completely evaporated. The resulting dried crude extracts were stored in airtight containers for subsequent bioassay experiments.

### Larvicidal Bioassay

Different concentrations of the crude plant extracts were prepared by dissolving 0.2 g of the dried extract in appropriate volumes of distilled water. The concentrations tested were 50, 150, 250, 350, and 450 ppm.

For each concentration, three replicates were prepared and labelled as R1, R2, and R3. Five *C. quinquefasciatus* larvae were introduced into each experimental cup using a dropper. The cups were placed in larger trays and covered with perforated newspaper to ensure adequate ventilation while preventing contamination.

Larval mortality was recorded after 24, 48, 72, and 96 hours of exposure. Dead larvae were counted at each observation interval, and the mortality data were recorded for further analysis.

### Statistical Analysis

The experimental data were analysed using Microsoft Excel 2020. Parameters such as mean larval mortality, standard error, regression coefficient, and $LC_{50}$ (median lethal concentration) values were calculated.

Probit analysis was performed following the method described by Finney (1952) to determine the $LC_{50}$ values of the plant extracts against *C. quinquefasciatus* larvae.

Regression analysis and correlation coefficients were also calculated to evaluate the relationship between extract concentration and larval mortality.

### Results

During the present experiment, the most common and locally available plant species leaf extracts were applied to the larvae of *C. quinquefasciatus* at different time intervals. Mosquito larvae also showed different morphological changes after exposure to different concentrations of five plants at different times. The results were presented in the following Tables 1, 2, and 3.

The percentage mortality of *Culex quinquefasciatus* larvae when exposed to different concentrations of *Foeniculum vulgare* extracts in water after 24 hours of treatment was 6.67,13.33,13.33,26.67, and 6.67%. After 48 hours of treatment was 6.67,13.33,20,46.67and 6.67%. After 72 hours of treatment, it was 6.67, 20, 20, 46.67 and 6.67%. After 96 hours of treatment was 20,33.33, 20, 53.33 and 13.33%. Similarly, in benzene, after 24 hours of treatment was 73.33, 60,80, 93.33 and 93.33%. After 48hrs of treatment was 93.33, 80, 93.33, 100 and 100%. After 72hrs of treatment was 100,100,100,100 and 100%. After 96hrs of treatment, it was 100, 100, 100, 100 and 100% (Table 1).

The percentage mortality of *Culex quinquefasciatus* larvae when exposed to different concentrations of *Brassica nigra* extracts in water after 24hrs of treatment was 46.67,13.33,6.67,13.33, and 26.67%. After 48 hours of treatment was 53.33,40,26.67,33.33 and 46.67%. After 72hrs of treatment was 53.33, 60, 33.33, 46.67 and 60%. After 96hrs of treatment was 60,60, 40, 60 and 60%. Similarly, in benzene, after 24hrs of treatment was 60, 80,86.67, 66.67 and 100%. After 48hrs of treatment, it was 86.67, 93.33, 100, 93.33 and 100%. After 72hrs of treatment was 100,100,100,100 and 100%. After 96hrs of treatment, it was 100, 100, 100, 100 and 100%**.** In the same concentrations of 50, 100, 150, 200, 250 and 300ppm, respectively (Table 1).

The result of the present study indicated that the mortality rate of *C. quinquefasciatus* larvae treated with crude extracts of spices was significantly higher than the mortality rates at 50 ppm,150ppm, 250ppm, 350 ppm and 450 ppm concentrations of each extract at 24, 48, 72 and 96 hrs of exposure (Table 3). The results of regression analysis revealed that the mortality rate (Y) was positively co-related with the period of exposure (X) with regression co-efficient close to 1. The result of log- probit analysis revealed that LC50 values gradually decreased with the exposure period with the lowest value at 96 hrs of exposure (Table 3). Relative mosquito larvicidal efficacy of the spices crude extract. The extract showed different mortality sequence, as follows: *Foeniculum vulgare* > *Brassica nigra* (Table- 3).

**Table 1:** Percentage mortality of *Culex quinquefasciatus* larvae when exposed to different concentrations of extract of *Foeniculum vulgare* at different time intervals

| | Concentration (ppm) | Mortality rate | | | |
|---|---|---|---|---|---|
| | | **24 hrs.** | **48 hrs.** | **72 hrs.** | **96 hrs.** |
| Water | 50 | 1.334±0.33 | 1.334±0.333 | 1.334±0.333 | 4.0±0.577 |
| | 150 | 2.666±0.333 | 2.666±0.333 | 4.0±0.577 | 6.667±0.666 |
| | 250 | 2.666±0.333 | 4.0±0.577 | 4.0±0.577 | 4.0±0.577 |
| | 350 | 5.334±0.333 | 9.334±0.333 | 9.334±0.333 | 10.666±0.333 |
| | 450 | 1.334±0.333 | 1.334±0.333 | 1.334±0.333 | 2.666±0.333 |

| | | | | | |
|---|---|---|---|---|---|
| Benzene | 50 | 14.666±0.666 | 18.667±0.666 | 20±0.666 | 20±0.577 |
| | 150 | 12±0.333 | 16±0.577 | 20±0.577 | 20±0.577 |
| | 250 | 16±0.333 | 18.667±0.88 | 20±0.88 | 20±1.154 |
| | 350 | 18.667±0.333 | 20±0.333 | 20±0.333 | 20±0.333 |
| | 450 | 18.667±0.333 | 20±0.333 | 20±0.577 | 20±0.577 |

**Table 2:** Percentage mortality of *culex quinquefasciatus* larvae when exposed to different concentration extract of *Brassica nigra* in different time intervals

| **Sample** | **Concentration (ppm)** | **Mortality rate** | | | |
|---|---|---|---|---|---|
| | | **24 hrs.** | **48 hrs.** | **72 hrs.** | **96 hrs.** |
| Water | 50 | 7±0.577 | 8±0.333 | 8±0 | 9±0 |
| | 150 | 2±0.577 | 6±0.577 | 9±0 | 9±0 |
| | 250 | 1±0.577 | 4±0.333 | 5±0 | 6±0 |
| | 350 | 2±0.333 | 5±0 | 7±0 | 9±0 |
| | 450 | 4±0.333 | 7±0 | 9±0 | 9±0 |
| Benzene | 50 | 9±0.577 | 13±0.333 | 15±0 | 15±0 |
| | 150 | 12±0.577 | 14±0.333 | 15±0 | 15±0 |
| | 250 | 13±0.666 | 15±0 | 15±0 | 15±0 |
| | 350 | 10±0.333 | 14±0.333 | 15±0 | 15±0 |
| | 450 | 15±0 | 15±0 | 15±0 | 150 |

**Table 3:** Log probit analysis and regression analysis of larvicidial activity of *Foeniculum vulgare and Brassica nigra* after 24,48,72 and 96hrs of exposure on *C. quinquefasciatus*

| **Plant used-*Foeniculum vulgare*** | | | | |
|---|---|---|---|---|
| **Solvent** | **Time** | **Regression equation** | **$r^2$ value** | **LC50** |
| Water | 24hrs | Y=1.71+0.98x | 0.76 | 2220.2995 |
| | 48hrs | Y=0.72+1.63x | 0.949 | 421.6969 |
| | 72hrs | Y=1.09+1.47x | 0.843 | 446.6836 |
| | 96hrs | Y=2.67+0.88x | 0.548 | 444.6319 |
| Benzene | 24hrs | Y=4.47+0.72x | 0.590 | 5.3283 |
| | 48hrs | Y=6.29+0.02x | 0.001 | 46.76572 |
| | 72hrs | Y=-4E-14x+8.95 | 0.5 | 0 |
| | 96hrs | Y=-4E-14x+8.95 | 0.5 | 0 |
| **Plant used-*Brassica nigra*** | | | | |
| **Solvent** | **Time** | **Regression equation** | **$r^2$ value** | **LC50** |
| Water | 24hrs | Y=4.84-0.32x | 0.036 | 1.62412E+12 |
| | 48hrs | Y=5.33-0.26x | 0.103 | 4.48503E+19 |
| | 72hrs | Y=5.09-0.01x | 0.0147 | 2.03496e+62 |
| | 96hrs | Y=5.16-0.01x | 0.0237 | 3.82857E+48 |
| Benzene | 24hrs | Y=5.19+0.22x | 0.062 | 51198.25168 |
| | 48hrs | Y=5.44+0.45x | 0.590 | 63270.47389 |
| | 72hrs | Y=-4E-14x+8.95 | 0.5 | 0 |
| | 96hrs | Y=-4E-14x+8.95 | 0.5 | 0 |

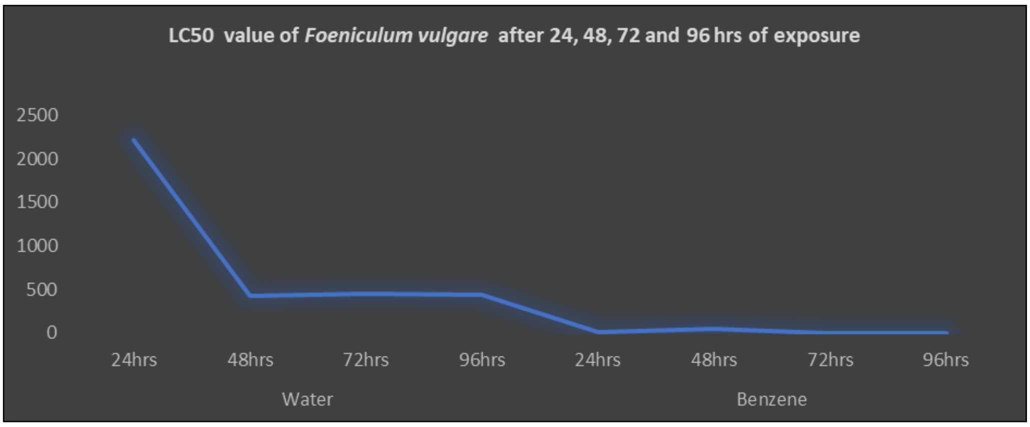


**Fig 1:** Graph showing LC50 values of *Foeniculum vulgare extract* with 2 different solvents

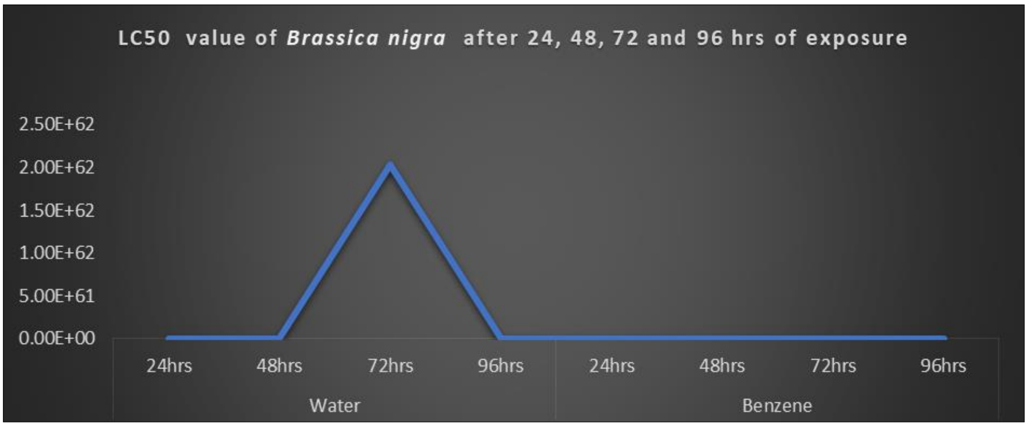


**Fig 2:** Graph showing LC50 values of *Brassica nigra extract* with 2 different solvents

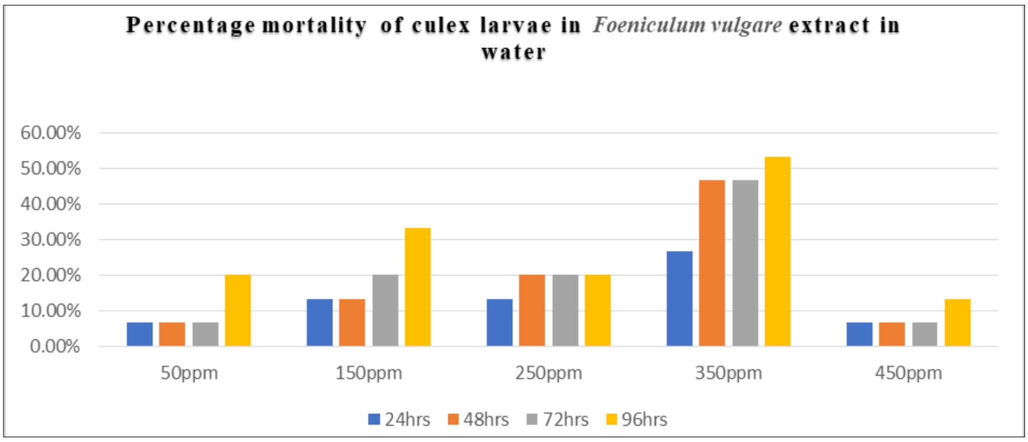


**Fig 3:** Percentage mortality of Culex larvae in *Foeniculum vulgare* extract in water

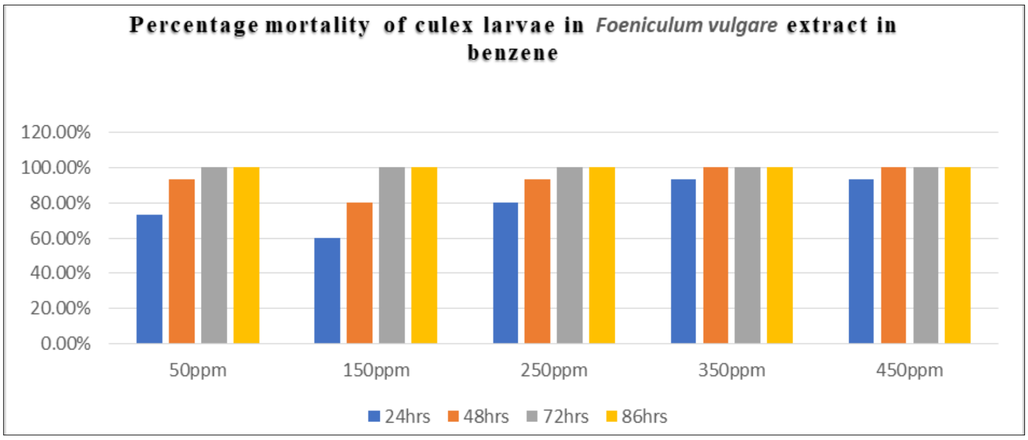


**Fig 4:** Percentage mortality of Culex larvae in *Foeniculum vulgare* extract in benzene

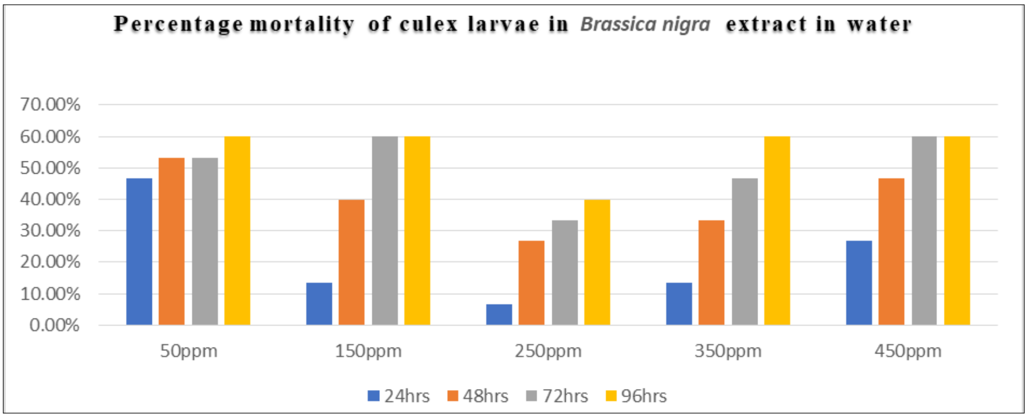


**Fig 5:** Percentage mortality of Culex larvae in *Brassica nigra* extract in water

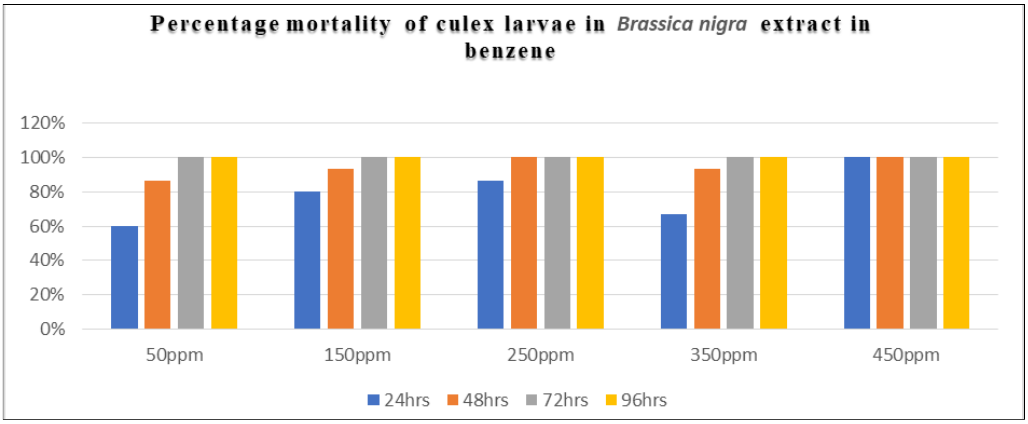


**Fig 6:** Percentage mortality of Culex larvae in *Brassica nigra* extract in benzene


**Fig:** Larvae after treatment with *Foeniculum vulgare and Brassica nigra* [A, B]: Water and [C, D]: Benzene

**Discussion**

The present study evaluated the larvicidal potential of *Foeniculum vulgare* (fennel) and *Brassica nigra* (black mustard) extracts prepared using benzene and water as extraction solvents. The extracts were tested at concentrations of 50, 150, 250, 350, and 450 ppm against mosquito larvae. Among all the extracts examined, the benzene extracts exhibited the highest larvicidal activity after 96 hours of exposure. The $LC_{50}$ values of *F. vulgare* and *B. nigra* extracts at 24, 48, 72, and 96 hours are presented in Table 3, indicating that larval mortality increased with both concentration and exposure time.

The findings of the present study are consistent with previous reports highlighting the larvicidal efficacy of plant-derived extracts against mosquito vectors. Kalimuthu *et al*. reported that the ethanol leaf extract of *Cadaba indica* Lam. showed the greatest larvicidal activity against *Aedes aegypti*, with an $LC_{50}$ value of 143.75 ppm, outperforming its hexane, chloroform, and petroleum ether extracts [9]. Similarly, Maheswaran *et al*. demonstrated that crude extracts of *Leucas aspera* were effective against *Aedes aegypti* and *Culex quinquefasciatus*, with the hexane extract showing the highest toxicity, followed by chloroform and ethanol extracts.

Warikoo and Kumar also reported significant bio-larvicidal and pupicidal activity of *Acalypha alnifolia* against different larval instars and pupae of *Culex quinquefasciatus*. Their study recorded $LC_{50}$ values of 5.388%, 6.233%, 6.884%, and 8.594% for the first, second, third, and fourth instar larvae, respectively, and 10.073% for pupae [10]. In another study, Vijayan *et al*. evaluated leaf extracts of *Euodia ridleyi* and observed considerable larvicidal activity against *Culex quinquefasciatus*. These studies collectively support the growing evidence that plant-based extracts can serve as effective and environmentally friendly alternatives to synthetic mosquito control agents [11].

The higher larvicidal activity of benzene extracts observed in this study can be attributed to the non-polar nature of the solvent, which favours extraction of lipophilic phytoconstituents. Several authors have noted that essential oils, terpenoids, and alkaloids with mosquito larvicidal properties are more soluble in non-polar or moderately polar solvents than in water. Ghosh *et al*. reviewed multiple plant species and concluded that hexane, petroleum ether, and benzene extracts frequently produce lower $LC_{50}$ values than aqueous or ethanol extracts due to better recovery of active volatiles and glycosides [12]. This pattern supports the present results, where benzene extracts of both *F. vulgare* and *B. nigra* outperformed aqueous extracts at all tested concentrations and exposure times.

The mechanism of larval toxicity for *F. vulgare* and *B. nigra* appears linked to their major volatile compounds. Traboulsi *et al*. reported that *F. vulgare* seed oil caused 100% mortality in Culex pipiens larvae within 24 h, and identified trans-anethole as the principal active component responsible for neurotoxic effects [13]. Similarly, Pavela demonstrated that seed extracts of *B. nigra* were highly toxic to *Culex quinquefasciatus* larvae, with allyl isothiocyanate disrupting acetylcholinesterase activity and membrane integrity in midgut epithelial cells [14]. These findings suggest that the larvicidal action recorded in the present study likely results from nervous system interference and cellular damage, leading to the concentration- and time-dependent mortality observed after 96 h.

**Conclusion**

The present investigation assessed the larvicidal activity of *Foeniculum vulgare* (fennel) and *Brassica nigra* (black mustard) extracts prepared using benzene and water. The extracts were tested at concentrations of 50, 150, 250, 350, and 450 ppm against *Culex* mosquito larvae. The results demonstrated significant larvicidal activity, with effectiveness varying according to the extraction solvent, concentration, and exposure period. Among the tested extracts, the benzene extracts showed superior larvicidal efficacy compared with the aqueous extracts.

These findings suggest that *F. vulgare* and *B. nigra* possess promising bioactive compounds that can be utilised in mosquito management programs. As natural, biodegradable, and potentially cost-effective resources, these plant species may contribute to the development of environmentally sustainable mosquito control strategies. Further studies focusing on the isolation of active compounds and field-level evaluations are recommended to explore their practical application in vector control programs.

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