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Mosquitocidal susceptibility and non-target effects of essential oil from *Brassica nigra* W.D.J.Koch seeds on immature stages of *Aedes aegypti* L., *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say

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ABSTRACT

Mosquito is a well-known insect belonging to the Culicidae family and is renowned for its global impact on infecting both animals and humans. The objective of the current research was to assess the effectiveness of essential oil (EO) from black mustard seeds in controlling *Anopheles, Culex* and *Aedes* mosquitoes. The results showed that the EO from black mustard seeds exhibited strong larvicidal, ovicidal and pupicidal effects against *Aedes, Culex* and *Anopheles* mosquito species. The tested EO produced 100 % mortality within 24 h in eggs of all the examined mosquito species. It also revealed strong larvicidal and pupicidal activity against *Aedes* (92 % and 84 %), *Culex* (96 % and 86 %) and *Anopheles* (98 % and 90 %) mosquitoes, respectively, at 24 h. Additionally, toxicity test and Suitability Index (SI) or Predator Safety Factor (PSF) revealed that the EO from mustard seeds had no harmful effects on non-target organisms (*P. reticulata* (predatory fish), *D. indicus* (water bug), *G. affinis* (predatory fish) and nymph (dragonfly larvae). The GC–MS analysis revealed the presence of eight compounds, with 1-hexyl-2-nitrocyclohexane (48.57 %) and cyclopentadecanone, 2-methyl (22.68 %) identified as the major probable constituents of the EO, which might play a significant role in their mosquitocidal activity. The formulation prepared from the EO showed strong activity (100 %) against the eggs of all treated mosquitoes. Additionally, it exhibited promising activity against larvae and pupae with LC_{50} values of *Aedes* (23.2 and 26.5 μl/l), *Culex* (19.3 and 26.7 μl/l) and *Anopheles* (17.4 and 23.0 μl/l) mosquitoes. The toxicity study confirmed that both the EO and its formulation were not harmful to the tested non-target organisms. Overall, our findings suggest that the EO from black mustard seeds could be effectively recommended for use in mosquito population control.

1. Introduction

The Culicidae family includes the small flying mosquitoes which are well-known for infecting both animals and humans worldwide. According to the World Health Organization (WHO, 1996), mosquitoes are considered 'public enemy number one.' Each year, over 7000,000 people die from vector-borne diseases caused by parasitic, bacterial or viral infections. Many diseases, some of which can have serious health consequences, are spread by mosquitoes (CDC, 2021; WHO, 2005; WHO, 2021). Moreover, the rapid reproduction rate and genetic adaptability of mosquitoes, along with the ongoing process of urbanization (André et al., 2019) and the extensive air travel networks across the globe, collectively contribute to the worldwide dissemination of these disease vectors and the pathogens they transmit (Luis et al., 2017). In India, three mosquito species that acts

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as vectors: are *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* (WHO, 2020). *Anopheles* mosquitoes are responsible for transmitting *Plasmodium* spp., the parasite responsible for malaria, as well as the Onyong-nyong virus. *Culex* species play a significant role in the transmission of the West Nile virus and filarial nematodes, including *Dirofilaria* spp. Additionally, *Aedes* mosquitoes are known vectors for a range of arboviruses, including those responsible for diseases such as dengue, chikungunya, West Nile, Zika, and yellow fever (Gao et al., 2020).

Effective vector management plays a crucial role as a preventive measure in this situation. Typically, organophosphates, insect growth inhibitors and microbiological agents are used to target mosquito larvae as control agents. In tropical nations, to reduce the spread of diseases carried by mosquitoes, strategies such as indoor residual spraying and the use of insecticide-treated bed nets are also implemented (Lees et al., 2014; Brown, 1986; Russell et al., 2009). Mosquito control is an essential public health activity worldwide, especially in tropical regions. Currently, synthetic pesticides are used to reduce mosquito populations due to their immediate effectiveness and ease of implementation. However, these substances pose risks to the environment, non-target creatures, and have led to the development of insect resistance (Jain et al., 2019; Shroff et al., 2020). The widespread use of insecticides has resulted in an increasing pesticide resistance in mosquito populations, raising concerns about the effectiveness of these control measures. Different mosquito species also vary in their vector competence for viral transmission (Fernandes et al., 2020).

Developing environmentally friendly and safe pesticides is essential for public health. Plant essential oils (EOs) are potential alternatives to synthetic pesticides in the fight against mosquito vectors (Vera et al., 2014; Pavela, 2015; Ganesan et al., 2023). In regions where mosquito-borne diseases are prevalent, plant-based pesticides are often locally available, considered environmentally friendly and have minimal impact on non-target species (Stroh et al., 1998; Pavela, 2014; Govindarajan et al., 2016; Vishnukumar et al., 2018; Shafey, 2020). Furthermore, EOs with multiple active ingredients can act on insects in diverse ways or target different areas, potentially reducing mosquito populations' resistance (Feng and Isman, 1995; Ganesan et al., 2023). Plant EOs have the potential to reduce mosquito populations and can be used either in combination with conventional mosquito pesticides or as bio-rational alternatives. The insecticidal properties of EOs and their ability to enhance pesticide effectiveness have been documented (Norris et al., 2018; O'Neal et al., 2019).

Mustard seeds belong to the Brassicaceae family. Three species of mustard seeds hold commercial value: brown (*Brassica juncea*), black (*Brassica nigra*), and yellow or white (*Brassica alba*) seeds (Hanley et al., 1990; Mejia-Garibayet al., 2015). Black mustard is cultivated in two distinct regions: the Mediterranean and the Middle East. It is grown globally, including America, Asia, Europe and Africa (Grygier, 2023). Extracts and seed meals from Brassica, along with their major chemical constituents, can serve as promising eco-friendly repellents and larvicides to control mosquitoes (Thangaraj et al., 2014; Flor-Weiler et al., 2023; Bekele and Petros, 2017). Black mustard seeds can be transformed into mustard oil through steam or water distillation when macerated in warm water. The crude oil is dark brown and highly concentrated in free fatty acids, while the refined oil has a dull light brown hue. Mustard oil derives its unique scent from EOs containing sulfur, a result of the hydrolysis of glucosides in the seeds. Its quality is influenced by the amount and ratio of fatty acids but is considered hazardous due to its high content of allyl isothiocyanate (Thomas et al., 2012). Previously, Mejía-Garibay et al. (2015) reported that EOs from black mustard seeds contain allylsulfide, allyl isothiocyanate, diallyl disulphide, allyl trisulfide, and 3-tert-butyl-4-hydroxyanisole compounds, which have antifungal activity. The objective of the current research was to assess the effectiveness of EO from black mustard seeds in controlling *Anopheles, Culex* and *Aedes* mosquitoes.

2. Materials and methods

2.1. Essential oil (EOs) extraction

Approximately I Kg of black mustard seeds (*Brassica nigra* W.D.J. Koch) were procured from the market located in Tirunelveli, Tamil Nadu, India. These acquired seeds were subjected to steam distillation using Clevenger apparatus. These were subsequently introduced into an extraction column linked to a round-bottomed distillation flask containing distilled water, at a proportion of approximately five times the amount of water and supplemented with 10 glass beads. The flask was then heated to around 100 °C and left to boil until the distillation process was finished (6–8 h). The resulting liquid oil was gathered with the assistance of a separating funnel. The amalgamated mixture, comprising distilled water and oil, was allowed to settle for duration of 1 day. This settling period gave rise to distinct layers of water and EO and the lower water layer was gradually withdrawn to obtain the EOs as detailed by Conti et al., 2012. The isolated oil (yield-2 ml) underwent a drying process over anhydrous sodium sulfate after which it was collected and stored within an amber-colored container at a temperature of 4 °C, for further mosquitocidal experiments.

2.2. Test mosquitoes

Eggs and larvae of *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus* mosquitoes were collected from various stagnant water bodies, comprising both clean and polluted sources in the vicinity of the laboratory. The mosquito culture was established and maintained in the laboratory at St. Xavier's College, Palayamkottai, Tamil Nadu, India. The larvae of mosquito were free from pathogens, repellents or insecticides and fed with dog biscuits and Brewer's yeast (3:2). Nurturing conditions for mosquitoes were 65–70 % relative humidity, 28 ± 1 °C temperatures and a photoperiod of 12 ± 0.5 h. The third instar larvae were used for the experiment (Samuel et al., 2023).

2.3. Ovicidal bioassay

A total of 20 eggs each of three species were treated with EOs from mustard at concentrations of 6.25, 12.5, 25 and 50 μl/l. Each concentration of the essential oils, with a sample size of five, was maintained. Control groups consisting of solvent, water and dimethyl Sulfoxide (249 ml water and 1 ml DMSO) were included. After 5 days of treatment, the microscope was utilized to observe the hatchability of the eggs (Elango et al., 2009). The following formula was used to calculate the percentage of ovicidal efficacy:

$$\text{Ovicidal activity } (\%) = \frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

2.4. Larvicidal and pupicidal assay

The mosquitocidal (larvae and pupae) assay of EOs from mustard seeds was conducted in accordance with the method outlined by the WHO (2005). Three species of mosquitoes were exposed to EO of mustard seed at 6.25, 12.5, 25 and 50 μl/l. Each concentration of the EOs was replicated five times. Control groups consisting of solvent, water and DMSO were included. After a 24-h exposure, the mortalities were observed. Mosquitoes were considered dead if they did not exhibit any response. The following formula as described by Abbott (1925) was used to calculate the proportion of larvicidal and pupicidal efficacy:

Corrected percentage of mortality:

$$1-\frac{n\ \mathrm{in}T\mathrm{aftertreatment}\times 100}{n\mathrm{in}C\mathrm{aftertreatment}}\times 100$$

Where n is the number of larvae and pupae, T is the number of treated larvae and pupae and C is the number of larvae and pupae in control. The corrected percentage mortality values for each concentration will be considered to estimate LC_{50} and LC_{90} values by using US EPA probit analysis software (version 1.5).

2.5. Toxicity study

The toxicity study of EOs on non-target organisms, specifically *P. reticulata* (predatory fish), *G. affinis*, nymph (dragonfly larvae) and *D. indicus* (water-bug), was assessed using the method of Maheswaran and Ignacimuthu (2012). One predator was introduced in each container, and 10 samples were maintained. The concentration used for larvicidal activity was applied in this study. After a 24-h exposure, the mortalities were observed. The LC_{50} values were calculated by US EPA software (Ganesan et al., 2018). The following formulae were used to evaluate the safety of the predators:

Suitability Index (SI)/Predatory Safety factor (PSF):
LC_{50} of non-target organism
LC_{50} of target vector species

2.6. Mosquitocidal activity of formulation

Formulations containing EOs were dissolved in mineral oil at a concentration of 1 ml/l, using DMSO as the dissolving agent. The resulting solution underwent thorough mixing with a magnetic stirrer for 30 min. The same procedures as those outlined in Sections 2.4 and 2.5 were used to assess the mosquitocidal activity and toxicity of the formulation

2.7. Gas chromatography-mass spectrometry (GC−MS) analysis

The EO of mustard seed was examined using gas chromatography-mass spectrometry (GC−MS) on an Agilent (GC−MS-5975) instrument (Palo Alto, CA, United States) to establish the phytocompounds (Ganesan et al., 2018). The column used was DB 5 ms Agilent, with dimensions of 30.0 m in length, 0.2 mm in internal diameter (ID), and a film thickness of 0.25 μm. The temperature program involved a range of 70−300 °C, with a heating rate of 10 °C/min. The injection temperature was set at 240 °C, and helium was used as the carrier gas at a flow rate of 1.51 ml/min. The GC−MS system was equipped with the GC−MS NIST-II library for data analysis.

2.8. Statistical analysis

The LC_{50} values were calculated by US EPA software (version 1.5). The SPSS software (version 11.5) was used to calculate upper and lower limits from the 95 % confidence limits and Chi-square values. ANOVA was used to assess the significance of the treatments.

3. Results

3.1. Ovicidal activity

The EOs demonstrated 100 % ovicidal activity against the eggs of all treated mosquito species (Supplementary Fig. 1) within 24 h of treatment at a concentration of 50 μl/l.

3.2. Larvicidal activity

The larvicidal effects of the EO indicated strong activity with LC_{50} and LC_{90} values of 21.5, 18.2, 16.3 and 61.0, 47.2 and 44.9 μl/l at 24 h (Table 1) against *Aedes, Culex* and *Anopheles* mosquitoes, respectively. Larvae were considered dead when they were unable to move or reach the surface.

3.3. Pupicidal activity

The EOs exhibited promising pupicidal activity with LC_{50} and LC_{90} values of 25.0, 24.9, 21.9 and 75.9, 76.4 and 66.6 μl/l, respectively, at 24 h against *Aedes, Culex* and *Anopheles* mosquitoes (Table 2). Pupae were considered dead when they were unable to move or reach the surface.

3.4. Toxicity of essential oil from black mustard seed against non-target organisms

The toxicity of the EOs from black mustard seeds was assessed against *P. reticulata, D. indicus, G. affinis* and nymph (dragonfly larvae) (Table 5). Among the tested organisms, larval predators exhibited the least susceptibility, with LC_{50} values of 209.3, 273.3 and 217.7 μl/l after 24 h of the experiment.

Table 1
Lethal concentrations (μl/l) of essential oil from Black mustard seed against larvae of three mosquito vectors.

Mosquito	LC_{50}	95 % Confidence Limit		LC_{90}	95 % Confidence Limit		Slope ± SE	Intercept ± SE	χ^2
		LL	UL		LL	UL			
Ae. aegypti	21.5	18.2	25.6	61.0	46.7	91.8	1.2 ± 0.4	2.8 ± 0.3	5.6*
Cx. quinquefasciatus	18.2	15.5	21.4	47.2	37.5	66.3	1.0 ± 0.4	3.0 ± 0.3	3.4*
An. stephensi	16.3	13.8	19.3	44.9	35.4	63.9	1.4 ± 0.4	2.9 ± 0.3	5.7*

LC_{50} lethal concentration that kills 50 % of the exposed larvae, LC_{90} lethal concentration that kills 90 % of the exposed larvae, LL lower limit (95 % confidence limit), and UL upper limit (95 % confidence limit). *$P \leq 0.05$, level of significance of chi-square values.

Table 2
Lethal concentrations (μl/l) of essential oil from black mustard seed against pupae of three mosquito vectors.

Mosquito	**LC_{50}**	**95 % Confidence Limit**		**LC_{90}**	**95 % Confidence Limit**		**Slope ± SE**	**Intercept ± SE**	**χ^2**
		LL	UL		LL	UL			
Ae. aegypti	25.0	21.0	30.5	75.9	55.9	123.4	1.2 ± 0.4	2.6 ± 0.3	2.7*
Cx. quinquefasciatus	24.9	20.9	30.5	76.4	56.1	124.9	1.3 ± 0.4	2.6 ± 0.3	5.7*
An. stephensi	21.9	18.4	26.5	66.6	49.8	104.8	1.4 ± 0.4	2.6 ± 0.3	5.7*

LC_{50} lethal concentration that kills 50 % of the exposed pupae, LC_{90} lethal concentration that kills 90 % of the exposed pupae, LL lower limit (95 % confidence limit), and UL upper limit (95 % confidence limit). *$P \leq 0.05$, level of significance of chi-square values.

Table 3
Lethal concentrations (μl/l) of essential oil-based formulation against larvae of three mosquito vectors.

Mosquito	LC_{50}	95 % Confidence Limit		LC_{90}	95 % Confidence Limit		Slope ± SE	Intercept ± SE	χ^2
		LL	UL		LL	UL			
Ae. aegypti	23.2	19.6	27.7	64.9	49.5	98.4	1.0 ± 0.4	2.8 ± 0.3	5.3*
Cx. quinquefasciatus	19.3	16.5	22.7	50.0	39.6	70.5	0.9 ± 0.4	3.1 ± 0.3	2.3*
An. stephensi	17.4	14.7	20.5	48.1	37.7	69.3	1.3 ± 0.4	2.9 ± 0.3	4.2*

LC_{50} lethal concentration that kills 50 % of the exposed larvae, LC_{90} lethal concentration that kills 90 % of the exposed larvae, LL lower limit (95 % confidence limit), and UL upper limit (95 % confidence limit). *$P \leq 0.05$, level of significance of chi-square values.

Table 4
Lethal concentrations (μl/l) of essential oil-based formulation against pupae of three mosquito vectors.

Mosquito	LC_{50}	95 % Confidence Limit		LC_{90}	95 % Confidence Limit		Slope ± SE	Intercept ± SE	χ^2
		LL	UL		LL	UL			
Ae. aegypti	26.5	22.1	32.8	83.3	60.1	141.2	1.3 ± 0.4	2.5 ± 0.3	1.6*
Cx. quinquefasciatus	26.7	22.3	33.1	83.3	60.3	140.8	1.2 ± 0.4	2.5 ± 0.3	3.5*
An. stephensi	23.0	19.3	28.1	72.1	53.1	116.9	1.4 ± 0.4	2.5 ± 0.3	5.9*

LC_{50} lethal concentration that kills 50 % of the exposed pupae, LC_{90} lethal concentration that kills 90 % of the exposed pupae, LL lower limit (95 % confidence limit), and UL upper limit (95 % confidence limit). *$P \leq 0.05$, level of significance of chi-square values.

Table 5
Toxicity of essential oil (μl/l) from black mustard seed against non-target organisms.

Treatment	*Poecilia reticulata*		*Diplonychus indicus*		Nymph (Dragonfly larvae)		*Gambusia affinis*	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Essential oil	209.3	1483.9	273.3	2526.8	217.735	1176.598	209.3	1483.9

3.5. Suitability index (SI) or predator safety factor (PSF) of non-target organisms with respect to the larval stages

SI/PSF showed that the EO extracted from black mustard seed did not cause harm to *P. reticulata, D. indicus* (water bug), *G. affinis* and nymph (dragon fly larvae) at the tested concentrations (Table 6). The day-to-day activities of the tested organisms remained normal, comparable to the control group, throughout the study period.

3.6. Phytochemical profile based on gas chromatography-mass spectrometry GC–MS

The GC–MS analysis of the EO extracted from black mustard seed revealed the presence of eight compounds (Supplementary Fig. 2). These included difluorophosphoric acid (6.47 %); carbamimidoylsulfanylacetic acid (1.14 %); n-hexadecanoic acid (9.2 %); docosanedioic acid, dimethyl ester (9.68 %); tetradecanoic acid, 12-methyl-, methyl ester, (*s*)- (1.21 %); 1-hexyl-2-nitrocyclohexane (48.57 %); cyclopentadecanone, 2-methyl (22.68 %) and octyl s-2-(dimethylamino) ethyl propylphosphonothiolate (1.01 %)

3.7. Mosquitocidal activity of essential oil-based formulation at laboratory conditions

The laboratory study clearly indicated that formulations prepared from the EO extracted from black mustard seeds exhibited promising activity. These formulations demonstrated 100 % (Supplementary Fig. 3.) ovicidal activity against the eggs of all treated mosquito species. Additionally, they showed good activity against larvae, with LC_{50} values of 23.2, 19.3 and 17.4 μl/l for *Aedes, Culex* and *Anopheles* mosquitoes. Furthermore, the formulations exhibited promising activity against pupae, with LC_{50} values of 26.5, 26.7 and 23.0 μl/l for *Aedes, Culex* and *Anopheles* mosquitoes (Tables 3 and 4).

3.8. Non-target effect of essential oil-based formulation

The findings of the study clearly demonstrated the safety of the prepared formulation on non-target organisms including *P. reticulata*, nymph (dragonfly larvae), *G. affinis* and *D. indicus*. These predators exhibited normal movement and behavior when exposed to the prepared formulation at a concentration of 50 μl/l. Throughout the observation period, the tested mosquito predators displayed regular survival rates and normal swimming activities.

4. Discussion

Essential oils (EOs) are highly promising metabolites that find applications in various human endeavors, including plant protection and the safeguarding of human and animal health from numerous vectors (Miresmailli and Isman, 2014; Gebashe et al., 2022). These complex mixtures consist of volatile organic compounds produced as secondary metabolites in plants. Plant EOs may prove valuable in controlling mosquitoes, as they are a rich source of bioactive compounds that can biodegrade into harmless products. They are well-suited for use in integrated management strategies for mosquito control (Hemingway and Ranson, 2000). Generally, EOs have demonstrated their effectiveness in controlling insect pests, such as

Table 6
Suitability Index (SI)/Predatory Safety factor (PSF) of non-target organisms with respect to the larval stages of three mosquito vectors exposed to essential oil of black mustard seed.

Predatory species	Essential oil		
	Aedes	*Culex*	*Anopheles*
Poecilia reticulata	9.7	11.5	12.8
Diplonychus indicus	12.7	15.01	16.7
Nymph (Dragonfly larvae)	10.1	11.9	13.3
Gambusia affinis	9.7	11.5	12.8

mosquitoes, when compared to synthetic pesticides (Muturi et al., 2017, 2020; ŞengulDemirak and Canpolat, 2022).

In the present study, EOs from mustard seeds exhibited strong larvicidal, ovicidal and pupicidal effects against *Aedes, Culex*, and *Anopheles* mosquito species. The tested EOs resulted in 100 % mortality within 24 h for the eggs of all the examined mosquito species. It also showed strong larvicidal and pupicidal activity against *Aedes, Culex* and *Anopheles* mosquito species, respectively. These results are comparable to a study by Huong et al. (2023) where EOs extracted from leaves and rhizomes of *Distichochlamys benenica* exhibited strong larvicidal activity with LC_{50} values ranging from 31.1 to 71.5 μg/ml and LC_{90} values ranging from 46.2 to 148.6 μg/ml against third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The main component identified in the GC–MS analysis was β-pinene (47.7 %), which may be responsible for the mosquitocidal activity. When compared to our study, the EOs from mustard seeds exhibited strong larvicidal activity with lower concentrations.

Wangrawa et al. (2022) conducted research on EOs (0.1 %) for larvicidal activities from *Lippia multiflora* Moldenke, *Lippia chevalieri* Moldenke, *Cymbopogon schoenanthus* (L.) Spreng, and *Lantana camara* L., testing them against *Anopheles funestus* and *Cx. quinquefasciatus*. The authors indicated larvicidal activity, with LC_{50} values of 23.3–54.1 μl/l against *Cx. quinquefasciatus* and 49.2–120.5 μl/l against *An. funestus*, respectively, at 24 h. Kweka et al. (2023) conducted a study on the larvicidal activity of *Feronia limonia* leaf EO against wild larvae of *An. arabiensis* and the GC–MS analysis revealed estragole (34.69 %) as one of the major compounds. The study revealed significant larvicidal activity against *An. arabiensis*, with LC_{50} values of 65.5 μl/l after a 24-h exposure period. Manh et al. (2020) investigated the larvicidal activity of EOs extracted from aromatic plants, namely *Cymbopogon citratus* (0.33 %), *Cymbopogon winterianus* (1.30 %), *Eucalyptus citriodora* (2.10 %) and *Eucalyptus camaldulensis* (0.61 %) grown in Vietnam against *Ae. aegypti* larvae. When compared to our findings, lower LC_{50} values were reported for *Cymbopogon citratus* (LC_{50}=120.6 μl/l), *Cymbopogon winterianus* (LC_{50} = 38.8 μl/l), *Eucalyptus citriodora* (LC_{50} = 104.4 μl/l) and *Eucalyptus camaldulensis* (LC_{50} = 33.7 μl/l).

Promising larvicidal activity have been observed in several studies with various EOs. For instance, Sheng et al. (2020) found LC_{50} values of 27.5, 31.6, 51.3 and 53.5 μl/l for the EOs of *Foeniculum vulgare* (fennel), *Thymus vulgaris* (thyme), *Citrus medica* (lime) and *Camellia sinensis* (green tea) against *Ae. albopictus* respectively. In a study by Dai et al. (2020), the EOs of *Litsea umbellata* (1.82 g) and *Litsea iteodaphne* (2.13 g) displayed larvicidal activity against *Cx. quinquefasciatus* (36.1 and 37.2 μg/ml) and *Ae. albopictus* (40.0 and 20.2 μg/ml) at 24 h. The GC–MS analysis showed that the major compounds were mostly terpenes (16–21 %). In the study by Kharoubi et al. (2021), the EOs of *Mentha piperita* (1.92 g) exhibited larvicidal activity against fourth-instar larvae of *Culex pipiens*, resulting in LC_{50} values of 448.7 μl/l. Additionally, the EO showed lower ovicidal activity, reducing the hatchability of *Culex pipiens* eggs by 53.67 %.The GC–MS analysis showed that the main components were menthol (28.66%), limonene (19.01%) and p-cymene (15.82 %). The present study reported a complete reduction of egg hatching when mustard EO was applied against three mosquito species. Vivekanandhan et al. (2020) reported comparative results in which the EO of *Eucalyptus globulus* exhibited strong larvicidal activity with LC_{50} values of 7.4- 30.1 μl/l against third instar larvae of *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus*. The authors reported that 1,8-cineol accounted for 71.7 % as the main component in the GC–MS analysis. The EO from *Amomum subulatum* L. (1.3 ml) demonstrated significant results against third instar larvae of *An. subpictus, Ae. albopictus* and *Cx. tritaeniorhynchus*, with LC_{50} values of 41.2 μl/l, 44.1 μl/l and 48.1 μl/l, respectively. As revealed by the authors, the primary constituents as determined by GC–MS analysis, were 1,8-cineole (39.8 %) and α-terpineol (11.5 %) (Govindarajan et al., 2018). Essential oil from *Carlina acaulis* exhibits strong larvicidal activity against *Cx. quinquefasciatus*, with an LC_{50} of 579.1 μl^{-1} at 24 h. The major compound was identified as carlina oxide (Pavela et al., 2021; Spinozzi et al., 2023). These reports of mosquitocidal activity support the findings of our study, indicating that the extract has useful control over the mosquito population.

Table 7
Gas Chromatography Mass Spectrometry (GC–MS) analysis of essential oil from black mustard seed.

Retention Time (min)	Area (%)	Compound
1.02	6.47	Difluorophosphoric acid
1.54	1.14	Carbamimidoylsulfanylacetic acid
17.52	9.2	N-Hexadecanoic acid
18.52	9.68	Docosanedioic Acid Dimethyl Ester
18.73	1.21	Tetradecanoic Acid, 12-Methyl-, Methyl Ester, (*S*)-
19.19	48.57	1-Hexyl-2-Nitrocyclohexane
19.31	22.68	Cyclopentadecanone, 2-Methyl
21.62	1.01	Octyl S-2-(Dimethylamino) Ethyl Propylphosphonothiolate

Ovicidal efficacy of 100 % was observed in EOs from Illiciaceae and Zingiberaceae families, such as *Alpinia galanga* and *Illicium verum*, against females of *Ae. albopictus* and *An. minimus* (Cotchakaew and Soonwera, 2018). The current study yielded similar results with 100 % of ovicidal efficacy. Kaura et al. (2019) reported the larvicidal and pupicidal efficacy of *Eucalyptus* and neem oil against *Aedes* mosquitoes. *Eucalyptus* oil exhibited good activity with LC_{50} values of 93.3 μl/l for larvae and 144.5 μl/l for pupae, while neem oil showed LC_{50} values of 7852 μl/l for larvae and 19,054 μl/l for pupae, respectively. Chantawee and Soonwera (2018) investigated the larvicidal and pupicidal activities of EOs from *Alpinia galanga* (L.) Willd rhizome, *Anethum graveolens* L. fruit, *Foeniculum vulgare* Mill. fruit and *Pimpinella anisum* L. fruit against *Ae. aegypti* and *An. graveolens*. Strong larvicidal and pupicidal activity was observed with LC_{50} values of <2.9 % against *An. graveolens* and *Ae. aegypti*. Tyagi et al. (2017) studied the pupicidal activity of EOs from clove, jasmine and camphor. Their research yielded promising pupicidal results with LC_{50} values of 46.4- 178.6 and 45.5- 218.0 μl/l for *An. stephensi* and *Ae. aegypti*. They reported that 1,3-ditert-butylbenzene, linalool, alpha-copane and *Artemisia* ketone present in the EO are responsible for the mosquitocidal activity. These reports offer a comparative perspective and support our study findings. Most importantly, when considering the individual activities of EOs, we may conclude that the observed activity can be attributed to individual compounds, if reported. Additionally, most of the results are either antagonistic or synergistic (Pavela, 2015; Ganesan et al., 2023)

The toxicity study revealed that the EOs had no adverse effects on *P. reticulata*, nymphs (dragonfly larvae), *G. affinis* and *D. indicus* as they exhibited normal movement. The Suitability Index (SI) or Predator Safety Factor (PSF) values, which assess the survival of non-target organisms, indicated that the EO derived from mustard seeds could be safely incorporated into mosquito control programs without posing a threat to these predators. Furthermore, the formulation based on mustard seed EO displayed potent activity against all treated mosquitoes and was found to be non-toxic to *P. reticulata*, nymphs (dragonfly larvae), *G. affinis* and *D. indicus*. These findings suggest the potential to utilise the essential oil-based formulation for mosquito population control without harming these natural predators (Table 8).

5. Conclusion

The current study unveiled the mosquito-killing potential of EO extracted from mustard seeds against various life stages of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti*. The results showcased the promising mosquito-killing properties of these EOs against the tested mosquito species. Furthermore, toxicity studies demonstrated no adverse effects on non-target organisms. The formulated EOs exhibited promising efficacy in laboratory treatments while ensuring the safety for the organisms involved in the study. Over all the extracted EO could serve as an environmentally friendly substitute for synthetic insecticides in mosquito control. However, future studies assessing the efficacy of mustard seed EO compared to synthetic pesticides at the field level to confirm their effectiveness, is pertinent.

Declaration of competing interest

The authors declare no conflict of interest.

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Supplementary materials

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