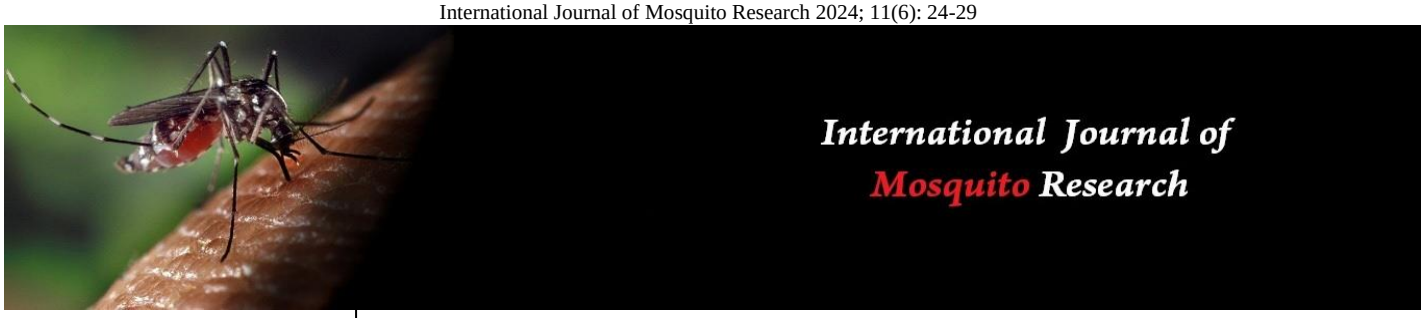

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Mosquito larvicidal effect of *Coriandrum sativum* root extract against *Aedes albopictus*

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Abstract
Mosquitoes are infamous creatures that spread numerous fatal diseases. Amongst several species, *Aedes albopictus* is one of the most dangerous ones, as it spreads dengue, yellow fever, and zika to name a few life-threatening diseases. Conventional control methods not only fail to control this pest but also are hazardous to the environment. Green mosquitocide is the next-generation mosquito control strategy. This study aims to analyze the larvicidal activity of methanol extract of *Coriandrum sativum* root against *Ae. albopictus*. This study also aims to identify plausible functional groups and secondary metabolites responsible for the toxicity through FT-IR and phytochemical analysis respectively. The effect on non-target organisms was evaluated to nullify the chances of eco hazards. Cent percent mortality was observed after 72 hours of treatment in all larval instars larvae at 200 ppm of concentration, while insignificant mortality was observed at LC_{50} and LC_{90} concentrations of the same when non-target organisms were exposed. After 72 hours of exposure, the third instar larva showed the lowest LC_{50} and LC_{90} values, which are 51.11 ppm and 109.90 ppm, respectively. Based on a three-way factorial ANOVA analysis, we have concluded that several characteristics, including time intervals, concentration, and instars, have a significant effect on larval mortality. This finding is statistically significant. Through phytochemical investigation, alkaloids, flavonoids, terpenoids, and coumarin were detected. FTIR examination revealed the presence of carboxylic acids, amides, alkanes, aldehydes, and organic alcohol. Current research suggests that *C. sativum* may be a perfect, low-cost, one-step, and environmentally acceptable source for controlling *Ae. albopictus* larvae. It is the first comprehensive report on the anti-mosquito activity of root extract from *C. sativum* against *Ae. albopictus*. As *C. sativum* methanol root extract is nontoxic to non-target animals, it is possible to employ this extract in integrated vector control programs in addition to mosquito predators. Our next endeavor will be to identify the active principle and do additional research on bioassay-guided fractionation to produce a more potent botanical pesticide.

Keywords: *Coriandrum sativum*, *Aedes albopictus*, larvicide, FT-IR analysis, phytochemicals, larvae

Introduction
Mosquitoes are responsible for the largest number of deaths from animals in the world [1]. These midge-like flies belong to the family Culicidae, class *Insecta*, and Phylum *Arthopoda*. More than 3500 different species of mosquitoes have been identified so far in every continent all over the world except Antarctica [2]. The prevalence of mosquito-borne diseases is being seen mainly in tropical countries. *Aedes albopictus* is an infamous mosquito species that is found in the Indian subcontinent and is responsible for spreading fatal diseases like Dengue, Yellow fever, Zika, etc [3]. Keeping in mind the accelerated speed of spread of these diseases as well as the vectors itself, it is high time to think about a sustainable long lasting mosquito control strategy. Hence, mosquito control targets should not only be towards adult mosquitoes but also towards mosquito larvae. Conventional mosquito control techniques include chemical control, physical control, and genetic control. While the scope of success from physical and genetic control is limited in a developing and population-rich country like India, Chemical control has its own side effects and is not very cost-effective. Mainly, organophosphate and insect growth regulators are used for chemical control at larval stages, which can seriously harm the ecosystem as many beneficial harmless flora and fauna have the same niche as mosquito larvae [4]. Besides, prolonged use of the same chemicals causes genetic changes in mosquitoes and gives rise to a drug-resistant mosquito population [5].

In this situation, green mosquito larvicides have a promising future as they are target-specific and environment-friendly. They are also economical to use, which is a great advantage for developing continents like Asia, Africa etc.

Herbs are being used as an important source of Ayurvedic medicine, as well as anti-microbial agents. Therefore, they could be a good choice for potential mosquito larvicides. *Coriandram sativum*, which is locally known as dhania in the Indian subcontinent, or cilantro, belongs to the family Apiaceae (umbelliferae). This plant is widely used as an herb all over the world [6]. Every part of this plant has medicinal as well as culinary value. This characteristic of coriander makes it a good candidate for potential mosquitocide. In fact, the essential oil extracted from the fruit of coriander has already been reported to have a larvicidal effect against *Aedes albopictus* Skuse species [7]. But this may not be a cost-effective approach as the fruit of coriander is of high demand already for spices. So, other parts that might have the same property should be tested.

In this paper, we examined the potential larvicidal activity of the methanol extract of the root of the *Coriandrum sativum* and the effect of the same on non-target organisms such as *Toxorhynchites splenders* and *Chironomus sp.* Probable functional groups responsible for the larvicidal activity of the extract and its secondary metabolites were also detected.

2. Materials and Method

2.1 Collection and maintenance of the mosquito larvae: *Aedes albopictus* larval population was maintained in a repellent, pathogen, and insecticide-free condition at Mosquito, Microbiology, and Nanotechnology Research Units, Department of Zoology, The University of Burdwan, Burdwan. The required larvae for all the experiments were collected here. Ideal temperature and humidity were maintained (temperature at 27±2 °C and the relative humidity: 80%-85%). 14:10 cycles of light and dark were maintained as per standard protocol [8]. Brewer yeast, dog biscuits, and algae were mixed in 3:1:1 and fed to the larvae for survival.

2.2 Collection of the plant: Fresh *Coriandrum sativum* was collected from the field of Bankura (23°15'0.00" N 87°04'12.00" E) with root. The roots were cut (white part only) and separated from the rest of the plant, and used for further processing.

2.3 Preparation of plant extract: The roots (white part only) of the freshly collected plant were cut from the rest and then washed thoroughly to remove all dirt. It was then tap-dried using a paper towel and shed-dried at room temperature. The dried root was cut into small pieces, and 200 gm of the sample was placed into the thimble of the Soxhlet apparatus. Non-polar to polar solvents (Petrolium ethar, ethyl acetate, acetone, chloroform:methanol {1:1}, methanol) were passed through the Soxhlet apparatus. Each solvent was measured at 2000 ml and loaded for extraction for 72 hours (8 hours per day). Extracts were collected in a separate beaker from the still pot and later concentrated using a rotary evaporator. The concentrated extractives were kept at 4° C for preservation and further use.

2.4 Larvicidal Bioassay: All the larvicidal bioassays were performed following the standard WHO protocol [9]. A preliminary screening larvicidal bioassay revealed methanol extract to have best larvicidal effect. Hence, methanol extract was selected for further assay and characterization.

Five concentrations (50, 75, 100, 150, and 200 ppm) of the methanol extract were selected for evaluation on four different larval instars of *Ae. albopictus*. 100 ml of tap water was taken in 250 ml sterilized glass beaker, and 25 larvae of each instar were put into each beaker. The larvae were subjected to the treatment of graded concentrations of the extract. The treatment continued for 72 hours at room temperature and standard humidity [(27±2°C) and (85±2 %)]. The result (mortality) was noted down for 24, 48, and 72 hours, respectively. The experiment was done in three batches on three separate days to minimize the standard error.

2.5 Phytochemical Analysis: phytochemical analysis revealed the presence or absence of secondary metabolites in the methanol extract of the *Coriandrum sativum* root. The standard method of Trease and Evans [10] Sofowara [11], and Harborne [12] were followed for the same.

2.5.1 Tannin detection test: A Ferric Chloride test was performed to detect tannin in the extract. 2 ml of sample was taken in a test tube, and 5-10 drops of $Fecl_3$ were mixed with it. The presence of tannin will turn the solution in a bluish-black color.

2.5.2 Terpenoids and steroids detection test: 1 ml glacial acetic acid (1N) was added to the test tube containing 1 ml of sample to acidify. Next, 1 ml of concentrated H_2SO_4 (4N) was added slowly through the wall of the test tube in the ice chamber. If the color changes to brown, it indicates the presence of steroids, while green indicates terpenoids.

2.5.3 Flavonoids detection test: Flavonoids is detected using the zinc hydrochloride test. 10 drops of 0.5 N HCL is gradually added to 1 ml of methanol extract. Then, a small amount of zinc was added to the same test tube. Reddish pink-colored precipitation confirms the presence of flavonoids.

2.5.4 Saponins detection test: 5 ml of methanol extract of the sample is taken in a test tube. A few drops of $NaHCO_3$ were added and shaken vigorously. Then, the test tube was kept still for 3-4 minutes. If honeycomb-like stable foam forms, it indicates the presence of saponin.

2.5.5 Alkaloids detection test: Mayer's and Wagner's tests were used to detect alkaloids in the extract. Mayer's reagent is the solution of 5g KI and 1.36 g of $HgCl_2$ in 100 ml of distilled water, whereas Wagner's reagent was prepared by adding potassium iodide to the iodine solution.

The sample was acidified first using glacial acetic acid, and then Mayer's reagent was added to 1 ml of the acidified extract. The formation of pale-yellow precipitation confirms the presence of alkaloids. The same method was repeated with Wagner's reagent, and reddish brown precipitation formed if alkaloids were present.

2.5.6 Coumarins detection test: 2 ml of the sample was taken in a test tube, and 3 ml of 10% NaOH was added to the same. The color would change to yellow instantly if coumarin is present in the sample.

2.5.7 Cardiac glycosides detection test: 2 ml of the sample

was mixed with 1 ml of glacial acetic acid in a test tube. 2 ml of ferric chloride was gradually added, followed by 2 ml of concentrated H_2SO_4. The presence of cardiac glycoside will turn the color brown.

2.5.8 Anthocyanins detection test: 2 ml of the sample, 1 ml of 2N HCL, and 1 ml of NH_3 is mixed in a test tube. The color change of pink-red to blue-violet indicates the presence of anthocyanin in the extract.

2.6 Test on the non-target organisms: There are many harmless flora and fauna that share common habitats with mosquito larvae. It is important to ensure that our extract is target-specific and does not harm other species. The effect of methanol extract of *Coriandrum sativum* root was studied on *Toxorhynchites splendens* and *Chironomus sp.* larvae. The concentration of LC_{50} of methanol extract against the third instar of *Aedes albopictus* after 24 hours of treatment was taken for the study. The percent mortality rate was recorded after 24 hours, 48 hours, and 72 hours of treatment, respectively.

2.8 Statistical Analysis: Abbott's formula [6] was used for percent mortality (%M) precision. The results were analyzed for statistical significance. LC_{50} and LC_{90} values were analyzed using STAT PLUS 2007 (Trial version). Regression equations and regression coefficient values were analyzed using MS Excel 2010. Three-way ANOVA analysis was done using SPSS 11.0 software.

3. Results:

Larval mortality against graded concentrations of methanol extract of *Coriandrum sativum* root has been presented in table 1. A positive linear relation was observed between larval mortality and time and larval mortality and concentration of the sample. Cent percent mortality was observed for 1st and 2nd instars larvae at 200 ppm concentration for 72 hours of treatment. For third instars, a cent percent mortality rate was achieved at 150 ppm concentration for 72 hours of treatment.

Lowest LC_{50} and LC_{90} value was observed for 3rd instar larva after 72 hours of exposure and the values are 51.11 ppm and 109.90 ppm respectively. Mortality was positively correlated with sample concentration. The regression coefficient was close to 1. [Table 2]

The three-way factorial ANOVA analysis revealed that our result was statistically significant, i.e. different factors like time intervals, concentrations, and instars have a significant influence on larval mortality (Table 3).

The results of the tests for the presence of phytochemicals have been recorded in table 4. Alkaloid, flavonoids, terpenoids, and coumarin were present in the methanol extract of coriander root.

The tests on non-target organisms revealed non-significant mortality rates [Table 5].

The presence of various functional groups was analysed by FT-IR analysis. The result has been tabulated in table 6 and figure 1. Presence of compounds containing functional groups like amide, alcohol, aldehyde, carboxylic acids, and alkenes was detected.

Table 1: Larval mortality of *Aedes albopictus* treated with graded concentrations (ppm) of methanol extracts of *Coriandrum sativum* root

Larval Instars	Concentrations (ppm)	Percent mortality (mean±SE)		
		24h	48h	72h
1st	50	21.33±0.33	26.67±0.33	54.67±0.88
	75	38.67±1.20	50.67±0.67	70.67±0.67
	100	45.33±0.88	60.00±0.58	80.00±1.15
	150	68.00±1.15	86.67±0.88	98.67±0.33
	200	81.33±0.88	93.33±1.20	100.00±0.00
2nd	50	22.67±0.33	28.00±0.57	54.67±0.33
	75	41.33±0.88	50.67±0.33	69.33±0.88
	100	42.67±0.88	58.67±0.88	84.00±1.00
	150	66.67±0.33	85.33±0.33	97.33±0.33
	200	82.67±0.33	96.00±0.57	100.00±0.00
3rd	50	24.00±0.57	32.00±0.57	56.00±0.57
	75	41.33±0.88	54.67±0.33	70.67±0.67
	100	53.33±0.67	64.00±0.57	80.00±0.57
	150	70.67±0.88	85.33±0.67	100.00±0.00
	200	84.00±0.57	96.00±0.57	100.00±0.00
4th	50	14.67±0.33	18.67±0.88	41.33±0.88
	75	24.00±1.15	33.33±1.33	56.00±1.15
	100	45.33±0.88	58.67±0.33	77.33±0.88
	150	56.00±1.00	73.33±0.88	84.00±0.57
	200	70.67±0.67	84.00±0.57	90.67±0.33

Table 2: Log probit and regression analyses of larval mortality of *Aedes albopictus* at various concentrations of methanol extracts of *Coriandrum sativum* root

Larval Instar	Period of Exposure (hour)	LC_{50} (ppm)	LC_{90} (ppm)	Regression Equation	R2- Value
1st	24	103.45	297.88	Y= 0.391x + 5.884	0.978
	48	79.91	186.10	Y= 0.434x + 13.45	0.926
	72	52.37	118.11	Y= 0.301x + 46.11	0.894
2nd	24	105.56	324.24	Y= 0.385x + 6.839	0.974
	48	78.43	179.73	Y= 0.442x + 12.81	0.953
	72	51.88	113.85	Y= 0.297x + 46.80	0.877
3rd	24	93.63	279.01	Y= 0.3862x + 10.251	0.9671
	48	72.34	173.92	Y= 0.4083x + 19.45	0.9436
	72	51.11	109.90	Y= 0.2988x + 46.967	0.8905
4th	24	129.41	383.97	Y= 0.3706x - 0.483	0.9483
	48	98.38	238.19	Y= 0.4331x + 3.7951	0.9157
	72	61.36	198.70	Y= 0.3145x + 33.696	0.8463

Table 3: Three way ANOVA analysis of mortality of *Aedes albopictus* larvae using treatment time, concentrations of methanol extracts of *Coriandrum sativum* root and instars as third parameters

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean of squares (MS)	F value	P-Level	F crit	squre
Instars (I)	253.75	3	84.5833	53.0488	0	5.7814	0.0359
Hours (H)	1538.6333	2	769.3167	482.4983	0	7.3211	0.2214
Concentration (C)	4769.4778	4	1192.3694	747.8275	0	7.3211	0.6867
I*H	3.9	6	0.65	0.4077	0.8728	4.0437	0
I*C	46.8333	12	3.9028	2.4477	0.0069	3.0162	0.004
H*C	115.2556	8	14.4069	9.0357	0	3.5519	0.0148
I*H*C	15.7667	24	0.6569	0.412	0.9929	0.412	0
Within Groups	191.3333	120	1.5944				
Total	6934.95	179	38.7427				
Omega squared for combined effect	0.9586						

Table 4: Different secondary metabolites present in methanol extracts of *Coriandrum sativum* root

Present (+)/ Absent (-)	Name of the Phytochemical							
	Alkaloid	Flavonoid	Tanin	Terpeniod	Steroid	Glycoside	Anthocyanin	Coumarine
	+	+	-	+	-	-	-	+

Table 5: mortality of non-target organisms due to treatment of methanol extracts of *Coriandrum sativum* root

Time of Exposure	Mortality	
	***Chironomous* sp.**	***Toxorhynchites* sp.**
24 hour	0.00±0.00	0.00±0.00
48 hour	0.00±0.00	0.00±0.00
72 hour	1.33±1.33	0.00±0.00

Table 6: Functional groups identified in methanol extracts of *Coriandrum sativum* root through FT-IR analysis

Absorption Spectra peaks	Probable Functional Group
3335.28 cm^{-1}	1. -OH stretching of organic alchol 2. NH strechinng of amides
2919.7 cm^{-1}	1. CH stretching of aldehyde 2. CH stretching of alkane
2682.5 cm^{-1}	1. CH stretching of aldehyde
1663.41 cm^{-1}	1. NH bending of Amides 2. C=O stretching of Amides
1102.12 cm^{-1}	1. CN stretching of amides
827.312 cm^{-1}	1. OH stretching of Carboxyl acid

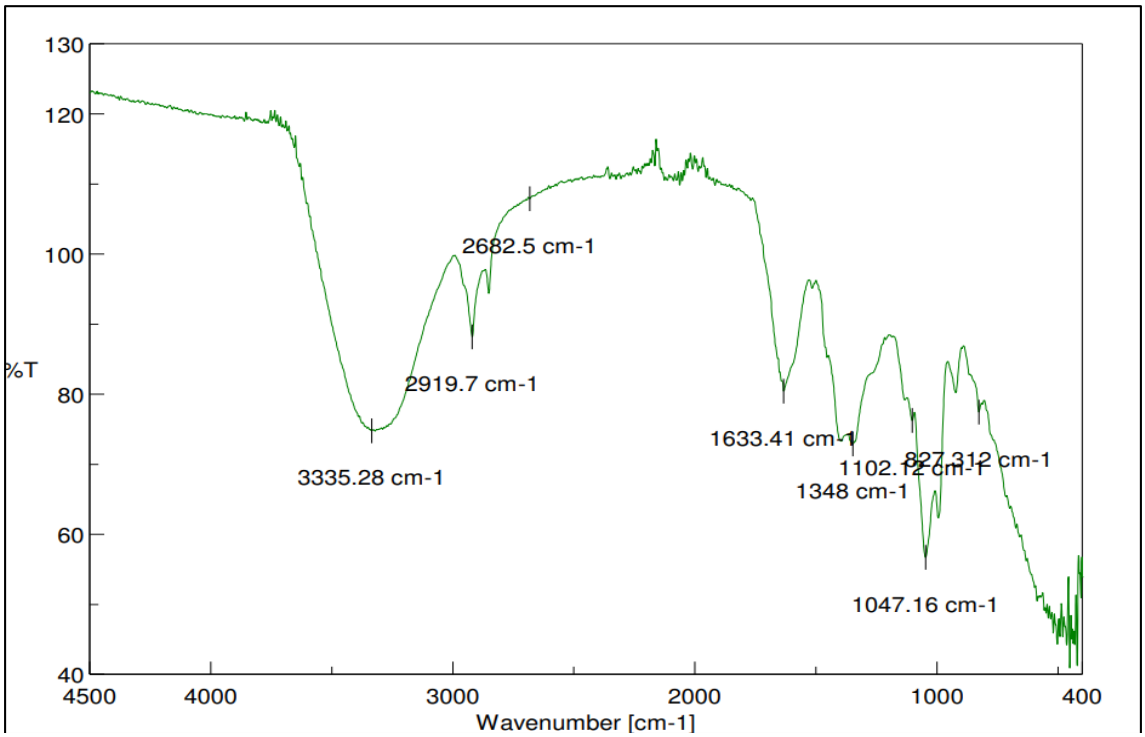

Fig 1: Fourier Transformation Infrared (FT-IR) spectroscopy analysis of methanol extract of *Coriandrum sativum*

4. Discussion

In the era of global warming and chronic pollution, using plant-based mosquitocide is a boon as it is cost-effective and has the least adverse effect on the environment. The best way to fight the mosquito population is to destroy it in the early stage of its life cycle, i.e., larval form. The confinement of this stage in an aquatic environment makes it an easy target. The larvicidal effects in plant comes mainly from the secondary metabolites present. The toxicity of these metabolites largely regulated by several factors such as extraction method, solvent used, season the plant is being harvested etc [13-15].

In our study cent percent mortality was observed at a significantly lower concentration of the solvent extract. The concentration was even lower in case of 3rd instar larvae than 1st, 2nd, and 4th instar. The regression analysis suggested a positive correlation between mortality and concentration, where the regression coefficient value was close to 1 in each case.

Previously reported data showed essential oil extracted from the seeds of *Coriandrum sativum* against *Anopheles stephensi* has LC_{50} and LC_{90} values similar to our findings or even higher [16]. Plant root extract having larvicidal activity was rare but not uncommon. The root extract of *Tragia involucrata* L. was reported for having larvicidal activity against *Culex quinquefasciatus* [17]. Hence our finding would be a novel addition to the same. Previously, methanol extract of *Typhonium trilobatum* showed cent percent mortality against *Culex quinquefasciatus* at 400 ppm concentration after 24 hours of treatment [18]. Methanol extract of fruits of *Diospyros kaki* showed larvicidal effect and cent percent mortality after 72 hours of treatment at 600 ppm concentration with LC_{50} and LC_{90} values as low as 46 ppm and 186 ppm [19].

Our report targeted *Aedes albopictus*, a relatively less reported species than other species of mosquito. The root part was not popularly used as a herb. Hence, the roots of *Coriandrum sativum* were more economically affordable than the other parts of the plant. Cent percent morality was achieved after 72 hours of treatment at 150 ppm of concentration against third-instar larvae. LC_{50} and LC_{90} values were 51.11 and 109.90, respectively.

Economic availability, relatively lower LC_{50} and LC_{90} value, cent percent mortality at a low concentration against a relatively less reported mosquito species, i.e., *Aedes albopictus* made our report a significant contribution towards mosquito research.

The presence of important secondary metabolites such as alkaloids, flavonoids, terpenoids, and coumarin further supported our argument. The significance of alkaloids extracted from another plant-based source against *Culex sp.* and *Anopheles sp.* was already reported [20, 21]. The flavonoids in *Millettia pinnata* seeds showed a positive larvicide effect on three different mosquito species [22]. Coumarin was also reported to be responsible for mosquito larvicide activity in plant extract [23]. The presence of such secondary metabolites in the methanol extract of the root of *Coriandrum sativum* suggested that they might be responsible for the larvicidal effect either singly or combinedly.

As the mortality of the non-target organisms was insignificant against the treatment of our extract, it can be assumed to be an environment-friendly larvicide.

5. Conclusion

This is a unique and first study of the effect of *Coriandrum sativum* root extract against *Aedes albopictus* larvae. Further research is needed to identify the bioactive compound responsible for the larvicide effect and to identify the mode of action. Nevertheless, our finding establishes methanol extract of *Coriandrum sativum* root to be a promising larvicidal agent. This will be a significant addition towards the green pesticide research field.

6. Acknowledgement

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7. Conflict of Interest

We have no conflict of interest.

8. Reference

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