Multi-Disciplinary Publishing Institute Pakistan **Vol. 4, NO. 1**

Submitted: 19 MAY 2025 Accepted: 16 JUL 2025 Published: 17 JUL 2025

Evaluation of Response Variation of Some Species of Mosquitoes to Various Plant Extracts

Original Article

Sobia Rani[1], Majid Khan[2], Sidra tul Muntaha[3], Safina Akbar[4], Raheela Noor Memon[5]

[1]Department of Zoology, University of Malakand, Chakdara Dir Lower, Khyber Pakhtunkhwa, Pakistan.

[2]Department of Zoology, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan.

[3,4]Government Girls Degree College Thana, District Malakand, Affiliated with University of Malakand, Department of Zoology, Chakdara Dir Lower, Khyber Pakhtunkhwa, Pakistan.

[5]Assistant Professor, Department of Zoology, Government Girls Zubaida College, Hyderabad, Sindh, Pakistan.

Corresponding author Email: abdussami@qurtuba.edu.pk

Citation

Rani, S., Khan, M., M.S. tul, Akbar, S., & Memon, R.N. (2025). Evaluation of response variation of some species of mosquitoes to various plant extracts. *Open Access Public Health and Health Administration Review*, 4(1), 29-49.

WEBSITE: www.mdpip.com
ISSN: Print: 2959-619X
ISSN: Online: 2959-6203
PUBLISHER: MDPIP

Abstract

This study aimed to discover the cheapest and non-toxic method to reduce the mosquito population at the larval stage. Several plant extracts were reported as biologically active against insects and pests. For a Larvicidal activity of some plant extracts, leaves of *Eucalyptus Lanceolatus*, *Olea Ferruginea*, *Melia Azedarach*, & *Pinus Roxburghii* were evaluated against the third and fourth instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* by preparing various concentrations of aqueous extract of leaves from selected plants. The concentration of these plants had an ethanolic extraction on *Culex quinquefasciatus* and *Aedes aegypti* was 500ppm, 1000ppm, 2000ppm, 3000ppm, 4000ppm, and 5000ppm concentration extract, after 24 hours, ten larvae (III and IV instar stage) were placed in each polyethylene plastic container with test solutions (100ml) at a room temperature of 25 to 30 °C. It has been observed that after 24 hours the percentage of mortality rate against 5000ppm, 4000ppm, 3000ppm, 2000ppm 1000ppm and 500ppm ethanolic extract concentration were recorded for *Pinus Roxburghii* extract against *Culex quinquefasciatus* were found the best, it was observed for Larvicidal activity after 24 hours for 5000ppm is 100%, and for 4000ppm the Larvicidal activity were also noted as 100%, Among all the tested plant extracts, the *Pinus Roxburghii* extract against *Culex quinquefasciatus for* 5000ppm and 4000ppm were also found the best as the mortality rate is 100% as well as the *Eucalyptus Lanceolatus* extract against *Aedes aegypti* were also found the best as the Larvicidal activity has been observed after 24 hours for 5000ppm is 100%, and for 4000ppm, the Larvicidal activity were also recorded as 100%, The mortality depends on time of exposure, plant species and chemical composition.

Keywords: Insecticides, Larvicidal Activity, Culex Quinquefasciatus, Eucalyptus Lanceolatus, Aedes Aegypti.

Introduction

Scientists have placed a significant emphasis on the Larvicidal qualities of plant extracts and essential oils over the past 20 years. Still, relatively few long-term therapy trials have been conducted on *Ae. albopictus* have been conducted (Uddin, Hossain, Reza, Nasrin, & Alam, 2022). Mosquitoes, also known as vectors, are flying insects with 3500 species worldwide. They belong to the Animalia kingdom and are a significant source of diseases. The average size is 0.12 to 0.75 inches, and female mosquitoes only bite and acquire blood for egg production (Zubair, McAlpine, & Gobeske, 2024). There are four phases in the life cycle of an adult mosquito: eggs, larvae, pupae, and adults. These flying insects require standing water to reproduce, which is why they can be found in both artificial and natural watering locations. The first stage to adulthood varies by species, climate, and food availability; under ideal circumstances, they can reach adulthood in 7–10 days (Joshi & Miller, 2021).

Synthetic insecticides, such as organochlorine and organophosphate compounds, are expensive, have adverse environmental effects, and are not biodegradable. As a result, their usage in mosquito control operations has recently decreased (Brühl *et al*., 2020). Female mosquitoes can survive both indoors and outdoors, and they have unique mouthparts that enable them to take blood. Compared to males, they live an average of two to four weeks longer. Temperature, humidity, species, and environment all affect how long they live (Pavela, 2015).

Melia Azedarach, also called the Chinaberry Tree, Pride of India Bead-tree, is a tree with a rounded crown that grows to a maximum height of 7–12 meters (20–40 feet). In exceptional cases, however, *M. Azedarach* can reach as high as 45 meters (150 feet). Farmers in Kenya have planted these trees for use as fodder trees. Cattle can be fed the leaves to increase milk output and farm earnings. Though leaves are extremely poisonous, they have been utilized as a natural pesticide to preserve food (Akram *et al*., 2022). Researchers are looking into sustainable and efficient mosquito control strategies. Using safer botanical insecticides is a straightforward and environmentally friendly substitute. Due to their extensive insecticidal qualities, botanicals may eventually supplant synthetic pesticides. This strategy could provide a new tool in the arsenal of synthetic insecticides and be a more environmentally friendly and sustainable method of controlling mosquitoes (Cafarchia *et al*., 2022).

The purpose and quest of the current study was to test the larvicidal activity of the *larvicides* on the different extracts of the various plants to demonstrate whether the results were environment-friendly or not, and to also come up with the easiest, cheapest, and least toxic method to control the population of the mosquitoes at the larval stage.

Review of Literature

Chinaberry fruit was used to prevent insect larvae from growing in the fruit. By placing the berries in drying apples (etc.) and keeping the fruit turned in the sun without damaging any of the chinaberry skin, the fruit will dry and not have insect larvae in the dried apples. Due to genetic resistance, ecological imbalances brought on by environmental pollution, and the killing of non-target organisms, human pesticide poisoning has resulted in higher expenses. The goal of integrated mosquito management is to create a successful plan that targets larval stages and uses plants as a substitute supply of environmentally friendly control chemicals. Since plants can serve as an alternate source of agents, developing an effective strategy that targets larval stages is essential for effective mosquito control (Usta, Guney, Ozturk, Selvi, & Mustafa, 2020).

The current mosquito control methods are mainly based on synthetic insecticides, usually considered as the first line of action, owing to their quick action. However, repeated application of chemical control often results in an unintended artificial selection of resistant mutants within the vector population. The use of plant extracts has several appealing features since they are less hazardous, safer for non-target organisms, rich in bioactive chemicals, and biodegradable (Dhama *et al.*, 2023). Mosquitoes are arthropods that can cause many more diseases than any other group of arthropods and can strike millions of people worldwide. This devours most of the life-threatening diseases, such as malaria, yellow fever, dengue fever, chikungunya fever, encephalitis, etc., all over the world. Mosquito control is vital to avoid transmission of mosquito-borne diseases, mitigate the quality of the environment, and promote the health of the people

(Lee, Halverson, & Ezinwa, 2018). An infusion of leaves and trees has been diluted and used in the past to induce relaxation of the uterus, as reported by Iqbal & Ashraf (2018).

Materials and Methods

The present study was conducted to find out the easiest, cheapest, and most nontoxic way to control mosquito population at its larval stage and to evaluate the larvicides in the various plant extracts to show whether the results are environment-friendly or not during 2021-2022. For this purpose, various plant extracts, i.e., 500ppm, 1000ppm, 2000ppm, 3000ppm, 4000ppm, and 5000ppm concentration, were used to observe the larvicidal response of target mosquitoes (*Culex and Aedes*) larvicidal. The experimental data obtained from the selected plants (*Eucalyptus Lanceolatus, Olea Ferruginea, Melia Azedarach & Pinus Roxburghii*) extracts were analyzed by using ANOVA, LC50, and LC90.

Collection of Plant Leaves

Fresh leaves *of Pinus Roxburghii, Melia Azedarach, Olea Ferruginea*, and *Eucalyptus Lanceolatus* were gathered from District Malakand and allowed to dry for a week. After being reduced to a powder and steeped in ethanol for three days, the leaves were extracted. For every plant, the ethanolic extract was kept in a different jar for three days.

Collection of Samples

The larvae of mosquitoes Culex quinquefasciatus and Aedes aegypti were tested in a laboratory trial using plant leaves. The concentration of ethanolic extraction against these mosquitoes was observed after 24 hours. After ten larvae were placed in jars with test solutions, the percentage of mortality against the extract concentrations was noted. The results showed promising results for mosquito control and were noted properly.

Figure 1
Total Arrangements of 100ml of Glass for all Plant Extraction.

Note: The mortality depends on the time of exposure, plant species, and chemical composition.

Table 1

Melia Azedarach, Olea Ferruginea, Pinus Roxburghii, and Eucalyptus Lanceolatus of ethanolic extraction against 500ppm, 1000ppm, 2000ppm, 3000ppm, 4000ppm, and 5000ppm

No	Botanical Name	Common Name	Part Used for Extraction	Quantity Fresh	Quantity after shade dried and grinding	Final Ethanolic Extraction	Chemicals used for Extraction
Plant-1	*Melia Azedarach*	Tora Shanda	Leaves Only	1 kg	0.210 g	98.74 g	Ethanol
Plant-2	*Olea Ferruginea*	Khonan	Leaves Only	1 kg	0.318 g	62.66 g	Ethanol
Plant-3	*Pinus Roxburghii*	Nakhtar	Leaves Only	1 kg	0.396 g	53.54 g	Ethanol
Plant-4	*Eucalyptus Lanceolatus*	Lachi	Leaves Only	1 kg	0.325 g	14.74 g	Ethanol

The following table for *Melia Azedarach, Olea Ferruginea, Pinus Roxburghii and Eucalyptus Lanceolatus* of ethanolic extraction against 500ppm, 1000ppm, 2000ppm, 3000ppm, 4000ppm and 5000ppm concentration extract were observed for larvicidal activity and mortality rate of *Aedes aegypti* and *Culex quinquefasciatus* after 24 hours.

Table 2

Per Leave Results for Melia Azedarach, Olea Ferruginea, Pinus Roxburghii, and Eucalyptus Lanceolatus.

Quantity	*Melia Azedarach*	*Olea Ferruginea*	*Pinus Roxburghii*	*Eucalyptus Lanceolatus*
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
500ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
1000ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
2000ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
3000ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
4000ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
5000ppm	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml
	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml	10 Larvae per 100ml

Figure 2
Leaves Dry in Shade at Room Temperature

Melia Azedarach *Olea Ferruginea*

Figure 3
Getting the Ethanolic Extraction for all selected plants

Pinus Roxburghii *Eucalyptus Lanceolatus*

Melia Azedarach Extraction --> *Olea Ferruginea* Extraction

Pinus Roxburghii Extraction *Eucalyptus Lanceolatus* Extraction

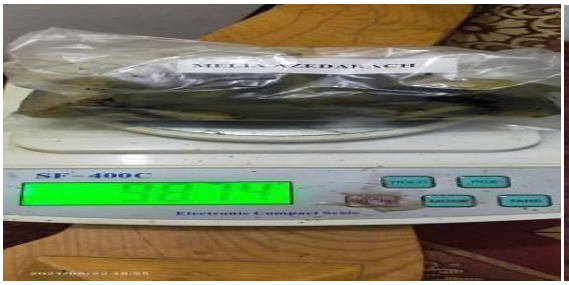
All plants grinded form placed in Ethanol

Final extract obtained

Figure 4

Final Ethanolic Extraction

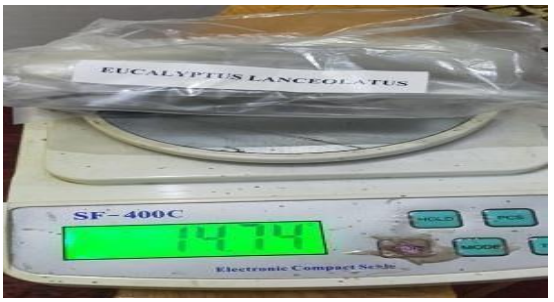
The final extraction obtained after drying the ethanolic extraction was weighed on a sensitive electronic scale model *SF-400C.* The following weights were observed.



Melia Azedarach final Extract weight

Olea Ferruginea final Extract Weight

Pinus Roxburghii final Extract weight



Eucalyptus Lanceolatus final Extract weight

Table 3
All plant final extraction weight

Botanical Name	Final Ethanolic Extraction
Melia Azedarach	98.74 g
Olea Ferruginea	62.66 g
Pinusroxburghii	53.54 g
Eucalyptus Lanceolatus	14.74 g

Results and Discussions

For each PPM 3 each 100 ml of disposable glass (jar) were used for data accuracy, called replication 1, replication 2 and replication 3 and by this way Ten larvae (III and IV instar stage) of *Culex quinquefasciatus* were placed in each polyethylene plastic disposable glass (jar) with test solutions (100 mL) at a temperature of 25°C to 30 °C the larvicidal activity and motility rate were noted for each glass of replication after 3-hour, 6-hour, 12 hours and 24 hours. *lanceolatus, Olea Ferruginea, Melia Azedarach* & *Pinus Roxburghii* extracts were assessed against the third and fourth instar larvae of *Culex quinquefasciatus* & *Aedes aegypti* by making different concentrations of aqueous extraction of leaves of the above plants.

Pinus Roxburghii extract against *Culex quinquefasciatus*

The study recorded the mortality rate of *Culex quinquefasciatus* against various extract concentrations *of Pinus Roxburghii* extract. The larvicidal activity was highest at 5000ppm, followed by 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm. The highest mortality rate was observed at 5000ppm, with a 100% larvicidal activity.

Figure 5
Pinus Roxburghii Extract Against Culex Quinquefasciatus

Eucalyptus Lanceolatus Extract against Culex Quinquefasciatus

The study found that Eucalyptus Lanceolatus extract showed larvicidal activity against Culex quinquefasciatus at various concentrations. The mortality rate was 63% at 5000ppm, 40% at 4000ppm, 26% at 3000ppm, 26% at 2000ppm, 16% at 1000ppm, and 16% at 500ppm after 24 hours. The extract concentrations also impacted larvicidal activity.

Figure 7
Eucalyptus Lanceolatus extract against *Culex Quinquefasciatus*

Olea Ferruginea extract against Culex Quinquefasciatus.

The study examined the larvicidal activity of Olea Ferruginea extract against Culex quinquefasciatus at different concentrations. After 24 hours, the larvicidal activity was found to be 43% at 5000ppm, 33% at 4000ppm, 23% at 3000ppm, 10% at 2000ppm, 3% at 1000ppm, and 3% at 500ppm. The results indicate that the extract has potential for effective pest control.

Figure 8
Olea Ferruginea extract against *Culex Quinquefasciatus*

1. Melia Azedarach extract against Culex Quinquefasciatus

The study examined the mortality rate of *Melia Azedarach* extract against Culex quinquefasciatus at different concentrations. Results showed that the extract had a mortality rate of 43% at 5000ppm, 33% at 4000ppm, 23% at 3000ppm, 13% at 2000ppm, 0% at 1000ppm, and 0% at 500ppm. The larvicidal activity was also observed after 24 hours.

Figure 9
Melia Azedarach extract against *Culex Quinquefasciatus*

2. *Pinus Roxburghii* extract against *Aedes aegypti*

After 24 hours, the mortality rate of *Aedes aegypti* was measured against various extract concentrations of *Pinus Roxburghii* extract. Results showed that the larvicidal activity was 100% for 5000ppm, 100% for 4000ppm, 86% for 3000ppm, 36% for 2000ppm, 30% for 1000ppm, and 20% for 500ppm.

Figure 10
Pinus Roxburghii extract against *Aedes aegypti*

3. Eucalyptus Lanceolatus* extract against *Aedes aegypti.

The study evaluated the larvicidal activity of *Eucalyptus Lanceolatus* extract against *Aedes aegypti* after 24 hours. The larvicidal activity was recorded at different concentrations, with 5000ppm showing 80% larvicidal activity, 4000ppm at 63%, 3000ppm at 60%, 2000ppm at 36%, 1000ppm at 33%, and 500ppm at 13%. The extract concentrations were 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm.

Figure 11
Eucalyptus Lanceolatus extract against *Aedes aegypti*

4. Olea Ferruginea* extract against *Aedes aegypti.

The study found that *Olea Ferruginea* extract, at different concentrations of 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm, showed a larvicidal rate of 6% against Aedes aegypti after 24 hours. The larvicidal activity was highest at 5000ppm at 80%, followed by 4000ppm at 76%, 3000ppm at 60%, 2000ppm at 33%, 1000ppm at 20%, and 500ppm at 6%.

Figure 12
Olea Ferruginea extract against Aedes aegypti

5. Melia Azedarach extract against *Aedes aegypti*

The study found that *Melia Azedarach* extract had a mortality rate of 46% against *Aedes aegypti* after 24 hours at different concentrations. The larvicidal activity was observed at 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm. The larvicidal ratios were 16% for 2000ppm, 6% for 1000ppm, and 3% for 500ppm. These findings suggest that *Melia Azedarach* extract may have potential as a natural larvicidal agent.

Figure 12
Melia Azedarach extract against *Aedes aegypti*

6. Olea Ferruginea* extract against *Aedes aegypti

The study found that *Olea Ferruginea* extract, at different concentrations of 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm, showed a larvicidal rate of 6% against Aedes aegypti after 24 hours. The larvicidal activity was highest at 5000ppm at 80%, followed by 4000ppm at 76%, 3000ppm at 60%, 2000ppm at 33%, 1000ppm at 20%, and 500ppm at 6%.

Figure 13
Olea Ferruginea extract against *Aedes aegypti*

7. Melia Azedarach* extract against *Aedes aegypti

The study found that *Melia Azedarach* extract had a mortality rate of 46% against Aedes aegypti after 24 hours at different concentrations. The larvicidal activity was observed at 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm,

and 500ppm. The larvicidal ratios were 16% for 2000ppm, 6% for 1000ppm, and 3% for 500ppm. These findings suggest that *Melia Azedarach* extract may have potential as a natural larvicidal agent.

Figure 14
Melia Azedarach extract against *Aedes aegypti*

Table 4
Larvicidal activity of Aedes against a 500 PPM concentration

Aedes-500 PPM			Mortality			
No	**Group**	**Replica**	**After 3 Hrs.**	**After 6 Hrs.**	**After 12 Hrs.**	**After 24 Hrs.**
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	10	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	0	0
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	0	0	0	0
7	*Olea Ferruginea*	1	0	10	0	0
8	*Olea Ferruginea*	2	0	0	0	0
9	*Olea Ferruginea*	3	0	10	10	10
10	Pinus Roxburghii	1	10	0	10	10
11	Pinus Roxburghii	2	0	10	10	20
12	Pinus Roxburghii	3	10	10	10	30
13	*Eucalyptus Lanceolatus*	1	0	10	20	20
14	*Eucalyptus Lanceolatus*	2	0	0	0	10
15	*Eucalyptus Lanceolatus*	3	0	0	10	10

Table 5

Larvicidal activity of Aedes against a 500 PPM concentration.

No	Aedes-500 PPM Group	Replica	Mortality After 3 Hrs.	After 6 Hrs.	After 12 Hrs.	After 24 Hrs.
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	10	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	0	0
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	0	0	0	0
7	*Olea Ferruginea*	1	0	10	0	0
8	*Olea Ferruginea*	2	0	0	0	0
9	*Olea Ferruginea*	3	0	10	10	10
10	Pinus Roxburghii	1	10	0	10	10
11	Pinus Roxburghii	2	0	10	10	20
12	Pinus Roxburghii	3	10	10	10	30
13	*Eucalyptus Lanceolatus*	1	0	10	20	20
14	*Eucalyptus Lanceolatus*	2	0	0	0	10
15	*Eucalyptus Lanceolatus*	3	0	0	10	10

Table 6

Larvicidal activity of Aedes against 1000 PPM concentration

No	Aedes-1000 PPM Group	Replica	Mortality After 3 Hrs.	After 6 Hrs.	After 12 Hrs.	After 24Hrs.
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	10	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	0	10
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	0	0	10	10
7	*Olea Ferruginea*	1	0	10	20	30
8	*Olea Ferruginea*	2	0	0	0	0
9	*Olea Ferruginea*	3	0	10	10	20
10	Pinus Roxburghii	1	20	40	40	50
11	Pinus Roxburghii	2	0	0	10	20
12	Pinus Roxburghii	3	10	30	30	40
13	*Eucalyptus Lanceolatus*	1	0	10	10	20
14	*Eucalyptus Lanceolatus*	2	0	0	20	40
15	*Eucalyptus Lanceolatus*	3	0	0	10	10

Table 7

Larvicidal activity of Aedes against 2000 PPM concentration

Aedes-2000 PPM			Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	10	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	10	10
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	10	20	30	40
7	*Olea Ferruginea*	1	10	30	30	40
8	*Olea Ferruginea*	2	10	20	20	30
9	*Olea Ferruginea*	3	10	10	30	30
10	Pinus Roxburghii	1	30	40	40	50
11	Pinus Roxburghii	2	0	20	30	50
12	Pinus Roxburghii	3	0	10	20	40
13	*Eucalyptus Lanceolatus*	1	10	30	30	40
14	*Eucalyptus Lanceolatus*	2	10	10	20	40
15	*Eucalyptus Lanceolatus*	3	10	10	30	40

Table 8

Larvicidal activity of Aedes against a 3000 PPM concentration.

Aedes-2000 PPM			Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	10	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	10	10
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	10	20	30	40
7	*Olea Ferruginea*	1	10	30	30	40
8	*Olea Ferruginea*	2	10	20	20	30
9	*Olea Ferruginea*	3	10	10	30	30
10	Pinus Roxburghii	1	30	40	40	50
11	Pinus Roxburghii	2	0	20	30	50
12	Pinus Roxburghii	3	0	10	20	40
13	*Eucalyptus Lanceolatus*	1	10	30	30	40
14	*Eucalyptus Lanceolatus*	2	10	10	20	40
15	*Eucalyptus Lanceolatus*	3	10	10	30	40

Table 9

Larvicidal activity of Aedes against a 4000 PPM concentration.

No	Aedes-4000 PPM Group	Replica	Mortality After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/Water	1	0	0	0	0
2	Control/Water	2	0	0	10	10
3	Control/Water	3	0	0	0	0
4	Melia Azeddarach	1	10	30	50	60
5	Melia Azeddarach	2	20	30	40	50
6	Melia Azeddarach	3	0	10	30	40
7	Olea Ferruginous	1	20	30	50	70
8	Olea Ferruginous	2	10	40	50	80
9	Olea Ferruginous	3	20	30	60	80
10	Pinus Roxburghii	1	30	60	100	100
11	Pinus Roxburghii	2	20	60	80	100
12	Pinus Roxburghii	3	40	60	70	100
13	*Eucalyptus Lanceolatus*	1	20	40	70	90
14	*Eucalyptus Lanceolatus*	2	0	30	50	70
15	*Eucalyptus Lanceolatus*	3	20	30	60	70

Table 10

Larvicidal activity of Aedes against a 5000 PPM concentration.

No	Aedes-5000 PPM Group	Replica	Mortality After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/Water	1	0	0	0	0
2	Control/Water	2	0	0	10	10
3	Control/Water	3	0	0	0	0
4	Melia Azeddarach	1	0	20	30	40
5	Melia Azeddarach	2	10	50	50	60
6	Melia Azeddarach	3	0	10	10	30
7	*Olea Ferruginea*	1	10	40	70	90
8	*Olea Ferruginea*	2	0	50	50	80
9	*Olea Ferruginea*	3	0	10	50	70
10	*Pinusroxburghii*	1	20	30	40	40
11	*Pinusroxburghii*	2	30	60	80	100
12	*Pinusroxburghii*	3	30	80	100	100
13	Eucalyptus Lanceolatus	1	20	50	70	80
14	Eucalyptus Lanceolatus	2	20	30	50	70
15	Eucalyptus Lanceolatus	3	0	30	40	80

Table 11
Larvicidal activity of Culex against a 500 PPM concentration.

	Culex-500 PPM		Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	10
2	Control/water	2	0	0	0	0
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	0	0
5	Melia Azeddarach	2	0	0	0	0
6	Melia Azeddarach	3	0	0	0	0
7	*Olea Ferruginea*	1	0	0	0	0
8	*Olea Ferruginea*	2	0	0	0	0
9	*Olea Ferruginea*	3	0	0	1	1
10	*Pinusroxburghii*	1	20	30	30	40
11	*Pinusroxburghii*	2	10	10	20	30
12	*Pinusroxburghii*	3	10	30	30	40
13	*Eucalyptus Lanceolatus*	1	0	10	10	10
14	*Eucalyptus Lanceolatus*	2	0	10	10	20
15	*Eucalyptus Lanceolatus*	3	0	0	0	20

Table 12
Larvicidal activity of Culex against 1000 PPM concentration.

	Culex-1000 PPM		Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	10
2	Control/water	2	0	0	0	0
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	0	0
5	Melia Azeddarach	2	0	0	0	0
6	Melia Azeddarach	3	0	0	0	0
7	*Olea Ferruginea*	1	0	0	0	0
8	*Olea Ferruginea*	2	0	0	0	0
9	*Olea Ferruginea*	3	0	10	10	10
10	*Pinusroxburghii*	1	30	40	60	70
11	*Pinusroxburghii*	2	20	20	50	70
12	*Pinusroxburghii*	3	10	20	30	30
13	*Eucalyptus Lanceolatus*	1	0	10	10	10
14	*Eucalyptus Lanceolatus*	2	10	10	20	20
15	*Eucalyptus Lanceolatus*	3	0	0	0	20

Table 13
Larvicidal activity of Culex against 2000 PPM concentration.

Culex-2000 PPM			Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	0	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	10	20
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	0	0	0	10
7	*Olea Ferruginea*	1	0	0	10	10
8	*Olea Ferruginea*	2	0	0	0	10
9	*Olea Ferruginea*	3	0	0	10	10
10	Pinus Roxburghii	1	40	40	60	60
11	Pinus Roxburghii	2	20	60	70	70
12	Pinus Roxburghii	3	20	30	50	80
13	*Eucalyptus Lanceolatus*	1	10	10	30	60
14	*Eucalyptus Lanceolatus*	2	0	10	10	30
15	*Eucalyptus Lanceolatus*	3	0	10	30	30

Table 14
Larvicidal activity of Culex against a 3000 PPM concentration.

Culex-3000 PPM			Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	0	0
3	Control/water	3	0	0	0	10
4	Melia Azeddarach	1	0	0	0	10
5	Melia Azeddarach	2	0	0	10	10
6	Melia Azeddarach	3	0	30	30	50
7	*Olea Ferruginea*	1	0	10	20	20
8	*Olea Ferruginea*	2	20	20	20	30
9	*Olea Ferruginea*	3	0	10	20	30
10	Pinus Roxburghii	1	10	20	70	70
11	Pinus Roxburghii	2	20	50	60	70
12	Pinus Roxburghii	3	30	60	70	70
13	*Eucalyptus Lanceolatus*	1	0	0	10	30
14	*Eucalyptus Lanceolatus*	2	0	0	0	20
15	*Eucalyptus Lanceolatus*	3	10	10	30	30

Table 15

Larvicidal activity of Culex against a 4000 PPM concentration

	Culex-4000 PPM		Mortality			
No	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	10
2	Control/water	2	0	0	0	0
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	0	10	20
5	Melia Azeddarach	2	10	30	30	40
6	Melia Azeddarach	3	0	10	10	40
7	*Olea Ferruginea*	1	10	10	30	40
8	*Olea Ferruginea*	2	10	10	10	30
9	*Olea Ferruginea*	3	0	20	30	30
10	Pinus Roxburghii	1	30	70	80	100
11	Pinus Roxburghii	2	10	30	70	100
12	Pinus Roxburghii	3	50	90	100	100
13	*Eucalyptus Lanceolatus*	1	0	0	20	30
14	*Eucalyptus Lanceolatus*	2	10	10	30	50
15	*Eucalyptus Lanceolatus*	3	20	30	40	40

Table 16

Larvicidal activity of Culex against a 5000 PPM concentration.

	Culex-5000PPM		Mortality			
No.	Group	Replica	After 3 Hrs	After 6 Hrs	After 12 Hrs	After 24 Hrs
1	Control/water	1	0	0	0	0
2	Control/water	2	0	0	0	10
3	Control/water	3	0	0	0	0
4	Melia Azeddarach	1	0	10	20	30
5	Melia Azeddarach	2	0	20	40	50
6	Melia Azeddarach	3	10	10	20	50
7	*Olea Ferruginea*	1	20	20	50	60
8	*Olea Ferruginea*	2	10	10	10	40
9	*Olea Ferruginea*	3	0	20	30	30
10	Pinus Roxburghii	1	50	100	100	100
11	Pinus Roxburghii	2	20	90	100	100
12	Pinus Roxburghii	3	50	90	100	100
13	*Eucalyptus Lanceolatus*	1	0	0	20	40
14	*Eucalyptus Lanceolatus*	2	10	30	50	90
15	*Eucalyptus Lanceolatus*	3	20	40	50	60

LC50 and LC90 Analysis

LC50 and LC90 Statistics against *Aedes aegypti*

All data collected for *Aedes aegypti* were applied against LC50 and LC90 statistics, and the following table we obtained. The data showed the least PPM required to kill 50 % & 90% of the *Aedes aegypti,* showing the most poisonous extract amongst all.

Table 17
Plant Extracts against Aedes Aegypti

Plant extracts	Against *Aedes aegypti*	
	LC 50 (ppm)	LC 90 (ppm)
M. Azedarach	5481.9	28430.8
O. Ferruginea	2382.4	7628.7
P. Roxburghii	1264.4	3425.1
E. Lanceolatus	2162.5	9449.1

LC50 and LC90 Statistics against *Aedes aegypti*

All data collected for *Culex quinquefasciatus* were applied against LC50 and LC90 statistics, and the following table we obtained. The data showed the least PPM required killing 50 % & 90% of the *Culex quinquefasciatus,* showing the most poisoning extract amongst all.

Table 18
Plant Extracts against Culex quinquefasciatus

Plant extracts	Against *Culex quinquefasciatus*	
	LC 50 (ppm)	LC 90% (ppm)
M. Azedarach	5482.3	14940.7
O. Ferruginea	6539.9	28329.9
P. Roxburghii	860.1	3762.7
E. Lanceolatus	4942.3	66570.3

The research aimed to determine a cost-effective method with low risk to control the population of mosquitoes when in the larval stage. Various quantities of ethanolic extract were used to examine various plant extracts on *Culex quinquefasciatus* and Aedes aegypti larvae. The results showed 100 percent of complete death rate against 5000ppm, 4000ppm, 3000ppm, 2000ppm, 1000ppm, and 500ppm. The extract also had better efficacy, which was pointed out when a hundred percent death of *Culex quinquefasciatus* and Aedes aegypti larvae was realized. As it depends on how long each animal is kept in contact with the plant, on the species of the plant, and on the available chemical composition of the extracts, the mortality rate varies. The study's aim was to confirm or approve the environmentally soundness of the extracts, as well as their ability to control mosquito densities. The research also stressed that it should be ensured that the extracts would be environmentally friendly and they should not lead to harm to the aquatic species.

Conclusion

The effective concentration of the tested extracts on the Larvicidal effect was dependent. The possible biological impact of the plant extracts was also well familiar, known to be attributed to the presence of several bioactive Phyto molecules present in the plant, such as alkaloids, Terpenoids, and phenolics. Nonetheless, the disparity of toxicity, which was presented by the various species and parts of the plants, was due to the quantitative and qualitative deviation in the chemical composition of the plant extracts. It was concluded that the *Pinus Roxburghii* induced full mortality (100%) against *Culex quinquefasciatus* & *Aedes aegypti,* ethanolic extraction of 5000ppm and 4000ppm. And as well as for the *Eucalyptus Lanceolatus* against *Culex quinquefasciatus* & *Aedes aegypti,* also induced full mortality (100%) was recorded against ethanolic extraction of 5000ppm and 4000ppm. When the concentration of ethanolic extraction in ppm increases, the mortality rate increases. Any larvae that failed to give any sign of movement were taken to be dead. Larvae that moved slightly and did not have any form of swimming were also classified to be moribund. The dead larvae were regarded as dead because these larvae could never come back to life. Mortality of the larvae also depended on extraction concentration, part of the plant, and exposure time.

Declarations

Ethical Approval and Consent to Participate: This study strictly adhered to the Declaration of Helsinki and relevant national and institutional ethical guidelines. Informed consent was not required, as secondary data available on websites was obtained for analysis. All procedures performed in this study were by the ethical standards of the Helsinki Declaration.

Consent for Publication: Not Applicable

Availability of Data and Materials: Data will be provided to the requesting researchers/bodies upon making a formal request from the corresponding author.

Competing Interest: The authors declare no competing interest.

Funding: Not Applicable

Authors' Contribution: RS, KM [Conceptualization and writeup, MS, AS, MRN [Data Analysis].

Acknowledgement: The researchers are obliged to the cooperation of fellow researchers, officials, and laboratory staff for the assistance they provided in conducting this research.

References

Akram, N. A., Saleem, M. H., Shafiq, S., Naz, H., Farid-ul-Haq, M., Ali, B., . . . Qureshi, K. A. (2022). Phytoextracts as crop biostimulants and natural protective agents—a critical review. *Sustainability*, 14(21), 14498.

Brühl, C. A., Després, L., Fror, O., Patil, C. D., Poulin, B., Tetreau, G., & Allgeier, S. (2020). Environmental and socioeconomic effects of mosquito control in Europe using the biocide Bacillus thuringiensis subsp. Israelensis (Bti*). Science of the Total Environment*, 724, 137800.

Cafarchia, C., Pellegrino, R., Romano, V., Friuli, M., Demitri, C., Pombi, M., . . . Otranto, D. (2022). Delivery and effectiveness of entomopathogenic fungi for mosquito and tick control: Current knowledge and research challenges. *Acta Tropica*, 234, 106627.

Dhama, K., Sharun, K., Gugjoo, M. B., Tiwari, R., Alagawany, M., Iqbal Yatoo, M., . . . Michalak, I. (2023). A comprehensive review on the chemical profile and pharmacological activities of Ocimum Basilicum. *Food Reviews International*, 39(1), 119-147.

Iqbal, M. N., & Ashraf, A. (2018). Recombinant Protein Production in Plants: Bio Factories for Therapeutics. International *Journal of Molecular Microbiology*, 1(1), 38-39.

Joshi, A., & Miller, C. (2021). Review of machine learning techniques for mosquito control in urban environments. *Ecological Informatics*, 61, 101241.

Lee, H., Halverson, S., & Ezinwa, N. (2018). Mosquito-borne diseases. *Primary Care: Clinics in Office Practice*, 45(3), 393-407.

Manzano, P., García, O. V., Malusín, J., Villamar, J., Quijano, M., Viteri, R., ...& Orellana-Manzano, A. (2020). Larvicidal activity of ethanolic extract of Azadirachta indica against Aedes aegypti larvae. *Revista Facultad Nacional de Agronomía Medellín*, *73*(3), 9315-9320.

Pavela, R. (2015). Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against Culex quinquefasciatus Say larvae. *Parasitology Research*, 114, 3835-3853.

Robert, S. H. (2001). Discover a native plant extract that repels and kills termites. *J. Econ. Entomol*, *9*, 1200-1208.

Russell, T. L., Kay, B. H., & Skilleter, G. A. (2009). Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. *Aquatic Biology*, *6*, 77-90.

Shaalan, E. A. S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., & Mansour, A. H. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment international*, *31*(8),1149-1166.

Shahzad, M. I., Ashraf, H., Iqbal, M. N., & Khanum, A. (2017). Medicinal Evaluation of Common Plants against Mouth Microflora. *PSM Microbiology*, *2*(2),34-40.

Singh, N., Hoette, Y., & Miller, D. R. (2002). *Tulsi: The mother medicine of nature*. International Institute of Herbal Medicine.

Teixeira, D., Fernandes, R., Prudêncio, C., & Vieira, M. (2016). 3-Nitrotyrosine quantification methods: Current concepts and future challenges. *Biochimie*, *125*, 1-11.

Tolle, Michael A. (2009). Malaria-borne diseases. Current Problems in Pediatric and Adolescent Health Care 3(4), 97-140

Uddin, A. N., Hossain, F., Reza, A. A., Nasrin, M. S., & Alam, A. K. (2022). Traditional uses, pharmacological activities, and phytochemical constituents of the genus Syzygium: A review. *Food Science & Nutrition*, 10(6), 1789-1819.

Ullah, M., Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Farooq, T. H., ...& Ali, M. (2018). Plants as Antidiabetic Agents: Traditional Knowledge to Pharmacological Validation. *PSM Biological Research*, *3*(3), 111-119.

Usta, A., Güney, İ., Öztürk, M., Selvi, E. K., & Mustafa, M. (2020). Toxicological and behavioral potency of different plant extracts on Aedes albopictus (Diptera: Culicidae) and their qualitative phytochemical analysis. *International Journal of Mosquito Research*, 7(5 Part A), 12-18.

Vargas-Miranda, K., Troyo, A., & Calderón-Arguedas, Ó. (2019). Resistencia de Aedes aegypti (diptera: culicidae) ainsecticidas organofosforados y piretroidesen la localidad de Orotina, Alajuela, Costa Rica. *Revista Costarricense de SaludPública*, *28*(1), 15-24.

Yadav, R., Tyagi, V., Tikar, S. N., Sharma, A. K., Mendki, M. J., Jain, A. K., & Sukumaran, D. (2014). Differential larval toxicity and oviposition-altering activity of some indigenous plant extracts against dengue and chikungunya vector Aedes albopictus. *Journal of Arthropod-borne Diseases*, *8*(2), 174.

Zubair, A. S., McAlpine, L. S., & Gobeske, K. T. (2024). Virology, ecology, epidemiology, pathology, and treatment of eastern equine encephalitis. *Journal of the Neurological Sciences*, 457, 122886

Note: Open Access Public Health and Health Administration Review is recognized by the Higher Education Commission of Pakistan in the Y category.

Disclaimer/ Publisher's Note: The statements, opinions, and data contained in all publications in this journal are solely those of the individual author(s) and not of the MDPIP and/ or the editor(s). MDPIP and editor(s) disclaim responsibility for any injury to the people or property resulting from any ideas, methods, instructions, or products referred to *in the content.*