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The relative Toxicity of Herbicides (*Adathoda vasica*, *Azadirachta indica*, *Brugmansia versicolor*, *Carica papaya*, *Calendula officinalis*) extract against larvae of Mosquito species

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Abstract: **The present study the larvicidal efficacy of five medicinal plant leaf extracts, *Adathoda vasica, Azadirachta indica, Brugmansia versicolor, Carica papaya*, and *Calendula officinalis* against various instar stages of the medically important mosquito *Aedes aegypti*. Larvae were exposed to a series of extract concentrations, and mortality rates were recorded after 24 hours. The median lethal concentration (LC_{50}/24 hours) for each plant extract was calculated using Finney's probit analysis method. Among the tested plant extracts, *Azadirachta indica* exhibited the highest toxicity with the lowest LC_{50} value of 2.3209%, followed by *Carica papaya* (2.4658%), *Adathoda vasica* (2.7907%), *Calendula officinalis* (5.1203%), and *Brugmansia versicolor* with the highest LC_{50} value of 6.8372%. A clear dose-dependent increase in larval mortality was observed for all extracts. These findings highlight the potential of plant-based larvicides in mosquito control programs, particularly *Azadirachta indica,* which demonstrated the most potent larvicidal activity against *Aedes aegypti* larvae.**

Keywords: **Azadiracta indica, Brugmansis versicolor, Carica papaya and Calendula officinalis etc.**

1. INTRODUCTION

Mosquitoes are important insects not only as nuisance biters. The World Health Organization (WHO) adopted mosquito control as the only method to prevent or control such diseases [14].*Aedes aegypti*, the primary vector for dengue, chikungunya, Zika, and yellow fever, poses a significant public health threat in tropical and subtropical regions worldwide. Traditional mosquito control strategies heavily rely on synthetic insecticides; however, their prolonged use has led to resistance in mosquito populations, environmental contamination, and adverse effects on non-target organisms [11]. In response, there is growing interest in eco-friendly and sustainable alternatives, such as plant-based larvicides derived from medicinal plants known for their bioactive compounds [1]. Various phytochemicals, including alkaloids, flavonoids, and terpenoids found in plants, have shown promising larvicidal activity against mosquito larvae [4]. In this context, the present study investigates the larvicidal potential of five medicinal plant extracts, *Adathoda vasica*, *Azadirachta indica*, *Brugmansia versicolor*, *Carica papaya*, and *Calendula officinalis*, against different instar larvae of *Aedes aegypti*, aiming to identify natural and effective alternatives to chemical insecticides.

2. MATERIALS AND METHODS

A field trial was conducted to compare the efficacy of different herbal extracts that mainly used against the mosquito larvae in and around Kannur city, during six month of duration from September-2023 to February-2024.

Test Animal

Developmental stages of (I to IV instar stages) of Aedes aegypti species of mosquitoes were used for experiments in the present study. The larvae were collected from the nearby drainage, brought to the laboratory and reared to became adult. They then identified and reared in cages (Kumar *et al.,*1991 and Lyimo *et al*.,1992). The eggs obtained from these stocks of mosquitoes were allowed to hatch. The larvae of I, II, III and IV instars were taken for the experiments.

Test Toxicants

This study evaluates the larvicidal efficacy of extracts from five different plants such as *Adathoda vasica, Azadirachta indica*, *Brugmansia versicolor*, *Carica papaya*, and *Calendula officinalis* against the larvae of mosquito *Aedes aegypti* species. Each plant contains unique bioactive compounds known for their potential insecticidal properties (Plate.1).

Plant Collection and Preparation of Extracts

1. *Adathoda vasica*: Leaves collected from mature plants.
2. *Azadirachta indica*: Leaves collected from mature trees.
3. *Brugmansia versicolor*: Flowers collected from mature plants.
4. *Carica papaya*: Seeds collected from ripe papaya fruits and mature plants.
5. *Calendula officinalis*: Flowers collected from mature plants.

The collected plant materials (leaves, seeds, and flowers) will be thoroughly washed with distilled water to remove dirt and contaminants. The plant materials will then be air-dried in the shade at room temperature for 7-10 days. Once dried, the plant materials will be ground into a fine powder using an electric grinder(Plate.1).

Preparation of Extracts

The powdered plant materials will be subjected to solvent extraction using water. For each plant, 100 grams of the powdered material will be soaked in 500ml of water for 72 hours with occasional shaking. After 72 hours, the mixture will be filtered through Whatman No. 1 filter paper to obtain the crude extract. The filtrate will be concentrated using a rotary evaporator under reduced pressure at 40°C to obtain a semi-solid extract. The concentrated extracts will be stored in labelled glass bottles at 4°C until further use.

Larvicidal Bioassay

Mosquito Larvae Collection

Mosquito eggs of *Aedes aegypti* were collected from local breeding sites or obtained from a mosquito rearing facility. Eggs will be hatched in dechlorinated water under laboratory conditions (27±2°C, 75-85% RH, 12:12 light/dark photoperiod). Newly hatched larvae will be reared to the third instar stage, which is typically used for bioassay experiments(Plate.2a).

Stock Preparation

Stock solutions of each plant extract will be prepared by dissolving the extracts in water. Serial dilutions will be made from the stock solutions to prepare test solutions with different concentrations (e.g., 50, 100, 150, 200, and 250 ppm).

Bioassay

Bioassay test were carried out for testing the efficiency of the herbicide concentration onthe larvae at I, II, III, IV instar stages of mosquito Aedes aegypti. Instructions of WHO (1960) for conductivity bioassay experiment with mosquito larval were carefully followed. Different concentrations from 0.001% to 0.07% of the test compound was prepared using unchlorinated, filtered tap water by serial dilution. Clean glass cups of 500 ml capacity were used as test containers (Plate 2b). Batches of 10 larvae at a particular stage of development were exposed to a series of concentration of the test solution (200 ml). The larvae were collected with an eye dropper placed on to a filter paper strip and immediately transferred to test cup containing a particular concentration of test solution.

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Mortality rates of larvae were recorded after 24 hours. Five more concentrations of a test compound giving between 0 and 100% mortality for larval different instar stages were tested. Parallel controls were maintained. Three replicates were conducted at each concentration and a particular stage of larvae. For computing LC_{50}, the mortality data were subjected to Finney's method of probit analysis as detailed by [13].

Mortality Calculation

Mortality rates will be calculated as the percentage of dead larvae in each treatment relative to the total number of larvae exposed.

Statistical Analysis

The data will be analyzed using probit analysis to determine the lethal concentration (LC_{50}) values, which represent the concentrations required to kill 50% of the larvae. The results will be expressed as mean ± standard error (SE) of the mean,the study aims to comprehensively evaluate the larvicidal efficacy of *Adathoda vasica*, *Azadirachta indica, Brugmansia versicolor*, *Carica papaya*, and *Calendula officinalis* extracts against mosquito larvae and assess their potential as eco-friendly alternatives to chemical insecticides.

3. RESULTS

The study evaluated the larvicidal efficacy of five medicinal plant leaf extracts, *Adathoda vasica*, *Azadirachta indica*, *Brugmansia versicolor*, *Carica papaya*, and *Calendula officinalis*, against different larval stages of the medically significant mosquito *Aedes aegypti*. Using Finney's probit analysis, the median lethal concentration (LC_{50}) values over 24 hours were calculated. Among the tested extracts, *Azadirachta indica* exhibited the highest toxicity with the lowest LC_{50}/24h value of 2.3209%, followed by *Carica papaya* (2.4658%) and *Adathoda vasica* (2.7907%), indicating their strong potential as natural larvicides. In contrast, *Calendula officinalis* (5.1203%) and *Brugmansia versicolor* (6.8372%) demonstrated comparatively lower toxicity, suggesting less larvicidal efficacy within the same exposure period. A clear dose-response relationship was observed in all cases, with increasing mortality rates corresponding to higher concentrations.

These findings underscore the potential of plant-based larvicides in integrated mosquito management, particularly the promising activity of *Azadirachta indica* (Neem), which is known for its bioactive compounds such as azadirachtin with insecticidal properties. The use of botanical insecticides offers an eco-friendly and biodegradable alternative to synthetic chemicals, minimizing environmental and public health risks. Future studies should assess long-term ecological impacts, synergistic effects between plant extracts, and field efficacy before recommending widespread application [8],[16](*Table.1-6 and Figure.1-6*).

Plate.1

Showing Test Toxicants

***Adathoda vasica* (Vasaka)**

***Azadirachta indica* (Neem)**

Brugmansia versicolor

Carica papaya (Papaya)

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***Calendula officinalis* (Marigold)**

Plate.2a

Showing Test Animal

Larvae of Aedes mosquito(I,II,III and IV Instars)

Plate.2b

Showing Experimental setup of Test Toxicants

Table.1 LC_{50}/24 hrs value (%) of Adathoda vasica to the larvae of Aedes aegypti

Dose %	Log10(dose %)	Mortality %	Probit of Mortality
2	0.30102	0%	0
2.2	0.342422681	0%	0
2.4	0.380211242	15%	3.96
2.6	0.414973348	20%	4.16
2.8	0.447158031	30%	4.48
3	0.477121255	40%	4.75
3.2	0.505149978	45%	4.87
3.4	0.531478917	55%	5.13

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Parameter	Value
LC_{50}	2.7907
Equations	
Equation	$Y = -3.7207 + \frac{63.4225 + 3.7207}{1 + (\frac{X}{2.7907})^{-8.3894}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{LC50})^{Hill\ coefficient}}$

Figure.1 LC_{50}/24 hrs value (%)of Adathoda vasica to the larvae of Aedes aegypti

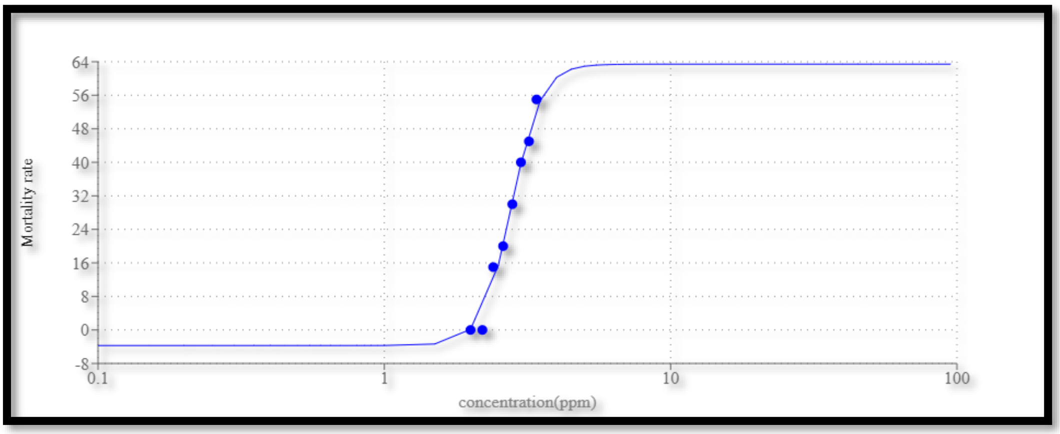

Table.2 LC_{50}/24 hrs value (%) of Azadirachta indica to the larvae of Aedes aegypti

Dose %	Log10(dose %)	Mortality %	Probit of Mortality
2	0.30102	45%	4.87
2.2	0.34242	60%	5.25
2.3	0.36172	65%	5.39
2.4	0.38021	80%	5.84
2.6	0.41497	95%	6.64
2.8	0.44715	95%	6.64

Parameter	Value
LC_{50}	2.3209
Equations	
Equation	$Y = 43.4025 + \frac{97.7396 - 43.4025}{1 + (\frac{X}{2.3209})^{-20.4228}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + (\frac{X}{LC50})^{Hill\ coefficient}}$

Figure.2 LC_{50}/24 hrs value (%) of Azadirachta indica to the larvae of Aedes aegypti

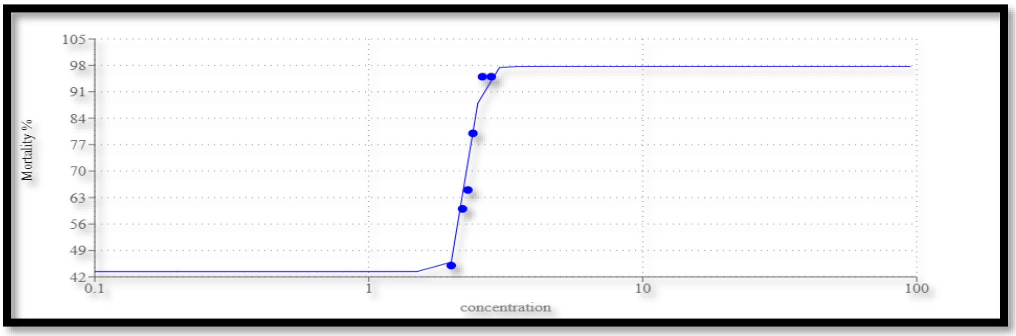

Table.3 LC_{50}/24 hrs value (%) of Brugmansia versicolor to the larvae of Aedes aegypti

Dose %	Log10(dose %)	Mortality %	Probit of Mortality
2	0.30102	0%	0
2.5	0.39794	0%	0
3	0.47712	5%	3.36
3.5	0.54406	25%	4.33
4	0.60205	30%	4.48
5.5	0.74036	40%	4.75
5	0.69897	45%	4.87
5.5	0.74036	60%	5.25
6	0.77815	65%	5.39
6.5	0.81291	75%	5.67

Parameter	Value
LC_{50}	6.8372
Equations	
Equation	$Y = -3.9928 + \frac{161.7096 + 3.9928}{1 + \left(\frac{x}{6.8372}\right)^{-2.8492}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + \left(\frac{x}{LC50}\right)^{Hill\ coefficient}}$

Figure.3 LC_{50}/24 hrs value (%) of Azadirachta indica to the larvae of Aedes aegypti

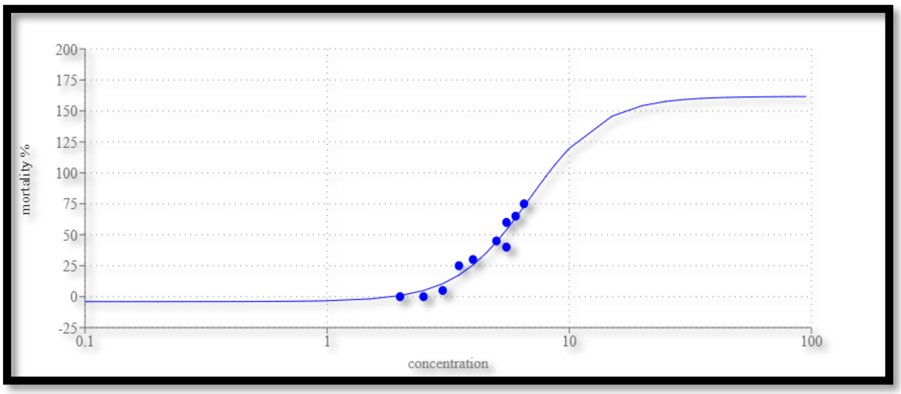

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Table.4 LC_{50}/24 hrs value (%) of Carica papaya to the larvae of Aedes aegypti

Dose %	Log10(dose %)	Mortality %	Probit of Mortality
2	0.30102	15%	3.96
2.2	0.34242	20%	4.16
2.4	0.38021	30%	4.48
2.6	0.41497	30%	4.48
2.8	0.44715	40%	4.75
3	0.47712	45%	4.87
3.2	0.50514	55%	5.13

Parameter	Value
LC_{50}	2.4658
Equations	
Equation	$Y = 5.8002 + \frac{53.0631 - 5.8002}{1 + \left(\frac{x}{2.4658}\right)^{-6.7808}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + \left(\frac{x}{LC50}\right)^{\text{Hill coefficient}}}$

Figure.4 LC_{50}/24 hrs value (%) of Azadirachta indica to the larvae of Aedes aegypti

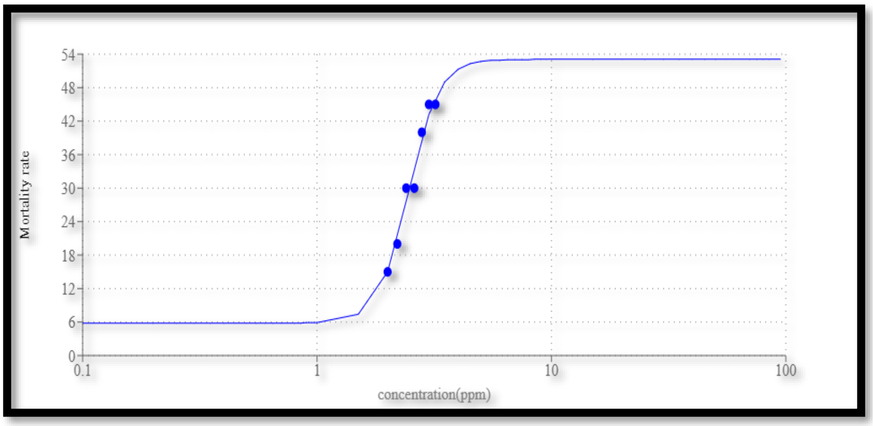

Table.5 LC_{50}/24 hrs value (%) of Calendula officinalis to the larvae of Aedes aegypti

Dose %	Log10(dose %)	Mortality %	Probit of Mortality
3.4	0.53147	20%	4.16
3.8	0.57978	20%	4.16
4.2	0.62324	25%	4.33
4.6	0.66275	30%	4.48
5	0.69897	40%	4.75
5.4	0.73239	60%	5.25
5.8	0.76342	65%	5.39
6	0.77815	70%	5.52

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Parameter	Value
LC_{50}	5.1203
Equations	
Equation	$Y = 20.5486 + \frac{73.7485 - 20.5486}{1 + \left(\frac{x}{5.1203}\right)^{-15.252}}$
Equation Form	$Y = Min + \frac{Max - Min}{1 + \left(\frac{x}{LC50}\right)^{\text{Hill coefficient}}}$

Figure.5 LC_{50}/24 hrs value (%) of Azadirachta indica to the larvae of Aedes aegypti

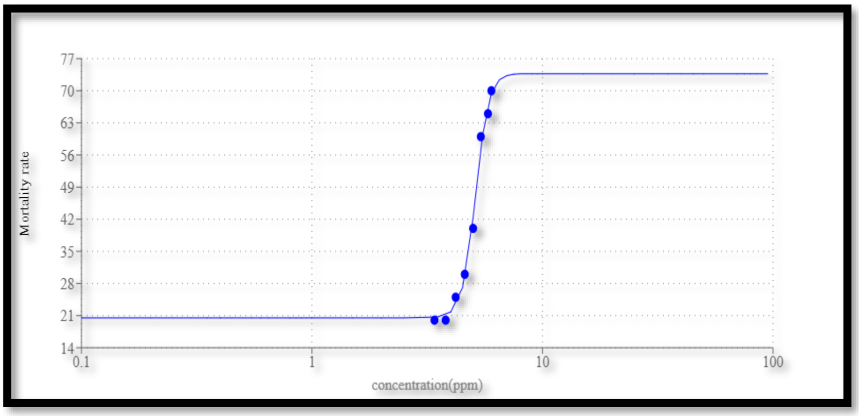
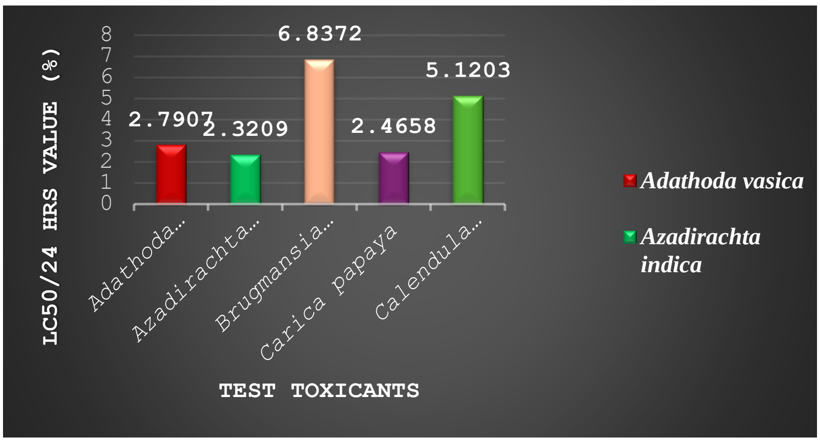


Table.6 LC_{50}/24 hrs value (%) of Test toxicants (Adathoda vasica, Azadirachta indica, Brugmansia versicolor, Carica papaya, and Calendula officinalis) against the larvae of Aedes aegypti

SL.NO	Test Toxicants	LC_{50}/24 hrs value (%)
1.	*Adathoda vasica*	2.7907
2.	*Azadirachta indica*	2.3209
3.	*Brugmansia versicolor*	6.8372
4.	*Carica papaya*	2.4658
5.	*Calendula officinalis*	5.1203

Figure.6 Showing LC_{50}/24 hrs value (%) of Test toxicants (Adathoda vasica, Azadirachta indica, Brugmansia versicolor, Carica papaya, and Calendula officinalis) against the larvae of Aedes aegypti



4. DISCUSSION

The study highlights the larvicidal efficacy of five medicinal plant extracts against *Aedes aegypti* larvae, with LC_{50}/24h values indicating varying levels of toxicity. *Azadirachta indica* (Neem) showed the highest potency (LC_{50} 2.3209%), followed by *Carica papaya* (2.4658%) and *Adathoda vasica* (2.7907%), which possess bioactive compounds like azadirachtin, papain, and vasicine known for their insecticidal properties. *Calendula officinalis* (5.1203%) and *Brugmansia versicolor* (6.8372%) were less toxic but still demonstrated potential as natural larvicides. The bioactivity of these plant extracts is primarily attributed to phytochemicals such as alkaloids, flavonoids, and terpenoids that disrupt larval development and physiological processes [5],[17].

Compared to synthetic insecticides or herbicides which may cause long-term environmental harm plant-based larvicides offer a safer and biodegradable alternative suitable for Integrated Pest Management (IPM) strategies. Unlike herbicides, which are ineffective and potentially hazardous in aquatic ecosystems, botanical larvicides have shown reduced toxicity to non-target species, faster biodegradability, and better community acceptance (Regnault-Roger *et al*., 2012; WHO, 2020). Continued research and field validation are essential to optimize these plant extracts for large-scale mosquito control programs, especially in regions facing mosquito-borne diseases like dengue, Zika, and chikungunya[1],[10].

5. CONCLUSION

A comparative larvicidal efficacy study was conducted on *Aedes aegypti* larvae using leaf extracts of five medicinal plants: *Adathoda vasica*, *Azadirachta indica*, *Brugmansia versicolor*, *Carica papaya*, and *Calendula officinalis*. The 24-hour median lethal concentration (LC_{50}/24hrs) was determined using probit analysis.

- *Azadirachta indica* (Neem) was found to be the most effective, with the lowest LC_{50} value of 2.3209%, indicating high toxicity to larvae.
- *Carica papaya* showed comparable efficacy with an LC_{50} of 2.4658%.
- *Adathoda vasica* demonstrated moderate toxicity with an LC_{50} of 2.7907%.
- *Calendula officinalis* had a higher LC_{50} of 5.1203%, suggesting lower larvicidal potential.
- *Brugmansia versicolor* showed the least toxicity with the highest LC_{50} of 6.8372%.

Overall, the results indicate that *Azadirachta indica*, *Carica papaya*, and *Adathoda vasica* possess promising larvicidal activity against *Aedes aegypti* and could be potential candidates for botanical mosquito control formulations.

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