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Larvicidal effects of aqueous leaf extracts of *Azadirachta indica*, *Tinospora cordifolia*, *Aloe barbadensis*, *Ageratum conyzoides*, and *Musa acuminata*, against *Aedes Aegypti (Diptera*: *Culicidae*)

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Abstract
Aedes Aegypti mosquito is a vector responsible for transmission of various viruses including dengue, which results into the development of an urgent need to develop safe and effective plant-based larvicides. In this research work, the effect of aqueous leaf extract of *Azadirachta indica, Aloe barbadensis, Tinospora cordifolia, Ageratum conyzoides,* and *Musa acuminata* leaves on larvae of *A. aegypt* mosquito collected from Lucknow, India was studied. The leaves were air-dried, made into aqueous extract, and tested at varying concentration ranges from 20%-100%. Mortality data were taken for ten consecutive days using a different concentration level of the extracts. The study revealed that all the extracts tested had significant activity but the extract of *Ageratum conyzoides* had the highest percentage mortality (92%) at 100% concentration, then *Aloe barbadensis* (90%), *Azadirachta indica* (83%), *Musa acuminata* leaf (80%), and *Tinospora cordifolia* (72%). Two-way ANOVA indicated that there was a significant effect of concentration and extract types ($p<0.05$).

Keywords: *Aedes Aegypti,* biolarvicides, *Azadirachta indica*, *Aloe barbadensis, Ageratum conyzoides, Tinospora cordifolia* and *Musa acuminata* leaves

Introduction
According to a WHO report in 2024, "Mosquitoes are the vectors of disease-causing agents and result in millions of deaths all around the world each year" (World Health Organization, 2024) [2]. The mosquitoes belong to the phylum Arthropoda. The family *Culicidae* contains 3,726 species, is further divided into two subfamilies that contains 216 genera. Some mosquito species spread vector-borne diseases that affect human, livestock, and wildlife health worldwide (Mathiarasan *et al.,* 2025) [4]. Mosquito-borne diseases cause over 700,000 deaths globally each year. India has 400 species of mosquitoes that is 17% of the world's species, and their spread is caused by the diverse climate, ecological conditions and monsoon patterns (Mathiarasan *et al.,* 2025) [4]. The primary vector of dengue, chikungunya, Zika and yellow fever is female *Aedes* mosquito (Hillary *et al.,* 2024) [1]. Dengue fever is an infection caused by four viral serotypes. This disease is mainly spread in tropical and subtropical regions, putting nearly a third of the human population, globally, at risk of infection (Khetarpal & Khanna, 2016) [7]. Monitoring these populations closely, along with using methods to manage the vector populations, is essential in reducing the amount of risk involved in contracting these diseases (Bakar *et al.,* 2024) [10]. In addition to developing effective management strategies, understanding the biological and ecological aspects of vectors will assist in managing their populations effectively (Giatropoulos, 2025). Repeatedly applying chemical pesticides will have negative effects on the environment, contribute to pollution, and create pesticide-resistant mosquitos. Bio-larvicides offer an alternative (Dwicahya *et al*., 2023) [21]. Mosquito populations can be managed through breaking the life-cycle of disease transmission through controlling larvae at breeding locations; however, synthetic products such as temephos have developed some degree of resistance and present a number of environmental concerns (Yadav *et al.,* 2025) [3].

When it comes to pest control and botanical insecticides, the *Azadirachta indica* is widely used. It has been effective in controlling insects of medical and veterinary importance by management of mosquitoes and their larval stages (Ferreira & Alves, 2021) [5]. *Tinospora cordifolia* is also called Guduchi which is a climbing shrub. It belongs to the family Menispermaceae. It is majorly used in Ayurvedic medicines. According to recent studies, it effectively kills mosquito larvae. Leaf extracts of Giloy have larvicidal qualities (Arya & Kumar, 2023) [6]. *Aloe barbadensis* (Aloe vera) is one of the most important larvicides. It has natural compounds like water, vitamins, minerals, enzymes, polysaccharides, and glycoproteins. Secondary metabolites such as saponins, tannins, flavonoids, and alkaloids are also the compounds present in aloe vera. It also shows larvicidal activity. (Lubi *et.al*., 2018) [14]. *Ageratum conyzoides* (Asteraceae) is a fragrant, annual plant and widespread weed often referred to as billygoat weed or goat weed. The species has been extensively researched due to its biological characteristics and its possible use in healthcare and farming (Rioba *et. al.,* 2017) [8]. *Musa acuminata* is a herbaceous plant that belongs to the family Musaceae. According to the studies this is used to treat stomach ulcer, hypertension, diabetes, dysentery and diarrhea. Many other studies show that its flowers, stem leaves and roots have antidiabetic effects. It shows antimicrobial effects also (Meenambigai *et al*., 2021) [9]

Methodology
Collection of Plant Materials
The leaves of *Azadirachta indica, Tinospora cordifolia, Aloe barbadensis, Ageratum conyzoides*, and *Musa spp.* leaves were collected from the campus of Isabella Thoburn College, Lucknow between October to November. All plants were authenticated in the various areas of Isabella Thoburn College, Lucknow. The leaves from healthy plants that were free of dirt, dust, and other contaminants were gathered and delivered to the lab for subsequent processing.

After being cleaned with distilled water, the collected plant parts were allowed to dry for 20 days in a dark environment at 30°C.

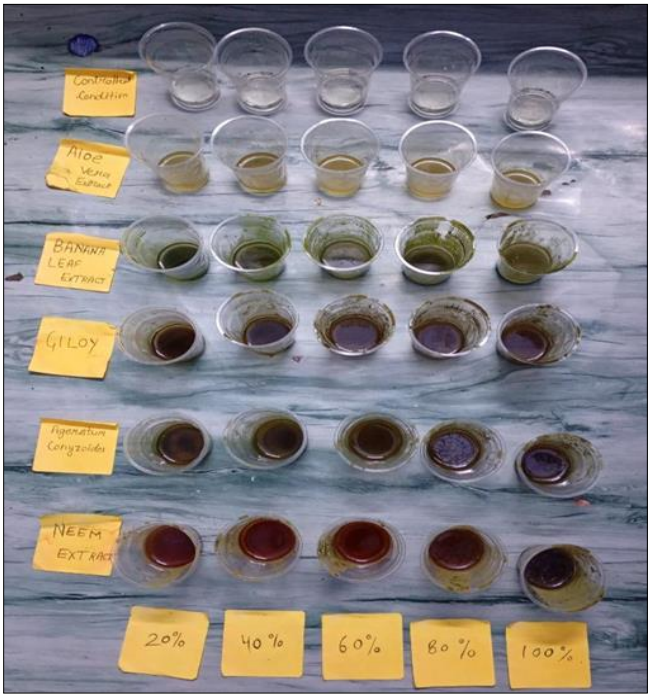


Fig 1: Image showing different plant extracts preparation of plant extracts

Tinospora cordifolia

Selected plant leaves and seeds were utilized to create aqueous extracts. In a beaker, 250 g of finely chopped plant material was processed into a thick paste. To dilute this mixture, distilled water was added to a beaker. After being stirred for 4 hours at 30°C, it was allowed to stand for 24 hours. The suspension was then thoroughly mixed and filtered using muslin cloth. Subsequently, a stock solution with a concentration of 10% was prepared. This stock solution was diluted with distilled water to achieve the desired concentration. Distilled water was used alone as a control. (Kumar & Arya, 2023) [6]

Agaratum conyzoides

In three Erlenmeyer flasks each containing ten (10 g) of Ageratum conyzoides leaf powder, 120 mL of distilled water. The aqueous mixtures obtained were stirred continuously for 15 min. They were then filtered and each filtrate is used to make different concentrations with distilled water. (Bamba & Ouattara *et. al.*, 2023) [12]

Aloe barbadensis

Leaves of Aloe barbadensis M. (Aloe vera) are washed thoroughly with clean water to remove dirt and debris. (Arianto *et al.* 2024) [13]. The leaves are then chopped or sliced into small pieces, often transversely, and sometimes dried in an oven or shade-dried before extraction. (Lubi *et al.*, 2018, Arianto *et al*., 2024) [13, 14] The material is soaked or percolated in distilled water (e.g., to make concentrations like 20- 100% extract), filtered through filter paper, and the resulting filtrate is used as the aqueous extract for bioassays. (Arianto *et. al.,* 2024) [13]

Azadirachta indica

Fifty grams of powdered dried leaves stem bark and fresh leaves paste were sucked in 200ml of distilled water respectively and kept for three days. The solutions were filtered using muslin cloth and concentrated under a water bath and then the concentrations were made. (Lawal *et al.,* 2021) [15]

***Musa acuminata* leaf extract**

For the preparation of the extracts, different strategies were carried out, including the use of different solvents (water and different hydroethanolic solutions) and different extraction methodologies, such as conventional, ultrasound-assisted, and pressurized-assisted extractions. For each extraction, 3% (w/v) of banana leaf powder was mixed with the respective extraction solvent i.e. water. (Gomes, 2024) [16]

Bioassays

The mosquito larvae were kept in a container. When all 5 concentrations of extracts are made then 100 mL of each are kept in the cups. Then the larvae were introduced in that cup carefully. Ten independent replicates were performed per concentration. One new cup (100 mL extract + 10 larvae) was prepared each day for 10 days. Each cup was observed for 24 hours, then discarded. Total larvae per concentration = 100. (10 Replicate × 10 larvae per replicate). We have taken 2nd to 3rd instar larvae. Death was recorded when no movement occurred after gentle prodding.

Mortality

Dead larvae were counted after 24 hours daily for 10 days. And the calculation of Mortality was done by following formula: -

$$\text{Mortality \%} = \frac{\text{Number of Dead Larvae}}{\text{Total Number of Larvae}} \times 100$$

The Statistical analysis was done by using two-way anova in microsoft excel ToolPak.

Results

Table 1: Showing effect of Different concentrations of extract on larva after 10 days

All Days						
Concentration	**Extracts**					
Concentration	***Aloe barbadensis***	***Azadirachta indica***	***Tinospora cordifolia***	***Ageratum conyzoides***	***Musa acuminata***	**Control**
20%	27	23	17	37	27	0
40%	45	38	26	65	36	0
60%	58	43	39	72	44	2
80%	70	56	58	83	63	3
100%	92	83	59	90	71	3
total population	292	243	199	347	241	8
mean	58.4	48.6	39.8	69.4	48.2	1.6
standard error	11.02	10.09	8.40	9.18	8.23	0.68

Table 2: Showing mortality of larva by Different extract on each day

Mortality in %						
Days	***Aloe barbadensis***	***Azadirachta indica***	***Tinospora cordifolia***	***Ageratum conyzoides***	***Musa acuminata***	**Control**
Day 1	16	16	14	82	40	0
Day 2	90	64	44	66	42	0
Day 3	28	20	30	78	48	0
Day 4	38	28	20	38	22	0
Day 5	50	38	26	48	30	2
Day 6	58	48	34	58	40	2
Day 7	66	56	44	68	50	2
Day 8	72	64	52	78	60	4
Day 9	80	72	62	86	70	2
Day 10	86	80	72	92	80	4
Mean	58.4	48.6	39.8	69.4	48.2	1.6
S. E	7.94	7.06	5.86	5.46	5.60	0.50

Table 3: Showing ANOVA Table

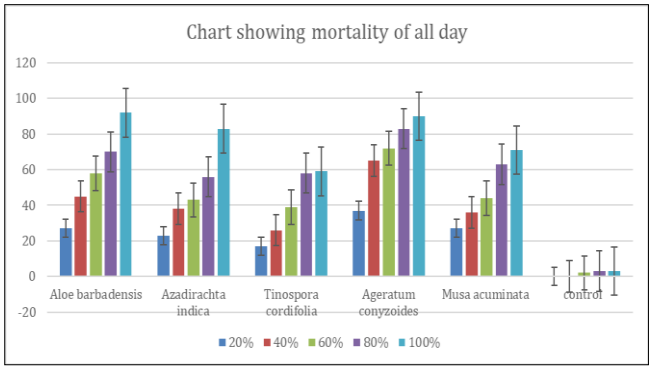
Source	df	Sum of Squares	Mean Square	F-statistics. (df1, df2)	P-value
Factor A - rows (A)	4	7223	1805.75	21.2017 (4,20)	5.82E-07
Factor B - columns (B)	5	13530.2667	2706.0533	31.7724 (5,20)	7.38E-09
Error	20	1703.4	85.17		
Total	29	22456.6667	774.3678		

In table one it is represented that the highest mortality rate was observed by *A. conyzoides* show its highest effect at 80% that have a Mortality of 83 slightly dipping at 92 at 100%. It is followed by Aloe barbadensis which highest effect observed is 92 at 100%. The neem comes next that shows its highest effect 83 followed by banana leaves extract with mortality of 71. The least effect is shown by *Tinospora cordifolia* which is 59 at highest concentration.

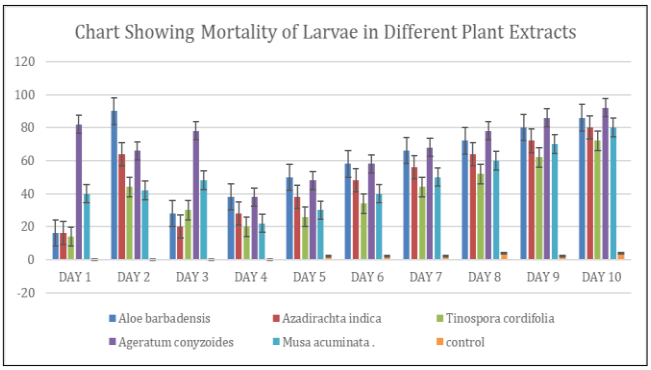
Table 2 shows that the Aloe barbadensis shows its highest mortality on day 2 which is 90 % and lowest on day 1 which is 16. The *A. indica* reaches its highest mortality on day 10 which is 80 % and lowest on day 1 which is 16. The effect of Tinospora cordifolia peaked on day 10 which is 72 % and lowest at day 1 i.e. 14. Followed by *A. conyzoides* that reaches its highest mortality on day 10 that is 92% and lowest on day 4 which is 38%. And *Musa* leaf extracts had the highest mortality on day 10 which was 80% and lowest on day 4, which was 22%.

Table 3 illustrates the two-way analysis of variance without interaction, looking at the impacts of two factors on a dependent variable, namely Factor A (rows) and Factor B (columns). For factor A, the value of F is very high, which is 21.2017, and there is a very low p-value of 5.82E-07, which shows that the impact of factor A-levels is statistically significant. For factor B, the p value is 7.38E-09, and the F value is also highly significant, which is 31.7724. Given that the p-values in both cases are very low than the significance level ($p < 0.05$). It is observed that there are statistically significant differences among means between both factors.

Graph 1 and 2 are showing effects of different concentration of plant extracts on *A. aegypti larvae.*



Graph 1: The chart showing the number of larval mortalities after 10 day



Graph 2: Chart showing mortality of larvae in different plant extracts

Discussion

Discussion Neem-based extracts have active ingredients like azadirachtin that kill 100% of larvae by controlling their growth and being toxic. These extracts also cause a lot of damage to the body, such as swelling of the digestive tract and darkening of the anal segments. (Kaur & Kocher, 2023) [17]. Research shows that Aloe barbadensis works well against larvae in the third instar stage. A study found that a high dosage of 10,000 parts per million of Aloe barbadensis is needed to kill 50% of the tested larvae, called the LC50 value. This means that 10,000 ppm of Aloe barbadensis can cause 50% of the larvae to die. (Wahyuni *et al*., 2019) [18]. Ageratum conyzoides flower extract exhibits significant, concentration-dependent toxicity against *Aedes Aegypti*

larvae, achieving a lethal time, i.e. LT50 at a 4% concentration that is statistically comparable to the bio-insecticide Spinosad. (Dawodu *et al*., 2025) [19]. According to studies the LC50 value for the aqueous extract of T. cordifolia was determined to be 380.18 ppm, while the LC 90 value was 707.94 ppm. A 100% larval mortality rate was achieved at a concentration of 1000 ppm within a 24-hour exposure period. Compared to other solvents used for T. cordifolia, the aqueous extract was the least potential (Kumar & Arya, 2023) [6]. The Musa acuminata Leaves aqueous extract had less effect on Anopheles. The LD50 value is 207.09 mg/L and the LC90 value of 1062.53 mg/L after 24 hours. After 72 hours of exposure, the highest mortality rate is 42% only (Chaudhary, *et al*., 2022) [20].

Conclusion

The mosquito are the vectors of many diseases and the findings of this study shows the aqueous extracts of plant are effective against *Aedes Aegypti* .

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