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Exploring plant extracts for sustainable larvicidal control of the dengue vector *Aedes aegypti*

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Abstract

Mosquitoes are notorious vectors for spreading deadly diseases like dengue and malaria. Chemical pesticides are commonly used for mosquito control but have led to resistance and environmental issues, prompting the exploration of botanical alternatives. This study aimed to evaluate the efficacy of plant extracts in their larvicidal potential, aiming to identify natural alternatives for mosquito control instead of synthetic agents. The *Aedes* larvae were collected from Faisalabad city and reared in controlled laboratory conditions. The plant materials *Cymbopogon citratus*, *Cinnamomum verum*, *Azadirachta indica*, *Ocimum basilicum* and *Allium cepa* were dried, ground into powder, and subjected to extract extraction using a Soxhlet apparatus and aqueous extraction methods. Standard stock solutions were prepared, and different concentrations (150 ppm, 250 ppm, and 500 ppm) were tested for their larvicidal. Mortality rates were calculated after 24, 48, and 72 hours. Data analysis involved calculating percentage mortality, LC₅₀, LC₉₀ and regression analysis. *A. cepa* was the most effective in ethanolic extracts, achieving 100% mortality at 500 ppm by 72 hours, followed by *O. basilicum* (95%) and *A. indica* (90%). *C. citratus* and *C. verum* showed moderate efficacy. In aqueous extracts, *A. cepa* demonstrated the highest mortality (85% at 500 ppm), followed by *O. basilicum* (75%). LC₅₀ and LC₉₀ values confirmed *A. cepa* superior potency, with an LC₅₀ of 24.664 ppm and LC₉₀ of 275.252 ppm in ethanolic extracts at 72 hours. For aqueous extracts, *A. cepa* had the lowest LC₅₀ (116.806 ppm) and LC₉₀ (565.726 ppm), with ethanolic extracts generally outperforming aqueous extracts. Results of this study shows that the ethanolic extract of *A. cepa* considered as a most potent source of a mosquito larvicidal agent. The findings indicate that the plant extracts hold promise as an environmentally friendly solution for controlling disease vectors. Also investigate the synergistic effects of combined plant extracts and optimize extraction methods to enhance their efficacy and potency against *Aedes* mosquitoes.

Keywords: *Aedes*, control, dengue, insecticides, resistance, Temephos

Introduction

Mosquitoes, belonging to the order Diptera and the family Culicidae, play a crucial role in transmitting several disease-causing microorganisms. These are also well recognized as a significant nuisance to millions of people, animals, and other creatures worldwide [1]. As a significant proportion of the human population in industrialized nations resides in urban areas, the presence of vector-borne illnesses in cities poses particular concern to epidemiologists and public health authorities. Mosquitoes provide significant risks to the environment as vector of several detrimental illnesses that impact both people and animals [2]. Multiple mosquito species act as vector for several parasitic illnesses such as malaria, dengue, yellow fever, zika, and chikungunya [3]. They contribute to illness and death, which eventually affect the economy in nations that are susceptible to disease [1].

Aedes aegypti serves as the primary vector of dengue, along with othillaryher arboviruses [4]. Dengue is a viral illness caused by the Flavivirus, which is largely spread by mosquitoes of the species *Ae. aegypti*, followed by *Ae. albopictus* and other species of the *Aedes* genus [5]. Dengue infection is a significant public health issue that has been documented in the Americas, Africa, Southeast Asia, Europe, Western Pacific, and Eastern Mediterranean areas. This arboviral illness is widespread in over 100 countries, with about 96 million infected persons experiencing symptoms of variable severity [6].

Since the commencement of 2023, there has been continuous spread of dengue, along with an unforeseen increase in cases, leading to a nearly record-breaking total of over five million illnesses and over 5000 fatalities caused by dengue. These cases and deaths have been documented in more than 80 countries/territories and throughout five regions as defined by the World Health Organization [7]. Pakistan is an endemic region for dengue fever, with transmission occurring throughout the year and seasonal increases in cases. Nevertheless, induced by the unprecedented floods that started in mid-June, the incidence of reported dengue cases in 2022 (from January to September) is much greater compared to the same time in the preceding four years [7].

Current methods for managing mosquitoes mostly depend on the use of chemical pesticides, including N, N-diethyl-metotoluamide (DEET), N, N-diethyl mandelic acid amide (DEM), and dimethyl phthalate (DMP). Nevertheless, the extensive use of chemical pesticides has led to resistance in vectors, pollution, bioaccumulation, and several health and environmental concerns [2]. Mosquitoes are controlled by synthetic pesticides, which have substantial environmental consequences [8].

Botanicals have historically been advocated as desirable alternatives to synthetic agrochemicals due to their alleged reduced environmental and human health impact in comparison to the latter [9]. Bioactive compounds, which have been utilized extensively in complementary and alternative medicine for decades, are prevalent in plants [10]. Plants such as Lemongrass (*C. citratus*), Neem (*A. indica*), Sweet Basil (*O. basilicum*), Cinnamon (*C. verum*) and Onion (*A. cepa*) exhibit a wide range of bioactive properties. *C. citratus* is prized for its citral-rich essential oil, with potent mosquito-repellent, antifungal, and insecticidal activities [11]. *A. indica* bioactive compounds, like azadirachtin, disrupt insect life cycles and possess antimicrobial and anti-inflammatory properties [12]. *O. basilicum* essential oils act as repellents and larvicides [13], while *C. verum*, known for its cinnamaldehyde, offers antimicrobial and pesticidal effects [14]. *A. cepa*, rich in quercetin and sulfur compounds, provide antioxidant, antimicrobial, and anti-diabetic benefits [15].

Previous research has established the critical role of larvicides in mitigating mosquito populations at breeding sites. However, the emergence of resistance in mosquito vectors to chemical insecticide, larvicides remains an unresolved challenge. In light of the increasing interest in botanical insecticides as eco-friendly and sustainable alternatives to synthetic chemicals, the present study seeks to address this gap by assessing the larvicidal efficacy of medicinal plant extracts. The investigation specifically targets medically significant mosquito species: *Aedes aegypti*, which is known vector of various human diseases. This study aimed to provide novel insights into the potential of plant-based larvicides as part of integrated vector management strategies, with a focus on overcoming resistance issues and reducing the environmental impact of chemical insecticides.

Materials and Methods

This study was carried out during 2023-2024 at Entomological Laboratory of Government College University Faisalabad. The purpose of our study was to evaluate the larvicidal efficacy of plant extract against *Aedes* larvae.






Rearing of mosquitos

Larvae and adult mosquitoes were collected from Faisalabad city. Standard dippers were used for collection and larvae of *Aedes*, larvae were stored in plastic bottles covered with muslin cloth. These were then taken to the Zoology Department at Government College University, Faisalabad, for sorting and rearing. The larvae were separated, larvae placed in rearing trays after identification. The laboratory conditions were maintained at $26\pm 1^\circ\text{C}$ and $75\pm 5\%$ RH. The larvae were fed fish diet [16]. For the study, 3rd instar larvae were used

Collection of plant materials

For the extract extraction, various selected plant materials (*C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa*) as shown in (Table 1). These plant materials were collected from Government College University, Faisalabad ($31^\circ 30' \text{N}$, $73^\circ 05' \text{E}$), while *C. verum* bark and *A. cepa* were purchased from the market and identified from a botanist.

Table 1: Plants and their parts used for extraction

Common names	Scientific names	Diagram	Parts
Lemongrass	<i>Cymbopogon citratus</i>		Branches and leaves
Cinnamon	<i>Cinnamomum verum</i>		Bark
Neem	<i>Azadirachta indica</i>		Branches and leaves
Niazbow	<i>Ocimum basilicum</i>		Branches and leaves
Onion	<i>Allium cepa</i>		Rhizome

Drying and grinding

The plant materials were washed with tap water to remove dust and dried in the shade at room temperature. After drying, they were ground into a powder using an electric grinder (RAF.7113) and stored in plastic bottles for oil extraction.

Extract preparation

Extract were extracted from the selected plant materials using a Soxhlet apparatus (J.P. Selecta, s.a., Cod: 6003286). Twenty-five grams of each powdered plant material and 250 ml of ethanol were used for the extraction, which took 8 to 24 hours. After extraction, a vacuum evaporator (Buchi Rotavapor, R-200) was used to remove the solvent, leaving the dehydrated filtrate. The extract was then stored in airtight jars [17].

Preparation of solution

Standard stock solutions were made by dissolving the residues in water at 1%. Different concentrations for larvicidal and repellent bioassays were prepared from this stock solution [18]. Concentrated solutions of 150 ppm, 250 ppm, and 500 ppm were formulated from the extract.

Larvicidal activity

Larvicidal bioassays were conducted in the laboratory following the WHO method for mosquitoes with transparency adjustments [18]. Five different concentrations of extract, including a control, were used, with each concentration replicated three times. Each glass beaker contained twenty-five 3rd instar larvae exposed to various oil solution concentrations: 150 ppm, 250 ppm, and 500 ppm [10]. Positive controls were treated with Temephos 50% EC (M/S TDC industries, ISO: 9001: 2015), and negative controls with distilled water. The experiments were conducted under controlled laboratory conditions at 27±2 °C and 65±5% relative humidity. As described by Khatoro *et al.* (2021) the percentage mortality was calculated from the average of three replicates using a formula [19]. Dead larvae were promptly removed from the beakers to prevent further mortality.

Percentage mortality= (Number of dead larvae/ Number of larvae tested) ×100

After the exposure of plant oils of 24, 48 and 72 hours mortality were verified.

Data analysis

The percentage mortality data were determined. Probit analysis was employed to calculate average larval death data, including LC₅₀ and LC₉₀ with 95% confidence intervals, Regression analysis and Chi-square tests using software. Statistical significance was set at $p < 0.05$ [20].

Results & Discussion

The present study was carried out to check the potential of five different botanical extracts to control *Aedes* mosquitoes under the laboratory conditions. For this, five different medicinal plants *C. citratus*, *C. verum*, *A. indica*, *O. basilicum*

and *A. cepa* were selected and were used with different concentrations 150 ppm, 250 ppm and 500 ppm to check the effect on larvae of *Aedes* mosquitoes. A control treatment was also run by using water and temephos.

Mortality (%) of *Aedes* larvae with ethanolic and aqueous plant extracts

The percentage mortality of *Aedes* third instar larvae treated with various ethanolic plant extracts (*C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa*) at concentrations of 150 ppm, 250 ppm, and 500 ppm was evaluated over 24, 48, and 72 hours as shown in (Fig. 1). At 150 ppm, *A. cepa* was the most effective, causing 60% mortality at 24 hours, rising to 70% by 72 hours. *O. basilicum* followed with 55% mortality at 24 hours and 65% at 72 hours. *A. indica* had moderate efficacy, reaching 60% by 72 hours, while *C. citratus* and *C. verum* were less effective, with 30-45% and 25-40% mortality, respectively. At 250 ppm, *A. cepa* achieved 75% mortality at 24 hours and 85% by 72 hours. *O. basilicum* reached 80% by 72 hours, and *A. idica* improved to 75%. At 500 ppm, *A. cepa* reached 90% mortality at 24 hours and 100% by 72 hours, making it the most effective overall. *O. basilicum* also performed well, achieving 95% at 72 hours. *A. indica* reached 90%, while *C. citratus* and *C. verum* showed improvements, with 80% and 75% mortality, respectively. The positive control caused 70-95% mortality, while the negative control showed minimal effects (5-10%), highlighting the effectiveness of the ethanolic extracts. Overall, *A. cepa* and *O. basilicum* were the most potent, with *C. citratus* and *C. verum* being the least effective, though all extracts displayed increased activity with higher concentrations and longer exposure times.

On the other hand, the effectiveness of various aqueous extracts on a biological assay, likely related to larvicidal activities against *Aedes* larvae (Fig. 2). At a concentration of 150 ppm, all extracts showed an increase in mortality rates over 72 hours, with *A. cepa* demonstrating the highest effectiveness at 65% mortality, followed by *O. basilicum* at 40%. As the concentration increased to 250 ppm, the mortality rates rose significantly across all extracts, with *A. cepa* reaching 70% and *O. basilicum* 55% at 72 hours. At the highest concentration of 500 ppm, *A. cepa* and *O. basilicum* proved to be the most effective, with mortality rates of 85% and 75%, respectively, after 72 hours. The positive control displayed a mortality rate of 95%, validating the assay's efficacy, while the negative control showed minimal effects. Overall, the results suggest that these aqueous extracts, particularly *A. cepa* and *O. basilicum*, exhibit significant biological activity against the target organisms, with effectiveness increasing in both concentration and exposure time.

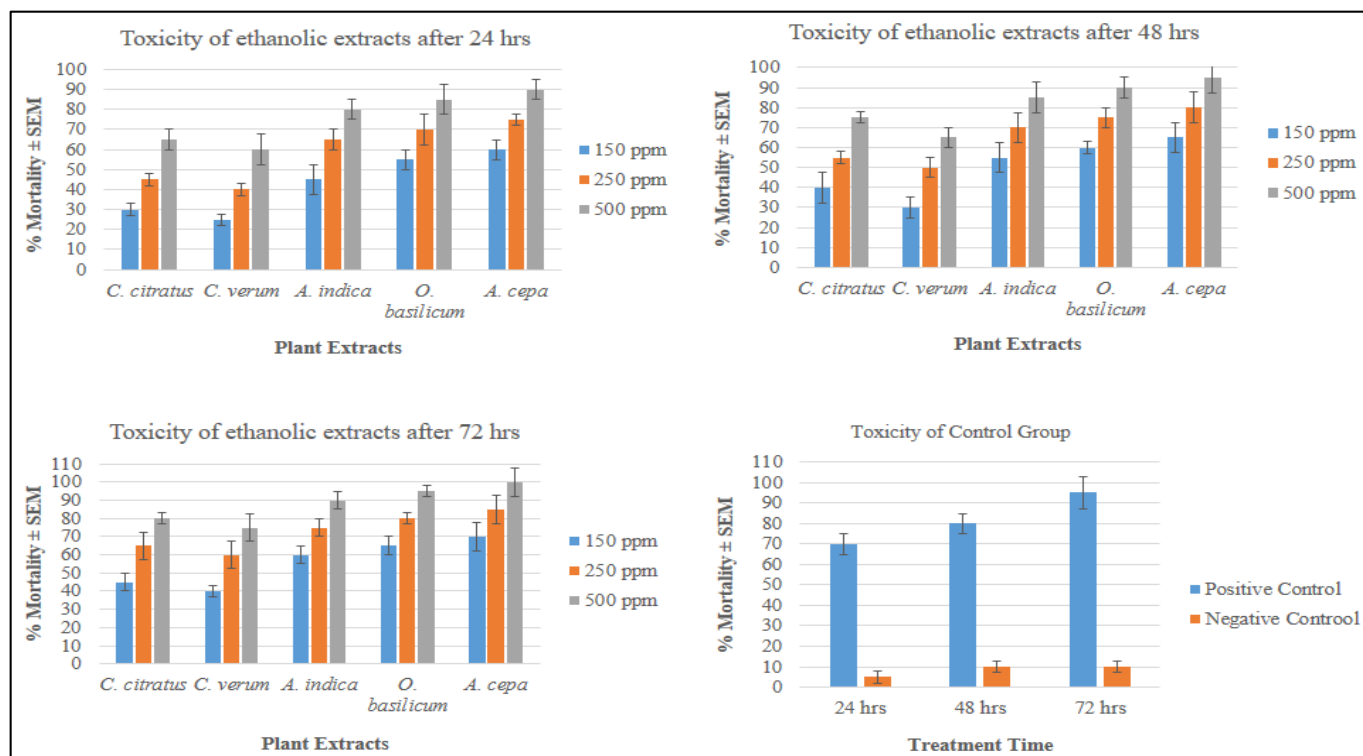


Fig 1: Percentage mortality ± SEM of *Aedes* larvae treated with ethanolic plant extracts and control group

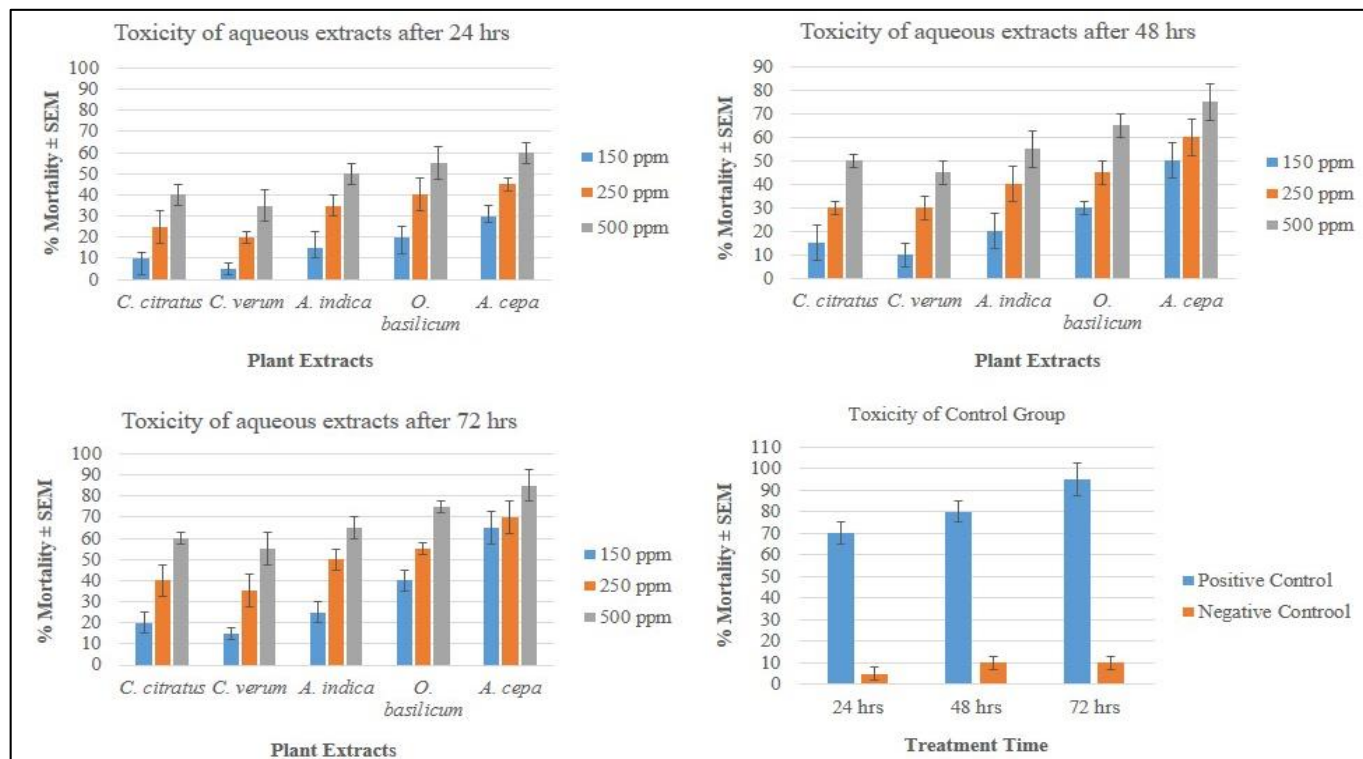


Fig 2: Percentage mortality ± SEM of *Aedes* larvae treated with aqueous plant extracts and control group

Toxicity of ethanolic and aqueous plant extracts against *Aedes* larvae

It was also observed that early life stages were more killed rather than late life stages i. e., 3rd instar (Table 2). At 24 hours, *A. cepa* extract was the most effective, with the lowest LC₅₀ (45.152 ppm) and LC₉₀ (487.169 ppm), followed by *A. indica* (LC₅₀: 159.326 ppm, LC₉₀: 647.33 ppm). *C. verum* was the least effective, with the highest LC₅₀ (388.399 ppm) and LC₉₀ (899.018 ppm). By 48 hours, *A. cepa* remained the most

effective (LC₅₀: 33.401 ppm, LC₉₀: 388.567 ppm), with *O. basilicum* and *C. citratus* showing improved efficacy. *C. verum* still required higher concentrations but showed better performance than at 24 hours. At 72 hours, *A. cepa* continued to demonstrate the highest efficacy (LC₅₀: 24.664 ppm, LC₉₀: 275.252 ppm), with *O. basilicum* and *A. indica* following closely. *C. verum*, while improved, remained the least effective overall. Overall, *A. cepa* consistently showed the greatest larvicidal effect, requiring the lowest concentrations.

Table 2: Lethal concentration (in ppm) of ethanolic plant extracts against *Aedes* larvae

Plant	Time (Hour)	LC ₅₀ (ppm)	95%Fiducial CI		LC ₉₀ (ppm)	95%Fiducial CI		Slope	Intercept	SE	R ²	Chi-test (χ ²) Sig
			Lower Limits	Upper Limits		Lower Limits	Upper Limits					
<i>C. citratus</i>	24	334.656	140.318	808.73	850.615	573.23	5629.471	0.019	3.56	0.003	0.975	0.7
<i>C. verum</i>		388.399	248.018	1142.899	899.018	603.918	5607.744	0.019	2.564	0.003	0.976	0.67
<i>A. indica</i>		159.326	100.22	286.159	647.33	450.237	3917.374	0.018	7.128	0.006	0.898	0.519
<i>O. basilicum</i>		77.749	44.77	222.368	583.063	400.816	12732.061	0.016	9.154	0.004	0.942	0.709
<i>A. cepa</i>		45.152	55.34	187.445	487.169	343.665	3283.632	0.016	10.154	0.004	0.942	0.739
<i>C. citratus</i>		228.568	134.44	377.981	726.706	500.442	4198	0.019	5.564	0.003	0.975	0.75
<i>C. verum</i>		317.664	38.282	847.93	855.977	569.33	8775.859	0.018	4.128	0.006	0.898	0.462
<i>A. indica</i>	48	77.749	44.77	222.368	583.063	400.816	12732.061	0.016	9.154	0.004	0.942	0.709
<i>O. basilicum</i>		45.152	55.34	187.445	487.169	343.665	3283.632	0.016	10.154	0.004	0.942	0.739
<i>A. cepa</i>		33.401	25.98	161.862	388.567	278.772	1406.015	0.016	11.154	0.004	0.942	0.811
<i>C. citratus</i>		159.326	55.87	286.159	647.33	450.237	3917.374	0.018	7.128	0.006	0.898	0.519
<i>C. verum</i>		208.587	77.81	352.835	723.77	493.797	5733.076	0.018	6.128	0.005	0.898	0.502
<i>A. indica</i>	72	45.152	33.21	187.445	487.169	343.665	3283.632	0.016	10.154	0.004	0.942	0.739
<i>O. basilicum</i>		33.401	25.98	161.862	388.567	278.772	1406.015	0.016	11.154	0.004	0.942	0.811
<i>A. cepa</i>		24.664	20.32	155.271	275.252	206.804	3691.638	0.016	12.154	0.04	0.942	0.7

On the other hand the LC₅₀ and LC₉₀ values reflected the lethal concentrations of plant extracts (*C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa*) needed to achieve 50% and 90% mortality of *Aedes* larvae through aqueous extract, respectively, at various time intervals (Table 3). At 24 hours, *A. cepa* showed the lowest LC₅₀ (317.664 ppm) and LC₉₀ (855.977 ppm), making it the most effective extract at this early stage. *A. indica* and *O. basilicum* followed with LC₅₀ values of 479.021 ppm and 428.018 ppm, respectively. *C. citratus* and *C. verum* were less effective, requiring higher concentrations to reach similar mortality levels, with *C. verum* having the highest LC₅₀ (615.241 ppm). After 48 hours, *A. cepa* continued to demonstrate the highest efficacy with a reduced LC₅₀ (208.587 ppm) and LC₉₀ (723.770 ppm). *O.*

basilicum followed closely, with a significantly improved LC₅₀ (334.656 ppm). *C. citratus* and *C. verum* showed some reduction in LC₅₀ but still required higher concentrations compared to *A. cepa* and *O. basilicum*. By 72 hours, *A. cepa* was the most effective, with the lowest LC₅₀ (116.806 ppm) and LC₉₀ (565.726 ppm), followed by *O. basilicum* (LC₅₀: 228.568 ppm). *A. indica* and *C. verum* required higher concentrations, with *C. verum* having the highest LC₅₀ (441.211 ppm) at 72 hours. Overall, *A. cepa* consistently showed the greatest larvicidal effect, with the lowest LC₅₀ and LC₉₀ values across all time points, while *C. verum* was the least potent. Overall, ethanolic extracts consistently outperformed than aqueous extracts.

Table 3: Lethal concentration (in ppm) of aqueous plant extracts against *Aedes* larvae

Plant	Time (Hour)	LC ₅₀ (ppm)	95%Fiducial CI		LC ₉₀ (ppm)	95%Fiducial CI		Slope	Intercept	SE	R ²	Chi-test (χ ²) Sig
			Lower Limits	Upper Limits		Lower Limits	Upper Limits					
<i>C. citratus</i>	24	583.079	419.181	4320.894	1086.138	705.720	12071.434	0.016	0.154	0.004	0.942	0.450
<i>C. verum</i>		615.241	453.108	2490.268	1054.629	709.105	5858.000	0.016	0.846	0.004	0.942	0.342
<i>A. indica</i>		479.021	345.200	1804.688	977.867	652.393	6151.981	0.018	1.128	0.006	0.898	0.356
<i>O. basilicum</i>		428.018	292.494	1730.514	950.146	629.304	7598.326	0.018	2.128	0.006	0.898	0.401
<i>A. cepa</i>		317.664	38.282	847.930	855.977	569.330	8775.859	0.018	4.128	0.006	0.900	0.462
<i>C. citratus</i>	48	488.349	358.621	1447.893	958.851	651.750	4402.800	0.019	0.564	0.003	0.975	0.580
<i>C. verum</i>		524.319	387.280	1702.133	988.663	669.524	4771.160	0.018	0.128	0.006	0.898	0.294
<i>A. indica</i>		428.018	292.494	1730.514	950.146	629.304	7598.326	0.018	2.128	0.006	0.898	0.401
<i>O. basilicum</i>		334.656	140.318	808.730	850.615	573.230	5629.471	0.019	3.564	0.003	0.975	0.700
<i>A. cepa</i>		208.587	139.760	352.835	723.770	493.797	5733.076	0.018	6.128	0.006	0.898	0.502
<i>C. citratus</i>	72	395.270	276.374	799.431	843.869	592.039	2861.747	0.022	1.538	0.005	0.942	0.459
<i>C. verum</i>		441.211	326.913	921.064	873.194	615.739	2759.047	0.022	0.538	0.005	0.942	0.407
<i>A. indica</i>		332.343	169.023	634.282	806.968	560.169	3298.925	0.021	3.103	0.008	0.858	0.307
<i>O. basilicum</i>		228.568	-373.715	377.981	726.706	500.442	4198.000	0.019	5.564	0.003	0.975	0.750
<i>A. cepa</i>		116.806	56.880	240.116	565.726	402.229	2527.572	0.018	8.128	0.006	0.898	0.538

Discussion

The continuous use of various insecticides has increased mosquitoes' resistance and caused environmental hazards, necessitating the development of a more potent and environmentally safe pesticide. This resistance reduces the effectiveness of insecticides and contributes to mosquito population growth. *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* have been used as growth regulators against many insect pests. Crude extracts of these plants are

eco-friendly and non-toxic to vertebrates. These plant extracts are less expensive and highly effective for controlling mosquitoes, which transmit many serious vector-borne diseases, compared to purified plant compounds or extracts [21]. The experimental results from this study show that various phytochemical classes in plants may work together or independently to produce larvicidal activity. Phytochemicals are a good alternative to synthetic insecticides because they are safe, inexpensive, and widely available.

Many of these plants are traditionally used to treat dengue fever, liver problems, and other symptoms caused by dengue infections. Several studies have explored using natural products to control mosquitoes as larvicides and insecticides, with varying results [22, 23].

Our results showed that the five plants were effective in killing mosquito larvae, though their effectiveness varied. This variation aligns with previous findings that different mosquito species respond differently to various plant extracts [24, 25]. Our study evaluated the effectiveness of five plant extract *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* against *Aedes* third instar larvae. *A. cepa* consistently demonstrated the highest efficacy, achieving up to 100% mortality in ethanolic extracts at 500 ppm and 85% in aqueous extracts at the same concentration. *O. basilicum* followed closely, showing 95% mortality in ethanolic and 75% in aqueous extracts. *A. indica* exhibited moderate effectiveness, with mortality rates improving over time, while lemongrass and *C. verum* were the least effective, requiring higher concentrations to achieve significant mortality. Overall, both ethanolic and aqueous extracts of *A. cepa* and *O. basilicum* were the most potent, indicating their potential for larvicidal applications. These findings are consistent with Rahman *et al.* (2019), who reported high larval mortality with Neem extracts [26]. Babu and Ashok (2021) also reported 50-90% mortality with *A. cepa* extracts at 100-500 ppm after 72 hours, consistent with our findings [27]. Additionally, Govindarajan *et al.* (2019) reported that essential oil of *O. basilicum* had remarkable larvicidal properties against *Aedes*, observing 90% mortality after 72 hours [28], which aligns with our study. Chaiphongpachara *et al.* (2020) found that *C. verum* and *C. citratus* had strong larvicidal activity against *Aedes*, with mortality increasing with higher concentrations [29], supporting our results.

Thomas *et al.* (2017) noted that wild-collected larvae had lower mortality rates compared to laboratory-reared larvae, likely due to previous exposure to insecticides and environmental contaminants, making them more tolerant [30]. Our study found that *A. cepa* extract was the most effective against *Aedes* larvae, consistently showing the lowest LC₅₀ and LC₉₀ values across all time points for both ethanolic and aqueous extracts. *O. basilicum* also demonstrated significant efficacy, particularly with lower LC₅₀ values over time. *A. indica* showed moderate effectiveness, while *C. verum* was the least potent, requiring the highest concentrations to achieve similar mortality levels. Overall, ethanolic extracts outperformed aqueous extracts in larvicidal activity. These findings are consistent with Muhammad *et al.* (2022), who reported an LC₅₀ of 0.284 ppm and an LC₉₀ of 17 ppm against 3rd instar larvae of *An. stephensi* after 72 hours [31]. Additionally, our study agrees with Panneerselvam *et al.* (2018), who found that botanical extracts exhibited larvicidal and pupicidal effects after 72 hours, with the highest mortality in the ethanolic extract of *Catharanthus roseus* against *Aedes* larvae, with LC₅₀ values of 3.34, 4.48, 5.90, and 8.17 ppm, and LC₉₀ values of 19.66, 24.55, 47.33, and 56.77 ppm, respectively [32]. Our findings are also consistent with Hidayatulfathi *et al.* (2018), who concluded that the LC₅₀ and LC₉₀ values decreased with increasing concentrations of plant extracts [33].

Application of plant extracts or pure phytochemicals -active principles can be administered in water holding tanks where mosquitoes breed. Nevertheless, the effective compounds

need to be isolated, its mechanism of action must be elucidated and it has to pass field tests before it can be advocated as mosquito control agents. Of all the potential candidates, plants may provide the most potential for larvicides since they contain bioactive compounds that pose little threat to humans and other animals. The use of these botanical derivatives in place of synthetic insecticides possibly led to reduced cost and minimal pollution. Further studies should be conducted to identify the active compounds and their impact on various life phases of the mosquito particularly on *A. aegypti*. Extracts of *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* phytochemicals can be utilized for biocide or insecticide formulation.

The use of extracts of *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* must extend beyond current limitations to improve its efficacy, and the effectiveness of the current formulations and delivery methods should be reassessed. Some real life uses includes natural larvicides, community level control measures, public health, and low chemical residues. The findings from this study are important to public health because they present an organic, environmentally friendly approach to mosquitoes larvae reduction. For sustainable vector control, this leads to supporting the community strategies with low chemical input and strengthens other comprehensive health interventions. The applicability of these extracts will therefore vary with the ease of extraction, storage and application of these extracts at different breeding sites of mosquitoes.

Further research is needed to determine how these extracts work. Understanding how *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* extracts affect mosquito larvae might help determine their larvicidal efficacy. This knowledge can also improve larvicidal product targeting and efficacy.

Conclusion

In conclusion, this study evaluates the potential role of medicinal plant extracts in controlling mosquitoes. Natural products are generally preferred in vector control due to their lower impact on non-target organisms and their biodegradability. As mosquito larvae have developed resistance against chemical insecticides, it is crucial to identify new active compounds from natural sources. The findings of this study suggest the need for further research into the larvicidal properties of medicinal plant extracts against mosquitoes. Extracts from *C. citratus*, *C. verum*, *A. indica*, *O. basilicum* and *A. cepa* showed promising ability in managing *Ae. aegypti* larvae. Onion, in particular, demonstrates strong larvicidal activity. Study the synergistic effects of combining different plant extracts to enhance their overall efficacy against *Aedes* mosquitoes and investigate and standardize different extraction methods to maximize the yield and potency of active compounds from plant materials.

Data availability

Data and materials have been provided with the manuscript as tables and figures. The datasets are available from the corresponding author on reasonable request.

Abbreviations

LC₅₀
Lethal concentrations 50
LC₉₀
Lethal concentrations 90

PPM
Parts per millions
WHO
World Health Organization

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References

- Aziz AT, Panneerselvam C, Edward-Sam E. Toxicity of plants as insecticides against human pathogenic mosquito vectors of Saudi Arabian strains-A review. *Entomol Res.* 2023;53(9):323-332. DOI:10.1111/1748-5967.12669.
- Hillary VE, Ceasar SA, Ignacimuthu S. Efficacy of plant products in controlling disease vector mosquitoes, a review. *Entomol Exp Appl.* 2024;34(3):2341-2354. DOI:10.1111/eea.13401.
- Muhammad I, Muhammad MA, Asher R, Shuaibu AB. *In vitro* assessment of the larvicidal activity of *Bacillus thuringiensis israelensis* (Vectobac 12AS formulation) on *Anopheles* mosquito larvae. *Cell Mol Biomed Rep.* 2024;4(1):9-16. DOI:10.55705/cnbr.2023.391224.1115.
- Romano CA, de Oliveira Neto JR, da Cunha LC, dos Santos AH, de Paula JR. Essential oil-based nanoemulsion of *Murraya koenigii* is an efficient larvicidal against *Aedes aegypti* under field conditions. *Ind Crops Prod.* 2024;208(5):11783-11791. DOI:10.1016/j.indcrop.2023.117836.
- Kumar P, Shakya R, Kumar V, Kumar D, Chauhan RPS, Singh H. Chemical constituents and strong larvicidal activity of *Solanum xanthocarpum* among selected plant extracts against the malaria, filaria, and dengue vectors. *J Vector Borne Dis.* 2023;60(1):18-31. DOI:10.4103/0972-9062.361177.
- Bhatt P, Sabeena SP, Varma M, Arunkumar G. Current understanding of the pathogenesis of dengue virus infection. *curr microbiol.* 2021;78(3):17-32. DOI:10.1007/s00284-020-02284-w.
- World Health Organization. Dengue and severe dengue. [Internet]. 2024 Apr 23. Available from: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>.
- Kaur R, Mavi GK, Raghav S, Khan I. Pesticides classification and its impact on environment. *Int J Curr Microbiol Appl Sci.* 2019;8(3):1889-1897. DOI: 10.20546/ijcmas.2019.803.224.
- Divekar P. Botanical pesticides: An eco-friendly approach for management of insect pests. *Acta Sci Agric.* 2023;7(2):1233-1244.
- Baz MM, Hegazy MM, Khater HF, El-Sayed YA. Comparative evaluation of five oil-resin plant extracts against the mosquito larvae, *Culex pipiens* Say (Diptera: Culicidae). *Pak Vet J.* 2021;41(2):2222-2234. DOI: 10.29261/pakvetj/2021.010.
- Mukarram M, Choudhary S, Khan MA, Poltronieri P, Khan MMA, Ali J, *et al.* Lemongrass essential oil components with antimicrobial and anticancer activities. *Antioxidants.* 2021;11(1):20-28. DOI: 10.3390/antiox11010020.
- Reddy IV, Neelima P. Neem (*Azadirachta indica*): A review on medicinal Kalpavriksha. *Int J Econ Plants.* 2022;9(1):59-63. DOI:10.23910/2/2021.0437d.
- Al-Snafi AE. Chemical constituents and pharmacological effects of *Ocimum basilicum*-A review. *Int J Pharm Res.* 2021;13(2):2997-3013. DOI:10.31838/ijpr/2021.13.02.388.
- Kowalska J, Tyburski J, Matysiak K, Jakubowska M, Łukaszuk J, Krzysińska J. Cinnamon as a useful preventive substance for the care of human and plant health. *Molecules.* 2021;26(17):5299-5306. DOI:10.3390/molecules26175299.
- Zhao XX, Lin FJ, Li H, Li HB, Wu DT, Geng F, *et al.* Recent advances in bioactive compounds, health functions, and safety concerns of onion (*Allium cepa* L.). *Front Nutr.* 2021;8(5):669-675. DOI: 10.3389/fnut.2021.669805.
- Shoukat RF, Shakeel M, Rizvi SAH, Zafar J, Zhang Y, Freed S, *et al.* Larvicidal, ovicidal, synergistic, and repellent activities of *Sophora alopecuroides* and its dominant constituents against *Aedes albopictus*. *Insects.* 2020;11(4):246-254. DOI:10.3390/insects11040246.
- Azeem M, Zaman T, Tahir M, Haris A, Iqbal Z, Binyameen M, *et al.* Chemical composition and repellent activity of native plants essential oils against dengue mosquito, *Aedes aegypti*. *Ind Crops Prod.*

- 2019;140(4):11160-11174.
DOI: 10.1016/j.indcrop.2019.111609.
18. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization; 2005 May 24. Available from: <https://iris.who.int/handle/10665/69101>.
 19. Khatoro RT, Yugi JO, Sudoi V. Ovicidal, larvicidal and pupicidal efficacy of crude methanol and hexane extract of *Urtica massaiica* Mildbri on *Anopheles gambiae* Giles. *J Biol Sci Jordan*. 2021;14(3):434.
DOI: 10.54319/jjbs/140308.
 20. Ochola JB, Muteru CM, Marubu RM, Haller BF, Hassanali A, Lwande W. Mosquitoes larvicidal activity of *Ocimum kilimandscharicum* oil formulation under laboratory and field-simulated conditions. *Insects*. 2022;13(2):203-213. doi: 10.3390/insects13020203.
 21. Nour AH, Sandanasamy JD, Nour AH. Larvicidal activity of extracts from different parts of Neem (*Azadirachta indica*) against *Aedes aegypti* mosquitoes' larvae. *Sci Res Essays*. 2022;7(31):2810-2815.
DOI: 10.5897/SRE12.133.
 22. Berhe M, Dugassa S, Shimelis S, Tekie H. Repellence and larvicidal effects of some selected plant extracts against adult *Anopheles arabiensis* and *Aedes aegypti* larvae under laboratory conditions. *Int J Trop Insect Sci*. 2021;5(2):1-8. DOI:10.1007/s42690-021-00446-2.
 23. Husna I, Setyaningrum E, Handayani TT, Kurnia Y, Palupi EK, Umam R, *et al.* Utilization of basil leaf extract as anti-mosquito repellent: A case study of total mosquito mortality (*Aedes aegypti* 3rd instar). *J Phys*. 2020;23(3):1456-1464.
DOI:10.1088/1742-6596/1467/1/012014.
 24. Hadidy DE, El Sayed AM, Tantawy ME, Alfay TE, Farag SM, Haleem DRA. Larvicidal and repellent potential of *Ageratum houstonianum* against *Culex pipiens*. *Sci Rep*. 2022;12(1):2141-2151.
DOI:10.1038/s41598-022-25939-z.
 25. Chellappandian M, Senthil-Nathan S, Karthi S, Vasanthasrinivasan P, Kalaivani K, Hunter WB, *et al.* Larvicidal and repellent activity of N-methyl-1-adamantylamine and oleic acid, a major derivative of bael tree ethanol leaf extracts, against dengue mosquito vector and their biosafety on natural predator. *Environ Sci Pollut Res*. 2021;2(1):1-10. DOI:10.1007/s11356-021-16219-w.
 26. Rahman K, Khan SU, Fahad S, Chang MX, Abbas A, Khan WU, *et al.* Nano-biotechnology: a new approach to treat and prevent malaria. *Int J Nanomedicine*. 2019;4(5):1401-1410. Available from: <https://www.tandfonline.com/doi/epdf/10.2147/IJN.S190692?needAccess=true>.
 27. Babu M, Ashok K. Larvicidal activity of onion (*Allium cepa*) peel extracts against *Anopheles stephensi*. *Int J Zoologic Investig*. 2021;7(2):230-237.
DOI: 10.33745/ijzi.2021.v07i02.042.
 28. Govindarajan M, Sivakumar R, Rajeswary M, Yagalakshmi K. Chemical composition and larvicidal activity of essential oil from *Ocimum basilicum* (L.) against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* (Diptera: Culicidae). *Exp Parasitol*. 2019;134(1):7-11. DOI:10.1016/j.exppara.2013.01.018.
 29. Chaiphongpachara T, Laojun S, Wassanasompong W. Screening seven commercial essential herb oils for larvicidal activity against the mosquito *Aedes aegypti* (Linnaeus), a vector of the dengue virus. *J Appl Pharm Sci*. 2020;10(7):43-50. DOI:10.7324/JAPS.2020.10706.
 30. Thomas A, Mazigo HD, Manjurano A, Morona D, Kweka EJ. Evaluation of active ingredients and larvicidal activity of clove and cinnamon essential oils against *Anopheles gambiae* (sensu lato). *Parasites Vectors*. 2017;10(1):1-7. DOI:10.1186/s13071-017-2355-6.
 31. Muhammad NA. Phytochemical screening and larvicidal activity of extracts of *Ocimum basilicum* (Lamiaceae) leaves against *Aedes aegypti*. *Bayero J Pure Appl Sci*. 2022;13(1):360-365.
 32. Paneershalvam C, Murugan K, Kovendan K, Kumar MP, Submaraniam J. Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae). *Asian Pac J Trop Med*. 2018;6(2):102-109. DOI:10.1016/S1995-7645(13)60003-6.
 33. Hidayatulfathi F, Akram W, Suhail A, Khan MA. Adulticidal action of tencitrus oils against *Aedes albopictus* (Diptera: Culicidae). *Pak J Agric Sci*. 2018;47(3):241-244.