

Revealing the Impact of Selected Weed Plants on Larval Mortality of *Culex Quinquefasciatus* (say) Diptera: Culicidae Juveniles

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Abstract

The prevalence of mosquito-borne diseases has precipitated significant global health concerns, particularly in tropical regions where millions of individuals remain at a heightened risk of infection. These diseases, often transmitted through the bites of infected mosquitoes, can lead to serious illnesses, such as malaria, dengue fever, Zika virus, and chikungunya. Given the substantial health and economic burden imposed by these diseases, there is an undeniable need to effectively address this issue. The efficiency of the selected weed plant extracts in water was evaluated for larval mortality of *Culex quinquefasciatus* (*Cu. quinquefasciatus*) after 24 h. The LC₅₀ and LC₉₅ values of the plant extracts of *Carthamus oxycantha*, *Trachyspermum ammi*, *Achyranthes aspera*, *Convolvulus arvensis*, and *Cynodon dactylon* were also determined. The experiment was conducted in a completely randomized design with six treatments, each replicated three times. The plant extracts were tested at five concentrations: 200, 300, 500, 700, and 1000 ppm. *Carthamus oxycantha* in the water extract yielded a higher mortality of 42% after 24 h. Both LC₅₀ and LC₉₅ values were maximum for *Cynodon dactylon* (66749.45) and lowest for *Carthamus oxycantha* (2535.34), whereas the LC₉₅ value was highest for *Cynodon dactylon*. The study recommends that these weed plant extracts be tested at lower concentrations, as higher concentrations yield 100% mortality of the pest. Natural bio-products are not only favored in vector control measures but also in resistance developed by the larvae. These findings highlight the potential of natural bioproducts for vector control and addressing larval resistance, emphasizing the need to identify new active compounds from cost-effective natural sources against mosquitoes.

Introduction

The mosquito species were *Cu. quinquefasciatus* (Say) is widespread across the globe, inhabiting regions characterized by tropical, subtropical, and warm temperate climates. What makes this particular mosquito one of the most successful of its kind is its exceptional adaptability to a wide range of habitats (Foster and Walker 2019). This adaptability is particularly evident in their ability to breed in diverse environments. These environments encompass not only stagnant water bodies but also other diverse niches, such as roadside ditches, freshwater ponds, riverbanks, and even natural streams. Remarkable success with *Cu. quinquefasciatus* can be attributed to its ability to exploit varied breeding grounds, demonstrating a high degree of ecological flexibility (Schneider et al. 2001). This adaptability allows the species to thrive and reproduce persistently across a broad spectrum of geographical and environmental conditions. By utilizing such an array of breeding habitats, this mosquito species secures survival and proliferation (Cantonati et al. 2020). The versatility of *Cu. quinquefasciatus* in selecting and thriving in these different habitats underscores its capacity to capitalize on available resources, irrespective of their nature or location. This adaptability not only highlights the evolutionary resilience of this species, but also emphasizes the challenges it poses in terms of vector control efforts, as its widespread presence and diverse breeding preferences make it a potential carrier of various diseases (Legros et al. 2021).

In Pakistan, it is found in abundance everywhere except in the northern parts of the country, which exhibit cold climatic conditions where the temperature ranges between – 3°C to -25°C (Ahmad et al. 2014). A diverse variety of mosquitoes are found in the southern parts of the country. Optimal temperature range for *Cu* survival. *Cu. quinquefasciatus* is 20°C to 30°C (Mara 2013). In Khyber Pakhtunkhwa, the mosquito flourishes easily under suitable environmental conditions (3°C to 49°C) throughout the province the mosquito flourishes easily. The

ability of mosquitoes to adapt to diverse environmental conditions has contributed to their survival (Gupta and Nair 2020).

The *Cu. quinquefasciatus* acts as a vector for *Wuchereria bancrofti*, which causes lymphatic filariasis, commonly known as elephantiasis, a painful disease that harms the skin extremely and persistently (Foster and Walker 2019). According to (Foster and Walker 2019) 120 million people in tropical and subtropical areas of the world suffer from lymphatic filariasis. Among these infected people, approximately 25 million men suffer from genital diseases, most commonly hydrocele, and 15 million (mostly women) suffer from lymphedema or elephantiasis of the leg. In 2023, confirmed cases of Tropical Pulmonary Eosinophilia (TPE) were reported in indigenous patients; however, this disease is rare in Pakistan (Pandian et al. 2023) (Fig. 1).

Effective mosquito control primarily focuses on targeting the mosquito population during the larval stage. This proactive approach aims to curtail mosquito numbers before they reach the adult phase, thereby reducing the potential transmission of diseases. One notable method employed for vector control, specifically during the larval stage, is the use of larvicides (Hussni Hasan et al. 2023).

Larvicides are substances designed to target and eliminate mosquito larvae present in breeding sites, which are often stagnant or slow-moving water bodies. By intervening in this early developmental stage, the growth and maturation of mosquitoes into disease-carrying adults can be impeded (Agyemang-Badu et al. 2023). The application of larvicides occurs in the breeding habitats themselves, preventing the larvae from progressing through their life cycle and becoming potential vectors of diseases, such as malaria, dengue fever, and Zika virus (Faraji and Unlu 2016).

The larval stage of mosquitoes is especially well suited for targeted control measures because of their habitat preferences. Because mosquito larvae develop in aquatic environments, they are confined to water bodies during this phase of their life cycle (Rejmánková et al. 2013). This restricted habitat makes them more susceptible to control efforts, as the application of larvicides to water sources can effectively reach and impact larvae. Furthermore, the confined nature of water bodies simplifies the delivery of larvicidal agents to target organisms (Walker and Lynch 2007).

Mosquito control strategies that target the larval stage offer a proactive approach to curbing disease transmission. Larvicides, when applied to breeding sites, capitalize on the larvae's aquatic habitat and vulnerability at this stage, preventing them from maturing into disease-transmitting adults. (Berube 2023). This approach not only minimizes the overall mosquito population but also reduces the risk of disease spread within communities.

Chemical methods are effective ways to control pests. Synthetic pesticides are used worldwide to control pests including mosquitoes. However, synthetic pesticides have residual effects and kill beneficial insect larvae, and most synthetic pesticides have environmental issues. Mosquitoes also acquire insecticide resistance (Ahmed et al. 2021). Essential oils and plant extracts have also been reported to control mosquito larvae by studying the effect of crude latex produced from the green parts of the plant against egg hatching and larval development of mosquitoes. The methanolic and aqueous extracts of fresh leaves showed good larvicidal properties against mosquitoes (Govindarajan et al. 2011).

The primary objective of this study was to assess the larvicidal properties of water-based extracts obtained from certain weed plants. The focus was specifically on evaluating how these extracts were performed against the mosquito species *Cu. quinquefasciatus*. In essence, this study sought to determine if these aqueous extracts could effectively eliminate or reduce the larval population of *Cu. quinquefasciatus*, a mosquito species. This research represents a critical step in understanding the potential of natural substances derived from weed plants to control mosquito populations, especially during their vulnerable larval stages.

Materials and Methods

This assessment took place within a controlled laboratory environment, where conditions could be carefully manipulated and observed to gain insights into the potential larvicidal effects of the weed plant extracts.

Selection of Plant Species

The plant species of interest, *Carthamus oxycantha*, *Trachyspermum ammi*, *Achyranthes aspera*, *Convolvulus arvensis*, and *Cynodon dactylon*, were identified by a taxonomist, Professor Dr. Mir Ajab Khan, Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan, and were selected for the extraction process.

Collection Location

Leaves were collected from appropriate sources, such as natural habitats or cultivated areas. The collection location, in this case, was Mardan, Pakistan. The collection site is located at a latitude of approximately 34.2019° N and has an altitude of approximately 283 m (929 ft) above sea level. The annual rainfall in Mardan, Pakistan varies from year to year and is influenced by seasonal and climatic factors. On average, Mardan receives approximately 600–800 mm (24 to 31 inches) of rainfall annually (Basharat et al. 2014).

Harvesting

Fresh and healthy green leaves of the selected plant species were carefully harvested. It is essential to select leaves that are free from damage, disease, or signs of stress because the quality of the extract depends on the quality of the plant material. The collection of leaves for plant extracts is a crucial initial step in the process, as the quality and purity of the starting material significantly influence the effectiveness of the extracted compounds. Proper care and attention during the collection and preparation of leaves are essential to ensure accurate and meaningful results in the subsequent extraction and experimentation processes (Liu 2011).

Cleaning

After harvesting, the leaves were thoroughly cleaned to remove any adhering dirt, debris, or contaminants. This step is crucial to ensure that the plant material used for extraction is clean and free of impurities.

Washing

The cleaned leaves were washed with sterile distilled water. This additional washing step helped eliminate any residual impurities and ensured that the extract was not contaminated by external factors.

Drying

After cleaning and washing, leaves were allowed to dry naturally. Drying is crucial for reducing the moisture content of leaves, which can affect the extraction process and the stability of the resulting extract.

Shade Drying

The leaves were dried in a shaded area at room temperature. This gentle drying method helps to preserve the bioactive compounds present in the leaves and prevents degradation due to excessive heat or direct sunlight.

Grinding

Once fully dried, the leaves were ground into fine powder using an electrical grinding machine (Faber FBG-460 K, Faber, Fabriano, Italy). This increases the surface area of the plant material, facilitating the extraction of bioactive compounds.

Storage

Powdered leaves were stored in sterile glass containers. Proper storage is essential for maintaining the integrity of the plant material and preventing degradation of bioactive compounds due to exposure to light, air, or moisture.

Preparation of Extracts

To prepare the test solutions, a total of 100 g of fine powder of each plant was put into conical flasks, and 1 L of ethanol (C_2H_5OH) was poured into the flask. The mixture was stirred every 24 h using a magnetic stirrer, in a process that lasted for 3 days. The mixture was then filtered using Whatman No. 1 filter paper to remove any solid particles or debris, yielding a clear solution and was evaporated in a rotary vacuum evaporator at 40°C to obtain the clear crude extract (Odey et al. 2012) (Fig. 2). A specific amount of the filtrate was measured and placed in petri dishes. These dishes were subsequently placed in a vacuum oven at a temperature of 45°C. The drying process was continued until the solvent, which included both water and ethanol, completely evaporated. The crude extracts were then stored at – 20°C in a sterile container. A sterile swab was used to streak the extract onto nutrient agar (NA), and it was then incubated for 24 h at 37°C to screen for contamination and determine the purity of the extract. This method ensured that the active components derived from the plant leaves were retained in a solid form for further analysis (Iqbal et al. 2015).

Mosquito larvae Collection

The *Cu. quinquefasciatus* larvae were collected from different breeding locations, such as irrigation channels, pits, pools, drainage channels, and river banks. The collection was placed in plastic tubs with 1 L of water to establish mosquito culture. Twenty-five early 3rd instar larvae of *Cu. quinquefasciatus* was pipetted out from the stock culture established in the laboratory and transferred to a plastic cup containing 100 ml water. Different concentrations of the plant extracts (200, 300, 500, 700 and 1000 ppm) were prepared and added to each plastic cup. Mortality was recorded every 24 h (Wang et al. 2023).

Larvicidal Bioassay

The regular World Health Organization (Organization 2005) recommendations for larval susceptibility test techniques (distilled water and plant solution) were followed while conducting larvicidal bioassays. A

homogenous population of twenty-five early 3rd instar *Cu. quinquefasciatus* larvae with a body length range of 5 mm to 6 mm was used for the bioassays. For each concentration, the bioassays were carried out three times with methanol CH₃OH serving as the control. Ground cat food was offered during the test period. Four concentrations were evaluated against the larvae in this study: 200 mg/L, 300 mg/L, 500 mg/L, 700 mg/L and 1000 mg/L. For each experimental replicate, the control solutions were made using 1 mL of distilled water and 10% of the appropriate methanol solvent.

The solvent control was used to make sure that the solvent was not the cause of the mortality data. Larvae mortalities were recorded after 24 h of experimentation at a room temperature of $28 \pm 2^\circ\text{C}$. Death was defined as complete immobility and the larvae's complete lack of movement, even when touched. Probit analysis was used to analyze the data and determine the LC₅₀, LC₉₅. Data after arcsine transformation was subjected to one-way ANOVA in order to distinguish between the various concentrations.

Microscopic Evaluation

Under a dissecting microscope, the *Cu. quinquefasciatus* larvae of the testing and control groups were compared for any physical differences.

Statistical Analysis

Statistical analysis was conducted to derive LC₅₀ and LC₉₅ values using the SPSS software (Rudayni et al. 2021). The experimental design followed a two-factor arrangement, which was replicated thrice to ensure the robustness and reliability of the results. This approach allowed for the accurate determination of the concentrations required to achieve 50% and 95% larval mortality, providing valuable insights into the potency of the tested weed plant extracts against the target mosquito species.

Results

The results showed that after 24 h of treatment, the mortality of *Cu quinquefasciatus* at 200 and 300 ppm of the plant extracts was significantly higher (26%) than that of *Carthamus oxycantha* and *Trachyspermum ammi*. No significant effect of the *Achyranthes aspera* leaf extract was observed at 200 ppm, but with the 400-ppm extract, 16% mortality was obtained. The mortality of the larvae was significantly higher in all treatments, ranging from (3.33–10.67%), compared to the control. At 500 ppm, larval mortality was significantly higher with *Carthamus oxycantha* (37.33%) and *Cynodon dactylon* (32%), but was lower with *Convolvulus arvensis* (24%). At 700 ppm, the mortality of the larvae was significantly higher with *Trachyspermum ammi* (61.33%) and *Carthamus oxycantha* (54.66%), whereas it was the lowest with *Cynodon dactylon*, showing a 36% increase. *Carthamus oxycantha* (66%) and *Trachyspermum ammi* (65%) caused significantly higher mortality at 1000 ppm, whereas *Cynodon dactylon* had the lowest mortality (41%). The lowest mortality (17.33%) of the larvae was recorded in the control group. Among the treatments, *Carthamus oxycantha* resulted in significantly higher (42.40%) mortality of the larvae, whereas the lowest mortality of 27.73% was recorded in *Convolvulus arvensis* (Table 1).

As compared to the larvae from the control groups, those treated with the plant extract usually appeared darker, with the thorax and upper abdominal segment standing out as being particularly dark in colour. The treated

larvae's lower abdominal segments were just as distinct as those of the control larvae. In addition, the treated larvae's anal gills were darker than those of the control group's larvae (Fig. 3).

Table 1
Effect of aqueous plant extracts on larval mortality of *Cu. quinquefasciatus* after 24 h.

Treatments (Plant extracts)	Mortality (%) of <i>Cu. quinquefasciatus</i> larvae at different concentration (ppm) of plant extracts					Mean
	200	300	500	700	1000	
<i>Trachyspermum ammi</i>	2.66pq	26.66hij	27.33hij	61.33ab	65.33a	36.67ab
<i>Convolvulus arvensis</i>	5.33opq	18.66kl	24ijk	40ef	50.66cd	27.73c
<i>Achyranthes aspera</i>	0p	16lm	30.66ghi	45.33de	53.33c	29.07bc
<i>Carthamus oxycantha</i>	26.67hij	26.67hij	37.33fg	54.66bc	66.66a	42.40ab
<i>Cynodon dactylon</i>	18.66kl	21.33jkl	32gh	36fg	41.33ef	29.87bc
Control	3.33opq	10.67mno	0q	9.33nop	17.33klm	8.13d
Mean	9.44d	20.00c	25.22c	41.11b	49.11a	

Means within a column followed by different letters are significantly different at 5% level of significance (LSD Test).

LSD value for treatment = 8.02

LSD value for concentration = 7.81

LSD value for means = 7.81.

The study revealed that larval mortality also increased with an increase in plant extract concentration.

LC₅₀ and LC₉₅ values of aqueous plant extracts against *Culex quinquefasciatus* after 24 hours

The LC₅₀ and LC₉₅ values at the 5% significance level for the aqueous plant extracts against *Cu. quinquefasciatus* larvae after 24h of incubation. The LC₅₀ value was highest (1593.96) for *Cynodon dactylon* and lowest (630.50) for *Carthamus oxycantha*, whereas the LC₉₅ value was the highest (66749.45) for *Cynodon dactylon* and lowest (2535.34) for *Trachyspermum ammi*. The slope was highest (2.783 + 0.318) for *Trachyspermum ammi* and lowest (1.014 + 0.281) for *Cynodon dactylon*. The Chi-square values for *Trachyspermum ammi* and *Cynodon dactylon* were 11.12 and 0.243, respectively. The results demonstrated that the mortality of *Carthamus oxycantha* was the highest; hence, the LC₅₀ value was the lowest. LC₉₅ value of *Trachyspermum ammi* was lower than that of the other treatments, showing greater toxicity against mosquito larvae. The lower the LC₅₀ and LC₉₀ values, the higher the toxic effect, and vice versa. Singh et al. (2003) reported larvicidal activity of different plants extract and *Cu. quinquefasciatus*. According to (Edwin et al.

2013) larvicidal activity of plant extracts against *Cu. quinquefasciatus* (LC₅₀ = 377.69, 207.61, 842.34, 623.80 ppm) (Table 1). Larvicidal activity of *Z. officinalis* (LC₅₀ = 50.78) against *Cu. quinquefasciatus* were also been reported by (Santhoshkumar et al. 2011) (Table 1–2 and Fig. 3).

Table 2
LC₅₀ and LC₉₅ values of aqueous plant extracts against *Cu. quinquefasciatus* after 48 hours

Treatment (Plant extracts)	LC ₅₀	95% confidence interval		LC ₉₅	95% confidence interval		Slope	Chi square
		Lower	Upper		Lower	Upper		
		Bound	Bound		Bound	Bound		
<i>Trachyspermum ammi</i>	421.31	335.14	516.77	5269.64	2725.32	20398.35	1.499 ± .27	1.89
<i>Convolvulus arvensis</i>	585.61	475.58	770.17	8107.09	3696.54	43533.55	1.441 ± .272	2.72
<i>Achyranthes aspera</i>	573.60	479.69	714.91	5365.67	2929.18	16993.98	1.694 ± .276	.494
<i>Carthamus oxycantha</i>	383.73	316.79	4451.36	2829.41	1854.75	6268.09	1.879 ± .277	1.34
<i>Cynodon dactylon</i>	490.80	377.82	653.75	11299.74	4226.61	129812.83	1.208 ± .266	421

Discussion

Larvicidal effects of various plant extracts on *Cu. quinquefasciatus* larvae provided valuable insights into the potency of these extracts at different concentrations (Santhoshkumar et al. 2011). After a 24-hour treatment period, the mortality rates of the mosquito larvae were subject to distinct variations based on the type and concentration of the plant extracts utilized.

At lower concentrations of 200 and 300 ppm, *Carthamus oxycantha* and *Trachyspermum ammi* exhibited significantly higher larval mortality rates, reaching 26%. In contrast, *A. aspera* extract at 200 ppm did not produce a significant effect, but at 400 ppm, a mortality rate of 16% was observed. The efficacy of these plant extracts was further highlighted by comparing their impact on mortality with that of the control group. All treatments demonstrated markedly higher mortality rates, ranging from 3.33–10.67%, than the control group. Supplementation with medicinal plant extracts, especially *Andrographis paniculata* and *Tinospora cordifolia*, can be used in poultry diets to improve production performance, carcass yield, and blood biochemical profiles in broiler chickens (Nety et al. 2017).

Evaluating the impact of 500 ppm concentrations, *Carthamus oxycantha* and *Cynodon dactylon* extracts led to significantly higher larval mortality rates of 37.33% and 32%, respectively, whereas *Convolvulus arvensis* yielded a lower rate of 24%. A concentration of 700 ppm further accentuated these trends, with *Trachyspermum ammi* and *Carthamus oxycantha* extracts resulting in 61.33% and 54.66% mortality rates, respectively, whereas *Cynodon dactylon* exhibited a lower increase of 36%. The highest concentration of 1000

ppm showed that *Carthamus oxycantha* and *Trachyspermum ammi* were the most effective, causing significantly higher mortality rates of 66% and 65%, respectively, with *Cynodon dactylon* showing the lowest rate of 41%. In comparison, the control group had the lowest mortality rate (17.33%). The effects of five temperatures (26, 28, 30, 32, and 34°C) on egg development time, percentage hatchability, and nymphal weight at hatching have been reported. Moreover, we evaluated the average weekly wet weight attained by *Ruspolia differens* and the percentage survival during growth when reared at 30°C on four different food plant diets (Ssepuuya et al. 2018).

Notably, *Carthamus oxycantha* has emerged as a standout treatment, resulting in a mortality rate of 42.40% among the larvae, underscoring its potential as an effective larvicidal agent. Conversely, *Convolvulus arvensis* exhibited the lowest mortality rate (27.73%) among the treatments. The results of this study indicate the potential of these plant extracts as viable options for mosquito control, with *Carthamus oxycantha* showing particularly promising outcomes in this context. Further investigations, including field studies, could shed light on the practicality and efficiency of incorporating these plant extracts into broader vector-control programs.

According to (Hamza 2016), *C. citrates* exhibits larvicidal activity against *Cu. quinquefasciatus* at 10000 ppm, whereas *Achyranthes aspera* extract resulted in 50% mortality at the same concentrations. Larvicidal activity of petroleum ether extracts of *Euphorbia hirta* and *Cu. quinquefasciatus* was also reported by (Agalya Priyadarshini et al. 2012).

In this study, we determined LC₅₀ and LC₉₅ values at a significance level of 5% for aqueous plant extracts in terms of their effects on *Cu. quinquefasciatus* larvae after a 24-hour incubation period. The calculated LC₅₀ value yielded the highest estimate of 1593.96 for *Cynodon dactylon*, whereas the lowest value of 630.50 was observed for *Carthamus oxycantha*. In a similar vein, the LC₉₅ value exhibited a similar trend, with *Cynodon dactylon* registering the highest value of 66749.45 and *Trachyspermum ammi* reflecting the lowest value of 2535.34. The variations in these values suggest differing degrees of effectiveness and toxicity across various plant extracts.

The slopes of the concentration-response curves also offer insights into the differential effects of plant extracts. *Trachyspermum ammi* exhibited the steepest slope at 2.783 ± 0.318 , indicating a rapid increase in larval mortality with increasing concentration. Conversely, *Cynodon dactylon* displayed the shallowest slope at 1.014 ± 0.281 , suggesting a slower response to increasing concentrations of the extract. This variation in slope reflects the diverse sensitivities of mosquito larvae to different plant extracts.

The Chi-square values for *Trachyspermum ammi* and *Cynodon dactylon* further substantiate the differences in their effects. *Trachyspermum ammi* recorded a higher Chi-square value of 11.12, indicating a stronger association between its concentrations and larval mortality. *Cynodon dactylon*, on the other hand, exhibited a lower Chi-square value of 0.243, suggesting a weaker correlation between its concentrations and the observed effects on larvae.

Comparative analysis of the results highlighted the significant efficacy of *Carthamus oxycantha* extract, leading to higher mortality rates. Consequently, the lowest recorded LC₅₀ value for *Carthamus oxycantha* indicates its potential for effective mosquito control. Similarly, *Trachyspermum ammi* demonstrated notable larvicidal activity, as evidenced by its lower LC₉₅ value, indicating higher toxicity towards mosquito larvae.

These findings align with those of a previous study (Mohammed et al. 2017) on the larvicidal activity of various plant extracts against *Cu. quinquefasciatus*, thereby reinforcing the significance of plant-derived compounds in mosquito control (Rajput et al. 2020) also investigated the plant extract-based larvicidal activity against *Cu. quinquefasciatus*, highlighting the diversity of potential sources for such control methods. In addition, (Sutthanont et al. 2019) reported the larvicidal potential of *Z. officinalis* oil against *Cu. quinquefasciatus*, further demonstrating the breadth of the natural compounds investigated.

The variations in LC_{50} , LC_{95} values, slopes, and chi-square values indicate the unique characteristics and potential applications of different plant extracts in mosquito control strategies. Furthermore, alignment with previous research underscores the importance of natural compounds as promising avenues for combating mosquito-borne diseases. Further investigations and field trials are warranted to fully evaluate the practical implications and applicability of these findings to real-world scenarios.

Conclusion and Future Perspectives

The aqueous extract of the weed *Carthamus oxycantha* caused significantly higher (42%) mortality of *Cu. quinquefasciatus* larvae after 24h as compared to the other treatments. Both LC_{50} and LC_{95} values were maximum for *Cynodon dactylon* and lowest for *Carthamus oxycantha*, while the LC_{95} value was maximum for *Cynodon dactylon* and lowest for *Trachyspermum ammi* after 24 h in water extracts. It is recommended that the plant extracts of these weeds be tested at lower concentrations, because many of the extracts yielded 100% mortality at higher concentrations. The aqueous extracts of these plants could be considered for their utilization in the countries with a lot of *Cu. quinquefasciatus* as recommended bio-insecticide and profitable repellents against mosquitoes, both individually and in combination with other natural products on a commercial scale. This study paves the way for future endeavors aimed at optimizing mosquito control strategies. The investigation of plant extracts as potential larvicidal agents, coupled with their application in repellent formulations, offers avenues for eco-friendly and effective approaches to combat mosquito-borne diseases. Further studies, including field trials and long-term efficacy assessments, are crucial for fully realizing the practical implications of these findings in real-world mosquito control programs.

Declarations

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Ethical Approval

No humans or animals were used in this study.

Competing interest

The author(s) of this manuscript declare that they have no competing interests.

Author's contribution

M.N. and N.A., performed the experiments and drafted the manuscript. B.I. reviewed and supervised the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

All the data used are already reflected in the article. Other relevant data may be available upon request from the authors.

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Figures



Adult male *Culex quinquefasciatus* mosquito.



Adult female *Culex quinquefasciatus* mosquito taking a blood meal.



Close up of *Culex quinquefasciatus* wings.



Culex quinquefasciatus mosquito eggs.



Culex quinquefasciatus mosquito four larval stages or instars.



Culex quinquefasciatus mosquito pupae in standing water.

Figure 1

Cu. quinquefasciatus Adults, Eggs, Larvae, and Pupae. Source: Center for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Vector-Borne Diseases (DVBD).



Figure 2
Various steps involved in the preparation of selected plants extracts.



Figure 3

Midgut color of *Cu. quinquefasciatus* control larvae (A) and those treated with aqueous plant extract (B) were different, with the plant extract (greenish) becoming visible in the treated larvae after 28 h.