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Research Article

Assessment of resistance and susceptibility in *Culex quinquefasciatus* larvae exposed to conventional and novel insecticides

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ABSTRACT

Mosquitoes belong to the order Diptera, suborder Nematocera, and family Culicidae. *Culex quinquefasciatus* Say is a medically significant mosquito and major pest species with a global distribution. The indiscriminate use of pesticides leads to environmental issues such as health risks, disruption of natural predators, ecosystem imbalances, and the development of resistance in insect pests. Therefore, the present study aimed to reduce the excessive use of pesticides by exploring novel alternative and chemical insecticides. The susceptibility (%) and resistance (%) of third instar larvae of *Culex quinquefasciatus* to conventional chemicals (lambda-cyhalothrin, permethrin and deltamethrin) and new chemicals (spinosad, ivermectin, and emamectin benzoate) were determined using different concentration rates (100, 125 and 150 ppm) and exposure times (24, 48, 72 h). In general, exposure time did not significantly affect the susceptibility or resistance percentages of *Culex quinquefasciatus*. However, changes in concentration directly influenced both susceptibility and resistance to both conventional and new chemicals. Lambda-cyhalothrin had the highest LD50 value (1.80), whereas spinosad had the lowest LD50 value (0.03). The LD90 value was highest for deltamethrin, whereas spinosad exhibited the lowest LD90 value across all treatments.

Keywords: *Culex quinquefasciatus*; insecticides; resistance; susceptibility; Lambda-cyhalothrin.

INTRODUCTION

Mosquitoes belong to the order Diptera, considered major insect pests of medical importance (Naik et al., 2025) (Rahman and Howlader 2018). From a medical perspective, they are thought to be a major contributor to the spread of various significant pathogens, germs, and parasites, such as bacteria, viruses and protozoa, which are largely responsible for the development of deadly diseases such as dengue, malaria, and chikungunya (Dhiman & Singh, 2024; Minwuyelet et al., 2025). In many parts of the world, they are a major contributor to human health problems. The three essential mosquito genera are *Anopheles*, *Aedes*, and *Culex* (Gyawali et al., 2025). Of these, Culex is regarded as one of the most important genera, acting as a vector for the development of numerous dangerous diseases in humans, birds, and other animals, such as filariasis, avian malaria, Japanese encephalitis, or St. Louis encephalitis, and West Nile virus (Bursali & Touray, 2024; Nebbak et al., 2022). They also act as nuisance pests by feeding on human blood using their piercing-sucking mouthparts, which can cause skin irritation. In both urban and rural

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locations, they are regarded as irritating mosquitoes, particularly in tropical and sub-tropical region (Tyagi et al., 2025). The deadliest disease transmitted by *Culex quinquefasciatus* is West Nile Virus, which was first reported in Harris County, Texas, in 2002 (Stark et al., 2017). WNV infection can impose a significant economic burden on the infected individual, and in the case of neuroinvasive WNV infection, treatment costs may range from $624 to $439,945 over an extended period (Staple et al., 2014). The viral infection may continue for up to eight years after infection (Murray et al., 2013).

For the majority of mosquito species, the developmental cycle lasts roughly two weeks during hot conditions (Agyekum et al., 2021). About a single female can lay 300 eggs on the water's surface in rafts (Mataba et al., 2024). Eggs can be laid in standing fresh water in a variety of containers, including spills, pools, ditches, cans, buckets, plastic bottles, and water storage tanks (Berube, 2023; Nanvyat & Luka, 2024). Adhesion forces cause little, cigar-shaped, dark brown eggs to adhere to one another. It is fairly easy to separate the eggs because they are not firmly linked. For eggs, hatching water is essential (Day, 2016). Simply by moving their bristly mouthparts, larvae maintain their posture and are primarily vertically oriented in the water. In order to swim, larvae move their bodies back and forth over the water's surface (Sarwar, 2020).

Insecticides such as organochlorines, organophosphates, carbamates, and pyrethroids are key agents as vector control programs worldwide (van den Berg et al., 2021). Over-dependence and large scale use of insecticides have created resistance against insecticides (World Health Organization (Ogunniran et al., 2024). As a matter of fact, insecticide resistance is not a new situation and is a terrible problem all around the world (Dentzman et al., 2025). Several cases have been reported from different regions of the world about the occurrence of resistance in *Cx. Quinquefasciatus* to a number of insecticides (Gul et al., 2025).

There are several control strategies for mosquitoes including adulticiding with ultra-low volume (ULV), fogging, thermal fogging, surface residual spray, or household insecticide products specially designed against adult mosquitoes (Anish et al., 2025; Muller et al., 2023). Generally, a number of cases have been reported from many urban area of Brazil regarding the high level of resistance against organochlorine and organophosphate. Larvicidal strategy is also used worldwide against *Cx. quinquefasciatus* (Lopes et al., 2019).

There are a number of concerns, hazards and harmful effects produced by the use of broad range conventional insecticides against mosquitoes (Hazarika & Krishnatreyya, 2025), that forces to use new chemistry insecticide that are efficient as well as safer to use, and less toxic to environment as compared to conventional insecticide (Korrat et al., 2012). However, new chemistry insecticides are much more specific and behave as specialists in pest control management program of particular pests (Bhatti et al., 2013; Ghimire & Joseph, 2025). In order to enhance the production of crops with multiple pest scenario, a mixture of more than one insecticide are used having distinct chemical groups are used (Bhatti et al., 2013; Vermelho et al., 2024). Insecticides mixtures in different compositions are assumed to increase the toxicity level in a synergistic fashion against the target pests (Akbar, Afzal, et al., 2024; Akbar et al., 2022). There is a need to develop better strategies to mitigate these challenges and implement management strategies of insecticide resistance to develop innovatory and novel vector control tools (Manzoor et al., 2025). Current studies of monitoring resistance status of field population of *Cx. quinquefasciatus* at district Haripur was planned for helping in the development of effective control tools and assess the resistance level of the prevailing mosquito populations against their insecticides. Considering the medical importance of *Cx. quinquefasciatus* and the rising concern of insecticide resistance, it is crucial to evaluate the efficacy of both conventional and new chemistry insecticides against its immature stages. In light of the increasing concern over insecticide resistance in *Cx. quinquefasciatus*, the present study was undertaken with specific objectives, including evaluation of the toxic effects of conventional and novel chemistry insecticides against wrigglers, monitoring of resistance in field populations, identification of the most effective insecticide, and determination of LC_{50} and LC_{90} values for the tested insecticides.

MATERIALS AND METHODS

Experimental Materials

This research work was conducted in Medical Entomology Laboratory at Nuclear Institute for Food & Agriculture (NIFA) Peshawar during 2019. Completely Randomized Design (CRD) having 10 replications was the planned design to conduct the experiment (Gul et al., 2025).

Collection and Rearing of Mosquito Larvae

Collection of mosquito larvae was done from four different locations i.e. Khanpur, Havelian, Refugee camp and University area at District Haripur. Iron dipper was used by six random dips from each breeding sites includes (standing water, Irrigation channel, road sites etc.) (Khan et al., 2014). The field-collected larvae were brought into laboratory for identification to species level. The culture was kept in larval trays at 26±1oC and 75±5% RH, in the laboratory. Continuously, larvae were fed with larval diet International Atomic Energy Agency (IAEA) at 1, 2 and 3% concentrations for larval development. After pupations, the pupae were collected and transferred into adults rearing cages (Table 1). After two days, the adults emerged and were provided with a 10% glucose solution, while females were offered albino mice as a blood source for egg laying. Third instars larvae of *Culex* mosquito was used for the experiment.

Table 1. Laboratory rearing and protocols (Kauffman et al., 2017).

Lab Conditions	Temperature 26±1ºC, relative humidity 75±5%
Larval Rearing.	Provided 3% with fish diet as per body requirement after hatching larvae according to their size and development rate.
Adult Rearing.	Solution of Sucrose and 2g sodium benzoate was used to rear adult.
Blood feeding	Blood was fed after five days of post emergence.
Schedule	Bovine blood was fed by using membrane to adults on Monday morning or at Friday afternoon for about 1 hour.
Egg collection	Egg cups were placed in adult cages after 2 days of t blood feeding. If Friday blood fed was done, then egg cups were placed in cages the same day after blood fed.
Egg Hatching	Rinse egg from blot paper to the water surface in the middle of a suspend wire, provided with pinch of diet to the medium for hatching larvae.

Insecticides used for Resistance Bioassays and Effectiveness

The insecticides were used in the experiment are lamda-cyhalothrin, permethrin, deltamethrin and three new chemistry insecticides i.e. spinosad; ivermectin and emamectin benzoate

Development of susceptible strain of *Cx. quinquefasciatus*

The susceptible laboratory strain of *Cx. quinquefasciatus* was developed in the Entomology Laboratory for comparison tests. For this purpose, collection was made from area of comparatively less selection pressure. Male and female pupae were separated based on pupation time and reared for successive generations to maintain a healthy laboratory colony.

Evaluation of different Insecticides

The following insecticides were used against *Cx. quinquefasciatus* larvae. They were used in three replications per treatment, Lamda-cyhalothrin, Permethrin, Deltamethrin and new chemistry insecticides Spinosad, Ivermectin and Emamectin benzoate.

Bioassays: Susceptibility (%) and Resistance (%)

The Susceptibility (%) and Resistance (%) of conventional and new chemistry insecticides were evaluated at various concentrations 0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 100, 125 and 150 ppm) against the Culex mosquito in accordance with the guidelines of World Health Organization 2004. Batches of 50 third instars larvae of *Cx. quinquefasciatus* were put in a small plastic disposable transparent cups having 100 ml distilled water and put in the netted area in the Laboratory room at 26±1oC, 75±5% relative humidity. For the control group, the mosquito larvae were exposed to only plain distilled water without addition of chemicals. Each tested concentration was repeated ten times. The insecticides were monitored carefully counting the susceptible and resistant ratio after 24, 48 and 72 hours. Susceptibility (%) and Resistance (%) were calculated by using the Abbot formula 1925 (Tikar et al., 2009).

Percent Susceptibility (%)= (Number of susceptible larvae)/ (Number of larvae introduce) X 100

Percent Resistance (%) = (Number of resistant larvae)/ (Number of larvae introduce) X 100

Statistical Analysis

The experiments were conducted in Completely Randomized Design (CRD). The mean values and SD was calculated from ten replications (Akbar et al., 2024a; Akbar et al., 2024 b). The susceptibility and resistance values were corrected by Abbott’s formula and the lethal concentration values of LC50 and LC90 were calculated by using probability analysis program polo plus (version 1.5) (Akbar et al., 2022; Akbar et al., 2024c).

RESULTS AND DISCUSSION

Concentration-Dependent Effects of Lamda-Cyhalothrin on *Cx. quinquefasciatus* larvae

Mean susceptibility (%) and resistance (%) values of *Cx. quinquefasciatus* against lambda-cyhalothrin are presented in Table 2. At higher concentrations (100, 125, and 150 ppm), *Cx. quinquefasciatus* exhibited complete susceptibility (100%) with no resistance recorded across 24 to 72 hours of exposure. At moderate concentrations (2 and 3 ppm), susceptibility ranged from 83.33% to 86.67%, followed by 80.00% susceptibility at 1 ppm, indicating partial effectiveness of the insecticide. In contrast, at lower concentrations (0.5, 0.25, and 0.125 ppm), susceptibility dropped sharply to 33.33%–36.67%, accompanied by higher resistance levels. The control group exhibited 0.00% susceptibility and 100% resistance. Overall, the results demonstrate that variations in concentration strongly influenced the response of Cx. quinquefasciatus to lambda-cyhalothrin, whereas exposure time had comparatively little effect.

Concentration-Dependent Effects of Permathrin on *Cx. quinquefasciatus* Larvae

Mean values regarding susceptibility (%) and Resistance (%) of *Cx. quinquefasciatus* against Permethrin are presented in Table (3). Higher susceptibility (%) level was shown by permethrin at 150ppm concentration which is 76.67 %, whereas, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150 ppm concentration. The susceptibility (%) of Culex against 125ppm concentration of permethrin was 73.33% which has been followed by 100ppm of concentration which is 70.00 % while the susceptibility (%) of *Cx. quinquefasciatus* against control is 0.00 %. *Cx. quinquefasciatus* showed 100% resistance in the control treatment. When treated with 100 ppm of permethrin, the resistance level decreased to 30.00%. At 125 ppm, resistance was further reduced to 26.67%, while the lowest resistance (23.33%) was observed at 150 ppm. Thus, resistance declined gradually with increasing concentrations of permethrin. In overall results, it was found that variation of concentrations directly affects the application of permathrin on *Cx. quinquefasciatus* rather than time exposure.

Concentration-Dependent Effects of Deltamethrin on *Cx. quinquefasciatus* Larvae

Mean values regarding susceptibility (%) and Resistance (%) of Cx. quinquefasciatus against deltamathrin are presented in Table (4). Higher susceptibility (%) level was shown by deltamathrin at 150ppm of concentration which is 93.33 %, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150 ppm concentration. The susceptibility (%) of *Cx. quinquefasciatus* against 125ppm concentration of deltamathrin were same as found in 150ppm concentration that's is 93.33% which has been followed by 100ppm of concentration which is 60.00 % during time duration from 24 to 48hrs while at 72hrs its susceptibility was reduced upto 56.00 %. The susceptibility (%) of *Cx. quinquefasciatus* against control is 0.00 %. *Culex* shows 100 % resistance against control which has been followed by the application of 100ppm concentration of deltamathrin that is 36.67 at 24 to 48hrs while the resistance % was increased upto 40.00 % during 72hrs, at 125 ppm concentration its resistance is 13.33 % at 150ppm concentration *Culex* shows the least 6.67 % resistance with respect to all the other concentrations of the application of deltamathrin. Overall, the results showed that susceptibility of *Culex* to deltamethrin increased at higher concentrations, while time had little effect except at lower concentrations.

Concentration-Dependent Effects of Spinosad on *Cx. quinquefasciatus* Larvae

Mean values regarding susceptibility (%) and Resistance (%) of Culex against spinosad are present in Table (5). Higher susceptibility (%) level was shown by spinosad at 150ppm of concentration which is 66.67 %, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 100 ppm concentration. The susceptibility (%) of *Cx. quinquefasciatus* against 125ppm concentration of spinosad were 67.67 % at 24hr while its susceptibility was change up to 60.00 % 48 and 72hrs of time interval. which is been followed by 100ppm of concentration that is 59.33 % while the susceptibility (%) of *Cx. quinquefasciatus* against control is 0.00 %. *Cx. quinquefasciatus* shows 100 % resistant against control which has been followed by the application of 100ppm concentration of spinosad that is 40.67 %, at 125 ppm concentration its resistance is 34.33 % to 33.33 % with the change of time interval while at 150ppm concentration *Culex* shows the least 33.33 % resistance with respect to all the other concentrations of the application of spinosad. In overall results, found that variation of concentrations directly affects the application of spinosad on Culex rather than time exposure.

Concentration-Dependent Effects of Emamectin benzoate on *Cx. quinquefasciatus* Larvae

Mean values regarding susceptibility (%) and Resistance (%) of *Culex* against emamectin are presented in Table (6). Emamectin benzoate exhibited maximum susceptibility (%) level at 150ppm of concentration which is 67.67 %, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150 ppm concentration. The susceptibility (%) of *Cx. quinquefasciatus* against 125ppm concentration of emamectin were 66.67% at 24hrs while it's were lower down up to 60.00 % during 48 & 72hrs of exposure time, which has been followed by 100ppm of concentration that is 62.67 % while the susceptibility (%) of *Culex* against control is 0.00 %. *Cx. quinquefasciatus* shows 100 % resistance against control which has been followed by the application of 100ppm concentration of emamectin that is 37.33 %, at

125 ppm concentration its resistant is 33.33 to 34.33% during the exposure of time from 24hrs to 72hrs while at 150ppm concentration *Culex* shows the least 32.33 % resistance with respect to all other concentrations of the application of emamectin.

Concentration-Dependent Effects of Ivermectin on *Cx. quinquefasciatus* Larvae

Mean values regarding susceptibility (%) and Résistance (%) of *Culex* against ivermectin are presented in Table (6). Susceptibility % of *Cx. quinquefasciatus* against all the concentrations (100, 125 & 150ppm) and at different time exposure (24, 48 & 72hrs) were 0.00 % while resistance % of *Cx. quinquefasciatus* against all the concentrations (100, 125 & 150ppm) and at different time exposure (24, 48 & 72hrs) were 100 %.

Table 2. Mean values of susceptibility (%) and resistance (%) of *Cx. quinquefasciatus* against different concentrations of lamda-cyhalothrin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
0.125	24	62.333 b	35.667 bc
	48	63.333 b	36.667 bc
	72	63.333 b	36.667 bc
0.25	24	64.000 b	36.667 bc
	48	66.667 b	36.667 bc
	72	68.667 b	36.667 bc
0.5	24	66.667 b	33.333 bc
	48	66.667 b	33.333 bc
	72	70.000 b	33.333 bc
1.0	24	80.00 ab	20.00 cd
	48	80.00 ab	20.00 cd
	72	80.00 ab	20.00 cd
2.0	24	83.333 ab	16.667cd
	48	83.333 ab	16.667 cd
	72	83.333 ab	16.667 cd
3.0	24	83.333 ab	13.333 cd
	48	86.667 ab	13.33 cd
	72	86.667 ab	13.33 cd
100	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
125	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
150	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
Control	24	0.00 c	100 a
	48	0.00 c	100 a
	72	0.00 c	100 a
Mean		72.556	26.00
CV		20.14	17.61

susceptibility critical value for comparison 14.16
resistance critical value for comparison 7.75

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Table 3. Mean values of susceptibility (%) and resistance (%) of *Cx. quinquefasciatus* against different concentrations of permethrin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	70.00 a	30.00 b
	48	70.00 a	30.00 b
	72	70.00 a	30.00 b
125	24	73.33 a	26.67 b
	48	73.33 a	26.67 b
	72	73.33 a	26.67 b
150	24	76.67 a	23.33 b
	48	76.67 a	23.33 b
	72	76.67 a	23.33 b
Control	24	0.000 b	100 a
	48	0.000 b	100 a
	72	0.000 b	100 a
Mean		52.16	44.83
CV		18.293	23.819

susceptibility critical value for comparison 22.98**;** resistance critical value for comparison 19.90

Table 4. Mean values of susceptibility (%) and resistance (%) of *Cx. quinquefasciatus* against different concentrations of deltamethrin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	56.00 a	40.00 b
	48	60.00 a	36.67 b
	72	60.00 a	36.67 b
125	24	93.33 a	13.33 b
	48	93.33 a	13.33 b
	72	93.33 a	13.33 b
150	24	93.33 a	6.67 b
	48	93.33 a	6.67 b
	72	93.33 a	6.67 b
Control	24	0.000 b	100.0 a
	48	0.000 b	100.0 a
	72	0.000 b	100.0 a
Mean		60.00	41.44
C V		21.08	18.94

susceptibility critical value for comparison 24.53**;** resistance critical value for comparison 21.25

Lethal Dose Concentration (Ppm)

Efficacy of both conventional (Lamda-cyhalothrin, Permathrin and Deltamathrin) and new chemistry chemicals (Spinosad, Ivermectin and Emamectin) were observed against 3rd instar Culex and LD50 and LD90 values observed at 12 hours' interval with the application of different concentrations of these chemicals and observe their affects. Showing lethal dose concentration (ppm) for different treatments. Lambda cyclothrine showing high value of CD50 (1.80) while spinosad showing low value of LD50 (0.03). Similarly, lambda cyhalothrin shows maximum percentage of 95%Cl (3.884) while spinosad shows minimum of 95%Cl (0.69). Value of LD90 is high in Deltamethrin and spinosad is lowest value in all treatments. The slop and Chi square (x2) value of lambda cyhalothrin is highest (14.787) in table while Emamectine show shows lowest value (0.01).

The present study provides a detailed evaluation of both conventional and novel chemistry insecticides against Cx. quinquefasciatus larvae, revealing significant variation in susceptibility and resistance profiles across different compounds and concentrations. Among conventional insecticides, lamda- cyhalothrin and deltamethrin demonstrated the highest efficacy, achieving near-complete susceptibility at concentrations of 125–150 ppm. These findings align

Table 5. Mean values of susceptibility (%) and resistance (%) of *Cx. quinquefasciatus* against different concentrations of spinosad at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	59.33 a	40.67
	48	59.33 a	40.67 b
	72	59.33 a	40.67 b
125	24	66.67 a	34.33 b
	48	66.00 a	34.00 b
	72	66.00 a	33.33 b
150	24	66.67 a	33.33 b
	48	66.67 a	33.33 b
	72	66.67 a	33.33 b
Control	24	0.00 b	100.0 a
	48	0.00 b	100.0 a
	72	0.00 b	100.0 a
Mean		48.056	51.972
C V		33.27	21.11

susceptibility critical value for comparison 25.84; resistance critical value for comparison 22.37

Table 6. Mean values of susceptibility (%) and Resistance (%) of *Cx. quinquefasciatus* against different concentrations of ememectin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	62.67 a	37.33 b
	48	62.67 a	37.33 b
	72	62.67 a	37.33 b
125	24	66.67 a	33.33 b
	48	66.00 a	34.00 b
	72	66.00 a	34.33 b
150	24	67.66 a	32.33 b
	48	67.66 a	32.33 b
	72	67.66 a	32.33 b
Control	24	0.00 b	100 a
	48	0.00 b	100 a
S	72	0.00 b	100 a
Mean		49.139	54.40
C V		33.34	28.40

susceptibility critical value for comparison 25.84; resistance critical value for comparison 22.37

Table 7. Mean values of susceptibility (%) and resistance (%) of *Cx. quinquefasciatus* against different concentrations of ivermectin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	0.00	100
	48	0.00	100
	72	0.00	100
125	24	0.00	100
	48	0.00	100
	72	0.00	100
150	24	0.00	100
	48	0.00	100
	72	0.00	100
Control	24	0.00	100
	48	0.00	100
	72	0.00	100
Mean		0.00	100

susceptibility critical value for comparison 00.00; resistance critical value for comparison 00.00

Table 8. Lethal dose concentration (ppm).

Treatments	Lethal dose concentration (ppm)					
	LD50	95% CL	LD90	95% CL	Slope	χ2
Lambda cyhalothrin	1.80	3.882	0.022	0.119	0.28	14.787
Deltamethrin	0.08	0.73	9.77	0.13	0.43	0.05
Permethrin	0.08	2.58	0.00	0.06	0.44	3.80
Emamectin	0.49	0.99	0.09	0.78	0.16	0.01
Ivermectin	0.09	0.12	0.06	0.11	0.23	2.39
Spinosad	0.03	0.09	0.00	0.00	0.16	0.132

with previous reports (N'Guessan et al., 2010; Tikar et al., 2009), confirming their continued effectiveness in mosquito control programs. The inverse relationship between susceptibility and resistance emphasizes the dose-dependent nature of these insecticides, with concentration exerting a more pronounced effect than exposure time (Frolova et al., 2025).

Permethrin showed moderate efficacy, with susceptibility levels ranging from 70–76.67% at higher concentrations, reflecting partial resistance in the tested field populations. This observation corroborates earlier studies indicating emerging pyrethroid resistance in *Cx. quinquefasciatus* (Corbel et al., 2007; Khan, 2019; Rasli et al., 2018). In contrast, the novel chemistry insecticides spinosad and emamectin exhibited substantial larvicidal activity, demonstrating their potential as alternative tools for managing resistant mosquito populations. These results are consistent with prior research highlighting spinosad's potent efficacy and environmentally friendly profile (Govindarajan, 2025; Santos et al., 2020; Yu et al., 2025).

Interestingly, ivermectin showed no larvicidal activity, with complete resistance observed across all concentrations and time points. This lack of effect may reflect innate tolerance mechanisms or potential cross-resistance, underscoring the necessity of careful pre-deployment resistance monitoring (Ouédraogo et al., 2025). Such findings highlight the critical importance of selecting insecticides based on localized susceptibility data to ensure effective vector control.

The observed concentration-dependent effects have important implications for integrated vector management (IVM) (Ullah et al., 2024). Optimizing insecticide application rates based on susceptibility patterns can enhance efficacy, reduce selection pressure for resistance, and prolong the operational lifespan of available compounds (Harun-Ur-Rashid & Imran, 2025). Incorporating both conventional and novel chemistry insecticides in rotation schemes could provide a strategic approach to managing resistance and sustaining long-term mosquito control outcomes.

This study provides novel insights into the resistance dynamics of *Cx. quinquefasciatus* larvae and identifies effective insecticide candidates for field application. The high efficacy of lamda- cyhalothrin, deltamethrin, spinosad, and emamectin, contrasted with the complete resistance to ivermectin, underscores the need for evidence-based insecticide selection in IVM programs. These results not only fill critical knowledge gaps but also have global relevance, offering guidance for regions experiencing increasing insecticide resistance and informing the development of next-generation, resistance-aware mosquito control strategies.

The present study highlights critical insights into the evolving insecticide resistance dynamics of *Culex quinquefasciatus* larvae, emphasizing the importance of concentration-dependent susceptibility as a key determinant of control efficacy. Rather than merely confirming known resistance trends, these findings underscore the need to integrate both conventional and novel chemistry insecticides within adaptive resistance management frameworks. The consistent effectiveness of lambda-cyhalothrin, deltamethrin, spinosad, and emamectin suggests their continued relevance for integrated vector management, whereas ivermectin's complete inefficacy reveals potential cross-resistance or physiological tolerance mechanisms that warrant further molecular investigation. Importantly, the study exposes a gap in understanding how field-evolved resistance interacts with new chemical modes of action under variable environmental conditions. Future work should therefore move beyond laboratory assays toward field validation, explore synergistic or rotational use of effective compounds, and incorporate resistance gene monitoring to design sustainable, evidence-based mosquito control strategies capable of adapting to rapidly shifting resistance landscapes.

CONCLUSION

The study evaluated the susceptibility and resistance of *Culex* larvae to both conventional (Lambda-cyhalothrin, Permethrin, Deltamethrin) and new-chemistry insecticides (Spinosad, Ivermectin, Emamectin benzoate) at varying concentrations and exposure times. Results showed that increasing concentration significantly influenced larval susceptibility and resistance, whereas exposure time had minimal effect. Among the tested insecticides, Lambda-cyhalothrin (conventional) and Emamectin benzoate (new chemistry) demonstrated the highest efficacy against Culex, while Ivermectin was completely ineffective, showing total resistance. These findings suggest that Lambda-cyhalothrin and Emamectin benzoate hold strong potential for controlling Culex populations. Future research should focus on evaluating their combined effects and conducting field-based validation trials to develop more effective and sustainable vector control strategies.

AUTHOR'S CONTRIBUTION

A Farid and R. Akbar Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing. S. W. Khan and B. Faheem: Conceptualization, Data curation, Visualization, Review & editing. G. Z. Khan: Conceptualization, Visualization, Data curation, Review. N Rafiq: Review & editing.

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AVAILABILITY OF DATA AND MATERIAL

The datasets supporting this study are included in the article. Extended methodological details and raw data can be accessed by contacting the corresponding author, subject to ethical and institutional guidelines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study did not involve human subjects or animal models. In accordance with institutional guidelines, ethical approval and informed consent requirements were formally waived for this research.

CONSENT FOR PUBLICATION

I, the undersigned, consent to the publication of my identifiable information.

CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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