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Comparative efficacy of *Catharanthus roseus* and *Phyla nodiflora* weed extracts for the sustainable management of *Culex quinquefasciatus*

Amina Siddiqua[1], Ali Raza[2], Syed Shakeel Shah[3], Hina Kanwal[1], Muhammad Saqlain Zaheer[4*], Kamran Ikram[4], Hafiz Haider Ali[5,6], Muhammad Saleem Kashif[5], Muhammad Waheed Riaz[7], Muhammad Aamir Manzoor[8] and Mohammad Yaqoob Sharafat[9*]

Abstract

Mosquitoes are among the most medically significant insects, causing more human suffering than any other organism by serving as vectors for numerous infectious diseases. This study examined the larvicidal effectiveness and phytochemical composition of aqueous and methanolic leaf extracts from *Catharanthus roseus* and *Phyla nodiflora* in District Narowal, Pakistan. Phytochemical screening revealed the presence of saponins, phenols, flavonoids, diterpenes, proteins, phytosterols, and alkaloids in the extracts. *Culex quinquefasciatus* larvae were used as test subjects for larvicidal activity. The larvae were exposed to *C. roseus* and *P. nodiflora* plant extracts with concentrations of 10, 20, 40, 80, and 160 ppm, and the mortality rate was measured after 24 and 48 h. Aqueous and methanolic extracts of *C. roseus* displayed LC_{50} and LC_{90} values ranging from 0.791 ppm to 83.35 ppm, while those of *P. nodiflora* ranged from 28.83 ppm to 521.3 ppm. Synthetic insecticides Cypermethrin and Deltamethrin were also used for checking resistance levels in *Cx. quinquefasciatus* with concentrations of 0.03125, 0.0625, 0.125, 0.25 and 0.5 ppm from 1 ppm stock solution. After 24 and 48 h, the LC_{50} values of Cypermethrin were 0.107 ppm, 0.051 ppm, and LC_{90} values were 0.707 ppm, 0.245 ppm, respectively. The LC_{50} and LC_{90} values for Deltamethrin were 0.066 ppm and 0.355 ppm after 24 h, and 0.022 ppm and 0.105 ppm after 48 h. The resistance level in *Cx. quinquefasciatus* larvae against synthetic insecticides were very rare in the district Narowal because they cannot be used here frequently, and mosquitoes are not repeatedly exposed to them. This study proved that aqueous and methanolic extracts of *C. roseus* and *P. nodiflora* had phytochemicals and can be used as effective larvicidal bioinsecticides to control mosquito larvae and displayed an effective response toward *Cx. quinquefasciatus* mosquito larvae.

Keywords *Catharanthus roseus*, *Culex quinquefasciatus* larvae, Cypermethrin, Deltamethrin, LC_{50}, LC_{90}, *Phyla nodiflora*

*Correspondence:
Muhammad Saqlain Zaheer
msaqlainzaheer@gmail.com
Mohammad Yaqoob Sharafat
m.yaqoob.sharafat@nu.edu.af

Full list of author information is available at the end of the article

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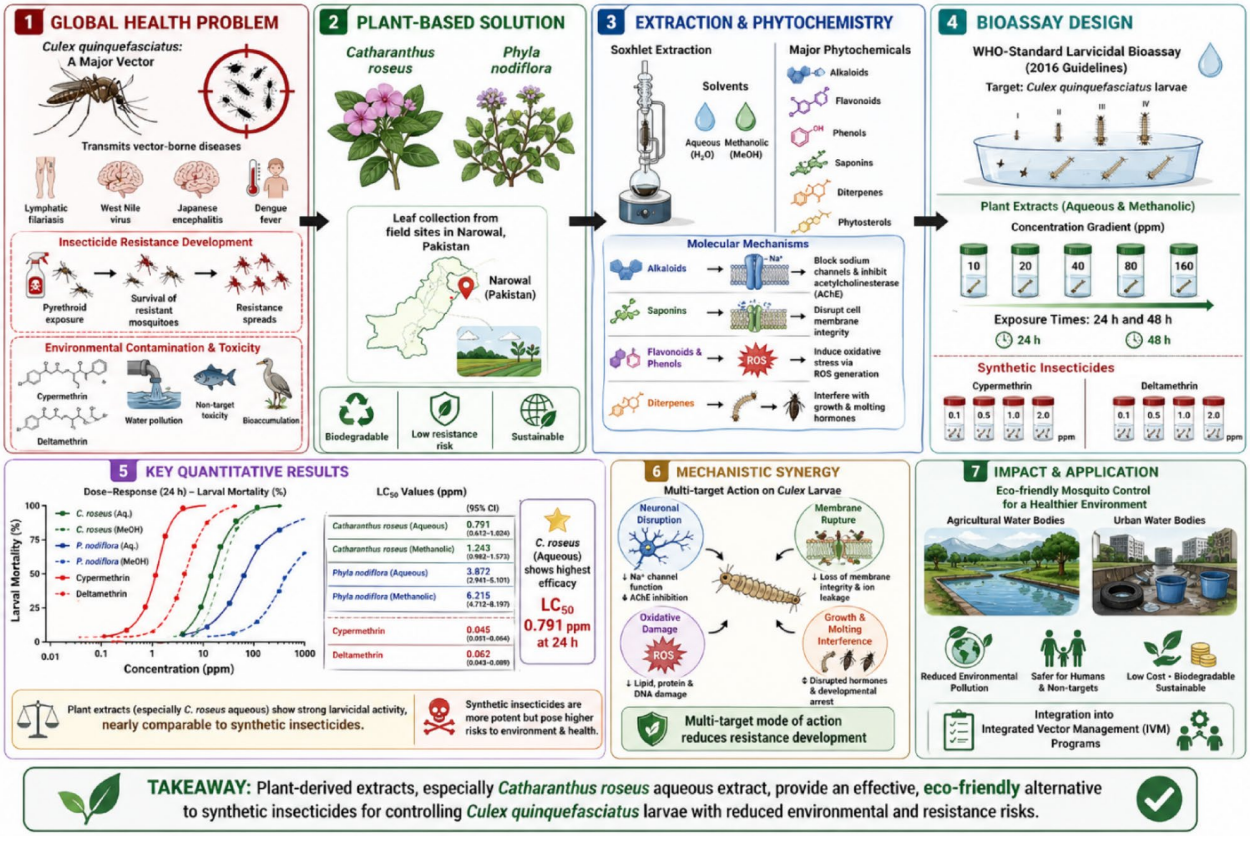
Graphical Abstract



Introduction

About 20% of all infectious diseases affecting people in developing countries are spread by insect vectors (Breedlove and Arguin 2016). Mosquitoes, dipterous insects belonging to the family Culicidae, are the chief disease carriers for a host of parasites (Samidurai 2012). The primary cause of public health issues worldwide is mosquitoes (Becker et al. 2020). Mosquitoes are little dipterous insects from the Culicidae family named little buzzing vampires" and "tiny assassins" (Samidurai 2012). According to a study (Samidurai 2012) this family contains the three genera *Anopheles* (human malaria vector), *Culex* (filarial worm's vector and West Nile virus), and *Aedes* (vector of dengue, yellow fever, chikungunya, and filarial infections) (David et al. 2000). More than a million people die every year because of mosquito-borne infectious diseases (Becker et al. 2020).

Mosquitoes have four stages in their life. They undergo full metamorphosis (Ghosh et al. 2012). It's also noted that the larval stages of mosquitoes are a preferred target due to their little movement during breeding environments and are easy to regulate in their environments (Ghosh et al. 2012). Synthetic pyrethroids such as Cypermethrin and Deltamethrin are commonly used for mosquito control due to their neurotoxic effects on insects. On neuronal membranes, pyrethroids block voltage-gated sodium channels (Krishnappa et al. 2012). This results in ongoing nerve stimulation, paralysis (or knockdown), and ultimately death of the insect (Chrustek et al. 2018). Several studies have used transcriptome sequencing to examine pyrethroid resistance in mosquitoes, including *Culex quinquefasciatus*. Despite their widespread use, synthetic pyrethroids have significant limitations. Unfortunately, due to rapid physiologic resistance development, synthetic pesticides are ineffective against disease vectors and cause long-lasting damage to the environment. Therefore, researchers have shifted their focus toward the creation and application of natural insecticides for the management of mosquitoes (Norris and Norris 2011).

Plants have evolved to produce a variety of chemical defenses against insects and dangerous microbes, and physiologically active chemical substances serve as insect repellents, poisons, feeding inhibitors, and growth regulators (War et al. 2012). An efficient plant-based insecticide can be made from a variety of higher plant components (leaves, roots, stems, seeds, bark, fruits, peels of fruit, etc.), tiny herbs as a whole, or a

combination of different plants (Şengül Demirak and Canpolat 2022). The potencies for the majority of these plants haven't been thoroughly examined for scientific validation, even though these medicinal plants are also identified to be unique, inexpensive, and effective methods of controlling vectors (Mhuji et al. 2017). Many announcements have demonstrated the effectiveness of extracts of the plant as active larvicides and repellents for mosquitoes without causing any effect on human health (Sharma 2018). Insecticides that are derived from plants affect both behavioral and physiological processes, due to which bioinsecticides have a very low possibility of developing resistance against these agents (Ghosh et al. 2012). The larvicidal activity of plant extracts is attributed to various bioactive phytochemicals that act through different mechanisms. Alkaloids are neurotoxins that interfere with sodium channels, acetylcholinesterase, and GABA receptors in insect nervous systems, leading to paralysis and death (Bloomquist 2003). Saponins exert their toxic effects by binding to cholesterol in insect cell membranes, causing pore formation, loss of membrane integrity, and subsequent cell lysis (Shakeel et al. 2025). Flavonoids and phenols induce oxidative stress in mosquito larvae by generating reactive oxygen species (ROS), which damage cellular proteins, lipids, and DNA (Messi et al. 2025). Diterpenes act as growth disruptors and feeding deterrents, interfering with molting and metamorphosis. The presence of multiple phytochemical classes in a single plant extract may produce synergistic effects, enhancing overall larvicidal potency while reducing the likelihood of resistance development (Qasim et al. 2024).

Among the numerous plant species with reported insecticidal properties, two common weeds - *Catharanthus roseus* and *Phyla nodiflora* - have shown promise as larvicidal agents. The recent study is concerned with the larvicidal effectiveness of the two common weed extracts, *Phyla nodiflora* and *Catharanthus roseus* against Culex larvae. It is also used in traditional herbal medicines to treat various diseases (Chettiar Kamatchi and Maheswaran 2016). Therefore, this study deals with evaluating the effects of *Phyla nodiflora* and *Catharanthus roseus* on *Culex quinquefasciatus* mosquito larvae.

Catharanthus roseus is a significant medicinal plant member of the Apocynaceae family; it is a dicotyledonous angiosperm (Guedes et al. 2024). According to (Kumar et al. 2012), white-yellow, white-red, pink-red, pink-white, pink-white and red-white are the five color variants of flowers and can reach a height of 1 m, grouped in opposing pairs, range in shape from oval to oblong, glossy green and hairless with a light midrib. *Phyla nodiflora* is a fast-growing plant included in the family Verbenaceae that is present in subtropical and tropical areas and forms mats on floodplains and in pastures (Chaudhary et al. 2016). The creeping annual plant *P. nodiflora*, also known as *Lippia nodiflora* and also called frog fruit in English and Bukkan in Hindi, grows locally in damp regions (Teoh et al. 2019). *P. nodiflora* collected from forestry places for regional local usage & originated as an astringent, febrifuge, parasiticide, diuretic, and can be used to treat many human diseases (Pham et al. 2024).

The current study was also used to evaluate the resistance of two pyrethroid insecticides (Deltamethrin and Cypermethrin) against mosquitoes (Sathantriphop et al. 2020). Esterase, glutathione S-transferasesand cytochrome P450s (Gan et al. 2021), for instance, have all been thought to have a role in metabolic resistance. Additionally, pyrethroid pesticides alter the physiological makeup of mosquitoes by thickening their epidermis, which inhibits their ability to absorb or penetrate poisons (Asadollahi et al. 2019).

Deltamethrin, a type II pyrethroid, is well-accepted and widely utilized in agriculture and forestry because of its strong action against a wide range of insect pests (Bakry et al. 2011). Preferred IUPAC name of Deltamethrin is ([(S)-cyano-(3-phenoxyphenyl)methyl](1R,3R)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane-1-carboxylate) (Farag et al. 2021). The human neurological system, reproductive system, immunological system, and skeletal muscles had adverse effects from Deltamethrin, which also led to oxidative stress in the body (Chrustek et al. 2018). Cypermethrin, which has the molecular formula C22H19Cl2NO3, is a class II type pyrethroid pesticide that is used in a variety of insecticides as well as other poisonous items.

Materials and methods

Collection and pretreatment of experimental weed plants

Fresh and natural weed plants, *Phyla nodiflora* and *Catharanthus roseus* (Fig. 1), were collected from different sites in the district of Narowal, Pakistan. The leaves of weed plants were separated washed, and then the leaves were shade-dried for a week at room temperature by being spread out on newspaper sheets. When sufficiently dried, crushed using an electric blender (Lazarjani et al. 2021). The plant specimens were identified and authenticated by one of our co-authors and an agronomist, Dr. Hafiz Haider Ali.

Phytochemical extraction and quantification of phytochemicals

The powdered form of *P. nodiflora* and *C. roseus* was used for extraction with distilled water and methanol in a Soxhlet apparatus as described (Redfern et al. 2014). 25 g plant powder was collected in a thimble in 250 ml solvents for 2 to 3 days, then the extractives were filtered with Whatman filter paper NO. 4. The extraction process was repeated three times with fresh solvent each time. The filtrates from the second and third extraction cycles

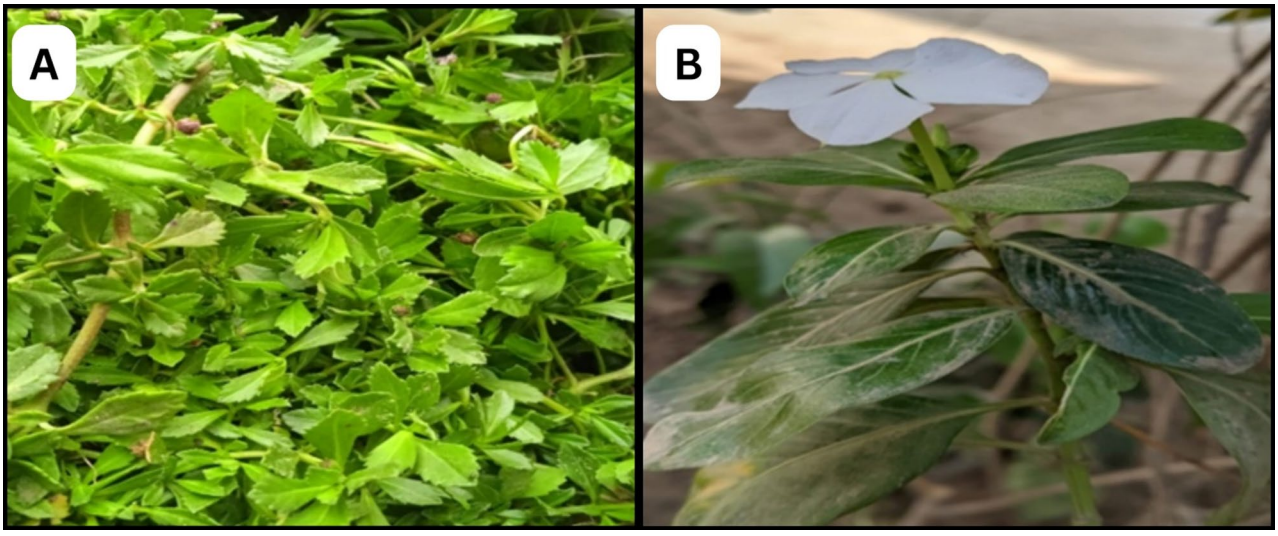


Fig. 1 Pictures of the **A***Phyla nodiflora* and **B***Catharanthus roseus* used in the experiment

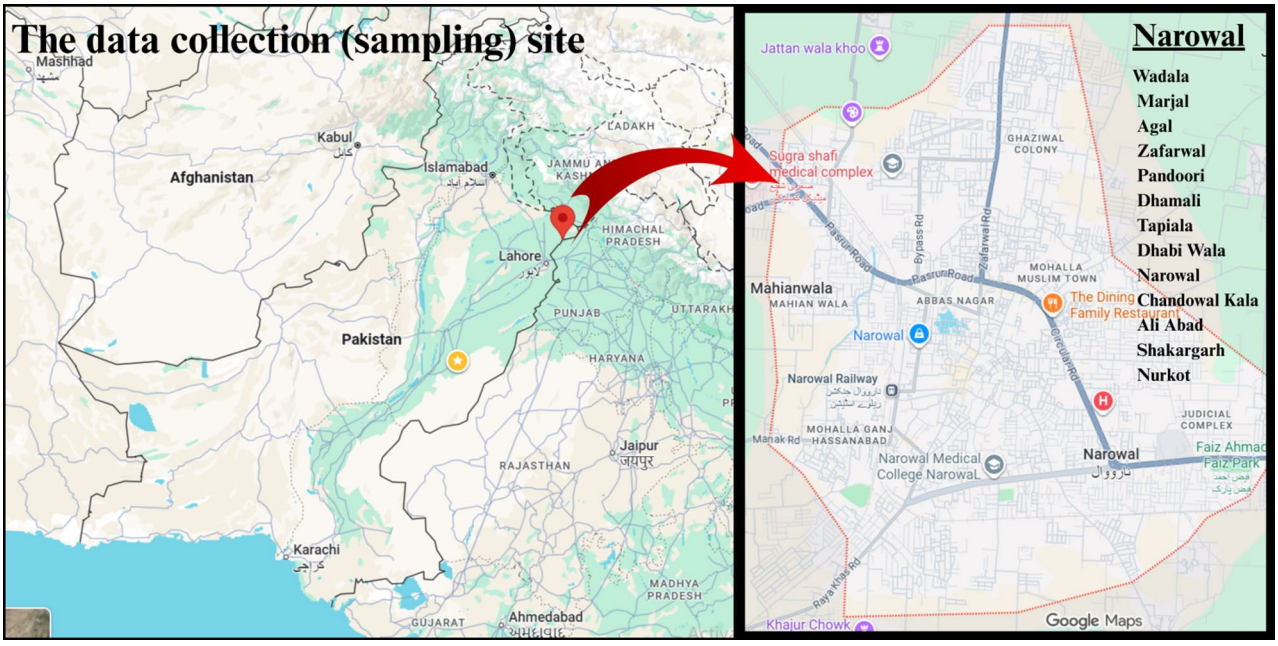


Fig. 2 Area of District Narowal from where Larvae Collected (Sampling site)

were combined with the first filtrate and then evaporated under reduced pressure using a rotary evaporator. Different tests were conducted for the detection of different phytochemicals (carbohydrates, saponins, phytosterols, phenol, flavonoids, proteins, diterpenes, and alkaloids) (Kotpal 2012), .

Collection and rearing of mosquito larvae

Mosquito larvae (*Culex quinquefasciatus* mosquito) were collected from different breeding areas of the district Narowal (Fig. 2). The collection was made from different water-holding places such as sewage ponds, gutters, agricultural lands, etc. The collected larvae were housed at 70–85% humidity and 26 ± 2 °C temperature in the Zoology Laboratory. The larvae were fed cat food in fine powdered form.

Concentrations preparation of plant extracts and synthetic insecticides

Five distinct concentrations, 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm, were prepared for plant extracts; A stock solution of 10,000 ppm (1%) was prepared by dissolving 10 g of plant extract powder in 1 L of distilled water or methanol. From this stock solution, working concentrations of 10, 20, 40, 80, and 160 ppm were prepared by serial dilution. Note that 1 ppm = 1 mg/L. For

synthetic insecticides, Cypermethrin and Deltamethrin were purchased from the local market. A stock solution of 1,000 ppm (0.1%) was prepared by dissolving 1 g of each insecticide in 1 L of distilled water. From this stock solution, five different working concentrations were prepared: 0.5 ppm, 0.25 ppm, 0.125 ppm, 0.0625 ppm, and 0.03125 ppm.

Larvicidal bioassay

The larvicidal bioassay was performed according to the World Health Organization guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005; WHO/CDS/WHOPES/GCDPP/2005.13) For experiment purposes, the collected larvae of mosquitoes were divided into different groups to calculate the mortality level as follows; Groups A and B (the larvae were exposed to different concentrations of aqueous and methanolic *C. roseus* extracts, Group C and D (the larvae were exposed to different concentrations of aqueous and methanolic *P. nodiflora* weed extracts, Group E (this group was exposed to the mixture of both *C. roseus* and *P. nodiflora* weed extracts with methanol and distilled water), and F and G (larvae were exposed to different concentrations of Cypermethrin and Deltamethrin). All the above experiments were repeated three times. Laboratory testing was done to determine whether crude extracts of several plants have larvicidal properties. 25 third and fourth instar larvae replicas were housed in plastic glasses during the bioassay at five different concentrations. After 24 and 48 h, the mortality rate was checked.

Calculations

Percentage mortality was calculated according to the following formulas:

$$\text{Percentage mortality} = (\text{Number of dead larvae/Number of larvae introduced}) \times 100.$$

Abbott's formula was used in cases where the mortality of the non-treated group of larvae is within the following range of 5–20% (Zahoor et al. 2023).

Statistical analysis

To determine the LC_{50} and LC_{90}, mean larval mortality data were submitted to Probit analysis. The standard deviation from the mean of LC_{50} and LC_{90} values was calculated and recorded. An ANOVA test was applied in the SPSS software version 29 to calculate the mortality rate of mosquitoes.

Results

For the detection of phytochemicals in *C. roseus* and *P. nodiflora* extracts, different tests i.e., Molish's, Benedict's, and Fehling's test for carbohydrates; froth and foam test for saponins; Salkowski's test for phytosterol; ferric chloride test for phytosterols; alkaline reagent, and lead acetate test for flavonoids; xanthoproteic test for proteins; lead acetate test for diterpenes; Mayer's, Wagner's, dragendorff's, and Hager's test for alkaloids were applied. The data for phytochemicals in *C. roseus* and *P. nodiflora* revealed that some phytochemicals were identified from aqueous extracts and not from the methanolic extracts, but some were identified in both, and some were not detected in either (Table 1). Tests for *P. nodiflora* phytochemicals revealed that saponins, phytosterols, phenol,

Table 1 Phytochemical detection results for *Phyla nodiflora* and *Catharanthus roseus* weed extracts

Phytochemicals	Tests	Aqueous extract of *Catharanthus roseus*	Methanol extract of *Catharanthus roseus*	Aqueous extract of *Phyla nodiflora*	Methanol extract of *Phyla nodiflora*
Carbohydrates	Molisch's test	-	-	-	-
	Benedict's test	-	-	-	-
	Fehling's test	-	-	-	-
Saponins	Froth test	+	-	+	+
	Foam test	+	-	+	-
Phytosterols	Salkowski's test	-	-	+	-
Phenol	Ferric Chloride test	+	+	+	+
Flavonoids	Alkaline Reagent test	+	+	+	+
	Lead Acetate test	+	-	+	+
Proteins	Xanthoproteic test	-	+	+	+
Diterpenes	Copper Acetate test	+	+	+	+
Alkaloids	Mayer's test	+	-	-	-
	Wagner's test	-	-	-	-
	Dragendorff's test	-	-	-	-
	Hager's test	-	+	-	-

(+) = presence of phytochemicals, (-) = absence of phytochemicals

flavonoids, proteins, and diterpenes were present in aqueous extract (Table 1).

Based on previous phytochemical studies, the alkaloids detected in C. roseus are primarily indole alkaloids, including vincristine ($C_{46}H_{56}N_4O_{10}$), vinblastine ($C_{46}H_{58}N_4O_9$), catharanthine ($C_{21}H_{24}N_2O_2$), vindoline ($C_{25}H_{32}N_2O_6$), ajmalicine ($C_{21}H_{24}N_2O_3$), and serpentine ($C_{21}H_{20}N_2O_3$). These alkaloids are well-documented neurotoxins that target voltage-gated sodium channels and acetylcholinesterase in insects. The flavonoid fraction of C. roseus includes quercetin ($C_{15}H_{10}O_7$), kaempferol ($C_{15}H_{10}O_6$), and catechin ($C_{15}H_{14}O_6$), which exert larvicidal effects through oxidative stress generation. Phenolic compounds such as caffeic acid ($C_9H_8O_4$), ferulic acid ($C_{10}H_{10}O_4$), and chlorogenic acid ($C_{16}H_{18}O_9$) contribute to protein denaturation and enzyme inhibition. For P. nodiflora, the major active compounds include the saponin lippioside A, the flavonoids nodiflorin and quercetin, and the phenylethanoid glycoside acteoside (verbascoside) with the formula $C_{29}H_{36}O_{15}$. While our phytochemical screening confirmed the presence of these compound classes, further high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) analysis is recommended to confirm the presence of these specific compounds in the studied plant populations from District Narowal, Pakistan.

For larvicidal bioassay, *Culex quinquefasciatus* larvae were exposed to 5 different concentrations of *C. roseus* and *P. nodiflora* i.e. 10, 20, 40, 80, and 160 ppm. The larvicidal activity of aqueous (distilled water) and methanolic extracts of the leaves of *C. roseus* and *P. nodiflora* reveals that both extracts were active, having a positive response against *Cx. quinquefasciatus* mosquito larvae. The aqueous extract of *C. roseus* after 24 h showed the highest mortality rate at 160 ppm (97.33%), the mortality rate at 10, 20, 40, 80 ppm, was 78.67%, 93.33%, 94.67%, and 92%, but after 48 h this extract showed highly effective results at every concentration, the mortality rate at 10 ppm was 88%, at 20 ppm was 98.67%, at 40 ppm was 97.33%, at 80 ppm was 98.67%, and at 160 ppm was 98.67%, respectively. By using Probit analysis LC_{50} and LC_{90} were also calculated i.e. LC_{50} was 0.791 ppm and LC_{90} was 30.79 ppm after 24 h, and LC_{50} and LC_{90} values were 0.44 and 13.00 ppm after 48 h, respectively (Table 2).

The results of the methanolic extract of *C. roseus* revealed that with the increase in concentration of plant extracts, the mortality rate also increased. The mortality rate after 24 h at 10, 20, 40, 80, and 160 ppm were 44%, 49.33%, 69.33%, 96%, and 100%, while after 48 h methanolic extract of *C. roseus* showed highly effective results. Probit analysis was also used to calculate LC_{50} and LC_{90} values after 24 and 48 h i.e. LC_{50}=18.59, LC_{90}=83.35 ppm, and 11.8, 57.53 ppm. respectively (Table 3). The aqueous extract of *P. nodiflora* after 24 and 48 h showed highly

Table 2 Efficacy of *Catharanthus roseus* aqueous extract against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Catharanthus roseus with aqueous leaf extracts after 24 and 48 h																	
Extract	Conc. (ppm)	Corrected % Mortality		Effectiveness of plant extract	Mosquito larval mortality (Mean ± SE)		LC_{50} ppm		LC_{90} ppm		95% confidence limits				Regression equation		X^2	
		24 h	48 h		24 h	48 h	24 h	48 h	24 h	48 h	24 h Lower	48 h Lower	24 h Upper	48 h Upper	24 h	48 h	24 h	48 h
Catharanthus roseus	10	78.67	88	Highly effective	19.33±0.33333	20.33±0.88192	0.791	0.44	30.79	13	-0.002	-0.002	0.003	0.003	Y=-0.562+.001X	Y=-0.528+.001X	5.712	3.134
	20	93.33	98.67		23.33±0.33333	23.67±0.33333												
	40	94.67	97.33		23.67±0.88192	24.33±0.33333												
	80	92	98.67		23± 0.57735	24.67±0.33333					-0.666	-0.631	-0.459	-0.426				
	160	97.33	98.67		24.33±0.33333	24.67±0.33333												

Table 3 Efficacy of *Catharanthus roseus* methanolic extract against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Catharanthus roseus with methanol leaf extracts after 24 and 48 h																	
Extract	**Conc. (ppm)**	**Corrected % Mortality**		**Effectiveness of plant extract**	**Mosquito larval mortality (Mean ± SE)**		**LC_{50} ppm**		**LC_{90} ppm**		**95% confidence limits**				**Regression equation**		**X^2**	
		24 h	**48 h**		**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
											Lower	**Lower**	**Upper**	**Upper**				
Catharanthus roseus	10	44	60	Highly effective	11 ± 6.55744	15 ± 4.04145	18.59	11.8	83.35	57.5	0.003	0.000	0.008	0.005	Y=-0.970+.004X	Y=-0.786+.003X	10.401	16.143
	20	49.33	56		12.33 ± 6.08276	14 ± 5.00000												
	40	69.33	90.67		17.33 ± 2.00000	22.67 ± 1.20185												
	80	96	100		24 ± 2.88675	25±0.00000					-1.308	-0.894	-1.067	-0.678				
	160	100	100		25±0.00000	25±0.00000												

effective results at 160 ppm concentration (98.67% and 100%), the mortality rate 24 h at 10, 20, 40, and 80 ppm was 28%, 36%, 41.333%, and 28%, but after 48 h were 32%, 50.67%, 52%, 40%, respectively. By using Probit's analysis, LC_{50} and LC_{90} values were calculated i.e. 61.39 and 521.3 ppm after 24 h and 50.28 and 511.7 ppm after 48 h, respectively (Table 4). The methanolic extract of *P. nodiflora* after 24 h showed highly effective results at 80 and 160 ppm concentration i.e. 100%, which means it is also 100% after 48 h while the mortality rate after 48 h at 10, 20, and 40 ppm was 32%, 48%, and 66.67%. The mean mortality ± SE of these concentrations after 48 h was 5 ± 2.08167, 9.67 ± 5.60753, 15 ± 3.00000, 25±0.00000, and 25±0.00000. By using Probit's analysis, LC_{50} and LC_{90} values were calculated i.e. 28.83 and 80.89 ppm after 24 h and 23.96 and 79.94 ppm after 48 h, respectively (Table 5).

When *Cx. quinquefasciatus* larvae were exposed to the mixture of both aqueous and methanolic extracts of *C. roseus* and *P. nodiflora*, these mixtures of extracts showed highly effective results at 80 and 160 ppm concentrations (94.67% and 100%), while the mortality rate at 10 ppm was 49.33%, at 20 ppm was 66.67%, and at 40 ppm was 36% after 24. After 48 h, results showed an effective mortality rate at all concentrations. The 95% lower and upper confidence limits after 24 huurs and 48 h with X^2 values of 70.903 and 1.809, as shown in Table 6. When larvae were exposed to different concentrations of Cypermethrin then, after 24 h, it showed highly effective results at 0.5 ppm concentration (89.99%), the mortality rate at 0.03125 ppm, 0.0625 ppm, 0.125 ppm, and 0.25 ppm was 32%, 36%, 58.67%, and 72%, respectively. While after 48 h, Cypermethrin showed highly effective results at 0.25 and 0.5 (94.67% and 98.67%) ppm and the mortality rate at 0.03125, 0.0625, and 0.125 ppm was 48%, 60%, and 72%. Probit's analysis was used to calculate LC_{50} and LC_{90} values i.e. 0.107 and 0.707 ppm after 24 h and the LC_{50} value was 0.051 and 0.245 ppm after 48 h, respectively (Table 7).

Deltamethrin after 24 h on *Cx. quinquefasciatus* larvae showed highly effective results at 0.25 and 0.5 ppm concentrations i.e. 86.67% and 92%. The mean mortality ± SE of Deltamethrin after 24 and 48 h. Probit's analysis was also used to calculate LC_{50} and LC_{90} values i.e. 0.066 and 0.355 ppm after 24 h, while 0.022 and 0.105 ppm, respectively. The 95% lower upper confidence limits with x2 values 2.556 and 3 after 24 and 48 h, as shown in Table 8. In the control group, when the absorbance level was checked with UV light in a spectrophotometer, the absorbance level at 190 nm wavelength was − 0.00796 and the absorbance level at 360 nm wavelength was 0.523422, respectively. UV light absorption was measured in a spectrophotometer at 0.0625 ppm of alive *Cx. quinquefasciatus* larvae after exposure to Cypermethrin, the

Table 4 Efficacy of *Phyla nodiflora* aqueous extracts against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Phyla nodiflora with aqueous leaf extracts after 24 and 48 h																			
Extract	Conc. (ppm)	Corrected % Mortality		Effectiveness of plant extract	Mosquito larval mortality (Mean ± SE)		LC_{50} ppm		LC_{90} ppm		95% confidence limits				Regression equation		X^2	
											24 h	48 h	24 h	48 h				
		24 h	48 h		24 h	48 h	24 h	48 h	24 h	48 h	Lower	Lower	Upper	Upper	24 h	48 h	24 h	48 h
Phyla nodiflora	10	28	32	Highly effective	7 ± 2.08167	8 ± 2.00000	61.391	50.28	521.3	512	0.003	0.002	0.008	0.007	Y=-1.393+.005X	Y=-1.241+.005X	69.750	57.688
	20	36	50.67		9 ± 2.64575	12.67 ± 4.33333												
	40	41.33	52		10.33 ± 5.17472	10.67 ± 4.84195												
	80	28	40		7 ± 3.05505	10 ± 3.51188					-1.522	-1.364	-1.264	-1.119				
	160	98.67	100		24.67±0.33333	25±0.00000												

Table 5 Efficacy of *Phyla nodiflora* methanolic extract against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Phyla nodiflora with methanolic leaf extracts after 24 and 48 h																			
Extract	Conc. (ppm)	Corrected % Mortality		Effectiveness of plant extract	Mosquito larval mortality (Mean ± SE)		LC_{50} ppm		LC_{90} ppm		95% confidence limits				Regression equation		X^2	
											24 h	48 h	24 h	48 h				
		24 h	48 h		24 h	48 h	24 h	48 h	24 h	48 h	Lower	Lower	Upper	Upper	24 h	48 h	24 h	48 h
Phyla nodiflora	10	20	32	Highly effective	5 ± 2.08167	8 ± 3.00000	28.829	23.96	80.89	79.9	0.003	0.002	0.008	0.007	Y=-1.188+.006X	Y=-1.043+.005X	8.876	8.411
	20	38.67	48		9.67 ± 5.60753	12 ± 5.77350												
	40	60	66.67		15 ± 3.00000	16.67 ± 2.33333												
	80	100	100		25±0.00000	25±0.00000					-1.308	-1.158	-1.067	-0.928				
	160	100	100		25±0.00000	25±0.00000												

Table 6 Efficacy of *Phyla nodiflora* and *Catharanthus roseus* extracts against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Phyla nodiflora + Catharanthus roseus with methanol & aqueous leaf extracts after 24 and 48 h																			
Extract	**Conc. (ppm)**	**Corrected % Mortality**		**Effectiveness of plant extract**	**Mosquito larval mortality (Mean ± SE)**		**LC_{50} ppm**		**LC_{90} ppm**		**95% confidence limits**				**Regression equation**		**X^2**	
											24 h	**48 h**	**24 h**	**48 h**				
		24 h	**48 h**		**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**Lower**	**Lower**	**Upper**	**Upper**	**24 h**	**48 h**	**24 h**	**48 h**
Phyla nodiflora + *Catharanthus roseus*	10	49.33	94.67	Highly effective	12.33 ± 2.84800	23.667±0.33333	18.203	0	143	0.9	0.001	-0.002	0.006	0.003	Y=-0.984+.004X	Y=-0.456+.000X	70.903	1.809
	20	66.67	100		16.67 ± 5.36449	25±0.00000												
	40	36	98.67		9 ± 1.52753	24.67±0.33333												
	80	94.67	98.67		23.66 ± 1.33333	24.67±0.33333					-1.097	-0.558	-0.871	-0.355				
	160	100	100		25±0.00000	25±0.00000												

Table 7 Efficacy of Cypermethrin against *Culex quinquefasciatus* larvae after 24 and 48 h

The larvicidal efficacy of Cypermethrin after 24 and 48 h																		
Extract	**Conc. (ppm)**	**Corrected % Mortality**		**Effectiveness of plant extract**	**Mosquito larval mortality (Mean ± SE)**		**LC_{50} ppm**		**LC_{90} ppm**		**95% confidence limits**				**Regression equation**		**X^2 (d.f)**	
											24 h	**48 h**	**24 h**	**48 h**				
		24 h	**48 h**		**24 h**	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**	**Lower**	**Lower**	**Upper**	**Upper**	**24 h**	**48 h**	**24 h**	**48 h**
Cypermethrin	0.03125	32	48	Highly effective	8 ± 2.00000	12 ± 1.52753	0.107	0.051	0.707	0.245	0.564	0.255	2.228	1.845	Y=-1.165 + 1.396X	Y=-8.082 + 2.589X	3.199	6.441
	0.0625	36	60		9 ± 2.08167	15 ± 1.52753												
	0.125	58.67	72		14.67 ± 3.38296	18 ± 3.05505												
	0.25	72	94.67		18 ± 4.04145	23.67±0.88192					-1.285	-1.002	-1.045	-0.782				
	0.5	89.33	98.67		22.33 ± 2.66667	24.67±0.33333												

absorbance level was – 0.02591 at 190 nm wavelength and 0.329377 at 360 nm wavelength. The alive *Cx. quinquefasciatus* larvae of 0.5 ppm Cypermethrin were exposed to the UV light in homogenate form, the absorbance level was – 0.00796 at 190 nm wavelength, and the absorbance level was 0.523422 at 350 nm wavelength, respectively. The alive larvae of 0.5 ppm Deltamethrin were exposed to the UV light in the spectrophotometer, the absorbance level at 190 nm wavelength was – 0.16059, and at 360 nm was 0.305696, respectively.

Discussion

It is best to take into account mosquitoes' whole life cycle, beginning with the larval stage, to control them quickly and effectively (Panneerselvam et al. 2013). Additionally, triterpenoids, saponins, and steroids in chloroform, hexane, and methanol extracts, tannins and alkaloids in methanol extracts had demonstrated their toxicity against *Aedes aegypti* and *Culex quinquefasciatus* larvae (Krishnappa et al. 2012). The toxicity of *Cx. quinquefasciatus* larvae in all instars has been documented to be caused by saponins and alkaloids (Gao et al. 2023). In this study, phytochemical screening of *C. roseus* and *P. nodiflora* revealed that saponins, phenol, flavonoids, diterpenes, proteins, phytosterols, and alkaloids were present in them, respectively (Ghosh et al. 2012). After phytochemical examination of the ethanolic extract of *C. roseus*, the presence of alkaloids, tannins, phenols, flavonoids, terpenoids, protein, and quinones was revealed, and the ovicidal activity of particular leaf extracts may have been enhanced due to different phytochemicals (Ghosh et al. 2012).

Aqueous and methanolic extracts of *C. roseus* showed an effective response towards *Cx. quinquefasciatus* mosquito larvae, but the aqueous extract showed more effective results. Subarani et al. (Subarani et al. 2013) reported LC_{50} values of 85.21 mg/mL for *C. roseus* methanolic extract against *Cx. quinquefasciatus*, which is substantially higher than the LC_{50} of 18.59 ppm observed in the present study. This considerable difference suggests that the *C. roseus* population in District Narowal, Pakistan, may contain higher concentrations of bioactive phytochemicals compared to specimens used in previous studiesOur aqueous extract of *C. roseus* demonstrated even greater potency (LC_{50} = 0.791 ppm after 24 h), indicating that polar compounds such as saponins, phenols, and flavonoids—which we detected in our phytochemical screening (Table 1) - are the primary drivers of larvicidal activity. The lower LC_{50} values obtained in this study compared to those reported by Subarani et al. (Subarani et al. 2013) highlight the potential of locally sourced *C. roseus* as an effective and inexpensive bioinsecticide for mosquito control programs. However, both showed more effective results with the aqueous extract of *C. roseus*, respectively. Our aqueous extract of C. roseus (LC_{50} = 0.791 ppm) showed activity against *Cx. quinquefasciatus* that is comparable to or better than the petroleum ether extract reported by Panneerselvam et al. (Panneerselvam et al. 2013) against Anopheles stephensi. This finding is significant because aqueous extraction is simpler, cheaper, and more environmentally friendly than organic solvent-based extraction methods, making it more suitable for community-based mosquito control programs in resource-limited settings.

The strong larvicidal activity of *C. roseus* aqueous extract (LC_{50} = 0.791 ppm) can be directly attributed to its phytochemical composition as detected in Table 1. This extract contained saponins, which likely disrupted the larval gut epithelium and respiratory membranes, leading to osmotic imbalance and cell lysis (Subarani et al. 2013). The presence of alkaloids (confirmed by Mayer's test) suggests neurotoxic activity, as alkaloids are known to block voltage-gated sodium channels in insect neurons, causing hyperexcitation followed by paralysis (Soderlund 2012). Flavonoids and phenols detected in the extract may have contributed through oxidative stress mechanisms, damaging larval tissues via reactive oxygen species generation (Kuljarusnont et al. 2024). The combination of these multiple phytochemicals with different modes of action likely produced a synergistic effect, explaining the exceptionally low LC_{50} value of 0.791 ppm. The moderate larvicidal activity of *P. nodiflora* methanolic extract (LC_{50} = 28.83 ppm) can be explained by its phytochemical profile (Table 1). This extract contained saponins, phenols, flavonoids, diterpenes, and proteins, but lacked alkaloids—a class of compounds known for potent neurotoxicity against insects. The absence of alkaloids likely explains why *P. nodiflora* extracts required higher concentrations to achieve similar mortality compared to *C. roseus*. However, the presence of saponins in *P. nodiflora* methanolic extract (froth test positive) contributed to its larvicidal activity through membrane-disrupting mechanisms. At the same time phenols and flavonoids provided additional oxidative stress-based toxicity. The superior activity of *C. roseus* aqueous extract is therefore attributed to the synergistic action of its phytochemical constituents, particularly alkaloids (neurotoxins), saponins (membrane disruptors), and flavonoids (oxidative stress inducers).

An extensive study of the larvicidal potential of plants in the Apocynaceae family, of which *C. roseus* was a member, was given (Panneerselvam et al. 2013, Subarani et al. 2013). Rajan and Varghese (Rajan and Varghese 2017) reported that exposure to 80 mg/100 mL of *C. roseus* aqueous extract for six hours resulted in 100% mortality of mosquito larvae. In the present study, exposure to 80 ppm (which is 0.008 mg/100 mL) of the same extract achieved 98.67% mortality after 24 h. This indicates that our *C. roseus* extract is approximately 10,000

times more potent on a concentration basis than that used by Rajan and Varghese (Rajan and Varghese 2017). This marked difference in potency may be attributed to several factors: (1) geographical variation in plant secondary metabolite production due to different climatic and soil conditions in District Narowal, Pakistan; (2) differences in extraction methods (Soxhlet extraction in our study versus maceration in their study); or (3) seasonal variation in phytochemical accumulation at the time of collection. The ethanolic leaf extract of *Catharanthus roseus* had LC_{50} values were 150, 145, and 160.57 mg/L against *A. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively (Chettiar Kamatchi and Maheswaran 2016). This study revealed that the LC_{50} value with methanolic extract of *C. roseus* was 18.59 ppm and LC_{90} was 83.35 ppm after 24 h. *A. stephensi* and *Cx. quinquefasciatus* were the larvicidal targets of *C. roseus* crude aqueous, ethyl acetate, and methanolic leaf extract having LC_{50} values were correspondingly 68.62, 82.47, 78.80, 85.21, 76.84, and 94.20 mg/mL (Subarani et al. 2013). Maximum larval mortality against *Cx. quinquefasciatus* larvae were found in the ethyl acetate extract of *Ae. fruticosa*, which had LC_{50} value of 253.08 ppm and LC_{90} value of 455.40 ppm, respectively. It was followed by a hexane extract of *C. roseus*, which had LC_{50} values of 645.33 ppm and 1452.88 ppm (Norris and Norris 2011). After being exposed to aqueous, ethyl acetate, and methanol extracts of *C. roseus* for 24 and 48 h, larval mortality was noted, and the LC_{50} values of these extracts against the fourth-instar larvae of *Cx. quinquefasciatus* were 85.21, 76.84, and 94.20 mg/mL (Subarani et al. 2013).

Irrusappan et al. (Irrusappan et al. 2022) reported LC_{50} and LC_{90} values of 25.80 ppm and 68.45 ppm for *P. nodiflora* leaf extracts against Anopheles stephensi. In the present study, the methanolic extract of *P. nodiflora* showed LC_{50} values of 28.83 ppm (24 h) and 23.96 ppm (48 h) against *Cx. quinquefasciatus*, which are remarkably similar to the values reported by Irrusappan et al. (Irrusappan et al. 2022)despite differences in mosquito species. This consistency suggests that the bioactive compounds in P. nodiflora responsible for larvicidal activity—namely saponins, phenols, flavonoids, and diterpenes (as detected in Table 1)—are equally effective against multiple mosquito genera. However, our aqueous extract of *P. nodiflora* (LC_{50} = 61.39 ppm) was less potent than the methanolic extract, indicating that the active compounds in this species are more efficiently extracted with organic solvents. This finding aligns with the phytochemical profile in Table 1, which shows that several compounds (e.g., phytosterols) were detected only in the methanolic extract of P. nodiflora.Methanol extract of *P. nodiflora* was used against *Ae. albopictus* and *Cx. quinquefasciatus* with LD_{50} and LD90 values of 190.00 ppm, and 301.60 ppm, this showed the lethal dose which kills 50% and 90% larvae and was much higher than the present study LC_{50} and LC_{90} values. After being exposed for 24 h to *Ad. aegypti* larvae, Citrus sinensis peels' hexane extract produced an LC_{50} value of 46.53 ppm, whereas alpha-Cypermethrin treatment produced an LC_{50} value of 0.0063 ppm (Kumar et al. 2016). The synthetic pyrethroids tested in this study—cypermethrin (LC_{50} = 0.107 ppm at 24 h) and deltamethrin (LC_{50} = 0.066 ppm at 24 h)—showed high potency against *Cx. quinquefasciatus* larvae. These values are slightly higher than the 0.0369 ppm reported by Mohan et al. (Mohan et al. 2007) for cypermethrin, suggesting that the *Cx. quinquefasciatus* population in District Narowal remains susceptible to these insecticides. Importantly, the aqueous extract of *C. roseus* (LC_{50} = 0.791 ppm) was only 7- to 12-fold less potent than the synthetic pyrethroids, yet offers significant advantages including environmental biodegradability, lower production costs, and reduced risk of resistance development (Dadzie et al. 2017). Given the well-documented negative effects of synthetic pyrethroids on human neurological, reproductive, and immune systems (Chrustek et al. 2018), *C. roseus* aqueous extract represents a promising, safer alternative for integrated vector management programs. Moreover, alpha-Cypermethrin had an effectiveness with an LC_{50} of 0.0005 ppm (Samidurai 2012), which was much lower than the current study, respectively.

Deltamethrin-related mortality after 24 h of exposure varied from 16.3% in Weija to 82.3% in Kade [45]. While in this study the % mortality is 92% at 0.5 ppm concentration, which was prepared from 1 ppm stock solution of Deltamethrin. According to studies, there is a good chance that the tested mosquitoes will develop resistance to Deltamethrin and Cypermethrin in all of the test locations (Teoh et al. 2019). The specific alkaloids reported in *C. roseus* - particularly catharanthine ($C_{21}H_{24}N_2O_2$), vindoline ($C_{25}H_{32}N_2O_6$), vincristine ($C_{46}H_{56}N_4O_{10}$), and vinblastine ($C_{46}H_{58}N_4O_9$) - act as neurotoxins by inhibiting acetylcholinesterase and disrupting microtubule formation, leading to paralysis and larval death. The flavonoid quercetin ($C_{15}H_{10}O_7$) detected in both plants generates reactive oxygen species causing oxidative tissue damage. For *P. nodiflora*, the saponin lippioside A and phenylethanoid glycoside acteoside ($C_{29}H_{36}O_{15}$) disrupt cell membrane integrity and induce apoptosis (Dadzie et al. 2017). The presence of multiple compounds with different mechanisms supports the synergistic larvicidal activity, particularly for *C. roseus* aqueous extract (LC_{50} = 0.791 ppm). The enzymatic assay of Cypermethrin in the present study represents a 0.329377 value at 360 nm wavelength which was close to the control group value (0.523422) and the value of Deltamethrin was 0.305696 at 360 nm wavelength. Thus, it concluded that the *Cx. quinquefasciatus* larvae were collected from a field area where there was no use of Deltamethrin and Cypermethrin as

insecticides. Thus, mosquitoes haven't developed resistance against these insecticides, respectively.

Conclusion

Under laboratory conditions, the aqueous extract of *Catharanthus roseus* demonstrated the strongest larvicidal activity (LC ~ 50 ~ = 0.791 ppm after 24 h) among all extracts tested against third and fourth instar *Culex quinquefasciatus* larvae. However, this finding requires validation in field settings before recommending *C. roseus* aqueous extract for sustained mosquito control programs. The methanolic extract of *C. roseus* (LC_{50} = 18.59 ppm) and both extracts of *Phyla nodiflora* (LC_{50} = 28.83–61.39 ppm) demonstrated moderate to weak larvicidal activity compared to the aqueous extract of C. roseus. Phytochemical screening revealed that the superior activity of *C. roseus* aqueous extract is associated with the presence of saponins, phenols, flavonoids, diterpenes, and alkaloids. Compared to synthetic pyrethroids (cypermethrin LC_{50} = 0.107 ppm; deltamethrin LC_{50} = 0.066 ppm), *C. roseus* aqueous extract was only 7- to 12-fold less potent but offers significant advantages including environmental biodegradability, low cost, and reduced risk of resistance development. Therefore, *C. roseus* aqueous extract is recommended as an effective, safe, and affordable bioinsecticide for integrated mosquito control programs. Future studies should focus on field evaluation, formulation development, and isolation of the specific bioactive compounds responsible for the observed larvicidal activity.

While this study provides promising laboratory-based evidence, certain limitations should be noted. The bioassays were conducted under controlled conditions that do not fully replicate natural mosquito breeding habitats, and the phytochemical analysis was qualitative without identification of specific active compounds. Additionally, as this study used plant and mosquito populations from a single geographic region (District Narowal, Pakistan) so the present findings should be considered preliminary, and there is need of further research in this topic.

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Data availability
The data will be available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
The collection of *Phyla nodiflora* and *Catharanthus roseus* for this study was conducted in accordance with local and international guidelines. Permissions for the collection of these plant species were obtained from the University of Sialkot, Pakistan. We ensured compliance with all legal and ethical standards for the collection and use of plant materials in scientific research.

Consent for publication
All authors have read and approved the final version of the manuscript and agree to its submission for publication. Furthermore, the authors consent to the publication of the manuscript and all accompanying data in "Biological Research", if accepted.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Zoology, University of Sialkot, Sialkot, Pakistan
[2]Department of Agronomy, University of Sargodha, Sargodha, Pakistan
[3]Department of Zoology, University of Narowal, Narowal, Pakistan
[4]Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
[5]Department of Agriculture, Government College University Lahore, Lahore 54000, Pakistan
[6]Department of Plant Sciences, Aberdeen Research & Extension Center, University of Idaho, Aberdeen, ID, USA
[7]Taizhou Liusui Agricultural Technology Co., Ltd., Taizhou, China
[8]Department of Plant Science, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, P. R. China
[9]Department of Water Resources and Environmental Engineering, Engineering Faculty, University of Nangarhar, Jalalabad, Afghanistan

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