

Comparative Efficacy of Selected Insecticides Against Adult Mosquitoes of Public Health Importance

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Abstract

Insecticide resistance is an increasing challenge to effective mosquito control and poses a serious threat to vector-borne disease management. The present study was conducted during 2024 at the National Agricultural Research Centre (NARC), Islamabad, to assess the susceptibility of selected mosquito populations from Khyber Pakhtunkhwa, Pakistan, to commonly used adulticides. *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* populations were evaluated using WHO diagnostic concentrations of DDT (4%), malathion (5%), and deltamethrin (0.05%). Marked differences in mortality were observed among mosquito populations. *An. stephensi* from Peshawar showed complete susceptibility, with 100% mortality against all three insecticides. Similarly, *Ae. aegypti* from Peshawar exhibited high susceptibility, with mortality ranging from 98% to 100%. In contrast, *An. stephensi* from Charsadda showed substantially reduced mortality to DDT (26.7%), malathion (19.2%), and deltamethrin (79.4%), indicating resistance. *Cx. quinquefasciatus* from Nowshera also demonstrated pronounced resistance to DDT (29.5%) and malathion (4.0%), while deltamethrin produced comparatively higher mortality (84.3%), requiring verification. Overall, the findings demonstrate considerable geographical and species-specific variation in insecticide susceptibility. The observed resistance, particularly to DDT and malathion, highlights the need for continuous insecticide-resistance surveillance, rational insecticide selection, and implementation of resistance-management strategies. These findings provide useful baseline information for improving mosquito-control programmes in Khyber Pakhtunkhwa and support the need for further investigation of the biochemical and molecular mechanisms underlying insecticide resistance.

Keywords: *Anopheles Stephensi*, *Aedes Aegypti*, *Culex Quinquefasciatus*, Insecticide Resistance, DDT, Malathion, Deltamethrin, Mosquito Control, Khyber Pakhtunkhwa

Introduction

Mosquitoes are among the most important vectors of infectious diseases, transmitting malaria, dengue, chikungunya, lymphatic filariasis, and several viral infections. Their widespread

distribution and close association with human settlements make mosquito control an essential component of disease prevention. Chemical insecticides remain one of the most effective tools for reducing mosquito populations, particularly through indoor residual spraying, insecticide-treated nets, and larval control. However, the extensive and repeated use of insecticides has contributed to the development and spread of insecticide resistance in many mosquito populations, reducing the effectiveness of conventional vector-control interventions (WHO, 2002; WHO, 2018). The major classes of insecticides used for mosquito control include organochlorines, organophosphates, carbamates, and pyrethroids. Resistance can develop through several mechanisms, including target-site mutations, increased metabolic detoxification, and reduced insecticide penetration. Resistance to pyrethroids is of particular concern because these compounds are widely used in public-health programs. The increasing prevalence of resistant mosquito populations highlights the need for alternative insecticides with different modes of action and for the continuous monitoring of susceptibility to commonly used compounds (WHO, 2018; Hemingway and Ranson, 2000). Neonicotinoids represent an important group of insecticides that may provide an alternative or complementary option for mosquito control. They act primarily on insect nicotinic acetylcholine receptors (nAChRs), causing persistent stimulation of the nervous system, followed by paralysis and death. Their distinct mode of action compared with pyrethroids, organophosphates, and carbamates makes them potentially useful in insecticide-resistance management programs. Imidacloprid, thiacloprid, and thiamethoxam are among the neonicotinoids that have demonstrated insecticidal activity against different mosquito species (Tomizawa and Casida, 2005; Jeschke and Nauen, 2008). Several studies have demonstrated the potential of neonicotinoids against medically important mosquitoes. Pridgeon et al. reported susceptibility of *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus* to imidacloprid, dinotefuran, and thiamethoxam, indicating that these compounds can affect multiple mosquito taxa. Similarly, Uragayala et al. evaluated imidacloprid, thiacloprid, and thiamethoxam against insecticide-susceptible and resistant mosquito strains and demonstrated their potential as both adulticides and larvicides. The efficacy of neonicotinoids against insecticide-resistant mosquitoes is particularly important because conventional insecticides may provide inadequate control where resistance is widespread. Darriet and Chandre demonstrated that several neonicotinoids were effective against pyrethroid-resistant *Aedes aegypti* and *Anopheles gambiae*, either alone or in combination with other insecticides. Such findings suggest that neonicotinoids may have value in resistance-management strategies, although their effectiveness can vary according to mosquito species, developmental stage, insecticide concentration, and resistance status. In Pakistan, insecticide resistance has increasingly been reported in mosquito populations, creating a need to evaluate alternative insecticides under local conditions. The emergence of resistance in important vectors such as *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* may compromise the effectiveness of existing control programs. Therefore, evaluating the adulticidal and larvicidal activity of neonicotinoids against local mosquito populations is important for identifying effective alternatives and developing evidence-based vector-control strategies (WHO, 2002; WHO, 2018). The present study was therefore undertaken to evaluate the efficacy of selected neonicotinoid insecticides against medically important mosquito species, with particular emphasis on their potential as alternative insecticides for controlling susceptible and resistant mosquito populations. The findings may contribute to improved insecticide-rotation and resistance-management strategies and support the development of more effective mosquito-control programs.

Materials and Methods

Study Site and Study Period

The study was conducted during 2024 at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Laboratory experiments were performed to evaluate the insecticidal efficacy of selected neonicotinoid insecticides against medically important mosquito species. Mosquito colonies were maintained under controlled laboratory conditions, and adulticidal bioassays were conducted following standard mosquito susceptibility-testing procedures.

Mosquito Collection and Identification

Mosquito larvae and/or adults were collected from selected breeding habitats and mosquito-infested areas and transported to the laboratory in suitable containers. Specimens were identified to species level using standard morphological identification keys. The mosquito species included in the study were *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. Following identification, larvae were transferred to clean rearing trays and maintained until adult emergence. Where required, adults were separated according to sex and used for subsequent bioassays.

Mosquito Rearing

Mosquitoes were maintained under laboratory conditions with appropriate temperature, relative humidity, and photoperiod. Larvae were provided with an appropriate larval diet, while adult mosquitoes were supplied with a carbohydrate source. Blood feeding was provided to females when required for colony maintenance. Eggs produced by adult females were collected and used to establish subsequent generations. Care was taken to maintain separate mosquito strains to prevent cross-contamination.

Insecticides

Selected neonicotinoid insecticides, including DDT, malathion, and deltamethrin, were evaluated for their insecticidal activity against the selected mosquito species. Stock solutions were prepared using appropriate solvents, and test concentrations were prepared freshly before each bioassay. The concentrations used were selected on the basis of preliminary tests and/or previously reported effective concentrations.

Adulticidal Bioassay

Adult susceptibility tests were performed using batches of healthy, non-blood-fed adult female mosquitoes of the appropriate age. Groups of mosquitoes were exposed to insecticide-treated surfaces or materials containing the predetermined concentrations of the test insecticides. Corresponding control groups were exposed to untreated materials or the solvent used for insecticide preparation.

Following exposure, mosquitoes were transferred to clean holding containers and provided with access to sugar solution. Mortality was recorded after the predetermined observation period. Mosquitoes were considered dead when they showed no movement or inability to stand or fly normally. Mortality in the treated groups was compared with that of the control groups.

Mortality and Efficacy Assessment

Percentage mortality was calculated using the number of dead mosquitoes relative to the total number exposed. Where mortality occurred in the control group, treatment mortality was corrected using Abbott's formula:

Corrected mortality (%) = $[(T - C) / (100 - C)] \times 100$

where **T** represents the percentage mortality in the treated group and **C** represents the percentage mortality in the control group. When control mortality exceeded the acceptable level, the test was repeated according to standard susceptibility-testing procedures.

Statistical Analysis

The experimental data were entered into a statistical software package and analysed to determine differences in mortality among insecticides, concentrations, and mosquito species. Dose-response relationships were evaluated where multiple concentrations were tested. Results were expressed as mortality percentages and, where appropriate, lethal concentration values with their corresponding confidence intervals. Differences were considered statistically significant at $p < 0.05$.

Quality Control

Appropriate control groups, replicates, and standardized mosquito-handling procedures were used throughout the experiments. All bioassays were performed under comparable laboratory conditions, and insecticide solutions were prepared carefully to ensure consistency among treatments. Mosquito identification and experimental observations were independently checked to minimize identification and recording errors.

Results

Mortality of Mosquito Populations Following Exposure to Diagnostic Concentrations of Insecticides

The susceptibility status of mosquito populations collected from selected areas of Khyber Pakhtunkhwa, Pakistan, was evaluated using the WHO adult mosquito susceptibility test against three commonly used insecticides, namely DDT (4%), malathion (5%) and deltamethrin (0.05%). The mortality response varied considerably among mosquito species and collection areas (Table 1). The *Anopheles stephensi* population from Peshawar demonstrated 100% mortality following exposure to all three insecticides. Mortality was 100% (101 mosquitoes exposed) with DDT, 100% (96 mosquitoes exposed) with malathion, and 100% (99 mosquitoes exposed) with deltamethrin. Based on the susceptibility criteria, the Peshawar population was classified as susceptible (S) to all three insecticides. In contrast, the *An. stephensi* population from Charsadda exhibited considerably lower mortality. Mortality following exposure to DDT was 26.7% (97 mosquitoes exposed), while malathion produced 19.2% mortality (99 mosquitoes exposed). Exposure to deltamethrin resulted in 79.4% mortality (83 mosquitoes exposed). The population was classified as resistant (R) to all three insecticides according to the classification used in the present study. The *Culex quinquefasciatus* population from Nowshera also showed a marked reduction in susceptibility to the tested insecticides. DDT produced 29.5% mortality (98 mosquitoes exposed), whereas malathion resulted in only 4.0% mortality (100 mosquitoes exposed). Both responses were classified as resistant. Deltamethrin produced a comparatively higher mortality of 84.3% (103 mosquitoes exposed); however, this response was categorized as verification required (VR)/tolerant, indicating that further investigation is required to confirm the susceptibility status of this population. The *Aedes aegypti* population from Peshawar showed high susceptibility to all three insecticides. Mortality was 98% (100 mosquitoes exposed) following DDT exposure and 100% (100 mosquitoes exposed) following exposure to both malathion and deltamethrin. The population was consequently classified as susceptible to all three insecticides.

Comparative Mortality among Mosquito Populations

A substantial difference in insecticide-induced mortality was observed between the mosquito populations. The highest mortality was recorded in *An. stephensi* from Peshawar and *Ae. aegypti* from Peshawar, both of which showed 98–100% mortality against the three tested insecticides. Conversely, *An. stephensi* from Charsadda and *Cx. quinquefasciatus* from Nowshera demonstrated substantially lower mortality, particularly following exposure to DDT and malathion. The lowest mortality recorded in the study was 4.0% in *Cx. quinquefasciatus* from Nowshera following malathion exposure, followed by 19.2% mortality in *An. stephensi* from Charsadda following malathion exposure. Deltamethrin generally produced higher mortality than DDT and malathion among the populations showing reduced susceptibility. In *An. stephensi* from Charsadda, deltamethrin produced 79.4% mortality compared with 26.7% for DDT and 19.2% for malathion. Similarly, *Cx. quinquefasciatus* from Nowshera showed 84.3% mortality with deltamethrin compared with 29.5% with DDT and 4.0% with malathion. Overall, the results demonstrate considerable variation in insecticide susceptibility among mosquito populations from different areas of Khyber Pakhtunkhwa. The findings indicate that susceptibility was population-specific, with some populations exhibiting complete susceptibility whereas others demonstrated marked resistance or reduced susceptibility to one or more insecticides.

Summary of Susceptibility Status

Of the four mosquito populations examined, *An. stephensi* from Peshawar and *Ae. aegypti* from Peshawar were susceptible to all three insecticides tested. The *An. stephensi* population from Charsadda showed resistance to DDT, malathion and deltamethrin, while *Cx. quinquefasciatus* from Nowshera showed resistance to DDT and malathion and a verification-required response to deltamethrin. The observed variation highlights the importance of continued insecticide susceptibility surveillance among mosquito populations in Khyber Pakhtunkhwa, particularly where insecticides are used repeatedly for vector control.

Table 1. Mortality of mosquito strains exposed to WHO diagnostic concentrations of selected adulticides

Species and Strain	DDT 4%	Status	Malathion 5%	Status	Deltamethrin	Status
<i>An. Stephensi</i> Peshawar	100% (101)	S	100% (96)	S	100% (99)	S
<i>An. Stephensi</i> Charsadda	26.7% (97)	R	19.2% (99)	R	79.4% (83)	R
<i>Cx. Quinquefasciatus</i> Nowshera	29.5% (98)	R	4.0% (100)	R	84.3% (103)	VR
<i>Ae. Aegypti</i> Peshawar	98% (100)	S	100% (100)	S	100% (100)	S

S = susceptible; R = resistant; VR = verification required/tolerant

Discussion

The present study demonstrated substantial variation in insecticide susceptibility among mosquito populations collected from different areas of Khyber Pakhtunkhwa. *Anopheles stephensi* and *Aedes aegypti* from Peshawar showed high susceptibility, with 98–100% mortality against DDT,

malathion, and deltamethrin. In contrast, *An. stephensi* from Charsadda and *Culex quinquefasciatus* from Nowshera showed marked reductions in susceptibility. Such geographical variation is expected because insecticide resistance can develop under different levels of selection pressure and insecticide exposure. WHO emphasizes that insecticide-resistance monitoring is essential for “selecting appropriate interventions” in mosquito-control programmes (WHO, 2022; WHO, 2018). The *An. stephensi* population from Charsadda showed only 26.7% mortality with DDT and 19.2% with malathion, while deltamethrin produced 79.4% mortality. Similarly, *Cx. quinquefasciatus* from Nowshera showed very low mortality with DDT (29.5%) and malathion (4.0%), whereas deltamethrin produced 84.3% mortality. These findings indicate that susceptibility varied considerably among insecticide classes. Previous research has established that insecticide resistance may involve multiple mechanisms, including “target-site insensitivity” and enhanced metabolic detoxification (Hemingway and Ranson, 2000; Hemingway et al., 2004). However, the mechanisms responsible for resistance in the present populations were not investigated and therefore require further molecular and biochemical studies. The particularly low mortality of *Cx. quinquefasciatus* to malathion indicates a serious reduction in susceptibility to this organophosphate insecticide. Conversely, the relatively higher mortality produced by deltamethrin suggests that this insecticide retained greater activity against the reduced-susceptibility populations tested. Similar variation in insecticide response has been reported in mosquito populations exposed to different insecticide classes (Hemingway and Ranson, 2000; Corbel, Guessan, 2013). Overall, the findings demonstrate population-specific differences in insecticide susceptibility and emphasize the importance of continuous resistance surveillance. Regular monitoring can help identify changes in susceptibility and support appropriate insecticide selection, rotation, and resistance-management strategies in Khyber Pakhtunkhwa (WHO, 2022; WHO, 2018).

Conclusion and Recommendations

The study revealed substantial variation in insecticide susceptibility among mosquito populations in Khyber Pakhtunkhwa. Peshawar populations remained susceptible, whereas Charsadda and Nowshera populations showed resistance, particularly to malathion and DDT. Continuous resistance surveillance, periodic susceptibility testing, and rotation of insecticides are recommended to improve mosquito-control effectiveness and delay resistance development.

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