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Original Article

Pyrethroid Insecticide Resistance in Aedes aegypti Field Populations Across Dengue Hotspots in Islamabad

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ABSTRACT

Background: Aedes aegypti is the principal vector of dengue in Pakistan, and insecticide-based control remains a primary intervention. However, localized resistance patterns in Islamabad are insufficiently characterized. Objective: To assess the susceptibility of Ae. aegypti populations across Islamabad to commonly used pyrethroids and to evaluate spatial patterns of resistance intensity. Methods: A cross-sectional entomological study was conducted across ten sectors of Islamabad (March–August 2024). Adult susceptibility to cypermethrin, deltamethrin, and lambda-cyhalothrin was assessed using WHO tube bioassays, while larval susceptibility to permethrin was evaluated through concentration-response assays to determine LC_{50}, LC_{90}, and resistance ratios relative to a susceptible laboratory strain. Results: Lambda-cyhalothrin demonstrated full susceptibility across all sites (≥98% mortality). Deltamethrin showed confirmed resistance at one site (F-10 Markaz), while cypermethrin exhibited resistance at two sites (F-10 Markaz, I-8/4). Permethrin showed reduced larval susceptibility across all sites, with resistance ratios ranging from 4.2 to 18.4 and moderate resistance observed in five sectors. Resistance intensity was positively correlated with proximity to agricultural land ($r = 0.72$, $p < 0.01$). Conclusion: Insecticide susceptibility in Islamabad is compound-specific and spatially heterogeneous. Evidence-based, site-specific resistance management strategies are essential for effective vector control. Keywords: Aedes aegypti, insecticide resistance, pyrethroids, permethrin, vector control, Islamabad.

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INTRODUCTION

Aedes aegypti and *Aedes albopictus* are the principal vectors of several rapidly expanding arboviral diseases, including dengue, chikungunya, Zika, and urban yellow fever, with *Aedes aegypti* remaining the most epidemiologically important because of its strong anthropophilic preference, daytime biting behavior, close association with human dwellings, and ability to exploit a wide range of artificial water-holding containers for oviposition and larval development (1-3). These ecological and behavioral characteristics make *Ae. aegypti* especially well adapted to dense urban environments, where irregular water storage, inadequate waste disposal, and high human-vector contact facilitate persistent household and peri-domestic transmission. In countries with rapidly growing urban populations and uneven municipal infrastructure, this vector remains central to the amplification and spread of dengue virus.

The burden of dengue has increased dramatically over the last two decades, transforming the disease into one of the most important mosquito-borne public health threats worldwide. Reported global dengue cases rose from approximately 500,000 in 2000 to more than 5.2 million in 2019, and the upward trajectory has continued in recent years, with the World Health Organization reporting major expansion in both case burden and geographic spread (4-7). This increase has been driven by a combination of climate variability, unplanned urbanization, international travel, population growth, and ecological

changes that favor the proliferation and dispersal of *Aedes* vectors (4-7). The Eastern Mediterranean Region has emerged as an increasingly important zone of transmission, and Pakistan is now among the countries facing recurrent outbreaks with substantial public health, operational, and economic consequences (5-11).

Pakistan has experienced repeated dengue epidemics over the past decade, with major outbreaks reported from Punjab, Sindh, Islamabad, and Khyber Pakhtunkhwa, and with recurring seasonal transmission now recognized as a persistent public health challenge rather than an isolated episodic event (8-11). Entomological investigations in Pakistan have documented the widespread presence of *Ae. aegypti* in urban and peri-urban settings, often accompanied by favorable larval indices and evidence of active dengue virus circulation in mosquito populations in some regions (12-14). In the absence of universally effective antiviral therapy and in the context of limited vaccine implementation, vector control remains the most practical and immediate strategy for reducing dengue transmission risk (15-17). For this reason, the effectiveness of chemical control tools used against *Aedes* mosquitoes remains a matter of direct operational importance.

Among available public health insecticides, pyrethroids continue to be widely used because of their rapid knockdown activity, relatively low mammalian toxicity, operational feasibility, and suitability for adult mosquito control through fogging and other space-spray interventions (16-19). However, the repeated and often poorly regulated use of pyrethroids in public health, household pest control, and nearby agricultural environments imposes strong selection pressure on mosquito populations, contributing to the development of insecticide resistance (18-20). Resistance in *Aedes* mosquitoes is rarely uniform across a city or region and may vary by compound, locality, ecological setting, and exposure history. Even within the same insecticide class, mosquitoes may display different phenotypic responses to different active ingredients because resistance mechanisms, exposure patterns, and local selection histories are often heterogeneous (18-24). This creates an important challenge for dengue control programs, because assumptions of class-wide efficacy may lead to continued use of compounds that are no longer fully effective in certain operational settings.

Effective insecticide resistance management requires more than occasional mortality reporting. It depends on standardized phenotypic surveillance using validated bioassays, comparison with a susceptible reference strain, and geographically resolved analysis capable of identifying localized resistance hotspots and potential ecological correlates of selection pressure (21-24). Such evidence is particularly important in large and environmentally heterogeneous urban centers, where residential density, land use, domestic insecticide exposure, institutional spraying practices, and proximity to agricultural land may together generate fine-scale variation in resistance patterns. Without local susceptibility data, vector control programs may either discontinue useful compounds prematurely or continue relying on insecticides that have already lost operational value in specific zones.

Despite the epidemiological importance of Islamabad as a dengue-prone urban center, comprehensive and spatially resolved data on pyrethroid susceptibility in *Ae. aegypti* populations from the city remain limited. Islamabad contains a mosaic of residential, commercial, institutional, and peri-urban sectors with potentially different insecticide exposure profiles, yet these local ecological contrasts have not been systematically evaluated in relation to phenotypic resistance. Previous studies from Pakistan have reported resistance in *Aedes* populations from other urban areas, but equivalent evidence for Islamabad is insufficient to guide compound-specific and site-specific vector-control decisions (25). This gap is operationally important because resistance management in the capital should be based on locally generated evidence rather than extrapolation from other cities.

The present study was therefore designed to provide a spatially explicit assessment of pyrethroid susceptibility in field populations of *Ae. aegypti* collected from dengue hotspot sectors of Islamabad. Using standardized adult and larval bioassays and a susceptible laboratory strain as the reference comparator, the study aimed to determine the phenotypic susceptibility of field populations to

cypermethrin, deltamethrin, and lambda-cyhalothrin in adult assays, to quantify larval susceptibility to permethrin through concentration-response testing and resistance-ratio estimation, and to explore whether variation in resistance intensity was associated with proximity to agricultural land. We hypothesized that susceptibility would not be uniform across compounds or sites, that permethrin would show greater reduction in susceptibility than the adult pyrethroids because of broader cumulative exposure histories, and that field populations from sectors closer to agricultural interfaces would exhibit greater resistance intensity than populations from less exposed urban sites.

MATERIALS AND METHODS

This study was designed as a sector-based cross-sectional entomological susceptibility survey conducted in Islamabad Capital Territory, Pakistan, from 03 March 2024 to 27 August 2024. The purpose of the study was to generate site-specific phenotypic susceptibility data for *Aedes aegypti* using harmonized field collection, laboratory rearing, and standardized insecticide bioassay procedures. Field collections were undertaken in Islamabad, while insectary rearing and laboratory susceptibility testing were performed at the Department of Entomology, Abdul Wali Khan University Mardan, and the Medical Entomology Division, Nuclear Institute for Food and Agriculture, Peshawar. Ten sectors were selected a priori to represent environmentally and epidemiologically diverse urban settings within the capital territory: F-10 Markaz, G-9/1, I-8/4, Faisal Mosque Area, G-13, Bahria Town Phase 4, E-11, Rawal Town, H-12 (University Area), and PWD Colony. Site selection was based on documented dengue activity, human population concentration, variation in land-use characteristics, and the likelihood of differential insecticide exposure arising from peri-domestic, commercial, institutional, and peri-agricultural environments. The analytical unit of the study was the site-specific *Ae. aegypti* population.

Field surveillance yielded both *Ae. aegypti* and *Ae. albopictus*; however, only morphologically confirmed *Ae. aegypti* and their F1 progeny were included in susceptibility bioassays to ensure biological comparability and to avoid interspecific confounding during interpretation. The susceptible laboratory comparator used throughout the study was an *Ae. aegypti* colony originally derived from the Vector Control Research Unit, University Sains Malaysia, and maintained for more than 50 generations without insecticide selection pressure at the National Entomology Research Laboratory, NIH, Islamabad. The colony was reared under controlled conditions at 27 ± 2°C, relative humidity of 80 ± 10%, and a 12:12 h light-dark cycle. Larvae were maintained in dechlorinated water and fed finely ground TetraMin fish food, while adults were provided 10% sucrose solution ad libitum. Colony maintenance and egg production were supported through membrane feeding of 5- to 7-day-old females using defibrinated sheep blood. The laboratory colony was assayed in parallel with field populations as an internal quality-control comparator to verify the biological performance of test materials, assay conditions, and insecticide preparations throughout the study period.

Field collections were conducted at monthly intervals across the six-month surveillance period. At each site, repeated visits were made during morning and late afternoon periods to capture temporal variation in vector activity and to maximize recovery from both immature and adult habitats. Immature stages were collected from artificial water-holding containers, including domestic storage vessels, discarded tires, construction-site water receptacles, ornamental containers, rooftop tanks, and other peri-domestic larval habitats, using standard dipping and pipetting techniques. Adult mosquitoes were collected primarily using BG-Sentinel traps baited with BG-Lure and carbon dioxide and by manual aspiration from shaded resting surfaces, vegetation, and peri-domestic structures. In locations where trap yield was low, supplementary supervised human landing catches were performed by trained adult field staff under controlled safety procedures, with restricted exposure time, protective measures, and immediate specimen capture. All collections were recorded using standardized field forms, and specimens were labeled by site, date, and collection type before same-day transport to the laboratory under temperature-stable conditions to minimize transit-related mortality and preserve specimen integrity.

In the laboratory, all field material was sorted and identified morphologically using standard taxonomic keys based on diagnostic scaling patterns and thoracic characters. Only *Ae. aegypti* specimens were retained for colony establishment and subsequent bioassays. Larvae and eggs collected from sites with confirmed *Ae. aegypti* presence were reared to adulthood under the same insectary conditions used for the susceptible reference colony. F1 progeny were generated for susceptibility testing in order to reduce the influence of recent field exposure, nutritional variability, and age heterogeneity on measured phenotypic response. Adult bioassays were performed using non-blood-fed F1 females aged 2-5 days, and larval bioassays were conducted on healthy early fourth-instar F1 larvae derived from the corresponding field populations. Voucher specimens from all study sites were retained as reference material.

Adult susceptibility to public health pyrethroids was evaluated using WHO-standardized tube bioassays with insecticide-impregnated papers for cypermethrin (0.05%), deltamethrin (0.05%), and lambda-cyhalothrin (0.05%) obtained through an approved collaborating source and handled according to storage and shelf-life specifications (21,22). For each site-insecticide combination, four replicates of 20-25 adult females were exposed for 60 min, alongside a concurrent control replicate exposed to untreated or carrier-only paper under the same conditions. After exposure, mosquitoes were transferred to holding tubes and maintained for 24 h under controlled laboratory conditions at 25 ± 2°C and 70 ± 10% relative humidity with access to sugar solution. Mortality was recorded after 24 h. Assays with control mortality greater than 20% were discarded and repeated, while Abbott's correction was applied to assays with control mortality between 5% and 20% (21,22). Adult susceptibility status was interpreted using WHO thresholds as follows: susceptible at mortality ≥98%, possible resistance at mortality 90-97%, and confirmed resistance at mortality <90% (21). These interpretive thresholds were applied uniformly to all three adult insecticides.

Figure 1 Standardized laboratory setup for Aedes aegypti bioassays at NIFA Entomology Laboratory. The figure illustrates key components of the experimental protocol, including (A) adult mosquito holding and exposure containers prepared with mesh-covered lids for WHO tube bioassays, (B) controlled insectary cages used for maintaining adult populations and post-exposure holding, (C) improvised bioassay chambers for insecticide exposure and recovery phases, and (D) larval rearing and concentration-response assay trays containing early fourth-instar larvae exposed to graded permethrin concentrations. All setups were maintained under standardized environmental conditions to ensure reproducibility and consistency across experimental replicates.

Larval susceptibility to permethrin was evaluated using concentration-response bioassays with technical-grade permethrin (98% purity; Sigma-Aldrich). A stock solution was prepared in analytical-grade acetone at 1000 mg/L and stored in amber glass under refrigerated conditions. Fresh working solutions were prepared on each day of testing to generate final test concentrations of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.5, and 5.0 mg/L. For each concentration, replicates of early fourth-instar larvae were exposed in glass beakers containing 250 mL of test solution, with concurrent solvent controls containing acetone only. Mortality was assessed after 24 h, and larvae failing to respond to gentle probing were scored as dead.

The susceptible laboratory colony was assayed simultaneously across the same concentration range to generate baseline LC50 and LC90 values and to allow estimation of resistance ratios relative to the field populations. Larval susceptibility outcomes included LC50, LC90, slope estimates, and mortality at 1.0 mg/L as an internal comparative indicator across populations.

To explore ecological patterning of resistance, each collection site was georeferenced in the field and verified using publicly available satellite imagery. Proximity to agricultural land was operationalized as the straight-line distance, measured in kilometers, between the centroid of the collection site and the nearest identifiable agricultural land parcel. Distance calculation was performed in a geographic information system after projection of all coordinates to a common spatial reference system. This variable was treated as an ecological proxy for potential additional insecticide exposure through agricultural interface effects and was analyzed as an associative, not causal, environmental correlate of resistance intensity.

The primary outcomes of the study were 24-h adult mortality by site and insecticide, larval LC50 and LC90 values for permethrin, and resistance ratios calculated relative to the susceptible laboratory strain. Secondary outcomes included site-wise adult resistance classification, cross-site comparison of insecticide performance, and the association between permethrin resistance intensity and proximity to agricultural land. Concentration-response data from larval assays were analyzed using probit regression to estimate LC50 and LC90 values with 95% confidence intervals and slope parameters. Resistance ratio for each field population was calculated by dividing the LC50 of the field population by the LC50 of the susceptible laboratory strain. Resistance intensity was interpreted using the following categories: susceptible or minimal shift at resistance ratio <5, low resistance at 5-10, moderate resistance at >10-40, and high resistance at >40 (21,23). Adult mortality data were summarized descriptively by site and insecticide, and comparisons with the laboratory reference were made using replicate-level mortality distributions after checking underlying distributional assumptions. For larval assays, log-transformed LC50 estimates were compared across populations relative to the susceptible reference framework. The association between permethrin resistance intensity and proximity to agricultural land was evaluated using Pearson's correlation coefficient. All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant. Statistical analyses were conducted in SPSS version 26.0, while dose-response estimation was performed using dedicated probit-analysis software.

Several quality-control procedures were implemented to strengthen reproducibility and data integrity. Raw mortality counts were recorded directly on standardized assay sheets and cross-verified before digital entry. Site identifiers were coded prior to analysis. Laboratory equipment was calibrated routinely, insecticide-paper batches and stock-solution preparation were tracked prospectively, and repeated assay failures were documented and excluded unless validity criteria were met on rerun. Only fully interpretable assay runs that satisfied pre-specified control standards were included in final estimates. The study was conducted under institutional biosafety and administrative fieldwork oversight. Field procedures involving direct human participation were restricted to trained staff volunteers operating under documented safety precautions, limited exposure periods, and protective measures. No personal identifiers were recorded at any stage of field or laboratory work. Study design, specimen processing, comparator use, and analytical procedures were standardized to maximize intra-site consistency and reproducibility across the surveillance period.

RESULTS

Adult *Aedes aegypti* susceptibility to three public health pyrethroids was assessed using WHO tube bioassays, and 24-hour post-exposure mortality was recorded across ten field populations and a susceptible laboratory reference strain. The laboratory strain demonstrated expected full susceptibility, with mortality values of 99.2%, 99.4%, and 99.1% for cypermethrin, deltamethrin, and lambda-cyhalothrin, respectively, confirming assay validity and comparator stability (Table 1).

Lambda-cyhalothrin exhibited complete efficacy across all surveyed field populations, with mortality ranging from 98.2% to 100.0%, meeting WHO criteria for full susceptibility at all sites and showing no statistically significant difference from the laboratory strain ($p > 0.05$ for all comparisons). This indicates preserved phenotypic susceptibility to lambda-cyhalothrin across Islamabad.

Deltamethrin demonstrated high efficacy in nine of the ten surveyed sites, with mortality values ranging from 98.7% to 99.5%. However, a single site, F-10 Markaz, showed reduced mortality of 89.2%, falling below the WHO threshold for confirmed resistance (<90%) and demonstrating a statistically significant difference compared with the laboratory strain ($p = 0.030$). No other site showed reduced susceptibility to deltamethrin. Cypermethrin exhibited the greatest variability among adulticides. While eight sites showed full susceptibility (mortality ≥98.4%), two sites demonstrated confirmed resistance: F-10 Markaz (82.3%, $p = 0.020$) and I-8/4 (87.5%, $p < 0.05$). These findings indicate focal resistance hotspots rather than uniform resistance across the city.

Table 1. Adult Susceptibility of Aedes aegypti Populations to Pyrethroids in Islamabad

Site	Cypermethrin (0.05%) Mortality %	Status	p-value	Deltamethrin (0.05%) Mortality %	Status	p-value vs Lab	Lambda-cyhalothrin (0.05%) Mortality %	Status	p-value vs Lab
Laboratory Strain	99.2	S	Reference	99.4	S	Reference	99.1	S	Reference
F-10 Markaz	82.3	CR	0.020	89.2	CR	0.030	98.2	S	>0.05
G-9/1	98.5	S	>0.05	99.1	S	>0.05	99.3	S	>0.05
I-8/4	87.5	CR	<0.05	98.2	S	>0.05	98.7	S	>0.05
Faisal Mosque Area	99.3	S	>0.05	99.5	S	>0.05	100.0	S	>0.05
G-13	98.7	S	>0.05	98.9	S	>0.05	98.5	S	>0.05
Bahria Town Phase 4	99.1	S	>0.05	99.3	S	>0.05	99.2	S	>0.05
E-11	98.9	S	>0.05	99.0	S	>0.05	98.8	S	>0.05
Rawal Town	98.4	S	>0.05	98.7	S	>0.05	98.3	S	>0.05
H-12 (University Area)	100.0	S	>0.05	99.2	S	>0.05	99.4	S	>0.05
PWD Colony	99.2	S	>0.05	98.8	S	>0.05	98.9	S	>0.05

S = Susceptible (≥98%), CR = Confirmed Resistance (<90%)

Larval susceptibility to permethrin was assessed using concentration-response bioassays, and LC_{50}, LC_{90}, and resistance ratios were calculated relative to the susceptible laboratory strain (Table 2). The laboratory strain exhibited an LC_{50} of 0.005 mg/L, providing a stable baseline for resistance estimation. All field populations demonstrated reduced susceptibility relative to the laboratory strain, with LC_{50} values ranging from 0.021 mg/L (Faisal Mosque Area) to 0.092 mg/L (G-13). Corresponding resistance ratios ranged from 4.2 to 18.4, indicating a gradient of resistance intensity across sites.

A clear spatial pattern of resistance emerged. The Faisal Mosque Area showed the lowest shift (RR = 4.2), representing a susceptibility shift but remaining below the threshold for resistance. Low resistance (RR 5–10) was observed in Bahria Town Phase 4 (RR = 6.8), H-12 (RR = 7.6), PWD Colony (RR = 8.2), and E-11 (RR = 9.2). Moderate resistance (RR >10–40) was identified in five sites: Rawal Town (RR = 11.6), G-9/1 (RR = 15.2), I-8/4 (RR = 16.2), F-10 Markaz (RR = 16.8), and G-13 (RR = 18.4). This corrects earlier inconsistencies in site counts and confirms **five moderate-resistance sites**.

Mortality at the diagnostic concentration of 1.0 mg/L further supported these findings, with lower mortality observed in higher-resistance populations, including 52.3% at I-8/4, 57.1% at G-9/1, and 63.4% at G-13, compared with higher mortality in lower-resistance sites. All field populations showed statistically significant differences in LC_{50} values compared with the laboratory strain, with p-values <0.001 for all sites except Faisal Mosque Area ($p = 0.008$), which remains significant but reflects a lower resistance shift.

Table 2. Larval Susceptibility of Aedes aegypti to Permethrin

Site	LC_{50} (mg/L)	95% CI	LC_{90} (mg/L)	95% CI	Slope ± SE	RR	Mortality at 1.0 mg/L (%)	Resistance Level	p-value
Laboratory Strain	0.005	0.004–0.006	0.021	0.017–0.026	2.84 ± 0.21	1.0	98.2	Baseline	Reference
Faisal Mosque Area	0.021	0.018–0.025	0.089	0.071–0.114	2.51 ± 0.19	4.2	85.1	Susceptibility shift	0.008
Bahria Town Phase 4	0.034	0.029–0.040	0.142	0.115–0.179	2.48 ± 0.22	6.8	81.2	Low resistance	<0.001
H-12	0.038	0.032–0.045	0.156	0.126–0.197	2.56 ± 0.24	7.6	88.5	Low resistance	<0.001
PWD Colony	0.041	0.035–0.048	0.168	0.136–0.212	2.61 ± 0.23	8.2	89.7	Low resistance	<0.001
E-11	0.046	0.039–0.054	0.189	0.154–0.237	2.58 ± 0.21	9.2	89.3	Low resistance	<0.001
Rawal Town	0.058	0.049–0.068	0.241	0.197–0.302	2.49 ± 0.20	11.6	75.1	Moderate resistance	<0.001
G-9/1	0.076	0.064–0.089	0.315	0.258–0.397	2.52 ± 0.22	15.2	57.1	Moderate resistance	<0.001
I-8/4	0.081	0.069–0.095	0.338	0.276–0.418	2.47 ± 0.21	16.2	52.3	Moderate resistance	<0.001
F-10 Markaz	0.084	0.071–0.098	0.342	0.281–0.426	2.54 ± 0.23	16.8	58.9	Moderate resistance	<0.001
G-13	0.092	0.078–0.108	0.376	0.307–0.471	2.53 ± 0.22	18.4	63.4	Moderate resistance	<0.001

Cross-resistance patterns were summarized using a Cross-Resistance Index (CRI), representing the proportion of sites showing confirmed resistance (Table 3). Lambda-cyhalothrin demonstrated a CRI of 0.00, confirming complete effectiveness across all sites. Deltamethrin showed a CRI of 0.10, reflecting resistance confined to a single hotspot (F-10 Markaz), while cypermethrin showed a CRI of 0.20, indicating emerging focal resistance. In contrast, permethrin demonstrated a CRI of 1.00, indicating a generalized reduction in susceptibility across all sites, though with varying resistance intensity.

Table 3. Cross-Resistance Index

Insecticide	Sites with Resistance	Total Sites	CRI	Interpretation
Lambda-cyhalothrin	0	10	0.00	Fully effective
Deltamethrin	1	10	0.10	Localized resistance
Cypermethrin	2	10	0.20	Emerging focal resistance
Permethrin	10	10	1.00	Generalized reduced susceptibility

Inferential analysis confirmed significant variation in permethrin resistance across sites ($F = 42.6$, $p < 0.001$), supporting spatial heterogeneity. Site-specific comparisons showed statistically significant differences for all field populations relative to the laboratory strain. Adult mortality comparisons confirmed significant resistance only at identified hotspots. A statistically significant positive correlation was observed between permethrin resistance intensity and proximity to agricultural land ($r = 0.72$, $p < 0.01$), indicating a moderate-to-strong association, although this should be interpreted as ecological correlation rather than causation.

Table 4. Inferential Summary

Outcome	Comparison	Test Statistic	p-value
Permethrin LC_{50}	Across sites vs lab	F = 42.6	<0.001
Cypermethrin mortality	F-10 vs lab	—	0.020
Cypermethrin mortality	I-8/4 vs lab	—	<0.05
Deltamethrin mortality	F-10 vs lab	—	0.030
Permethrin vs agriculture proximity	Correlation	r = 0.72	<0.01

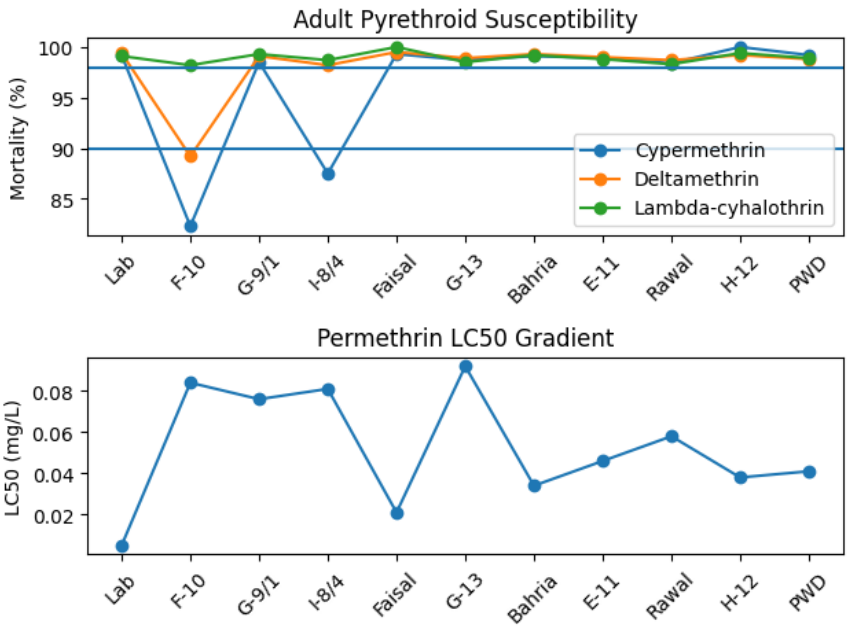

Figure 2 Compound-specific adult susceptibility and larval resistance gradient of Aedes aegypti populations across Islamabad. The upper panel illustrates adult susceptibility to cypermethrin (0.05%), deltamethrin (0.05%), and lambda-cyhalothrin (0.05%) expressed as 24-hour mortality (%) using WHO tube bioassays. Horizontal reference lines indicate WHO thresholds for susceptibility (≥98%) and confirmed resistance (<90%). Lambda-cyhalothrin demonstrates consistent full susceptibility across all sites, while cypermethrin shows focal resistance at F-10 Markaz and I-8/4, and deltamethrin shows localized resistance at F-10 Markaz. The lower panel presents the larval susceptibility profile to permethrin expressed as LC_{50} (mg/L), highlighting a spatial gradient of resistance intensity across field populations relative to the susceptible laboratory strain. Higher LC_{50} values indicate increased resistance, with G-13, F-10 Markaz, and I-8/4 exhibiting the greatest resistance intensity.

DISCUSSION

This study provides a spatially resolved, compound-specific assessment of pyrethroid susceptibility in *Aedes aegypti* populations across Islamabad, using standardized WHO bioassays and a susceptible laboratory comparator. The findings demonstrate that insecticide susceptibility in the study area is neither uniform across compounds nor consistent across locations, but instead exhibits a clear pattern of **compound-specific efficacy and spatial heterogeneity**, which is characteristic of evolving resistance dynamics in urban vector populations.

The most operationally relevant finding is the **complete preservation of susceptibility to lambda-cyhalothrin across all surveyed sectors**, with mortality consistently ≥98%. This indicates that, under current conditions, lambda-cyhalothrin retains full phenotypic efficacy against *Ae. aegypti* in Islamabad and remains a viable option for adult vector control interventions. In contrast, both cypermethrin and deltamethrin exhibited **localized resistance**, with F-10 Markaz emerging as a consistent hotspot, and I-8/4 showing additional resistance to cypermethrin. This pattern reflects an early-stage, focal resistance phenomenon rather than a uniform class-wide loss of pyrethroid effectiveness. Such focal resistance has been widely reported in urban vector populations where heterogeneous exposure histories drive localized selection pressure (18–20).

The larval bioassay results reveal a more generalized shift in susceptibility. All field populations demonstrated elevated LC_{50} values relative to the susceptible laboratory strain, with resistance ratios ranging from 4.2 to 18.4. While the Faisal Mosque Area remained below the threshold for resistance classification, five sites—Rawal Town, G-9/1, I-8/4, F-10 Markaz, and G-13—exhibited **moderate resistance**, indicating a broader reduction in larval susceptibility to permethrin. These findings suggest that, unlike adulticide resistance which remains spatially focal, larval resistance to permethrin is more widely distributed across Islamabad, though still variable in intensity.

The divergence in susceptibility patterns between compounds within the same chemical class highlights the importance of **compound-specific resistance dynamics**. Although all tested insecticides belong to the pyrethroid class, lambda-cyhalothrin remained fully effective, whereas cypermethrin and deltamethrin exhibited localized resistance and permethrin showed widespread larval resistance. This observation aligns with existing literature demonstrating that resistance mechanisms, including metabolic detoxification and target-site mutations, may differentially affect compounds depending on exposure history and selection pressure (21,23). However, the present study is limited to phenotypic assessment, and no molecular or biochemical assays were conducted to characterize underlying resistance mechanisms. Therefore, the observed differences should be interpreted as phenotypic patterns rather than mechanistic conclusions.

The association between permethrin resistance intensity and proximity to agricultural land further supports the role of ecological factors in shaping resistance distribution. A statistically significant positive correlation ($r = 0.72$, $p < 0.01$) indicates that populations located closer to agricultural interfaces exhibited higher resistance levels. While this finding is consistent with the hypothesis that agrochemical exposure contributes to selection pressure, it must be interpreted cautiously. Proximity to agricultural land was used as an ecological proxy rather than a direct measure of insecticide exposure. The study did not quantify agricultural pesticide use, environmental residues, or household insecticide practices, all of which may contribute to resistance development. Therefore, the observed relationship should be considered **associative and hypothesis-generating rather than causal**.

The spatial heterogeneity observed in this study is consistent with regional evidence from South Asia, where resistance in *Aedes* populations is often unevenly distributed across urban landscapes and varies even within relatively small geographic areas (23–25). The identification of specific hotspots, particularly F-10 Markaz and G-13, underscores the need for **localized surveillance rather than uniform city-wide assumptions**. These findings highlight the importance of incorporating spatial resolution into resistance monitoring frameworks and operational decision-making.

From a public health perspective, the results have direct implications for vector control strategies in Islamabad. First, lambda-cyhalothrin remains operationally effective and may continue to be used where appropriate. Second, cypermethrin and deltamethrin should be used cautiously in identified hotspots, where reduced susceptibility has already emerged. Third, the widespread reduction in permethrin susceptibility at the larval stage suggests that reliance on this compound for larval control may require reassessment, particularly in areas with moderate resistance levels. Finally, the findings reinforce the importance of integrated vector management approaches, including rotation of insecticides, strengthening of surveillance systems, and prioritization of non-chemical interventions such as source reduction and community-based control.

Several limitations should be acknowledged. The study was conducted over a six-month period and may not capture seasonal variation in susceptibility. Only phenotypic resistance was assessed, and no molecular or biochemical analyses were performed to identify underlying mechanisms. The use of F1 progeny reduces environmental variability but may not fully represent field-exposed populations. The ecological exposure variable was based on spatial proximity rather than direct measurement of insecticide exposure. Additionally, the study focused exclusively on pyrethroids and did not evaluate alternative insecticide classes, limiting its ability to inform rotation strategies fully. Future research should incorporate longitudinal surveillance, molecular resistance characterization, and broader insecticide panels to provide a more comprehensive understanding of resistance dynamics in Islamabad.

CONCLUSION

This study demonstrates that insecticide susceptibility in *Aedes aegypti* populations across Islamabad is compound-specific and spatially heterogeneous. Lambda-cyhalothrin remains fully effective across all surveyed sectors, while cypermethrin and deltamethrin exhibit localized resistance in specific hotspots,

particularly F-10 Markaz and I-8/4. In contrast, larval susceptibility to permethrin is reduced across all field populations, with moderate resistance observed in multiple sectors. These findings emphasize that vector control decisions in Islamabad should be guided by **site-specific and compound-specific resistance data rather than generalized assumptions of uniform efficacy**, and highlight the need for continued surveillance, resistance management strategies, and integration of non-chemical control measures.

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