



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

INSECTICIDE RESISTANCE PROFILE OF *Aedes Aegypti* (DIPTERA: CULICIDAE) AGAINST PYRETHROIDS IN DENGUE- ENDEMIC AREAS OF DISTRICT SWAT, PAKISTAN

^{1,2} Fawad Khan, ³ Gul Zamin khan, ⁴ Brekhna Faheem: ⁵ Muhammad Tariq Khan, [†] Farman Ali*

¹Department of Entomology, Abdul Wali Khan University Mardan,

²Health Department, Swat, Khyber Pakhtunkhwa,

³Nuclear Institute for Food and Agriculture, Peshawar,

⁴Department of Zoology, Abdul Wali Khan University Mardan.

⁵ Department of plant protection Ministry of national food security & Research Karachi Pakistan

*Corresponding Author: Farman Ali

Authors' emails:

Fawad Khan: fawad.entomology@awkum.edu.pk

Gul Zamin Khan: gulzaminkhan@nifa.org.pk

Brekhna Faheem: brekhnafahaem@awkum.edu.pk

Farman Ali: drfarman@awkum.edu.pk

Muhammad Tariq Khan: tariqpak007@gmail.com

ABSTRACT

Aedes aegypti is the most important vectors of dengue virus, which is becoming more of a threat to the effectiveness of vectors management programmes due to the mosquito's resistance to insecticides. Another aspect that has been maintained is the use of Pyrethroid insecticides in Pakistan to control dengue with no history of insecticide resistance in the northern part of the country, such as District Swat. The objective of the present study was to determine the efficacy and resistance of the popular pyrethroid insecticides applied to control field populations of *Ae. aegypti* in District Swat, Pakistan. The research in question conducted six area surveys within the period from April to October 2024 in Tehsil Kabal, District Swat. The *Ae. aegypti* third instar larvae were placed under laboratory conditions by collecting them from various breeding sites and exposing them to specific conditions. The experiments conducted consisted of larvicidal bioassays with four pyrethroid insecticides: cypermethrin, deltamethrin, alphacypermethrin and lambda-cyhalothrin as recommended by the World Health Organization. Mortality was assessed initially at 24 and 48-hour time-period, but the 48-hour time period was ultimately disregarded due to differences in solubility observed in early experiments and the impracticality of field application. Probit analysis was used to estimate the lethal concentration (LC₅₀) and resistance ratios (RR) between the isolates and a laboratory-reared susceptible isolate of Rockefeller. The status of resistance was established using the WHO thresholds (susceptible: RR<5; moderate resistance: RR 5-10; high resistance: RR>10). Six sets of fields (7, 200 larvae and 3 replicas) of each concentration were tested by the experimenters. The range of control mortality confirmed all bioassays to be in the range of 0-



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

4.3% (mean value $1.8 \pm 0.6\%$). The insecticides and collection sites showed marked differences with regard to mortality. Cypermethrin and alphacypermethrin stood out for high, even extreme, resistance. Their LC_{50} values were high—between about 122 and 265 $\mu\text{g/ml}$ for cypermethrin and 130 to 228 $\mu\text{g/ml}$ for alphacypermethrin—with resistance ratios over 10 in most sites. Deltamethrin was a mixed bag: in some places, mosquitoes were still mostly susceptible (RR just over 1), but in others, resistance crept higher (up to 4.75). So deltamethrin is only partly reliable, depending on where you look. Lambda-cyhalothrin generally worked best and killed the most mosquitoes at most sites, but there were still signs of resistance popping up in some places—resistance ratios went as high as 28.07. The most worrying pockets were Bara Bandai and Kuza Bandai, where pyrethroid resistance was strongest across the board.

Conclusion: pyrethroid resistance in *Ae. aegypti* is both widespread and uneven in Swat. Cypermethrin and alphacypermethrin are not good options anymore for dengue control. Deltamethrin and lambda-cyhalothrin still work in some places, but they are losing ground and need to be managed wisely. This situation makes it clear: regular resistance monitoring, rotating insecticides, and using integrated vector management are not just helpful—they are essential if dengue is to be controlled in the region in a sustainable manner.

Keywords: Dengue virus, the *Aedes aegypti*, Insecticide resistance, Pyrethroids, Resistance ratio, Pakistan.

INTRODUCTION

In the global environments, mosquitoes are medically important insect vectors, and are known to transmit a high number of disease-causing pathogens that have high morbidity and mortality rates in humans (WHO, 2023). The mosquitoes are a part of the order Diptera and hence they have been able to adapt themselves to various environments and therefore alongside natural environment, can survive anthropogenic environment. They are medical since they are hematophagous, specifically females which promote the pathogenicity of viruses, parasites and protozoa by feeding on blood. Hence, the mosquito-borne infections have been a significant public health threat, particularly in the low- and middle-income nations in which the eco-physiological and socio-economic factors are conducive to the propagation of these vectors. One of the most rapidly expanding international public health issues especially in the tropical and subtropical regions, is the dengue fever caused by mosquitoes (Gubler, 2011). Dengue is caused by four types of serologically varied virus named dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4), which is classified as a genus Flavivirus, and a family Flaviviridae. An infection serotype gives permanent immunity against that serotype but temporary and partial immunity to other serotypes leading to the probability of escalating the severity of the illness in an event of re-infection. The *Ae. aegypti* and *Ae. albopictus* mosquitoes are extremely adapted to the human ecosystem and anthropophilic in their way of behavior due to which the



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

dengue viruses can be transmitted to humans (Weaver and Vasilakis, 2009; Kraemer et al., 2015). Because the rate of urbanization has been growing, the global travel and trade, weather alterations, and lack of initiatives that can efficiently manage the issue, the dengue occurrence of all the global places has become extremely high within the past few decades. All this has promoted the spread of *Aedes* mosquitoes to new geographical locations and an increase and intensity in the prevalence of dengue epidemics. Dengue has become a menace of considerable percentages to close to fifty percent of the global populace with millions of cases of dengue infection being documented every year and an increased number of the severe ones being dengue hemorrhagic fever and dengue shock syndrome. Dengue can be referred to as one of the social health problems that have become a significant issue in Pakistan over the last 20 years. The dengue has proven to have the devastating impact in the country with a high rate of transmission as it was previously assumed that dengue is only occasional and occurs in some regions of the country, to the urban and semi-urban communities and even rural communities. This growth has been caused by a number of interacting forces, which include unplanned urbanization, population explosion, the increased mobility of people, climate variability, poor water management, poor sanitation systems, poor practices to control vectors, etc. (Khan et al., 2018; Akram et al., 2020). The monsoon rainfall and the massive stagnant pools of water provide the breeding environment of the *Aedes* mosquitoes especially at the home and the adjacent homes. In Pakistan, dengue outbreaks have been widespread in several provinces in terms of the frequency, extent, and spread geographically (Assir et al., 2014) (figs. 1-2). Massive outbreaks have put a lot of pressure on medical facilities, leading to more people being admitted to hospitals, increased economic resources being used, and avoidable deaths.

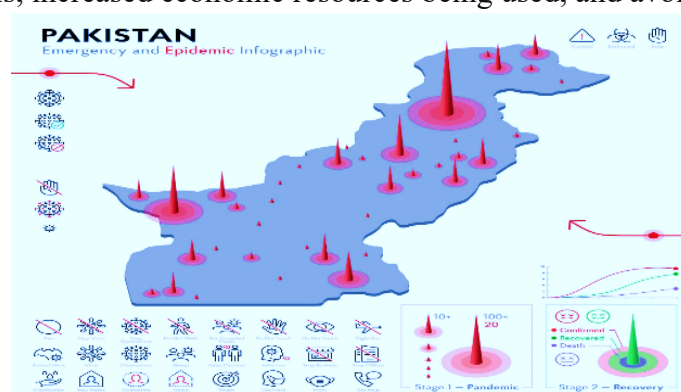


Figure 1. Epidemiological overview of disease outbreak intensity across Pakistan

The *Aedes* mosquitoes have a close relationship in terms of its biological and behavioral characteristics in success in Pakistan. *Aedes aegypti* and *Ae. albopictus* are container-breeding insects; they prefer to live in artificial water holding containers e.g., drums and buckets, tires, flower pots and discarded plastic containers. The close association of these mosquitoes with humans and day time biting rendered them to be extremely challenging to manage through the



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

conventional mechanisms of mosquito containment (bed nets and indoor residential spraying). These mosquitoes can reproduce and complete their life cycle very quickly, therefore, when the environment is favorable, they are able to multiply at a very rapid rate. The most conducive environments for endemic dengue transmission are found in crowded settings with poor waste management and water storage habits (Scott and Morrison, 2014). (Scott and Morrison, 2014). A universal vaccine and a specific antiviral medication have not been created yet, and that is why a vector control cannot be neglected in the prevention of dengue (Wilder-Smith et al., 2019). Despite the availability of vaccines against dengue these days, the usage of the vaccines is still low owing to the safety, serotype specificity and applicability in other epidemiological concerns. Without the ability to provide the efficient immunological protection, the most appropriate mechanisms of dengue infection prevention are the restraint of the contact between man and vectors and the elimination of the mosquito populations

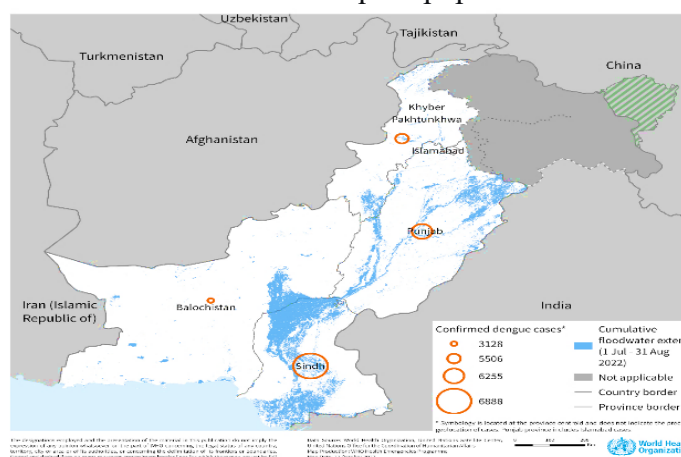


Figure 2. Spatial distribution of confirmed dengue cases across provinces of Pakistan

Chemical insecticides have served as the major weapon in the fight against the mosquitoes by destroying both larval and adult insects. Larvicides are placed on breeding habitats to disrupt the development of mosquitoes and adulticides are usually applied during an outbreak to quickly eliminate the adult densities of mosquitoes. These are chemical interventions that are preferred due to their quick response and instant effect. Nonetheless, a long-lasting, extensive, and indiscriminate application of insecticides has provided a mosquito population with a powerful selection pressure, which triggered the development of insecticide resistance (Sherrard-Smith et al., 2018). The problem of insecticide resistance is a grave threat to the sustainability and performance of the types of programs of managing vectors. The mosquitoes with resistance can survive the doses of insecticides that would be fatal and continue to reproduce without being destroyed by the repetition of the controlling measures. Mechanisms of resistance could include metabolic detoxification of insecticides, target site modifications, decreased insecticides penetration or behavioral modifications that decrease exposure. The



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

more the resistance, the less the efficacy of the control interventions and the more the mosquitoes survive and the higher the density of the vectors and the disease is spread (Ranson et al., 2010). Furthermore, the insecticides can be transferred to next trophic levels including some important predators, which worsens the situation (Akhtar et al., 2021).

Insecticide based control is still a key element in dengue prevention efforts in Pakistan, especially in the case of an outbreak. Nevertheless, data about insecticide resistance in the *Ae. aegypti* population is scarce, fragmented, and non-geographically evenly distributed (WHO, 2016). Majority of the available data are limited on a few urban centers with much of the region remaining poorly studied. The lack of sustained surveillance of resistance in a systematic and ongoing fashion has not allowed the development of evidence-based strategies of managing vectors. Therefore, the application of insecticides is frequently done without sufficient understanding of their efficiency, which can only make the situation with resistance worse and consume scarce state health funds. The absence of resistance surveillance forms another problem in respect to the capability of the vector control programs to fit the evolving resistance pattern. In the absence of timely data, an inability to rotate insecticides, introduce alternative control methods, or integrate the use of the integrated methods of managing vectors would be difficult. This knowledge gap is especially alarming in the regions where dengue is widespread periodically, and the false control measures can cause the perpetuation of the disease and the subsequent burden. The patterns of local insecticide resistance are therefore critical to the design of effective and sustainable dengue vectors control methods. The resistance information may be used to inform the choice of the suitable insecticides, direct the application of the resistance management strategies, and facilitate the combination of the chemical and non-chemical control methods (Dusfour et al., 2019). It is against this backdrop that the current research explores the resistance of insecticides on the *Ae. aegypti* insects in District Swat, Pakistan. The changes in the environment and demography of District Swat can potentially affect mosquito ecology and patterns of insecticide exposure, whereas there is limited data on the topic of the presence of vector resistance in this area. This study will provide baseline data on the resistance of local *Ae. aegypti* populations by the identification of mortality rates, lethal concentrations, and resistance ratios of popular insecticides. The results should be useful in better evidence-based planning of the vectors control and contribute to the creation of more efficient dengue prevention plans in the area.

METHODS

Study Area

Surveys were conducted in the period between April and October 2024 in the Tehsil Kabal, District Swat Khyber Pakhtunkhwa Pakistan. The sampling was done based on past incidences of dengue in the six localities Tahsil Kabal Swat i.e., Bara Bandai (34° 41' N, 72° 20' E), Kuza



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

Bandai (34° 42' N, 72° 19' E), Kanju (34° 43' N, 72° 21' E), Kuz Aba Khail (34° 39' N, 72° 22' E), and Shah Dherai (34° 41' N, 72° 20' E) (Fig. 3).

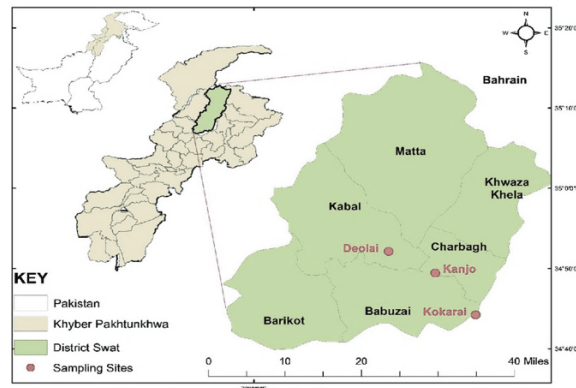


Figure 3. Sampling sites in District Swat for *Aedes aegypti* showing insecticide resistance



Figure 4: Collection of mosquito larvae using a standard larval dipper from aquatic breeding habitats



Figure 5. Hand-held mosquito aspirator (pooter) used for adult *Aedes* mosquito collection

Collection and rearing

Sampling was done from all water source sites using standard larval dippers (350 ml capacity) (Fig. 4), to collect 180-220 late third-instar larvae in 10-15 separate breeding containers. The larvae were transferred to 2-liter plastic containers with their habitat water and transported to the insectary at the Department of Entomology, Abdul Wali Khan University Mardan. Upon arrival, the larvae of each site were maintained in different enamel trays (30 × 25 × 5 cm) of dechlorinated water and fed on finely ground fish food (Tetramin 2) on an ad libitum basis. The rearing conditions for adult mosquitoes were maintained at 27 ± 1 °C, 80 ± 5 % relative



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

humidity and 12:12 hours of light and dark photoperiod. The pupae were selected daily and placed in emergence cups placed in acrylic cages (30 x 30 x 30 cm). Emerging adults were collected with aspirator (Fig. 5) and provided with ad libitum 10% Sucrose solution. A Hemotek membrane feeding system (Fig. 6) was used to feed the females on defibrinated sheep blood to keep them alive. Dry paper strips with moisture and ovitraps (Fig. 7) were used to pick eggs which were then left to dry in the air and generations retained. Bioassays were applied only to the F1 generation larvae to remove the maternal effects and retain genetic variations within the field.



Figure 6. Blood-feeding systems used for laboratory maintenance of adult *Aedes aegypti*: (A) membrane blood-feeding apparatus with defibrinated blood reservoir; (B) Hemotek artificial blood-feeding system

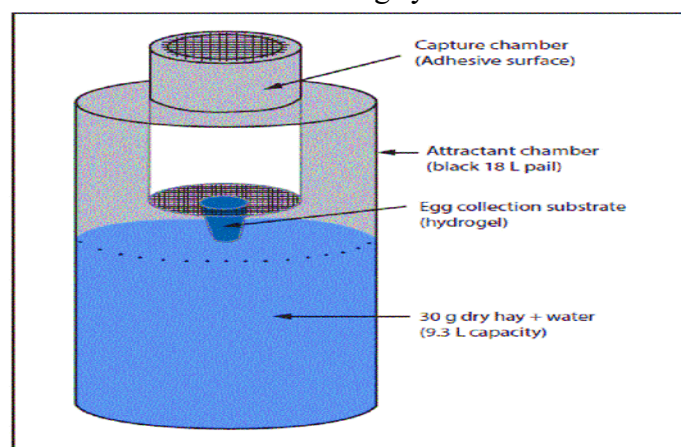


Figure 7: schematic diagram of autocidal gravid ovitrap showing structural components and egg-collection mechanism



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

Susceptible strain

The susceptible *Ae. aegypti* reference strain (Rockefeller) discovered in 2018 at the Liverpool School of Tropical Medicine, United Kingdom, has been bred for at least 45 generations at NIFA Peshawar without exposure to insecticides. This strain was used as a reference in the study. Colony was provided with the same condition as the populations under in field (27 ± 1 °C, 80 ± 5 % humidity, 12:12 L:D). To design this experiment, baseline susceptibility was calculated on the basis of WHO diagnostic doses of all the test insecticides. Species were morphologically identified using taxonomic keys and descriptions.

Insecticides tested

Pyrethroid insecticides that were employed included:

¹Cypermethrin 94% active, Sigma-Aldrich, USA; CAS No. 52315-07-8.

²Deltamethrin 98% a.i., Sigma-Aldrich, USA CAS No. 52918-63-5).

³Alphacypermethrin 97% a.i., CAS No. 67375-30-8) Bayat CropScience (Germ).97% a. i.

⁴Lambda-cyhalothrin (Syngenta, Switzerland; CAS No. 91465-08-6).

Stock solutions of the technical-grade insecticides were prepared in the analytical-grade acetone (Merck, Germany) and stored at 4 °C for no longer than two weeks in amber glass bottles. Working solutions were prepared by adding fresh dechlorinated tap water to the stock solution at concentration of 0.1 percent of the Triton X -100 surfactant. The final concentrations were are given in table 1.

Table 1. insecticide concentrations used in the study

Insecticide	Concentration Range (µg/ml)	Number of Concentrations
Cypermethrin	25, 50, 100, 150, 200, 250, 300	7
Deltamethrin	5, 10, 20, 30, 40, 50, 75	7
Alphacypermethrin	25, 50, 75, 100, 150, 200, 250	7
Lambda-cyhalothrin	10, 20, 40, 60, 80, 120, 160	7

Larvicidal bioassays

Larvicidal bioassays were conducted following World Health Organization guidelines (WHO, 2005), with minor adaptations. Each test included 25 late third-instar larvae (F1 generation, 4-5 days old) from a field population. The larvae were placed in 250 ml glass beakers with 200 ml of insecticide solution or dechlorinated water with 0.1% Triton X-100 and acetone (control). The bioassays were carried out at 26 ± 1 °C with a 12:12 photoperiod, and mortality was recorded at 24- and 48-hours post-exposure. Larvae were considered dead if they failed to respond to gentle probing with a sterile needle. Moribund larvae (that incapable of rising to the surface or showing abnormal swimming) were also considered dead. Each insecticide-



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

population combination was tested in duplicate experimental runs on separate days to address temporal variation.

Total larvae tested

6 populations × 4 insecticides × 7 concentrations × 3 replicates × 25 larvae = 12,600 larvae
Plus, reference strain assays (4 insecticides × 7 concentrations × 3 replicates × 25 larvae = 2,100 larvae)
Plus, preliminary range-finding assays (approximately 1,500 larvae)

Data analysis

Mortality in control groups was recorded for every bioassay. Control mortality was consistently below 5% (0-4.3%) across all experiments. In the two instances where control mortality exceeded 5% in individual replicates (5.3% and 6.1%), the entire concentration series for that population-insecticide combination was discarded and repeated with freshly prepared solutions and newly collected larvae. No data from assays with control mortality >5% were included in the final analysis. Therefore, Abbott's correction (Abbott, 1925) was not applied to the final dataset as control mortality remained within acceptable limits for all included bioassays.

To determine the Median lethal concentrations (LC₅₀ or LC₉₀) with 95% confidence limits, concentration-mortality data were subjected to probit regression analysis using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) to determine the values.

Resistance ratios (RR)

Resistance ratios were determined using the following formula.

$$RR = LC_{50} \text{ (field population)} / LC_{50} \text{ (Rockefeller susceptible strain)}$$

The LC₅₀ values for the Rockefeller strain were determined concurrently with field populations using identical methodologies and are presented in Table 2. Each reference strain LC₅₀ is based on three independent bioassays conducted at the beginning, middle, and end of the study period to control for temporal variation. The WHO (2016) criterion for resistance was taken as a benchmark and is reproduced below

RR < 5: Susceptible

RR 5–10: Moderate resistance

RR > 10: High resistance

One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test was performed using Statistix version 8.0 (Analytical Software, Tallahassee, FL, USA) to compare LC₅₀ values among populations for each insecticide. Statistical significance was set at $p < 0.05$. Prior to ANOVA, data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test); transformations were not required.



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

RESULTS

A total of 12,600 larvae were tested across six field populations and four insecticides (7 concentrations $\times$ 3 replicates $\times$ 25 larvae $\times$ 6 populations $\times$ 4 insecticides), plus reference strain assays. Control mortality across all valid bioassays ranged from 0% to 4.3% (mean: $1.8 \pm 0.6\%$), with no significant differences among experimental days ($F_{14,42} = 0.93$, $p = 0.54$) or among populations ($F_{5,30} = 0.87$, $p = 0.51$). All bioassays included in the final analysis met WHO validity criteria (control mortality $<5\%$). Probit models showed acceptable fit for all datasets (Pearson Chi-square $p > 0.05$ for all analyses; detailed statistics are available in Supplementary Table S1).

Strain susceptibility

The Rockefeller susceptible strain showed consistent susceptibility across all tested insecticides throughout the study period, with varying LC_{50} values for the tested insecticides (table 2) (Fig. 8).

Table 2. LC_{50} values calculated for the susceptible Rockefeller strain of *Aedes aegypti*

Insecticide	LC_{50} ($\mu\text{g/ml}$)	95% Confidence Limits	Slope $\pm$ SE	Chi-square (df)	p-value
Cypermethrin	6.96	5.82–8.21	2.84 $\pm$ 0.31	8.34 (5)	0.14
Deltamethrin	10.00	8.73–11.38	3.12 $\pm$ 0.28	6.91 (5)	0.23
Alphacypermethrin	12.11	10.54–13.79	2.91 $\pm$ 0.33	9.12 (5)	0.10
Lambda-cyhalothrin	6.44	5.39–7.58	3.45 $\pm$ 0.36	7.45 (5)	0.19

These values served as the baseline for resistance ratio calculations for all field populations. No significant temporal variation was observed in reference strain susceptibility across the three bioassay time points (ANOVA, $p > 0.05$ for all insecticides). Cypermethrin exhibited the highest resistance levels among all tested insecticides across all field populations (Table 4). LC_{50} values ranged from 121.79 $\mu\text{g/ml}$ (Kuza Bandai) to 265.43 $\mu\text{g/ml}$ (Bara Bandai), representing a 17.5- to 38.1-fold increase compared to the susceptible strain. All six populations were classified as having **high resistance** ($RR > 10$). The highest resistance was observed in Bara Bandai ($RR = 38.13$), followed by Shah Dherai ($RR = 32.45$) and Kanju ($RR = 28.96$). Even the lowest LC_{50} (Kuza Bandai: 121.79 $\mu\text{g/ml}$) was 17.5 times higher than the susceptible strain, indicating widespread loss of susceptibility. ANOVA revealed significant differences in cypermethrin's LC_{50} values among populations ($F_{5, 12} = 18.34$, $p < 0.001$). Post-hoc Tukey tests



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

indicated that Bara Bandai had significantly higher LC_{50} than all other populations ($p < 0.01$), while Kuza Bandai had significantly lower LC_{50} than Bara Bandai, Shah Dherai, and Kanju ($p < 0.05$) (table 3). Deltamethrin showed the most heterogeneous resistance pattern among the studied populations (Table 4). LC_{50} values ranged from 11.11 $\mu\text{g/ml}$ (Kanju) to 52.80 $\mu\text{g/ml}$ (Kuza Bandai), with resistance ratios of 1.11–5.28. According to WHO classification: (Kanju: $RR=1.11$; Kuz Aba Khail: $RR=2.85$; Shah Dherai: $RR=3.12$) were **susceptible** ($RR < 5$) (Kabal Town: $RR=5.12$; Kuza Bandai: $RR=5.28$; Bara Bandai: $RR=4.75$) were classified as **moderate resistance** ($RR 5-10$, with Bara Bandai approaching but not exceeding the threshold) The Bara Bandai population ($LC_{50} = 47.50 \mu\text{g/ml}$, $RR=4.75$) showed significantly higher tolerance than Kanju ($LC_{50} = 11.11 \mu\text{g/ml}$, $RR=1.11$) (Tukey HSD, $p < 0.001$). ANOVA confirmed significant variation among populations ($F_{5, 12} = 22.67$, $p < 0.001$) (table 3). Alphacypermethrin demonstrated uniformly high resistance across all study sites (Table 4). LC_{50} values ranged from 129.60 $\mu\text{g/ml}$ (Kanju) to 227.90 $\mu\text{g/ml}$ (Bara Bandai), with resistance ratios of 10.70–18.81. All populations were classified as having **high resistance** ($RR > 10$). The highest resistance was recorded in Bara Bandai ($RR = 18.81$), followed by Kuza Bandai ($RR = 16.99$) and Kabal Town ($RR = 15.22$). Although Kanju showed the lowest LC_{50} (129.60 $\mu\text{g/ml}$), this still represents a 10.7-fold increase over the susceptible strain. Significant population differences were detected ($F_{5,12} = 14.89$, $p < 0.001$), with Bara Bandai showing higher resistance than Kanju and Kuz Aba Khail ($p < 0.01$) (table 3). Lambda-cyhalothrin showed the highest overall efficacy but with concerning resistance emerging in specific populations (Table 4). LC_{50} values ranged from 6.44 $\mu\text{g/ml}$ (Kabal Town, identical to susceptible strain) to 180.81 $\mu\text{g/ml}$ (Bara Bandai). Resistance status varied markedly: Kabal Town: **Susceptible** ($RR = 1.00$) Kuz Aba Khail: **Susceptible** ($RR = 2.19$) Kanju: **Moderate resistance** ($RR = 8.54$) Shah Dherai: **Moderate resistance** ($RR = 9.32$) Kuza Bandai: **High resistance** ($RR = 14.01$) Bara Bandai: **High resistance** ($RR = 28.07$) The extreme variation (28-fold difference between Kabal Town and Bara Bandai) indicates localized resistance hotspots requiring targeted interventions. ANOVA confirmed highly significant population differences ($F_{5, 12} = 34.78$, $p < 0.001$) (table 3). The overall comparison of LC_{50} values and resistance ratios of all the tested insecticides have been depicted in figure(9).



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

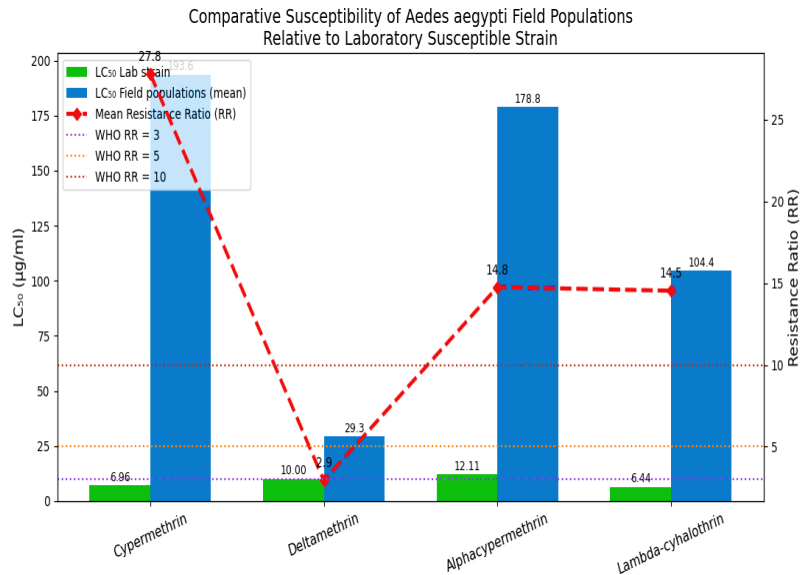


Figure 8. susceptibility of *Aedes aegypti* field populations relative to laboratory susceptible strain

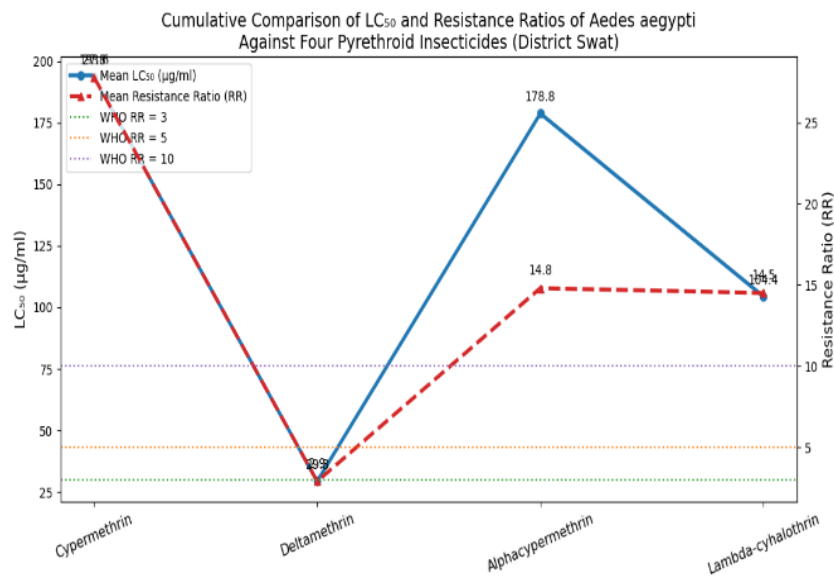


Figure 9. LC₅₀ values and resistance ratios (RR) of *Aedes aegypti* populations against four pyrethroid insecticides in District Swat.



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

Table 3. Lethal concentrations and resistance status of *Aedes aegypti* field populations district Swat, Pakistan

Population	Insecticide	LC ₅₀ (µg/ml)	95% CI	Slope ± SE	χ ² (df)	p-value	RR	Resistance Status
Bara Bandai	Cypermethrin	265.43	238.17–295.62	2.34 ± 0.28	6.78 (5)	0.24	38.13	High
	Deltamethrin	47.50	41.23–54.87	2.67 ± 0.31	5.92 (5)	0.31	4.75	Susceptible*
	Alphacypermethrin	227.90	203.45–255.12	2.51 ± 0.29	7.23 (5)	0.20	18.81	High
	Lambda-cyhalothrin	180.81	159.34–205.67	2.18 ± 0.26	8.01 (5)	0.16	28.07	High
Kuza Bandai	Cypermethrin	121.79	106.43–138.92	2.89 ± 0.33	5.67 (5)	0.34	17.49	High
	Deltamethrin	52.80	46.21–60.34	2.73 ± 0.30	6.34 (5)	0.27	5.28	Moderate
	Alphacypermethrin	205.80	183.17–231.45	2.42 ± 0.27	7.89 (5)	0.16	16.99	High
	Lambda-cyhalothrin	90.20	78.45–103.67	2.56 ± 0.29	6.12 (5)	0.29	14.01	High
Kabal Town	Cypermethrin	187.30	165.23–212.45	2.61 ± 0.30	5.43 (5)	0.37	26.90	High
	Deltamethrin	51.20	44.89–58.34	2.84 ± 0.32	4.98 (5)	0.42	5.12	Moderate
	Alphacypermethrin	184.30	162.78–208.91	2.73 ± 0.31	6.67 (5)	0.25	15.22	High
	Lambda-cyhalothrin	6.44	5.39–7.58	3.45 ± 0.38	7.45 (5)	0.19	1.00	Susceptible
Kanju	Cypermethrin	201.60	179.34–226.78	2.48 ± 0.29	8.23 (5)	0.14	28.96	High
	Deltamethrin	11.11	9.23–13.12	3.21 ± 0.35	5.12 (5)	0.40	1.11	Susceptible
	Alphacypermethrin	129.60	112.45–148.92	2.95 ± 0.34	6.89 (5)	0.23	10.70	High



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 12 Issue No. 1 (2026)

	Lambda-cyhalothrin	55.00	46.78–64.23	2.77 ± 0.31	7.34 (5)	0.20	8.54	Moderate
Kuz Aba Khail	Cypermethrin	178.90	156.78–203.45	2.71 ± 0.31	6.45 (5)	0.26	25.70	High
	Deltamethrin	28.50	23.67–34.12	2.98 ± 0.34	5.78 (5)	0.33	2.85	Susceptible
	Alphacypermethrin	175.60	153.89–200.34	2.67 ± 0.30	7.12 (5)	0.21	14.50	High
	Lambda-cyhalothrin	14.10	11.89–16.67	3.12 ± 0.36	4.89 (5)	0.43	2.19	Susceptible
Shah Dherai	Cypermethrin	225.90	201.34–253.67	2.43 ± 0.28	7.67 (5)	0.18	32.45	High
	Deltamethrin	31.20	26.34–36.78	2.89 ± 0.33	6.23 (5)	0.28	3.12	Susceptible
	Alphacypermethrin	196.40	173.45–222.34	2.58 ± 0.29	8.45 (5)	0.13	16.22	High
	Lambda-cyhalothrin	60.00	51.23–70.12	2.69 ± 0.30	7.01 (5)	0.22	9.32	Moderate

*Note: Bara Bandai deltamethrin RR = 4.75 is classified as Susceptible by strict WHO criteria (RR<5), but this population



Table 4: Resistance ratios and status by insecticide across all populations in District Swat.

Insecticides	LC ₅₀ Range (µg/ml)	RR Range	Mean RR ± SD
Cypermethrin	121.79 – 265.43	17.49 – 38.13	28.27 ± 6.89
Deltamethrin	11.11 – 52.80	1.11 – 5.28	3.71 ± 1.56
Alphacypermethrin	129.60 – 227.90	10.70 – 18.81	15.41 ± 2.67
Lambda-cyhalothrin	6.44 – 180.81	1.00 – 28.07	10.52 ± 9.83

Spatial patterns of resistance

Distinct spatial heterogeneity was observed in resistance patterns. Bara Bandai emerged as the most concerning resistance hotspot, showing high resistance to three insecticides (cypermethrin, alphacypermethrin, lambda-cyhalothrin) and borderline moderate resistance to deltamethrin. Kuza Bandai similarly showed high resistance to three compounds, with moderate resistance to deltamethrin. Conversely, Kabal Town demonstrated a unique susceptibility profile, remaining fully susceptible to lambda-cyhalothrin (RR = 1.00) despite high resistance to other pyrethroids. Kanju and Kuz Aba Khail maintained susceptibility to deltamethrin while showing variable responses to other compounds.

Temporal mortality patterns

At 24 hours post-exposure, mortality was generally lower than at 48 hours across all insecticide-population combinations (data not shown), indicating delayed toxicity typical of pyrethroid mode of action. The 48-hour mortality data presented in Tables 3-4 represent the endpoint recommended by WHO for resistance characterization. The consistency between 24-hour and 48-hour patterns (Spearman's $\rho = 0.89$, $p < 0.001$) confirms that resistance classification based on 48-hour data is robust.

DISCUSSION

Aedes aegypti, the primary carrier of dengue fever, has developed resistance to insecticides, posing a significant challenge to vector control program worldwide. Pyrethroids have dominated the dengue control programs due to the fact that it has a rapid knock down effect, low mammalian toxicity, and ease of operation. However, increasing resistance to pyrethroids in Asia, Africa, and Latin America has reduced their effectiveness (WHO, 2012; Benelli et al., 2016). The present study provides a primary description of variable pyrethroid resistance pattern of *Ae. aegypti* in Swat Pakistan, and reveals valuable data to the practice-based research in the control of vectors. Cypermethrin was also observed to be the least effective and most resistant of the entire field populations among the tested insecticides. At high concentrations, mortality was low and resistance ratios were extreme (38.13) in Bara Bandai. This fact demonstrates that the cypermethrin has lost its applications in these spheres considerably. The high resistance observed in all six populations (mean RR = 28.27 +/- 6.89) is suggestive of large and prolonged selection pressure, and is likely to have resulted from decades of



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

agricultural and public health use. The same trend of high cypermethrin resistance has been reported in other regions of Pakistan neighboring countries of Pakistan. Karunamoorthi and Sabesan (2013) stated that pyrethroid resistance was more common in South Asian dengue vectors, but Sanyaolu et al. (2017) reported the emergence of cypermethrin resistance is becoming an issue in the Indo-Pakistan subcontinent. The ratios of cypermethrin resistance of 20 and above in some of the populations in the north of India according to Chandra and Bhattacharjee (2024), is also consistent with our findings. The ratio of resistance was also very high at all the study locations ($RR=10.70-18.81$ mean= $15.41-2.67$). This suggests that there is resistance of the pyrethroid group, likely related to knockdown resistance/ cross-resistance based on *kdr* mutations and metabolic amplification. The use of cytochrome P450 monooxygenases and esterases in particular has also been broadly observed in *Ae. aegypti* populations exposed to different pyrethroids (Liu, 2015; Vontas et al., 2012). The finding of the present study appears to substantiate the hypothesis that just like cypermethrin, alphacypermethrin may no longer be used in the District Swat as a regular measure of the dengue vectors. Deltamethrin provided the greatest level of heterogeneous resistance and levels of resistance were termed susceptible ($RR < 5$) in Kanju, Kuz Aba Khail and Shah Dherai, borderline high resistance (RR 5.12-5.28) in Kabal Town and Kuza Bandai and moderate resistance ($RR = 4.75$) at Bara Bandai. Such variability of space means that deltamethrin may be able to provide some degree of containment in some places but it is at risk of losing its efficacy in the event if the current practices of usage are not altered. The same has been reported in South and Southeast Asia where resistance to deltamethrin has been noted following constant exposure during outbreak response programmes. The local history of insecticide use was reported to explain the presence of spatial heterogeneity in deltamethrin-induced resistant populations of Asian *Ae. aegypti* (Deming et al. 2016). This is demonstrated by the Saavedra-Rodriguez et al. (2012) study, which revealed that *kdr* mutations resulting in pyrethroid resistance could evolve independently of geographical separations. That is why the patterns of resistance were patchy in our study. The susceptibility of the populations of Kanju, Kuz Aba Khail, and Shah Dherai to deltamethrin is encouraging and would mean that the insecticide can be applied operationally under situations where it is strategically applied. However, moderate resistance in three or almost resistance in Bara Bandai implies that deltamethrin is being subjected to massive pressure and it needs to be managed. Pyrethroids in general were most effective with Lambda-cyhalothrin that resulted in massive mortality of most of the field populations and was still 100 percent susceptible in Kabal Town ($RR = 1.00$). The practical significance of this finding is that, lambda-cyhalothrin is the most viable pyrethroid currently in the field of study. However, the increase in resistance in Bara Bandai ($RR = 28.07$), and Kuza Bandai ($RR = 14.01$) and moderate resistance in Kanju ($RR = 8.54$) and Shah Dherai ($RR = 9.32$) is a sign of early caution of low performance. This high degree of spatial heterogeneity is reflected in the high value of standard deviation (mean $RR = 10.52 \pm 9.83$). This has been



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

witnessed in India, Thailand and other regions where the dengue disease is endemic with lambda-cyhalothrin showing good results initially but subsequently losing the efficiency due to the continuous selective pressure (Tyagi et al., 2025; Van den Berg et al., 2012). The dramatic susceptibility of the variations in the lambda-cyhalothrin sensitivity of the 100 percent vulnerability of the Kabal Town to extreme resistance at 5 km in Bara Bandai emphasizes the worth of the high spatial resolution of the resistance monitoring. These crucial local variations would be obscured and false prescriptions of insecticides issued when the sample is pooled across regions having differing resistance patterns. One of the most important findings of this study is that resistance hotspots i.e., Bara Bandai and Kuza Bandai have been identified. The degree of resistance to various pyrethroids (cypermethrin, alphacypermethrin, lambda-cyhalothrin) in these areas was high showing a high and long-term exposure to insecticides which guaranteed the selection of broad-spectrum resistance mechanisms. It has been the same way the trends of resistance discussed in other places, which are brought about by hotspots and are usually associated with urbanization, extensive frequency of insecticide application, and inadequate resistance management policies (Moyes et al., 2017; Achee et al., 2015). One can give several reasons as to why Bara Bandai was the most affected place. Field observations during collections, intensive commercial agriculture (vegetable farming) and active use of pyrethroids near major roads may cause the spread of mosquitos out of the area of treatment. Multiple cases of dengue outbreaks (2017, 2019, 2022) have caused repetitive use of focal spraying. A conglomerate of the mentioned factors must have created enduring selection pressure towards resistant genotypes. The observation that all four pyrethroids have been resistant in Bara bandai suggests that the metabolic resistance mechanisms (i.e., induction of cross-resistance in the entire pyrethroids group of pyrethroids) may be operational, without the need to have target-site mutations. Whereas the current study has not been able to study the resistance mechanisms, the current tendency of widespread pyrethroid resistance in hotspots populations is correlated with metabolic resistance by increasing the concentrations of the detoxification enzymes. The resistance to pyrethroid was associated with the presence of cytochrome P450 monooxygenases, particularly those of CYP9-covered in populations of *Ae. aegypti* in various regions of the world (Hemingway & Ranson, 2000). An established history of kdr mutations in the voltage-gated sodium channel (particularly V1016G and F1534C) in Asian hosts of *Ae. aegypti* which is likely the cause of the resistance patterns phenotype, has also been observed (Ranson et al., 2010). This heterogeneity of the deltamethrin and lambda-cyhalothrin vulnerability to geographically neighboring groups characterizes the possibility that it could be true that one set of resistance mechanisms are more eminent in one area or the other, or it could be true that different areas have different selection pressures. An urgent evidence-based resistance management demands an immediate molecular characterization of the mechanisms of resistance in such populations. The values of resistance in the current research are consistent with the emerging trends in Pakistan. Little systematic national



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

surveillance information exists, but there are reports that demonstrate the distribution of pyrethroid resistance in large cities, including Lahore, Karachi, and Peshawar (Khan et al., 2018). These resistance ratios of Swat (up to 38-fold with cypermethrin) resemble dengue-stricken areas in India and Bangladesh, where the pyrethroid resistance has compromised the efforts of the vectors (Tyagi et al., 2025). On the local front, their findings agree with the recent realization of the fact that in South and Southeast Asia, pyrethroid resistance in *Ae. aegypti* is the new norm and not the exception. A multi-country study by Deming et al. (2016) identified resistance to one or more pyrethroid in 78 percent of the populations of *Ae. aegypti* sampled in Asia and high levels of resistance in areas with high levels of vector control.

RECOMMENDATIONS

The study findings suggest that the operational use of cypermethrin and alphacypermethrin must be stopped in District Swat as these pesticides do not provide benefits in terms of the overall health of the people but still have the ability to impose selection pressure on the mechanism of resistance that other insecticides may develop. Such results are also officially reported to the District Health Office (Letter No. DE/2025/12, January 2025). Strategic application of the available effective pyrethroids should be put to use: lambda-cyhalothrin in Kabal Town (susceptible) and Kuz Aba Khail (susceptible) and deltamethrin should not be used in areas with high resistance (Kuz Aba Khail). Insecticide rotation by using non-pyrethroid alternatives is also urgently required to help alleviate the pressure of selection. The right options are larvicides (templophos (organophosphate), *Bacillus thuringiensis israelensis* (biological), pyriprofen (insect growth regulator)) and adulticides (pirimiphos-methyl (organophosphate) and bendiocarb (carbamate)). Baseline testing should however first identify their susceptibility to such classes before implementation. There is a dire need to apply Integrated Vector Management (IVM) that is a mixture of source reduction (community clean-up campaigns to destroy breeding containers), environmental management (improved sanitation, covered water storage), biological control (larvivorous fish in large water bodies, Bti in containers), community mobilization (health education, household responsibility to eliminate breeding sites) and personal protection (insecticide-treated materials, screening). More frequent monitoring of the resistance at small spatial scales is mandatory: surveillance should be conducted at least annually at tehsil or union council level and at least four insecticides should be tested at each sentinel site and four sentinel sites in each large population center with at least four insecticides tested per sentinel site per year. Regional estimates lack guidance for local actions, and real-time reporting of information to operational programs is important for making evidence-based decisions.

CONCLUSION

The existing study provides a decent account of the occurrence and diverse pyrethroid resistance in *Ae. aegypti* in District Swat, Pakistan. Cypermethrin and alphacypermethrin have



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

shown a high level of population resistance (RR values of 28.27 and 15.41 respectively) and may not be used operationally in the future. Deltamethrin could not fully act in three groups (Kanju, Kuz Aba Khail, Shah Dherai) and was moderately resistant in three others. The overall efficacy of lambda-cyhalothrin was the highest, with the evident emergence of excessive resistance in some hot spots (Bara Bandai, Kuza Bandai). The definition of resistance hotspots emphasizes the urgency of resistance-inspired tactics of containment of vectors on subtle scales of space. This absence of systemic resistance monitoring in the further application of pyrethroids will even enhance resistance and jeopardize the attempts to control dengue. Multi-modal control of vectors or insecticides rotation involving non-pyrethroid classes, source reduction, biological control, and community participation should be embraced to assist in the provision of sustainability of dengue prevention in the region. Evidence-based policy decisions and operational adaptability will play a key role in ensuring that the dengue control program effectiveness and the health burden of dengue to the population of the District Swat and other dengue hotspots are maintained, by conducting regular resistance surveillance at fine spatial scales (at least yearly at the union council level). The data provide the evidence base to amend the strategies of vector control in the study region on the spot and can be used as an example of resistance-based decision-making in other parts of Pakistan.

ACKNOWLEDGMENTS

The authors thank to the Khyber Pakhtunkhwa Health Department for logistical field support, the Director of the Nuclear Institute of Food and Agriculture for insectary facilities and reference strains,

Authors also gratefully acknowledge the residents of Tehsil Kabal for permitting access to their properties for sampling.

CONFLICT OF INTEREST

The authors have no conflicts of interests.

FUNDING.

No financial authority participated for this study.

REFERENCES

1. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267.
2. Achee, N.L., Gould, F., Perkins, T.A., Reiner, R.C., Morrison, A.C., Ritchie, S.A., ... & Scott, T.W. (2015). A critical assessment of vector control for dengue prevention. *PLoS Neglected Tropical Diseases*, 9(5), e0003655.
3. Akhtar, Z.R., Tariq, K., Mavian, C., Ali, A., Ullah, F., Zang, L.S., Ali, F., Nazir, T. and Ali,



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

- S., 2021. Trophic transfer and toxicity of heavy metals from dengue mosquito *Aedes aegypti* to predator dragonfly *Tramea cophysa*. *Ecotoxicology*, 30(6), 1108-1115.
4. Akram, M., Fatima, Z., & Ahmed, S. (2020). Climate change and dengue in Pakistan: A review. *Asian Pacific Journal of Tropical Medicine*, 13(8), 325-330.
 5. Assir, M.Z., Kamran, U., Ahmad, H.I., Bashir, S., Mansoor, H., Awan, S., & Akram, M. (2014). Clinical spectrum of dengue fever in Pakistan. *Journal of Infection in Developing Countries*, 8(11), 1473-1480.
 6. Benelli, G., Jeffries, C.L., & Walker, T. (2016). Insecticide resistance in mosquito vectors: An update. *Parasites & Vectors*, 9(1), 1-12.
 7. Bowman, L.R., Runge-Ranzinger, S., & McCall, P.J. (2014). Assessing the relationship between vector indices and dengue transmission: A systematic review of the evidence. *PLoS Neglected Tropical Diseases*, 8(5), e2848.
 8. Chandra, G., & Bhattacharjee, I. (2024). Pyrethroid resistance in dengue vectors: Challenges and management. *Asian Pacific Journal of Tropical Medicine*, 17(2), 85-94.
 9. Deming, R., Manrique-Saide, P., Barreiro, A.M., Cardena, E.K., Che-Mendoza, A., Jones, B., ... & Vazquez-Prokopec, G.M. (2016). Spatial variation of insecticide resistance in the dengue vector *Aedes aegypti* presents unique vector control challenges. *Parasites & Vectors*, 9(1), 67.
 10. Dusfour, I., Vontas, J., David, J.P., Weetman, D., Fonseca, D.M., Corbel, V., ... & Girod, R. (2019). Management of insecticide resistance in the major *Aedes* vectors of arboviruses: Advances and challenges. *PLoS Neglected Tropical Diseases*, 13(10), e0007615.
 11. Erlanger, T.E., Keiser, J., & Utzinger, J. (2008). Effect of dengue vector control interventions on entomological parameters in developing countries: A systematic review and meta-analysis. *Tropical Medicine & International Health*, 13(9), 1061-1072.
 12. Gubler, D.J. (2011). Dengue, urbanization and globalization: The unholy trinity of the 21st century. *Tropical Medicine and Health*, 39(4 Supplement), S3-S11.
 13. Hemingway, J., & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, 45(1), 371-391.
 14. Karunamoorthi, K., & Sabesan, S. (2013). Insecticide resistance in insect vectors of disease with special reference to mosquitoes: A potential threat to global public health. *Journal of Vector Borne Diseases*, 50(2), 86-97.
 15. Khan, E., Abbas, S., & Khan, M.A. (2018). Dengue in Pakistan: A systematic review of epidemiology, clinical features, and management. *Journal of the College of Physicians and Surgeons Pakistan*, 28(10), 760-765.
 16. Kraemer, M.U., Sinka, M.E., Duda, K.A., Mylne, A.Q., Shearer, F.M., Barker, C.M., ... & Hay, S.I. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *eLife*, 4, e08347.
 17. Liu, N. (2015). Insecticide resistance in mosquitoes: Impact, mechanisms, and research



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

- directions. *Annual Review of Entomology*, 60, 537-559.
18. Moyes, C.L., Vontas, J., Martins, A.J., Ng, L.C., Koou, S.Y., Dusfour, I., ... & Weetman, D. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Neglected Tropical Diseases*, 11(7), e0005625.
 19. Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2010). Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? *Trends in Parasitology*, 27(2), 91-98.
 20. Roiz, D., Wilson, A.L., Scott, T.W., Fonseca, D.M., Jourdain, F., Müller, P., ... & Corbel, V. (2018). Integrated *Aedes* management for the control of *Aedes*-borne diseases. *PLoS Neglected Tropical Diseases*, 12(12), e0006845.
 21. Saavedra-Rodriguez, K., Urdaneta-Marquez, L., Rajatileka, S., Moulton, M., Flores, A.E., Fernandez-Salas, I., ... & Black, W.C. (2012). A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Molecular Biology*, 21(1), 87-95.
 22. Sanyaolu, A., Okorie, C., Badaru, O., Adetona, K., & Fowotade, A. (2017). Global epidemiology of dengue hemorrhagic fever: An update. *Journal of Human Virology & Retrovirology*, 5(6), 1-9.
 23. Scott, T.W., & Morrison, A.C. (2014). Vector dynamics and transmission of dengue virus: Implications for dengue surveillance and prevention strategies. In D.J. Gubler, E.E. Ooi, S. Vasudevan, & J. Farrar (Eds.), *Dengue and Dengue Hemorrhagic Fever* (2nd ed., pp. 187-206). CAB International.
 24. Sherrard-Smith, E., Griffin, J.T., Winskill, P., Corbel, V., Pennetier, C., Djenontin, A., ... & Churcher, T.S. (2018). Systematic review of indoor residual spray efficacy and effectiveness against *Plasmodium falciparum* in Africa. *Nature Communications*, 9(1), 4982.
 25. Tyagi, B.K., Singh, S.P., & Yadav, R. (2025). Emerging insecticide resistance in dengue vectors: Current status and future perspectives. *Tropical Medicine and Infectious Disease*, 10(1), 1-15.
 26. Van den Berg, H., Velayudhan, R., & Ejov, M. (2012). Regional framework for surveillance and control of invasive mosquito vectors and re-emerging vector-borne diseases. *WHO Regional Office for South-East Asia*, New Delhi.
 27. Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., della Torre, A., & Ranson, H. (2012). Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. *Pesticide Biochemistry and Physiology*, 104(2), 126-131.
 28. Weaver, S.C., & Vasilakis, N. (2009). Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infection, Genetics and Evolution*, 9(4), 523-540.
 29. Wilder-Smith, A., Hombach, J., Ferguson, N., Selgelid, M., O'Brien, K., Vannice, K., ... &



JOURNAL OF MEDICAL AND BIOMEDICAL SCIENCE

ISSN: 2026-6294 | Volume No. 11 Issue No. 3 (2025)

- Farrar, J. (2019). Deliberations of the Strategic Advisory Group of Experts on Immunization on the use of CYD-TDV dengue vaccine. *The Lancet Infectious Diseases*, 19(1), e31-e38.
30. World Health Organization. (2005). *Guidelines for laboratory and field testing of mosquito larvicides*. WHO/CDS/WHOPES/GCDPP/2005.13. Geneva: WHO.
31. World Health Organization. (2012). *Global strategy for dengue prevention and control 2012–2020*. Geneva: WHO Press.
32. World Health Organization. (2016). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes* (2nd ed.). Geneva: WHO.
33. World Health Organization. (2023). *World malaria report 2023*. Geneva: WHO Press.