Original Article

Organophosphate and Pyrethroid Resistance Status of Invasive *Aedes aegypti* (Diptera: Culicidae) from Iran

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(Received 04 Sep 2024; accepted 03 Dec 2024)

Abstract

Background: The growing concerns regarding the recent invasion of *Aedes aegypti* in Iran and the potential outbreak of dengue fever, chikungunya and Zika viruses in the country highlight the importance of assessing the susceptibility of this vector to different insecticides.

Methods: The study assessed the resistance status of *Ae. aegypti* resistance to insecticides such as deltamethrin, permethrin, malathion, and temephos in Bandar Abbas City, Hormozgan Province, Iran. The research followed WHO standard testing procedures for adult mosquitoes. Adult susceptibility tests were conducted using 1X the discriminating concentrations to determine the frequency and status of insecticide resistance. Additionally, 5X and 10X the discriminating concentration were used to evaluate the intensity of resistance. Larval susceptibility to temephos was tested using concentrations of 156.25, 31.25, 6.25, 1.25, and 0.25 mg/l of temephos.

Results: Adults were resistant to all three tested insecticides at WHO-recommended diagnostic concentrations (DCs). In terms of resistance intensity, *Ae. aegypti* exhibited low-intensity resistance to malathion and deltamethrin, while resistance to permethrin was high-intensity. Dose-response analysis regarding the susceptibility of larvae to temephos showed LC_{50}, LC_{90}, and LC_{99} values of 0.013, 0.065, and 0.238 mg/l, respectively. These values indicate resistance when compared to the WHO diagnostic dose for temephos resistance of 0.012 mg/l.

Conclusion: The results of this study highlight the need for an urgent strategy to manage resistance to insecticides and strengthen the integrated management program of *Ae. aegypti*. This fact emphasizes the importance of reducing larval sources and promoting research on alternative methods and products.

Keywords: *Aedes aegypti*; Insecticide resistance; Dengue fever; Vector control; Iran

Introduction

Aedes aegypti (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1895) are two of the most important mosquito vector species worldwide. *Aedes aegypti* is the primary vector of the dengue virus. This species is closely associated with humans and their environment (1, 2) and feeds on multiple individuals during each blood meal. The frequent feeding behavior may contribute to the rapid and sudden spread of diseases in its geographical range (2, 3).

Aedes aegypti is distributed worldwide between the latitudes of 35°N and 35°S (2). Currently, *Ae. aegypti* has been established in 167 countries (4). Inadequate urban planning and

sanitation contribute to the proliferation of breeding sites for *Ae. aegypti* (5). The vector's "ecological flexibility" is a key factor in its global spread and effectiveness as a vector of human diseases (3).

Prevention and control of dengue fever depend on managing mosquitoes that transmit the disease. The control of *Ae. aegypti* mosquitoes depend on insecticides, primarily through the use of larvicides, space spraying of pyrethroids and organophosphates, and community participation for source reduction (6). The primary reasons insecticides are the preferred method for mosquito control are their ease of use, accessibility, and immediate visible impact (2).

The lengthy time needed to develop new control tools implies that existing insecticide-based methods will remain crucial in managing *Ae. aegypti* in the coming years. This is because new control strategies are currently limited in availability and have only been tested in a few locations globally (7–9).

Insecticides are essential in controlling dengue fever, but mosquito resistance reduces the effectiveness of these interventions (10). The control of *Ae. aegypti* using neurotoxic insecticides has been intense in the past decades. According to review studies, this has led to the appearance of resistant strains of the vector to this group of insecticides in Asia, Africa, and America (11, 12). *Aedes aegypti* resistance to pyrethroids and organophosphates has been reported in WHO South-East Asia Region countries such as Thailand, Timor-Leste, Indonesia, India, and Bangladesh (13). Generally, insecticide resistance in *Aedes* mosquitoes is mainly due to mutations in the target site and increased detoxification (11). Since the problem of *Ae. aegypti* resistance to insecticides is spreading worldwide, and insecticide resistance poses a main risk in interventions to control vector-borne diseases, the detection of insecticide resistance in disease vectors helps formulate a global strategy in this field (10).

Iran is one of the countries in the Eastern Mediterranean Region (EMRO) of the World Health Organization (WHO). In this region, outbreaks of dengue have been recorded in Saudi Arabia, Sudan, Pakistan, and Yemen. The highest burden of dengue disease is related to Pakistan, a neighbor of Iran. Both *Aedes* species are abundant in Pakistan, with *Ae. aegypti* is the most common in urban regions. Insecticide-resistant populations of *Ae. aegypti* and *Ae. albopictus* has recently been reported in Pakistan (14–17). In 2020, Afghanistan's surveillance system detected locally acquired DENV cases for the first time in provinces bordering Pakistan. Both invasive *Aedes* species are also found in Afghanistan, another neighboring country of Iran (18).

Aedes aegypti was reported in Hormozgan Province, Iran in 2022 (19). Currently, the presence of this species has been confirmed in Hormozgan, Sistan Baluchistan, Bushehr, and Khuzestan Provinces, while *Ae. albopictus* has been identified in Guilan, Mazandaran, East Azarbaijan, and Ardebil Provinces, also, verified in Zanjan and Qazvin Provinces (20, 21). The presence of *Ae. aegypti* in the southern regions and *Ae. albopictus* in the north of the country, along with frequent travel to and from Pakistan, Afghanistan, and other countries with dengue fever cases, have implications for Iran. In 2024, locally acquired cases of DENV were reported from Lengeh Port and Chabahar (20).

Pyrethroids are one of the most important classes of insecticides that are widely used in mosquito control. These insecticides are popular due to their affordability, established safety, and effectiveness in both indoor and outdoor settings (22–25). Pyrethroids are known for being more stable and potent than other insecticides, with low toxicity to mammals (26). In Iran, deltamethrin and permethrin are the most widely used pyrethroids for vector control. The development of pyrethroid resistance in mosquitoes poses a significant challenge for vector control efforts.

Using a single type of insecticide for an extended period or continuously can lead to *Ae. aegypti* developing resistance to that insecti-

cide (27, 28). Over several decades, the consistent use of organophosphorus and pyrethroids insecticides to control both the larval and adult stages of *Ae. aegypti* has resulted in a reduced susceptibility of these mosquitoes to these compounds (29).

Temephos or abate and malathion are organophosphate compounds that have been used worldwide for about 40 years against larvae and adult mosquitoes. Reports on the susceptibility status of dengue vectors to these insecticides vary globally, indicating susceptibility, tolerance, and resistance of invasive *Aedes* to insecticides (11, 12). Malathion is extensively used for pest control in health, agriculture, livestock, and household pest control due to its toxicity is low for humans and other mammals (30). Since 1973, malathion has been employed for fogging to prevent dengue fever (2). Temephos is a non-systemic organophosphate insecticide used primarily as a larvicide for mosquito control, including in household water containers and those used to store drinking water (31).

In designing effective vector control interventions, it is necessary to determine the susceptibility or resistance state of the vector to insecticides. Therefore, it is crucial to monitor the resistance of vector species before and during the implementation of vector control interventions. Control programs should establish insecticide resistance monitoring and management programs (32).

Understanding the resistance type of the vector population, the status of phenotypic resistance, and resistance mechanisms in each region, as well as the level of insecticide resistance, is crucial for current or future planning in selecting effective vector control interventions. Since 2020, *Ae. aegypti* has been established in Hormozgan Province, southern Iran (19, 33). For the control of *Ae. aegypti*, environmental management, and source reduction following insecticide-based interventions are recommended (33). Therefore, it is required to assess the susceptibility or resistance of the vector to the insecticides used in this area. Determining phenotypic resistance is done using bioassay tests, which are the best and most reliable way to assess the susceptibility or resistance of mosquitoes to insecticides. By using papers impregnated with discriminating concentrations of 5X and 10X of the desired insecticide, the intensity of resistance is also determined (32). In the early stages of *Ae. aegypti* entry into Iran, to identify the susceptibility or resistance of the vector to insecticides for the implementation of effective control interventions, molecular studies of resistance to pyrethroids were carried out (34). By setting up an insectary for *Ae. aegypti* rearing in Bandar Abbas, it became possible to perform bioassay tests.

Insecticide resistance can decrease the effectiveness of controlling *Ae. aegypti* mosquitoes. This study aimed to assess the resistance status of *Ae. aegypti* to deltamethrin, permethrin, malathion, and temephos compounds using WHO susceptibility tests in Bandar Abbas City, Hormozgan Province, Iran. These findings will improve our understanding of *Ae. aegypti*'s insecticide resistance and assist in monitoring vector control initiatives.

Materials and Methods

Study area

Bandar Abbas is the capital city of Hormozgan Province of Iran. Bandar Abbas is a port on the southern coast of the country and the edge of the Persian Gulf. The city is 9.8 meters above sea level, located at 27°11′46″N 56°17′16″E. Bandar Abbas has a subtropical hot desert climate. The area receives low precipitation (168 mm) with high variance. The average relative humidity ranges from 60% to 70% throughout the year, occasionally reaching 100%. Summer temperatures can rise to 49 °C, while winter temperatures can drop to 5 °C.

Rearing *Aedes aegypti* in insectary

Three zones of Shahed, Suroo, and Elahieh

in Bandar Abbas City were selected for sampling. In these zones, *Ae. aegypti* eggs, larvae, and adults were collected using ovitraps, droppers, and aspirators, respectively. We examined the F1 generation of specimens, caught by different traps, using the identification key, after confirming the identity and ensuring that all samples were *Ae. aegypti* species (35), the samples were transferred to the insectary for rearing. The larvae were placed in plastic trays measuring 28×37×8 centimeters and were fed with fish food. The air temperature and humidity were kept between 27–28 ºC and 65–75%, respectively, and 12:12 hours light: dark photoperiod was provided. The pupae emergences were then transferred to a 50×50×50 centimeter mosquito cage. The adults were fed with a 10% water-sugar solution through a leaked tissue paper. Fresh human blood (obtained from the Iranian Blood Transfusion Organization) was used as artificial nutrition twice a week to feed female mosquitoes (36). To investigate the potential infection of field-collected samples with dengue virus, we tested the F1 generation using the NS1 kit. The SD Bioline NS1 antigen kit (Standards Diagnostic, Gyeonggi-do, Republic of Korea) was used to test for dengue antigen in the mosquito abdomen. The abdomens of blood-fed mosquitoes were used for dengue virus antigen detection assay. 50 µl PBS was added to the abdomens of mosquitoes, homogenized, and centrifuged. The supernatant was added to the well of the test kit. After 15 minutes, only the control band was observed, so the samples were negative for dengue virus infection. Also, some samples were sent to the Arbovirus Reference Laboratory, and RNA extraction and multiplex RT-PCR were done (37). After confirming the absence of infection, the rearing of *Ae. aegypti* continued in the insectary. Egg laying commenced on the third day after blood feeding. Following a dry period, the eggs were submerged in water to hatch.

The samples collected from three zones (Shahed, Suroo, and Elahieh in Bandar Abbas City), were mixed for bioassays.

Bioassay survey

The susceptibility of *Ae. aegypti* to two pyrethroid class insecticides, deltamethrin and permethrin (≥98% purity), and two organophosphate compounds, malathion (>98% purity), and temephos (technical-grade 97.5%) were evaluated.

***Aedes aegypti* adult stage susceptibility test using the WHO guidelines (standard tube test)**

The World Health Organization susceptibility bioassay test guideline was utilized to determine the resistance status of *Ae. aegypti* using impregnated papers (32). These papers were prepared at Mazandaran University of Medical Sciences, Iran, in March 2024, following the WHO standard protocol (38). Insecticide discriminating concentrations for WHO susceptibility bioassays with *Ae. aegypti* include deltamethrin 0.03%, permethrin 0.4%, and malathion 1.5% (39). The paper was left to air dry for 24 hours before use. Papers impregnated only with solvent were utilized as the control (Organophosphate control: olive oil and acetone; Pyrethroids control: silicone oil and acetone) (38).

Adult susceptibility tests were conducted on 3 to 5-day-old female mosquitoes (non-blood-fed) that were fed with 10% sugar water. The sugar water meal was removed approximately 6 hours before the test. A total of 150 mosquitoes were required to test each insecticide using the WHO tube test. In this bioassay, mosquitoes were exposed for 1 hour to filter papers treated with an insecticide at the discriminating concentration (DC). The number of treatment and control replicates per test were 4 and 2 respectively (32, 39).

In the test tubes, the mosquitoes were exposed to impregnated papers for 1 hour. The number of knocked-down *Ae. aegypti* was noted every five minutes.

After the exposure time ended, the mosquitoes were transferred to holding tubes. A piece

of cotton soaked in a 10% water-sugar solution was placed on the net of each holding tube. The mosquitoes were then kept in the insectary for 24 hours at a temperature of 26±1 °C and a relative humidity of 70±5%. After 24 hours, the number of dead mosquitoes and those alive but unable to move in the holding tubes were considered susceptible. The surviving adults were considered resistant, and their numbers were recorded in the appropriate forms.

Resistance intensity tests

In case resistance to any of the insecticides was detected in the vector population, to measure the intensity of *Ae. aegypti* adults' resistance to insecticides, WHO intensity bioassay tests were used in this study. Mosquitoes were exposed to concentrations of 5X and then 10X the DC (discriminating concentrations) following WHO testing guidelines to assess whether the intensity of resistance was low, moderate, or high (32).

***Aedes aegypti* larval stage susceptibility test using WHO guidelines**

Concentrations of 156.25, 31.25, 6.25, 1.25, and 0.25 mg/L of temephos, technical-grade 97.5 % (Pestanal Sigma-Aldrich), were used to evaluate the susceptibility status of larvae (40). Four repetitions were conducted for each concentration, and two repetitions were designated as controls. Initially, in the test replicates, 25 mL of dechlorinated water was added to each 50 mL glass beaker, and 20 larvae at the end of the 3rd instar or the beginning of the 4th instar were placed in them. A one-hour rest period was allowed to replace any weak or dead larvae with healthy ones. Subsequently, 74 mL of dechlorinated water was added to each of the 250 mL glass beakers, and then 1 mL of each temephos concentration was added to the beakers using separate pipettes, mixed thoroughly, and labeled. Carefully and slowly, the 25 mL glass beakers containing larvae were added to each of the 250 mL beakers containing the insecticide. The time was recorded, and the setup was placed in the insectary at a temperature of 26±1 °C and a relative humidity of 70±5% for 24 hours. Subsequently, after the recovery period, the mortality results were recorded, and mortality rates for treated and control larvae were calculated (41).

Data analysis

The mortality rates of adults and larvae, as well as the percentage of mortality, were estimated based on the number of dead adults and larvae after 24 hours of contact. If the mortality rate of the control group was found to be ≥5% and ≤20%, Abbott's formula was used to correct the observed mortality in insecticide-exposed mosquitoes (42).

Probit analysis was conducted using SPSS software version 26, and dose-response assays were used to determine the lethal concentrations (LCs) of temephos. The LC_{50} and LC_{90} values were calculated, and a regression line was plotted. Furthermore, the LC_{99} value was compared with the diagnostic resistance value to temephos provided by the WHO (41).

Interpretation of susceptibility bioassays

Confirmed resistance: If mortality (after correction) is less than 90%, the mosquito population is considered resistant to insecticides.

Possible resistance: If mortality (after correction) is greater than or equal to 90% but less than 98%, the existence of resistance is possible but not confirmed. The test should be repeated, and results confirmed with a new sample from the same mosquito population. Resistance is confirmed if two tests consistently show less than 98% mortality.

Susceptibility: If mortality (after correction) is equal to or greater than 98%, the vector population is susceptible to the insecticide (32).

Results

Mortality rates for adult mosquitoes and larvae were calculated based on the number of dead mosquitoes and larvae after 24 hours of exposure to the insecticide.

Bioassays with adulticides

Test results of different insecticides (1× the

diagnostic concentration) on adult *Ae. aegypti* within one hour, using the WHO tube test, indicated that, *Ae. aegypti* mosquitoes were resistant to all three tested insecticides at the doses recommended by the WHO.

Aedes aegypti mosquitoes that were resistant to 1.5% malathion and 0.03% deltamethrin, did not survive the bioassay performed at 5X the DC, this means that the intensity of resistance of *Ae. aegypti* to these insecticides is low (Fig. 1). However, to determine the intensity of resistance of these mosquitoes to permethrin, it was necessary to perform a susceptibility bioassay with 10X the DC of this insecticide. Therefore, a permethrin paper at 10X the DC was prepared, and the test was conducted using this paper following the same procedure as in the previous steps.

The mortality rate of *Ae. aegypti* exposed to 4% permethrin being less than 98% indicates that these mosquitoes demonstrate high-intensity resistance to permethrin (Fig. 1).

Bioassay survey showed that the knockdown time of 50% (KDT_{50}) of *Ae. aegypti* after exposure to 1X diagnostic dose of deltamethrin was 49.69 minutes (ranging from 45.49 to 55.91 minutes). Figure 2 illustrates the trend of knockdown mosquito numbers during one hour of exposure to a 1X diagnostic dose of deltamethrin and permethrin. Malathion did not exhibit a knockdown effect.

Bioassay with temephos

Table 1 demonstrates that as the dose of insecticide increases, there is a corresponding rise in the mortality rate of larvae. Hence, the dose-effect is deemed significant (p-value< 0.001).

Pearson goodness-of-fit test showed that the probability value of the test, p-value= 0.282, was greater than 0.15, confirming the hypothesis of the suitability of the probit regression model. Moreover, as the significance level exceeds 0.15, there is no heterogeneity factor in the computation of confidence limits.

Dose-response tests showed the lethal concentrations (LCs) for temephos in *Ae. aegypti* from Bandar Abbas, Iran. The LC_{50} and LC_{90} were 0.013 and 0.065 mg/l, respectively. Probit analysis of temephos revealed that the LC_{99} value was 0.238 mg/l. Comparing this with the WHO diagnostic dose for the insecticide temephos (0.012 mg/l), it was evident that *Ae. aegypti* was resistant (Table 2 and Fig. 3) (41).

Table 1. Dose–response assays using bioassay data after exposing *Aedes aegypti* to temephos concentrations (156.25, 31.25, 6.25, 1.25, and 0.25 mg/L) in Bandar Abbas, Iran, 2024

						95% Confidence Interval	
	Parameter	Estimate	Std. Error	Z	Sig.	Lower Bound	Upper Bound
PROBIT	Dose	1.856	.310	5.982	.000	1.248	2.464
	Intercept	3.484	.606	5.749	.000	2.878	4.090

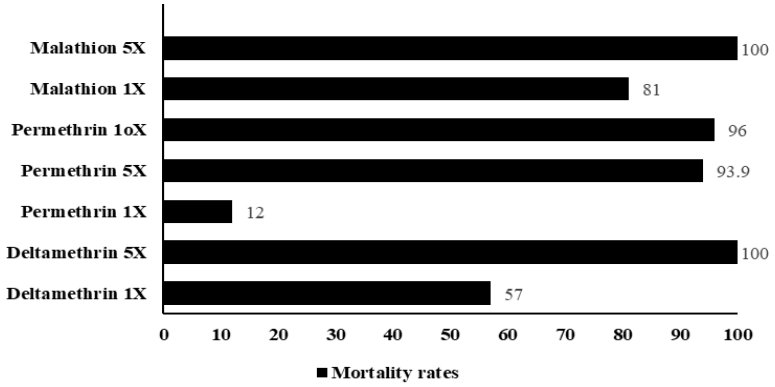

Fig. 1. The mortality rate of adult *Aedes aegypti* after exposure to 1X and 5X diagnostic doses of deltamethrin (0.03% and 0.15%), permethrin (0.4% and 2%), malathion (1.5% and 7.5%) and 10X permethrin (4%), Bandar Abbas, Iran, 2024

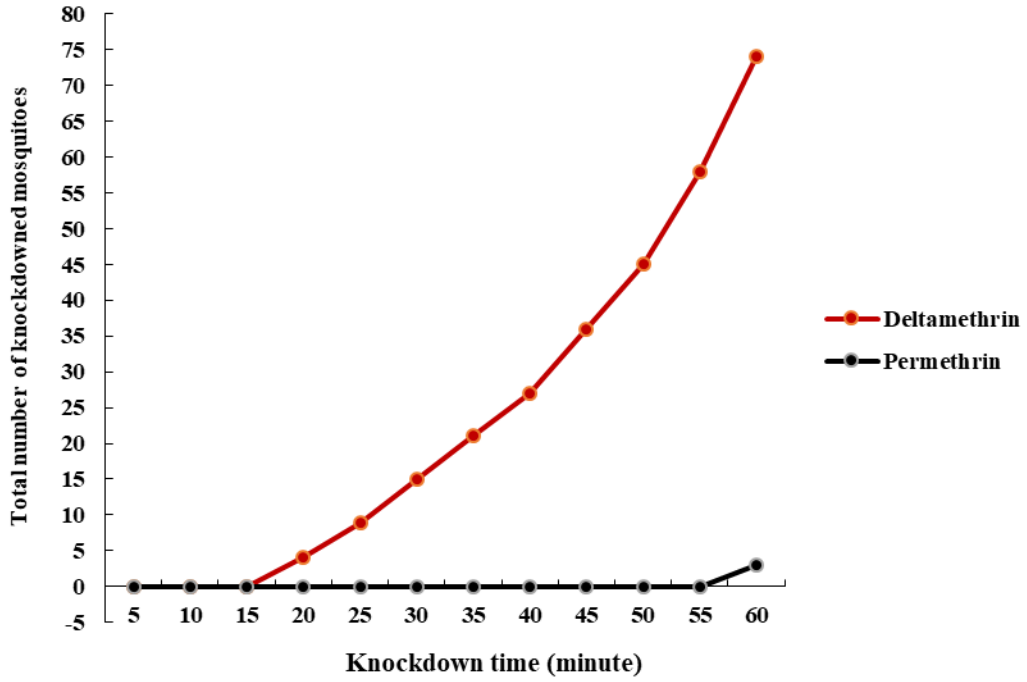

Fig. 2. The trend of the knockdown of adult *Aedes aegypti* numbers over a 60-minute of exposure to deltamethrin 0.03% and permethrin 0.4% insecticides using WHO tube test including 150 mosquitoes and four replicates for each insecticide, Bandar Abbas, Iran, 2024

Table 2. Susceptibility profile of *Aedes aegypti* to doses of the larvicide organophosphate temephos concentrations (156.25, 31.25, 6.25, 1.25, and 0.25 mg/L) in Bandar Abbas, Iran, 2024

Population	LC_{50} (CI95%)	LC_{90} (CI95%)	LC_{99} (CI95%)
Aedes aegypti	0.013 (0.009-0.021)	0.065 (0.038-0.163)	0.238 (0.107-1.056)

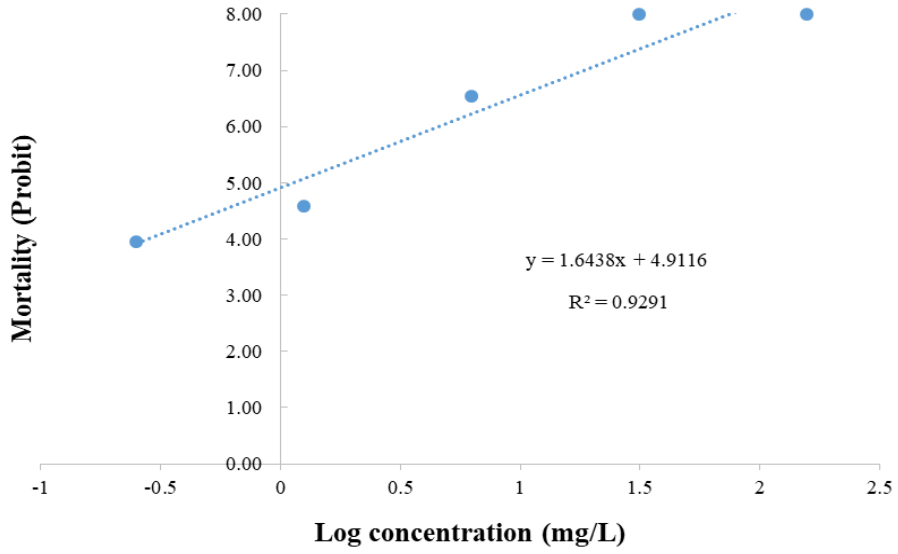

Fig. 3. *Aedes aegypti* larval stage susceptibility test using WHO guidelines. The mortality (probit) of *Aedes aegypti* late 3rd/early 4th instar larvae after 24 hours of exposure to concentrations of temephos (156.25, 31.25, 6.25, 1.25, and 0.25 mg/L) with four replicates for each concentration, in Bandar Abbas, Iran, 2024

Discussion

Monitoring the susceptibility of *Ae. aegypti* to pyrethroid and organophosphate insecticides is an essential routine to assess the status of susceptible and resistant vector populations. This information is crucial before implementing chemical control measures using selective insecticides in Iran.

The results of our study showed varying the intensity of resistance of *Ae. aegypti* adults to different concentrations of three insecticide compounds in Bandar Abbas. *Aedes aegypti* showed resistance to 1X the DC of the insecticide compounds: malathion 1.5%, deltamethrin 0.03%, and permethrin 0.4%. Therefore, it exhibited resistance to the WHO diagnostic doses of the three insecticides, with the level of resistance different for each insecticide. Permethrin exhibited lower mortality rates compared to deltamethrin and malathion. Based on the results obtained with 5X the DC and 10X the DC papers, the intensity of resistance of *Ae. aegypti* to malathion and deltamethrin were low, but these mosquitoes showed high-intensity resistance to permethrin insecticide. These findings are consistent with the results of Enayati et al. (34), where the frequency of the F1534C mutation in their study was higher than other knockdown resistance (kdr) mutations. Regarding the correlation between the kdr mutation and resistance to permethrin, they suggested that, *Ae. aegypti* resistance to permethrin is likely high. In the same study, four other kdr mutations, including V410L, S989P, V1016G, and V1016I, were also identified in *Ae. aegypti* in Iran with different frequencies (34). High resistance to permethrin could potentially lead to cross-resistance to other insecticides, including organochlorines and other pyrethroids such as deltamethrin (43). This was also confirmed by the results of our study, as the high resistance of *Ae. aegypti* mosquitoes to permethrin have reduced their susceptibility to deltamethrin.

Aedes aegypti's resistance to pyrethroids poses a significant global challenge (11–13, 44–46). Different levels of resistance to deltamethrin and permethrin have been reported in Pakistan (17, 47). Moreover, high resistance to permethrin and deltamethrin has been observed in *Ae. aegypti* populations in Jeddah and Makkah (48). Selection pressure is an important factor that influences the rate of resistance development (49).

The high intensity of resistance in *Ae. aegypti* to permethrin could be due to its presence in several commercial insecticide formulations for household use (50, 51). Given that the species has recently been reported in Iran and has not been exposed to prolonged local selection pressure, the observed high frequency of kdr mutations strongly implies that pyrethroid resistance may have initiated in the original country of *Ae. aegypti* (34).

Acute toxicity of a substance is determined using the LC_{50} value, while the LC_{99} value indicates a diagnostic value for resistance. In our study, the LC_{99} value of temephos was 0.238 mg/l, resulting in the death of 99% of *Ae. aegypti* larvae at this concentration. Comparison with the WHO diagnostic dose revealed that, *Ae. aegypti* larvae showed resistance to temephos (41). Temephos at a dosage of 0.012 mg/l was ineffective against *Ae. aegypti* larvae in Malaysia. Conversely, a dosage of 1 mg/l led to complete mortality of the larvae (52).

In our study, the dose-response assays showed that as the concentration of temephos increased, mortality also increased. Temephos is an insecticide compound that easily penetrates cuticular surfaces or spiracles due to its lipophilic nature. The increased toxicity at higher concentrations of temephos may be due to its enhanced penetration through the larval cuticle (53).

Temephos is an effective and low-cost insecticide for larval control in many regions of the world. Consequently, it has led to the development and spread of resistance in mosquitoes. However, temephos is one of the most widely used larvicides in various regions globally (53). Reports of *Ae. aegypti* resistance to

temephos has emerged from Latin American countries like Peru and Mexico, as well as Southeast Asian countries such as Thailand, Malaysia, and India (13, 54, 55). A recent study in Mexico revealed widespread resistance to temephos in *Ae. aegypti* populations when subjected to 5 times the DC of temephos, showing a moderate to high intensity of resistance (56). In Lahore, Pakistan, *Ae. aegypti* and *Ae. albopictus* were still susceptible to temephos (16), but the presence of resistance was confirmed in field strains of *Ae. aegypti* from Punjab (17). In the Jazan region of Saudi Arabia, larvae exhibited high resistance to temephos (57). Conversely, in the Western Region of Saudi Arabia, populations in Jeddah and Makkah showed full susceptibility to temephos (58). Resistance to insecticides can increase when insecticides are heavily used, leading to a higher percentage of resistant insects. In such cases, the susceptible strain is eliminated by the insecticide, causing an imbalance between susceptible and resistant strains and rendering the insecticide ineffective (59). However, when there is no selection pressure, a decrease in resistance has been observed in laboratory and natural populations of *Ae. aegypti* (60, 61).

To effectively manage insecticide resistance in public health, it is important to use appropriate doses of insecticides. Various strategies such as mosaics, rotations, and mixtures of insecticides with different modes of action can be employed to reduce the risk of developing resistance. However, insecticide resistance management against dengue vectors has been unsuccessful (8, 43, 62). Organophosphates were substituted for pyrethroids after resistance was detected in Singapore and Brazil. Nevertheless, high resistance to pyrethroids persisted in field populations even after 9–10 years of discontinuation (63, 64). This persistence may be attributed to the fact that resistance had already been established when insecticide resistance management was initiated (43). Furthermore, the maintenance of resistance could also be linked to the exposure of larvae and adults to agricultural and household insecticides or other pollutants (65–67).

The relatively high levels of insecticide resistance in *Ae. aegypti* emphasizes the need for planning and implementing insecticide resistance management programs. Although other control strategies and non-chemical interventions in the context of integrated vector control should always be promoted, procuring alternative chemicals should also be planned for efficient vector control. On the other hand, control strategies using insecticides should be combined with non-insecticide methods to limit the evolution of resistance to pesticides (68). This strategy requires the training and development of new tools to control disease vectors (43). Vector control tools other than chemical insecticides, such as *Bacillus thuringiensis* for larval control, *Wolbachia*-infected mosquito release, the sterile insect technique (SIT), and genetically modified mosquitoes, are needed for effective vector control (69–72). Further research is required to understand the emergence of resistance genes leading to metabolic resistance or the role of detoxifying enzymes in organophosphate resistance in *Ae. aegypti* from Iran.

Conclusion

Dose-response assays are suitable for estimating resistance levels. The main strength of our study lies in providing essential information about the susceptibility status of *Ae. aegypti* to pyrethroids and organophosphates in Bandar Abbas of Iran. Because the natural selection pressure can vary in different regions of the country, it is necessary to conduct routine susceptibility tests in all geographically distributed areas of the vector. The findings of this study will help select insecticides for effective control of *Ae. aegypti*. A high level of pyrethroid (permethrin) resistance necessitates the use of alternative insecticides from different insecticide classes and modes of action.

Acknowledgments

The authors are grateful to the head and staff of Bandar Abbas Health Education and Research Station. This research has been supported by Tehran University of Medical Sciences and Health Services grant No.: 1402-2-99-67144.

Ethical Considerations

The authors declare the ethical approval code as No.: IR.TUMS.MEDICINE.REC.1402.029.

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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