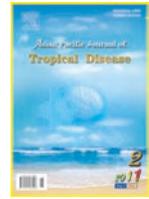




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Insecticide susceptibility of the dengue vector *Aedes aegypti* (Diptera: culicidae) in Makkah City, Saudi Arabia

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ABSTRACT

Objective: To examine the insecticide susceptibility of *Aedes aegypti* (*Ae. aegypti*) from various sites in Makkah City, Saudi Arabia. **Methods:** This was examined based on WHO standard procedures. **Results:** The larvae of *Ae. aegypti* were susceptible to all larvicides examined, but this susceptibility was more pronounced in wild populations, which tended to show tolerance to icon. Icon was the most effective larvicide with LC₅₀ values of 0.007 ppm and 0.012 ppm for the laboratory and field strains, respectively. *Ae. aegypti* adults exposed to lambda-cyhalothrin showed a low mortality rate in comparison with those exposed to deltamethrin and cyfluthrin. **Conclusions:** The results of the present study indicate differential susceptibility between field and laboratory larval populations. Wild larvae are less susceptible to insecticide treatments than their laboratory-bred counterparts. Taken together, these results suggest that tolerance and the tendency toward resistance to commonly used insecticides are present in *Ae. aegypti* populations throughout Makkah City, Saudi Arabia.

1. Introduction

With the increased development of transportation, there is a concern among epidemiologists regarding the eventual effects of the movements of humans on the evolution of arboviral infections^[1]. Diseases most likely to become a public health threat include dengue, outbreaks of which are now possible anywhere and at any time^[2,3]. Reasons for these outbreaks include unplanned urban growth, which has resulted into the proliferation of breeding sites. There has also been an increase in diversity of serotypes, *i.e.*, DEN-1, DEN-2, DEN-3, and DEN-4^[4], being introduced into new regions^[5].

In Saudi Arabia, *Aedes* mosquitoes have been implicated in many arboviral infection epidemics including outbreaks of dengue^[6,7]. Three serotypes of dengue (DEN-1, DEN-2, DEN-3) were first detected in Jeddah in 1994^[8]. Concomitant with these disease occurrences, there has

been an increase in the distribution of *Aedes aegypti* (*Ae. aegypti*) throughout the country. El-Badry *et al* reported the recent establishment of viable populations in Al-Madinah Al-Munawwarah where the mosquito was previously unknown^[9]. This mosquito has recently been incriminated in dengue epidemics in some areas, including Makkah, a city in Western Saudi Arabia. Fifty-five cases of dengue were reported in this city in 2008^[10], with a marked increase in the incidence of the disease thereafter^[11]. As the city holds the Kaaba, the most sacred site in Islam, it is a pilgrimage point for Muslims worldwide. Millions of Muslim pilgrims visit Makkah annually^[12]. Thus, the huge influx of visitors from dengue-endemic areas and the presence of ecological features conducive to the spread of *Ae. aegypti* (*i.e.*, uncovered domestic water storage, warm climate, well-developed transport network) have crucial public health significance, and there is a concern with respect to the possibility of large-scale dengue outbreaks.

Current efforts to control mosquito-borne diseases rely heavily on insecticides, the mainstream vector control strategy in many countries, including the Kingdom of Saudi Arabia. In this country, the application of larvicides

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(actellic, icon, and bactilod) and the spraying of adulticides (deltamethrin, cyfluthrin, lambda-cyhalothrin) are the primary strategies for combating mosquito-borne diseases. Although insecticide use has been sometimes effective, intermittent dengue cases are reminders of both the continued threat of this disease and the inefficiency of the existing chemical control arsenal. In Saudi Arabia, control operations are undertaken by the Ministry of Health (MOH) following outbreaks. It has been reported that misbranded insecticides are being sold by several entrepreneurs in Jeddah^[13]. Despite these practices, which may contribute to the development of resistance, monitoring susceptibility to insecticides has attracted little interest. Although some previous studies have indicated high levels of resistance to some commonly used insecticides^[14,15], these are not recent and were unrelated to *Ae. aegypti*. To address this issue, the World Health Organization (WHO)^[16] has argued for the necessity of continuous insecticide resistance monitoring, as this plays a key role in modifying control programs to increase effectiveness. Therefore, we examined the susceptibility of laboratory and field strains of *Ae. aegypti* to insecticides currently in use in Makkah City, Saudi Arabia.

2. Materials and methods

2.1. Study and mosquito collection sites

Mosquito larvae were collected in 2008 from indoor and outdoor containers around homes throughout Makkah City, Saudi Arabia (Figure 1), located between latitude 21 ° 25'N and longitude 39 ° 49'E. Sites for sampling were selected in relation to the intensity of insecticide use.

2.2. Mosquitoes

Ae. aegypti mosquitoes utilized in this study were collected as larvae from different localities in Makkah City in April 2008 and kept in the laboratory of MOH in Makkah under conditions of controlled temperature (27±1) °C and relative humidity (70±5)% with a constant photoperiod (light:dark, 14 h:10 h). Pupae were transferred from water medium to standard mosquito rearing cages (30 cm × 30 cm × 30 cm). Resulting adults were kept in similar cages and provided with a cotton wick soaked with 10% glucose solution for post-emergence. After a period of 4 days, sugar-fed females were starved for 24 h prior to blood feeding using pigeons. Blood-fed females were allowed to assimilate the blood meals for 48 h. Gravid females were given access to oviposition sites consisting of small glass containers (23 cm × 17 cm × 8 cm) lined with filter paper as egg deposition sites. Eggs were dried under laboratory conditions.

2.3. Experimental mosquitoes

Samples of eggs from filial generation 11 were hatched in cool boiled water. Newly eclosed larvae were reared in

plastic trays and fed every two days with a powdered mixture of biscuits, dried yeast, and fat-free milk (1:1:1). Late 3rd or early 4th instar larvae of generation 12 were used for larval bioassay testing. Adult bioassays were conducted using sugar-fed (10% glucose solution) 3–5-day-old adults derived from wild larvae after one generation under laboratory conditions.

2.4. Insecticides

The organophosphate actellic (5% pirimiphos-methyl; Syngenta Group Co., Basel, Switzerland), 2.5% the pyrethroid icon EC (Syngenta), and the bacterial insecticide bactilod [1 200 ITU/mg wettable powder formulation of *Bacillus thuringiensis* var. *israelensis* (*Bti*), (Long Xiong Co., Shenzhen, China)] were used for larval bioassay. For adult bioassay, 0.05% pyrethroids deltamethrin (PS-2071; Supelco, Bellefonte, PA), 95.5% technical-grade cyfluthrin and 0.05% lambda-cyhalothrin.

2.5. Larval bioassay

The tests were conducted in accordance with the previously published instructions^[17]. Briefly, batches of 20 larvae were added to glass beakers filled with 100 mL of water containing different concentrations of three insecticides: *i.e.*, actellic, icon, and bactilod. When larvae were introduced into the beakers, 0.02 g of the powdered mixture was added to avoid death by starvation. The concentrations applied were 0.020–0.120, 0.030–0.150, 0.004–0.020, 0.006–0.040, 0.100–0.450, and 0.100–0.600. These concentrations of each insecticide were tested in quintuplet for field-collected larvae reared to early 3rd or 4th instar in the laboratory as well as laboratory-adapted larvae. In each case, the same number of glass beakers with the same treatment but without insecticide served as controls. Beakers were inspected 24 h after introduction of larvae and the numbers of dead larvae were recorded.

2.6. Adult bioassay

Adult susceptibility tests were carried out according to the method described previously^[18]. Batches of 25 sugar-fed 3–5-day-old adults were exposed to paper impregnated with three pyrethroids (0.05% deltamethrin, 0.15% cyfluthrin, 0.05% lambda-cyhalothrin) in WHO standard tubes. Exposure was performed in quadruplicate at diagnostic dosages under conditions of controlled temperature (27±1) °C and relative humidity (70±5)% with a constant photoperiod (light:dark, 14 h:10 h). Tubes containing the same number of mosquitoes but with insecticide-free paper were used as controls. After 24 h of exposure, mosquitoes were transferred to new tubes for recovery. Mortality was monitored after 24 h of recovery.

2.7. Data collection and analysis

In the larval bioassay experiment, the numbers of dead

larvae were determined by counting the numbers of dead and moribund larvae. Based on the WHO criteria^[19], larvae incapable of reaching the water surface for oxygen and those showing no diving reaction characteristics when the water was disturbed were considered moribund. In the adult test experiment, the numbers of dead adults were counted from both test and control tubes after 24 h of sugar feeding (10% glucose solution from a cotton wick). The numbers of dead mosquitoes (larvae and adults) were used to calculate percentage mortality rates by dividing the number of dead mosquitoes by the number of exposed mosquitoes.

In the control settings in both experiments, the results were excluded from analysis if mortality rate was above 20%. In addition, if the percentage ranged between 5% and 20%, the mortality was corrected using the Abbott formula^[20]. Data from both larval and adult bioassays were subjected to probit analysis^[21]. The concentrations of agents that killed 50% and 90% of mosquito larvae in 24 h (LC_{50} and LC_{90} , respectively) were used to judge the larvicidal activities of the insecticides examined. Log concentration–probability regression lines ($LC-p$ lines) were drawn for the insecticides used in the larval bioassay. Statistical parameters were also calculated according the method of Litchfield and Wilcoxon^[22]. The resistance status was determined according to WHO criteria^[18].

3. Results

3.1. Larval susceptibility

The different parameters of larval susceptibility were summarized in Table 1. Larval *Ae. aegypti* showed various percentage mortalities after exposure to larvicides for 24 h. The lowest percentage mortality (13%) was recorded among wild larvae exposed to bactilod, whereas the highest mortality rate (98%) was observed for laboratory–adapted larvae exposed to icon. The percentage mortality rates of *Ae. aegypti* exposed to larvicides were lower in the field populations than in the laboratory strain. The LC_{50} of actellic for the laboratory strain was 0.040 mg/L, which was 1.5–fold lower than that of the field strain. The LC_{50} of icon for the laboratory strain was 0.007 mg/L, which was 1.7–fold lower than that of the field strain. The LC_{50} of bactilod for the laboratory strain was 0.200 mg/L, which was 1.3–fold lower than that of the field strain. Icon was the most effective larvicide in both field and laboratory strains, followed by actellic. The slopes of the concentration–mortality curves varied considerably between strains (Figure 2, 3, and 4). The slopes were greater for the laboratory strain than the wild strain for actellic (3.9 vs. 2.9, respectively), icon (4.3 vs. 3.6, respectively), and bactilod (4.2 vs. 3.4, respectively), indicating homogeneity of response to the tested larvicides. It is clear that the wild larvae were less susceptible to the larvicides than the laboratory–adapted larvae.



Figure 1. Sites of *Ae. aegypti* collections from indoor/domestic containers throughout Makkah City in 2008.

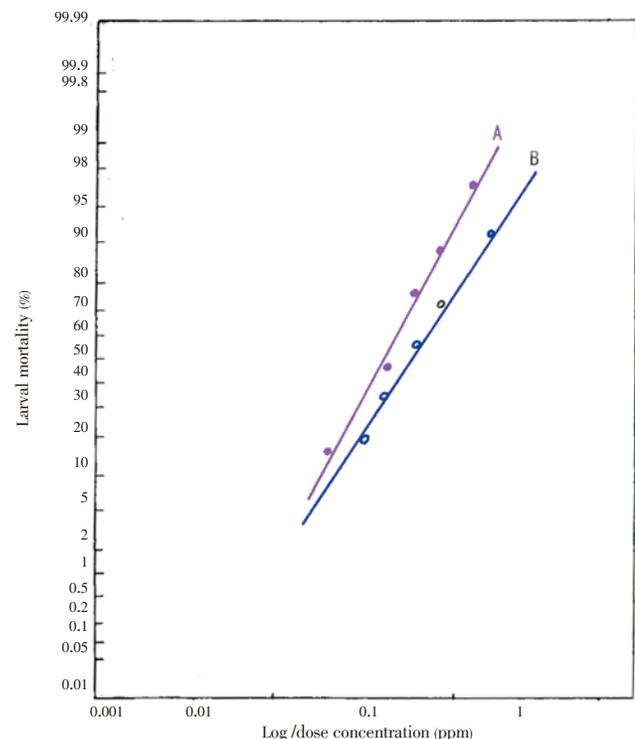


Figure 2. Regression lines ($LC-p$ lines) resulting from actellic bioassay of larvae of the laboratory (A) and field (B) strains of *Ae. aegypti*.

Table 1

Susceptibility of *Ae. aegypti* larvae (lab and field strains) to chemical insecticides actellic, icon and the bacterial insecticide (bacilod) following continuous exposure for 24 h.

Insecticide	Mosquito strain	Effective concentrations (ppm)	Larval mortality (%) ^a	Statistical parameters ^b		
				LC ₅₀ (mg/L)	Slope function	Slope
Actellic	Lab St.	0.020–0.120	15–97	0.040	0.80	3.9
	Field St.	0.030–0.150	18–91	0.058	2.20	2.9
Icon	Lab St.	0.004–0.020	17–98	0.007	1.70	4.3
	Field St.	0.006–0.040	15–97	0.012	1.90	3.6
Bacilod	Lab St.	0.100–0.450	18–92	0.200	1.73	4.2
	Field St.	0.100–0.600	13–90	0.250	1.96	3.4

a: Five replicates, 20 larvae each; control mortalities ranged from 0.0%–3.0%. b: Litchfield and Wilcoxon (1949).

Table 2

Results of susceptibility tests performed against the adults of *Ae. aegypti* derived from wild larvae after one generation using three pyrethroid insecticides.

Adulticides	Diagnostic dosages (%)	No. of mosquitoes exposed*	Mortality (%)	Susceptibility status**
Deltamethrin	0.05	100	86	Tolerant
Lambdacyhalothrin	0.05	100	77	Resistant
Cyfluthrin	0.15	100	90	Tolerant

*Four replicates; 25 mosquito females each; control mortalities ranged from 2%–3%.

**WHO (1981).

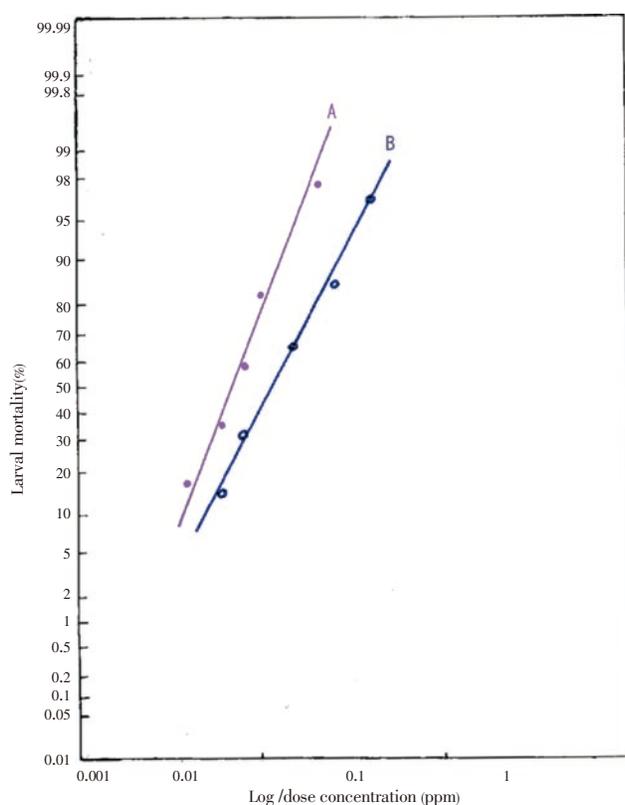


Figure 3. Regression lines (LC-p lines) resulting from icon bioassay of larvae of the laboratory (A) and field (B) strains of *Ae. aegypti*.

3.2. Adult susceptibility

The susceptibility results of 100 wild *Ae. aegypti* adults exposed to diagnostic dosages of deltamethrin (0.05%), lambda-cyhalothrin (0.05%), and cyfluthrin (0.15%) were observed (Table 2). The percentage mortality rate (77%) during the 24 h exposure period was lowest in the group treated with lambda-cyhalothrin. In the other adulticide

treatments, the percent mortality rate of *Ae. aegypti* in the group exposed to cyfluthrin (90%) was higher than that in the deltamethrin-exposed group (86%). The number of survivors was highest in the lambda-cyhalothrin-treated group, intermediate in the deltamethrin-treated group, and lowest in the cyfluthrin-treated group. With reference to the WHO criteria of adult susceptibility, it seems likely that the wild *Ae. aegypti* populations tested here possess high tolerance to deltamethrin and cyfluthrin as well as resistance to lambda-cyhalothrin.

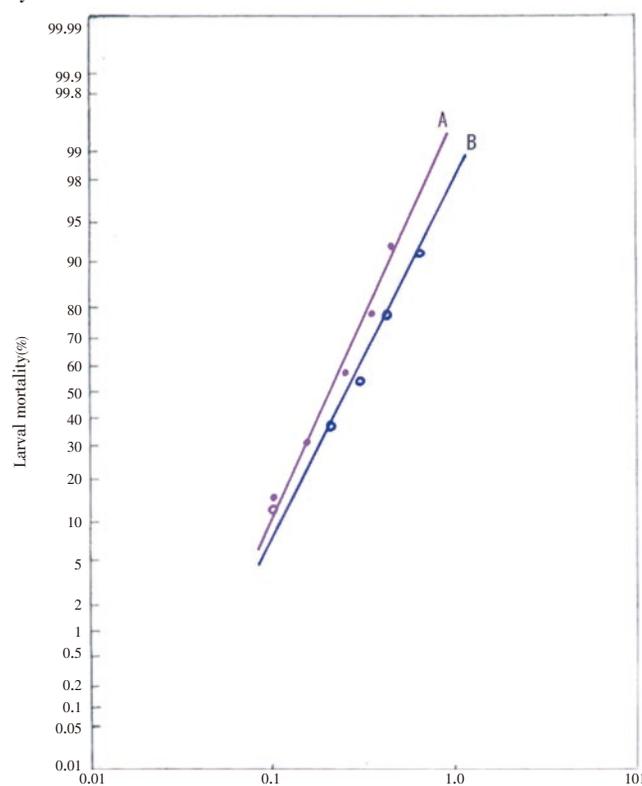


Figure 4. Regression lines (LC-p lines) resulting from bacilod bioassay of larvae of the laboratory (A) and field (B) strains of *Ae. aegypti*.

4. Discussion

The present study was performed to determine the susceptibility of *Ae. aegypti* to commonly used insecticides in Makkah City, Saudi Arabia. The most essential observation in this study was that wild larval populations were less susceptible to various agents than their laboratory-adapted counterparts. The LC₅₀ values of the larvicides used, particularly icon and actellic, were markedly lower among the laboratory-adapted larvae. In addition, mortality rates after exposure of adults to three pyrethroids indicated that cyfluthrin was the most effective adulticide followed by deltamethrin.

We observed a critical effect of strain on susceptibility. Field-collected larvae reared to the early 3rd or 4th instar stage in the laboratory were less susceptible to all larvicides tested than the laboratory-adapted strain. Similar observations have been reported previously in a study in which temephos was tested against larvae derived from wild *Ae. aegypti* mosquitoes after one generation and laboratory-adapted larvae^[23]. The results indicated that this organophosphate insecticide had a greater effect on the latter group. The authors suggested that the frequent use of insecticides in mosquito and agricultural pest control operations has contributed to selection for resistance in the natural populations. In a related study, Ocampo *et al*^[24] investigated the population dynamics of *Ae. aegypti* in relation to insecticide resistance and its enzymatic mechanism. They reported variability in levels of mixed function oxidases, which were attributed to differing levels of insecticide selection pressure at the sites from which their experimental mosquitoes were collected. For insect pests, selection pressure that leads to the acquisition of the ability to tolerate insecticides is often associated with the frequency of insecticide use^[25–35]. However, many other factors also stimulate the occurrence of insecticide tolerance. For example, frequent use of insecticides with the same mode of action can accelerate the development of resistance^[36]. In Makkah City, larviciding and house-to-house spraying of adulticides are common practices after dengue outbreaks or when adult mosquito densities are high (Aziz, pers. com). Most common insecticides (icon, deltamethrin, cyfluthrin, and lambda-cyhalothrin) have pyrethroids as their active constituents, which is clearly conducive to insecticide persistence in the environment. Although we did not investigate insecticide resistance in the present study, by the year this study was conducted. The ancient generations of *Ae. aegypti* used would have come into contact with pyrethroids, and therefore, the low susceptibility observed in the wild strain was similar to the result of previous contact with insecticides persisting in the environment.

Both the larvae of the laboratory and field strains of *Ae. aegypti* exposed to the pyrethroid icon tended to die at greater rates than those exposed to the organophosphate actellic or the bacterial insecticide bactilod. In addition,

exposure of adults to the pyrethroids cyfluthrin, deltamethrin, and lambda-cyhalothrin resulted in increased mortality rates. Adult bioassays were performed using individuals derived from wild larvae after one generation under laboratory conditions. Taken together, these results strongly suggest a high level of pyrethroid tolerance in the wild population.

Our study emphasized the susceptibility status of *Ae. aegypti* to frequently used insecticides in Makkah City. Especially, our results indicated an increased level of tolerance to operational dosages of pyrethroids in wild populations of *Ae. aegypti*, which may preface the emergence of resistance. The major factors influencing the development of insecticide resistance include the frequency of application, the mode of action of the insecticides applied, and dispersal potential of the target insect. The incidence rates of arboviral infections have increased in Saudi Arabia over the past several years^[6–8]. In response to dengue outbreaks, the MOH has implemented operational controls using an arsenal of insecticides in which the pyrethroid family accounts for a high proportion. In addition, many entrepreneurs have been found to be selling misbranded insecticides^[13], meaning that not only the recommended insecticides are being used. These observations combined with the increasing spread of *Ae. aegypti*^[9] may lead to large-scale problems of insecticide resistance. Therefore, the results of the present study suggest the need for mosquito control professionals to search for strategies to prevent or delay the development of insecticide resistance in Makkah City, and this may be valuable to other areas with similar problems. Frequent planned changes of chemical families of the insecticide arsenal used have the potential to defer or avert the development of resistance. This concept is seemingly most important with mixtures where insecticides from the pyrethroid family are present in lower proportions, as these result in not only the rapid development of resistance, but also cross-resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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