

Resistance Status of *Aedes aegypti* to Insecticides in the Jazan Region of Saudi Arabia

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The present study was designed to provide information on the susceptibility status of the adult and larvae of *Aedes aegypti* mosquitoes in Jazan region of Saudi Arabia. Bioassay tests were performed on adults and larvae by using WHO recommended concentrations and test kits. Adults of *Ae. aegypti* mosquitoes were exposed to test papers impregnated with Lambda-cyhalothrin (0.05%), Cyfluthrin (0.15%), Deltamethrin (0.05%), Permethrin (0.75%), Fenitrothion (1%), Bendiocarb (0.1%) and DDT (4%) insecticides. *Ae. aegypti* mosquitoes were found to be susceptible only to Cyfluthrin; (mortality rate was 100 %), whereas variable resistances were observed from the rest of the other insecticides tested (mortality rates ranged between 93.6 and 17%). Larvae were subjected to different concentrations of Diflubenzuron, Methoprene (IGRs) and Temephos (Organophosphate). The inhibition of adult emergence (IE_{50} and IE_{95}) values for the IGRs and the (LC_{50} and LC_{95}) for Temephos have been determined by log-probit regression analysis. The *Ae. aegypti* larvae were resistant to Temephos (LC_{50} 61.8 - LC_{95} 35600.1 mg/l) and showed high susceptibility to Methoprene than Diflubenzuron (IE_{50} 0.49 - IE_{95} 10.9 mg/l) and (IE_{50} 0.86 and IE_{95} 93.8 mg/l), respectively. The larvae were more susceptible to Methoprene than Diflubenzuron by 1.8 folds.

Key words: Insecticides, resistance, *Aedes aegypti*, Jazan region, Saudi Arabia.

Dengue virus (DENV, Flaviviridae, Flavivirus) and Chikungunya virus (CHIKV, Togaviridae, Alphavirus) are the most significant human viral pathogen spread by the bite of an infected mosquito in most tropical regions. The annual reported cases are estimated to be 50-100 million, including 500,000 severe cases of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS)¹. DENV are the most prevalent mosquito-borne human virus worldwide.² Globally, *Aedes aegypti* and *Aedes albopictus* are the main epidemic vectors of DENV and CHIKV.^{3,4}

With no vaccine or antiviral therapy currently available, disease prevention relies largely on surveillance and mosquito control by using insecticides.

Pyrethroids are still the mainstay of vector control program. The use of pyrethroid insecticides has, however, increased and that of the organochlorines and some of the more toxic organophosphate compounds has decreased in recent years.

The extensive use of insecticide in agriculture, house spraying and space spraying result in the appearing of insect that can survive and make resistance to insecticides. Appropriate monitoring of vector resistance to insecticides is an integral part of planning and evaluation of insecticide uses in control programs.

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Unfortunately, many vector control programs are threatened by the development of insecticide resistance in *Ae. aegypti* and *Ae. Albopictus*.^{5,6} Resistance to multiple insecticides (e.g. pyrethroids and organophosphates) has been reported in *Ae. Aegypti* in South America and the Caribbean.^{7,6} DDT resistance is now widely distributed in *Ae. aegypti* throughout northern Thailand and it is also resistant to the pyrethroids, permethrin and deltamethrin, in many areas.^{8,9}

In India, Most of the studies reviewed have revealed that the adult *Aedes aegypti* and *Aedes albopictus* mosquitoes are resistant to DDT, but generally, remain susceptible to malathion, temephos, propoxur and fenitrothion. The larval stages of *Aedes aegypti* and *Aedes albopictus* have also been found to be resistant to DDT, but susceptible to larvicides, namely temephos, fenthion, and malathion.¹⁰

In South India, workers¹¹ reported that adult *Aedes aegypti* and *Ae. albopictus* were resistant to DDT and dieldrin, but susceptible to propoxur, fenitrothion, malathion, deltamethrin, permethrin and lambda-cyhalothrin. The resistance to pyrethroids (permethrin and deltamethrin) has been developed in *Ae. aegypti* in most of the collected areas of Thailand.¹² Workers have tested *Aedes aegypti*, which is the main dengue vector in Baan Suan community (Thailand) with deltamethrin, permethrin and fenitrothion.⁹ Their results showed that dengue mosquitoes are clearly resistant to permethrin and tolerant to deltamethrin, but were 100% susceptible to fenitrothion.

In Costa Rica, the *Ae. aegypti* larvae showed higher resistance levels to the organophosphate (OP) temephos and the adult were resistant to deltamethrin, but susceptible to bendiocarb, chlorpyrifos, and cypermethrin.¹³ In Havana city, a high resistance of *Ae. aegypti* larvae to temephos and fenthion was observed.¹⁴

Currently, *Ae. aegypti* resistance to temephos is widespread in the Americas.¹⁵ Acquired resistance by *Ae. aegypti* to temephos was reported in Brazil¹⁶⁻¹⁸, Thailand¹⁹, Bolivia and Argentina.²⁰ Recently, incipient resistance to temephos was also reported in *Ae. aegypti* in Argentina.²¹

In Malaysia, *Aedes* larvae tolerance against temephos has been reported and have

exhibited moderate resistance toward methoprene and low resistance toward pyriproxyfen^{22, 23}, but susceptible to diflubenzuron, cyromazine, and novaluron.²⁴

During the past two decades, considerable progress has been made in the development of natural and synthetic compounds known as insect growth regulators (IGRs) which are capable of interfering with the process of growth, development, and metamorphosis of the target mosquito species. Two types of IGRs are available, one which inhibit the growth of larvae (Methoprene and Pyriproxyfen) due to juvenile hormone-like action and known as JH mimics or analogs and the other type of IGR compound (Diflubenzuron) which interferes with chitin production leading to moulting disturbances, resulting in the death of the insect.

In Jeddah, Saudi Arabia, the LC₅₀ of five insecticides against *Ae.aegypti* larvae have been evaluated.²⁵ The results revealed that the larvae proved to be more susceptible to Lambda-cyhalothrin (0.01 ppm) than Snap >γPermethrin 11% + Tetramethrin 1% + Piperonyl butoxide 11% >γ (0.048 ppm) and Bacilod >γ*Bacillus thuringiensis israelensis*?γ (0.3 ppm) by about 4.8 and 30 folds, respectively. Moreover, Baycidal >γTriflumuron 25WP?γ (0.0007 ppm) proved to be more effective against *Ae. aegypti* than Sumilarv >γPyriproxyfen 0.5g ?γ(0.003 ppm) by about 4.3 folds.

In Makkah city, Saudi Arabia, it was found that the Lambdacyhalothrin was the most effective larvicide against *Ae. aegypti* with LC₅₀ values of 0.007 ppm and 0.012ppm for the laboratory and field strains, respectively.²⁶

In Jazan region of Saudi Arabia, there are no available data on pesticide resistance for *Ae. aegypti* against IGR and chemical insecticides used in the area. In order to implement effective and sustainable arbovirus vector control measures, there is an urgent need to determine the susceptibility of the major vector of DENV and CHIK to insecticides commonly used for mosquito control. We, therefore, examined the insecticide susceptibility for the adult and Larvae of *Ae. aegypti* populations in Jazan region using WHOPES-approved procedures.^{27, 28}

MATERIALS AND METHODS

This study was carried out in Jazan region which is located in Southwest Saudi Arabia, lies between 16°-12, and 18°-25, N latitude. The Jazan region is situated in the subtropical zone and has an average monthly temperature ranging between 25.8°C in January to 33.4°C in July and average relative humidity ranges between 55% and 72.5%, with an average rain of 77 - 56.7 mm, (August and October), respectively.²⁹

Mosquitoes

Aedes aegypti larvae were collected from different parts of Jazan region and brought to the insectary of the National Center for Vector-Borne Disease in Gizan city. The larvae were kept under control conditions of temperature (25 ± 2) °C and relative humidity (70-80%) with a photo period of 12-hour darkness and 12-hour light. Pupae from water medium were transferred to mosquito rearing cages (30 cm³). Resulting mosquito adults were provided with 10% glucose solution supplemented with 1% vitamin B complex soaked in cotton wool.

Female mosquitoes were permitted to blood-feed on pigeons after 5 days of adult emergence. After 3 days of feeding, a piece of moist filter paper in a porcelain bowl half filled with water was introduced for egg deposition.

Eggs from filial generation (F₁) were hatched and larvae were reared in plastic trays and fed every alternate day with a powdered mixture of wheat, yeast and milk (1:1:1). Late third or early fourth instar larvae were used for larval bioassay testing. While 3-days sugar-fed adults derived from wild larvae after one generation under laboratory conditions were used for adult bioassay testing.

Insecticides

The insecticides used in this study for the larval and adult susceptibility tests were the diagnostic dosages as specified in the WHO standard methods.^{30, 31} The insecticides were obtained from WHO Collaborating Centre in Malaysia (Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Penang). Larvae were tested against two Insect Growth Regulators (IGRs); Diflubenzuron (20 mg/l, 4 mg/l, 0.8 mg/l, 0.16 mg/l and 0.032 mg/l) and Methoprene (20 mg/l, 4 mg/l, 0.8 mg/l, 0.16 mg/l and 0.032 mg/l) and organophosphate; Temephos (0.005, 0.025, 0.125 and 0.625 mg/L). While adults

were tested against organochlorine (DDT 4%), organophosphate (Fenitrothion 1%), four pyrethroids (Permethrin 0.75%, Lambda-cyhalothrin 0.05%, Deltamethrin 0.05%, Cyfluthrin 0.15%), and Carbamate (Bendiocarb 0.1%).

Larval Bioassay

Lots of 20 (third and fourth instar) larvae were placed in glass beakers filled with 249 ml of distilled water and 1 ml of each concentration of Diflubenzuron, Methoprene and Temephos. Five replicates of 20 larvae for each concentration and for control trials were used. For the Temephos, the numbers of dead larvae were recorded after 24 hours of the introduction of larvae to the beakers. While for the two IGRs, the duration of the observation period was that required for complete adult emergence in the control batches.

Percent inhibition of adult emergence (IE) was calculated and IE₅₀ and IE₉₅ values (effective concentration required for 50 and 95% emergence inhibition) were calculated for each of Diflubenzuron and Methoprene. Whereas, lethal concentration (LC) was calculated for Temephos (LC₅₀ and LC₉₅).

Adult bioassay

Sugar-fed, 3-5 days old-female mosquitoes were tested. A batch of 25 adults was introduced into holding tube before being exposed to insecticide-impregnated papers. Equal numbers of control tests were also carried out by exposing mosquitoes to insecticide-free papers. The experiment was replicated four times. After the respective period of exposure, all mosquitoes were transferred to new tubes, provided with 10% sugar solution and held for 24 hours recovery period. Mortality was recorded and resistance status was determined as per WHO criteria²⁸; a population is considered susceptible if the mortality rate is 98-100%, the possibility of resistance (80-97%) and resistant (<80%).

Data analysis

Data were analyzed using computerized probit analysis program (Biostat 5) to determine the LC₅₀ - LC₉₅, and IE₅₀ - IE₉₅ in the larval bioassay.

RESULTS AND DISCUSSION

Larval susceptibility

The efficacy of Methoprene and Diflubenzuron against *Ae. aegypti* Larvae is shown

in Table 1. The result shows a high resistance of *Ae. aegypti* larvae to Temephos ($LC_{50} = 61.8$ mg/l., and the best percentage mortality was obtained at the highest diagnostic dose of 0.625 mg/l was only 10%). Prolonged use of Temephos as a larvicide in Jazan region since 1986 could be one possible reason. Resistance to Temephos was detected in the region at 2003. Many authors around the world have reported resistance to Temephos in Havana¹⁴, Costa Rica¹³, Brazil¹⁶⁻¹⁸, and Thailand¹⁹. Similar results were also recorded in Bolivia and Argentina.^{20,21} Contradictory, these findings are in contrast to the results reported in India¹⁰, where *Ae. aegypti* larvae were found susceptible to the Temephos. While in Malaysia, *Ae. aegypti* larvae showed to be tolerant to Temephos.^{22,23}

The development of a new class of synthetic insect growth regulators (IGRs) Benzoylphenylureas (Chitin synthesis inhibitors) was a successful step forward towards non-hazardous and eco-safe Integrated Pest management (IPM).³²

The results of the efficacy of two IGRs, Diflubenzuron and Methoprene, against *Ae. aegypti* larvae are shown in Table 2.

At the maximum dose of 20 mg/l, Methoprene and Diflubenzuron produced (100% and 87.5%) mortality against *Ae. aegypti* larvae, respectively. The IE_{50} of the Methoprene and Diflubenzuron were 0.49 and 0.86 mg/l, respectively. This result indicates that mosquito larvae of *Ae. aegypti* were more susceptible to Methoprene than Diflubenzuron by 1.8 folds.

Table 1. Efficacy of Temephos against *Ae. aegypti* larvae

Insecticide	Concentration (mg/l)	Larval Mortality (%)	LC_{50} (LC_{95}) (mg/l)	95% Confidence limit	
				Lower LC_{50} (LC_{95})	Upper LC_{50} (LC_{95})
Temephos	0.005 – 0.625	0 - 10	61.8 (35600.1)	3.2 (101.9)	8.2 (1.31E+46)

Table 2. Efficacy of Diflubenzuron and Methoprene against *Ae. aegypti* larvae

Insecticide	Concentration (mg/l)	Larval Mortality (%)	LC_{50} (LC_{95}) (mg/l)	95% Confidence limit	
				Lower LC_{50} (LC_{95})	Upper LC_{50} (LC_{95})
Diflubenzuron	0.032 - 20	17 - 87.5	0.86 (93.8)	0.48 (30.9)	1.52 (583.1)
Methoprene	0.032 - 20	18 - 100	0.49 (10.9)	0.0002 (1.49)	81.9 (7.57)

*IE = Inhibition of adult Emergence

Table 3. Mortality of *adult Aedes aegypti* 24 Hours after Exposure to Deltamethrin, Permethrin, Cyfluthrin, Lambda-cyhalothrin, Fenitrothion, Bendiocarb, and DDT

Insecticide	Concentration (%)	Exposure period (Hours)	Number of Mosquitoes Tested	Mortality (%)	Resistance status
Deltamethrin	0.05	1	120	93.3	Possibility of resistance
Permethrin	0.75	1	120	86.8	Possibility of resistance
Cyfluthrin	0.15	1	120	100	Susceptible
Lambda-cyhalothrin	0.05	1	120	76.5	Resistant
DDT	4	1	120	56.3	Resistant
Fenitrothion	1	1	100	93.6	Possibility of resistance
Bendiocarb	0.1	1	100	17	Resistant

The efficacy of Diflubenzuron against *Ae. aegypti* in this study is in contradiction with that of Nusrat *et al.*³³ and Seccacini *et al.*³⁴ who noted that the Diflubenzuron was highly effective for inhibition of adult emergence, and (100%) adult emergence inhibition was observed in the presence of 0.1 ppm.

With low concentration (0.032 mg/l), Methoprene was found to be less effective in reducing the emergence of adult *Ae. aegypti* mosquito (Table 2), a result that matches the findings of Nayar *et al.*³⁵

The IE_{50} - IE_{95} range (0.86 – 93.8 mg/l) post exposure (Table 2) against Diflubenzuron indicated less effect at larval stages as compared with Methoprene (0.49 - 10.9 mg/l). This finding of the effectiveness of the Methoprene against *Ae. aegypti* larvae in Jazan region is more relevant to the results obtained by other workers.^{34, 36-40}

In Malaysia, it was found that the larvae of *Ae. aegypti* exhibited moderate resistance toward Methoprene and low resistance toward Pyriproxyfen, with resistance ratios of 12.7 and 1.4, respectively, but susceptibility to Diflubenzuron.²⁴

In this study, comparing the larvicidal activity of Methoprene with Diflubenzuron and Temephos (Table 1 and 2) it can be inferred that Methoprene is more effective against *Ae. aegypti* and can be used as an alternative larvicide in the areas where the low efficacy of Diflubenzuron and high resistance to organophosphate compounds have been detected.

Adult susceptibility

The mortality of *Aedes aegypti* 24 hours after exposure to Deltamethrin, Permethrin, Cyfluthrin, Lambda-cyhalothrin, and DDT is presented in Table 3.

The adult susceptibility from the different parts of Jazan revealed that the mosquitoes were susceptible to Cyfluthrin (100% mortality) and having a possibility of resistance to each of Fenitrothion, Deltamethrin, and Permethrin (93.6%, 93.3%, and 86.8%, respectively). While, mosquitoes were resistant to Lambda-cyhalothrin (76.5%) and DDT (56.3%), as well as, they were highly resistant to Bendiocarb (17%).

It can be seen that *Ae. aegypti* showed the possibility of resistance to Deltamethrin and Permethrin (mortality 93.3%, 86.8%, respectively) in Jazan region. This result is in accordance with

the findings of other authors in Thailand who reported the resistance of *Ae. aegypti* to Permethrin and Deltamethrin¹². Whereas, the possibility of resistance of *Ae. aegypti* to the two insecticides is contradicted with the work of authors in India who found that adults *Aedes aegypti* and *Ae. albopictus* were susceptible to Deltamethrin and Permethrin¹¹.

Deltamethrin and Permethrin could have resulted from cross-resistance with DDT. Moreover, the use of household aerosol insecticides where the main active ingredient is Permethrin might have contributed to the resistance of the species.

The development of high resistance against Bendiocarb (17%) and possibility of resistance against Fenitrothion (93.6%) could be due to the selection for resistance in the mosquitoes resulting from municipal and agricultural applications.⁴¹ In addition, Fenitrothion has been used in the region as an interdomiliary residual spray between 1987 and 1994. However, the high resistance to Bendiocarb in our study is contrasting the findings of workers in Costa Rica, where *Ae. aegypti* was susceptible to it.¹³

In this study, *Ae. aegypti* mosquitoes showed resistance to Lambda-cyhalothrin and DDT, a result which was similarly obtained by other authors in India¹¹ with the exception only to the susceptibility of *Ae. aegypti* to Lambda-cyhalothrin. Conversely, when taking the LC_{50} into consideration, workers in Jeddah found the larvae of *Ae. aegypti* to be susceptible to Lambda-cyhalothrin at a concentration of 0.01 ppm.²⁵ In Jazan region, the mosquito larvae have exhibited resistance to DDT as early as 1987 where the insecticide has long been used to control the vector of malaria.

Our findings show the appearance of resistance to multiple insecticides (e.g. Pyrethroids and Organochlorines) in *Ae. aegypti*. The same findings of multiple resistances between Pyrethroids and another group (Organophosphates) have been reported in South-East Asia¹², South America and the Caribbean.^{6,7}

The extensively successive use of Pyrethroids (Lambda-cyhalothrin, Deltamethrin, and Permethrin) in the region to control Malaria, Dengue and Rift valley mosquito vectors by the Ministries of Health, Municipality and Agriculture, respectively, might have been contributed to

accelerating the resistance to this group of insecticides.

This study revealed the effectiveness of Cyfluthrin against the adults of *Ae. aegypti* (100% mortality). This result may be attributed to the fact that Cyfluthrin is newly introduced to the region.

The resistance of *Ae. aegypti* against the tested insecticides decreased in the order of:

Bendiocarb > DDT > Lambda-cyhalothrin > Permethrin > Deltamethrin > Fenitrothion > Cyfluthrin.

CONCLUSION

In this study, Cyfluthrin was found to be the only effective Pyrethroid used in Jazan region against the adult of *Ae. aegypti*. Methoprene on other hand proved to be an effective IGR against the larvae of *Ae. aegypti* in the region.

These results suggest that continuous resistance monitoring should be conducted regularly to identify the efficacy of compounds for dengue control. Well-managed rotation of the effective insecticides, community awareness and participation, and public health campaigns to reduce larval breeding sites are recommended strategies to control dengue infections.

Moreover, the use of IGRs should be considered as an alternative when the larvae of *Ae. aegypti* develop resistance to conventional insecticides.

Further studies should be carried out to provide more information about resistance status and to identify the resistance mechanism by PCR test in order to confirm and supplement the bioassay results.

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