J. Egypt. Soc. Parasitol. (JESP), 46(1), 2016: 209- 216

STUDIES ON *AEDES AEGYPTI* RESISTANCE TO SOME INSECTICIDES IN THE JAZAN DISTRICT, SAUDI ARABIA

By

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Abstract

The present study provided 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). Adult emergence inhibition (IE_{50} & IE_{95}) values for the IGRs and the (LC_{50} & LC_{95}) for Temephos were determined by log-probit regression analysis. *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. Larvae were more susceptible to Methoprene than Diflubenzuron by 1.8 folds.

Key words: Jazan Region, Saudi Arabia, Insecticides, Resistance, *Aedes aegypti*.

Introduction

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 (WHO, 2009). DENV are the most prevalent mosquito-borne human virus worldwide (Gubler, 2002). Globally, *Aedes aegypti* and *Aedes albopictus* are the main epidemic vectors of DENV and CHIKV (Kow *et al*, 2001; Peyrefitte *et al*, 2007).

Without 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 (El-Bahnasawy *et al*, 2014a). Nowadays, use of pyrethroid however, increased and that of organochlorines and some of more toxic organophosphate compounds decreased.

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.

Generally, many vector control programs were threatened by the development of insecticide resistance (Adasi and Hemingway, 2008). This is also the case with Ae. aegypti and *Ae. albopictus* (Marcombe *et al*, 2009; Chediak *et al*, 2016). Besides, resistance to multiple insecticides (pyrethroids & organophosphates) was also reported in *Ae. aegypti* (Marcombe *et al*, 2009; Sivan *et al*, 2015).

Sharma et al. (2004) in India studied the susceptibility status in aquatic and adult stages of *Aedes aegypti* and *Ae. albopictus* at International Airports of Thiruvananthapuram and Cochin located in southern India using WHO standard test kits to conduct insecticide susceptibility tests against various organophosphates, organochlorines, carbamates and synthetic pyrethroids. They found that adult *Ae. aegypti* and *Ae. albopictus* were resistant to DDT and dieldrin, but susceptible to propoxur, fenitrothion, malathion, deltamethrin, permethrin and lambdacyhalothrin. The susceptibility test conducted on immature stages of *Ae. aegypti* and *Ae. albopictus* revealed that they are susceptible to the larvicides commonly used under the National Vector Borne Diseases Control Programme viz. Temephos (0.02 ppm), Fenthion (0.05 ppm) Malathion (1.0 ppm) and Fenitrothion (0.06 ppm).

Kushwah *et al*. (2015a) stated that control of Ae.aegypti, the mosquito vector of dengue, chikungunya and yellow fever was a challenging task. Pyrethroid insecticides had emerged as a preferred choice for vector control but are threatened by the emergence of resistance. They reported a focus of pyrethroid resistance and presence of two kdr mutations--F1534C and a novel mutation T1520I, in *Ae. aegypti* from Delhi, India. They concluded that *Ae. aegypti* population of Delhi is resistant to DDT, deltamethrin and permethrin. Two kdr mutations, F1534C and a novel mutation T1520I, were identified in this population. This is the first report of kdr mutations being present in the Indian *Ae. aegypti* population. Highly specific PCR-RFLP assays were developed for discrimination of alleles at both kdr loci. A positive association of F1534C mutation with DDT and deltamethrin resistance was confirmed. Kushwah *et al.* (2015b) reported insecticide susceptibility status of *Ae. albopictus* to DDT and pyrethroids in some Indian populations and status of presence of knockdown resistance (kdr) mutations. They concluded that *Ae. albopictus* developed resistance against DDT and that there was emergence of incipient resistance against pyrethroids in some populations. So far, there was no evidence of presence of knockdown resistance (kdr) mutation in *Ae. albopictus*.

Jirakanjanakit *et al*. (2007) in Thailand used the diagnostic dose subsequently to evaluate the susceptibility/resistance status in F1 progenies of field-collected samples from Bangkok and allover Thailand. They found that *Ae. aegypti* of one collection site each in Bangkok, Nakhon Sawan (north-central), and Nakhon Ratchasrima (north-east) were resistant to temephos, with mortality ranging from 50.5 to 71.4%. Also, there was a trend of resistance to temephos among *Ae. aegypti* populations of all studied districts. They added that various levels of temephos susceptibility were however, found in Bangkok populations, including resistance and incipient resistance. They added that in Chonburi Province, all were susceptible to temephos with an indication of tolerance in one sample. Besides, *Aedes* from Songkhla (south), Chiang Rai (north), Kanchanaburi (west), and Chanthaburi (east) remained susceptible to temephos during the sample collecting period. Bioassay tests on Ae. albopictus populations from Nakhon Sawan, Nakorn Ratchasima, Songkhla, and Kanchanaburi showed high susceptibility to temephos. They concluded that although temephos was potentially effective in many areas of the country, a noticeable trend of resistance indicated that alternative vector control methods should be periodically applied.

Bisset *et al*. (2013) in Costa Rica studied the levels of insecticide resistance and metabolic resistance mechanisms involved in two *Ae. aegypti* strains collected from Puntarenas and Limon. Bioassays with larvae were performed according to WHO guidelines and adult resistance was measured by standard bottle assays. Beta-esterases, cytochrome P450 monooxygenases, and glutathione S-transferases (GST), were assayed by synergists and biochemical tests, wherein the threshold criteria for each enzyme using the susceptible Rockefeller strain. The results showed higher resistance levels to organophosphate; temephos and pyrethroid; deltamethrin in larvae. Efficacy of commercial formulations of temephos against *Ae. aegypti* populations was 100% mortality up to 11 & 12 day post-treatment with daily water replacements in test containers. Temephos and deltamethrin resistance in larvae were associated with high esterase activity, but not to cytochrome P450 monooxygenase or GST activities. Adult mosquitoes were resistant to deltamethrin, and susceptible to bendiocarb, chlorpyrifos, and cypermethrin. Because temephos and deltamethrin resistance are emerging at the studied sites, alternative insecticides should be considered. The insecticides chlorpyrifos and cypermethrin could be good candidates to use as alternatives for *Ae. aegypti* control. Besides, *Ae. aegypti* resistance to temephos was reported in the Americas (WHO, 1992), Brazil (Lima *et al*, 2003; Macoris *et al*, 2003; Braga *et al*, 2004), Bolivia and Argentina (Biber *et al*, 2006), Cuba (Magdalena *et al*, 2004). and incipient resistance to temephos in Argentina (Seccacini *et al*, 2007).

In Malaysia, *Aedes* larvae tolerance against temephos was reported and exhibited moderate resistance toward methoprene and low resistance toward pyriproxyfen (Lee and Lime, 1989; Chen *et al*, 2005), but susceptible to diflubenzuron, cyromazine, and novaluron (Lau *et al*, 2015).

During the past two decades, considerable progress in the development of natural and synthetic compounds; as insect growth regulators (IGRs) interfered with the process of growth, development, and metamorphosis of the target mosquitoes. Two types of IGRs are available, one inhibited the larval growth (Methoprene and proxyfen) by the juvenile hormone-like action and known as JH mimics or analogs and the second (Diflubenzuron) interferes with chitin production leading to moulting disturbances and death.

In Jeddah, Saudi Arabia, the LC_{50} of five insecticides against *Ae. aegypti* larvae were evaluated (Alghamdi *et al*, 2008). The larvae proved to be more susceptible to Lambda-cyhalothrin (0.01 ppm) than Snap (Permethrin 11% + Tetramethrin 1% + Piperonyl butoxide 11%; 0.048ppm) and Bacilod (*Bacillus thuringiensis israelensis*; 0.3ppm) by about 4.8 and 30 folds, respectively. Moreover, Baycidal (Triflumuron 25WP; 0.0007ppm) proved to be more effective against *Ae. aegypti* than sumilarv (Pyriproxyfen 0.5g; 0.003ppm) by about 4.3 folds. In Makkah City, the Lambda-cyhalothrin was the most effective larvicide against *Ae. aegypti* with LC_{50} values of 0.007ppm & 0.012ppm for lab and field strains, respectively (Althbiani *et al*, 2011).

In Jazan Region, non-data on pesticide resistance for *Ae. aegypti* against IGR and chemical insecticides used. In order to implement effective and sustainable arbovirus vector control measures, and there was an urgent need to determine susceptibility of the major vector of dengue, Rift Valley fever and Chikungunya to insecticides commonly used for mosquito control.

The present study aimed to evaluate the insecticide susceptibility for adult and larvae of *Ae. aegypti* populations in Jazan region by using WHOPES-approved procedures (WHO 2005; WHO, 2006).

Materials and Methods

This study was carried out in Jazan region which is located in Southwest Saudi Arabia, lies between 16^o-12, and 18^o-25, N latitude. The Jazan region is situated in the subtropical zone and has an average monthly temperature ranging between 25.8^oc in January to 33.4^oc 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 (Al-Sheikh, 2011).

Aedes aegypti larvae were collected from different areas allover Jazan and immediately transported to the insectory of the National Center for Vector-Borne Disease. The larvae were kept under control conditions of 25±2C° and relative humidity; 70-80% with a photo period of 12-hour darkness and 12-hour light. Emerging pupae were transferred to mosquito rearing cages ($30x30cm^3$). The emerging adults were provided with 10% glucose solution supplemented with 1% vitamin B complex soaked in cotton wool.

Females 5 days old were feed on clean laboratory-bred pigeons, three days later, a piece of moist filter paper in a porcelain bowl half-filled was introduced for egg deposition. Eggs from filial generation (F_1) hatched and larvae were reared in plastic trays and fed every other day with a powdered mixture of wheat, yeast and milk (1:1:1). Larvae were used for bioassay testing. Three-days sugar-fed adults from wild larvae after one generation under laboratory conditions were used for bioassay.

Insecticides used were diagnostic dosages (WHO, 1981) obtained from WHO Collaborating Centre in Malaysia. Larvae were tested against 2 insect growth regulators (IGRs); Diflubenzuron (20mg/l, 4mg/l, 0.8 mg/l, 0.16 mg/l & 0.032 mg/l); Methoprene (20 mg/l, 4 mg/l, 0.8mg/l, 0.16 mg/l & 0.032 mg/l) and Temephos (0.005, 0.025, 0.125 & 0.625mg/L). Adults were tested against DDT 4%, Fenitrothion 1%, Permethrin 0.75%, Lambdacyhalothrin 0.05%, Deltamethrin 0.05%, Cyfluthrin 0.15%, and Bendiocarb 0.1%.

Larval Bioassay: Twenty 3rd & 4th larvae were placed in labeled Pyrex glass beakers filled with 249ml of distilled water and 1ml of each of Diflubenzuron, Methoprene and Temephos, using five replicates of 20 larvae for each concentration and for control. For Temephos, dead larvae were recorded after 24 hours (Abbott, 1925). While for the two IGRs, the duration of the observation period was that required for complete adult emergence in the control batches.

Inhibition% of adult emergence (IE) was calculated and IE_{50} and IE_{95} 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 (LC_{50} & LC_{95}).

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 the insecticide-impregnated papers. Equal numbers of control tests were also carried out by exposing mosquitoes to insecticides–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 hrs recovery period. Mortality was recorded and resistance status was determine; a population is susceptible if mortality rate is 98-100%, possibility of resistance (80-97%) and resistant (<80%).

Statistical analysis: Data were analyzed using computerized Probit analysis program **(**Biostat 5) to determine the LC_{50} & LC_{95}, and IE_{50} & IE_{95} in larval bioassay.

Results

The results are shown in tables (1, 2 & 3).

Table 1. Efficacy of Temephos against *Ae. aegypti* larvae (95% Confidence limit).

Insecticide	Concentration (mg/l)	Larval mortality (%)	LC_{50} & LC_{95} (mg/l)	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 (95% Confidence limit).

Insecticides (IGRs)	Concentration (mg/l)	Larval Mortality (%)	*IE_{50} (IE_{95}) (mg/l)	Lower IE_{50} (IE_{95})	Upper IE_{50} (IE_{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)	Mosquitoes Tested	Mortality (%)	Resistance status
Deltamethrin	0.05	1	120	93.3	Possibility
Permethrin	0.75	1	120	86.8	Possibility
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
Bendiocarb	0.1	1	100	17	Resistant

Discussion

No doubt, 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) (Tunaz and Uygun, 2004).

In the present study, \larvae high resistance was found to Temephos (LC_{50}= 61.8 mg/l., with best mortality %, but at diagnostic dose of 0.625 mg/l was 10%). Prolonged use of Temephos as a larvicide in Jazan region since 1986 could be a possible reason. Resistance to Temephos was detected in the region at 2003. Resistance to Temephos was reported in Havana (Magdalena *et al*, 2004), Costa Rica (Bisset *et al*, 2013), Brazil (Lima *et al*, 2003; Macoris *et al*, 2003; Braga *et al*, 2004), and Thailand (Jirakanjanakit *et al*, 2007), as well as in Bolivia and Argentina (Biber *et al*, 2006; Seccacini *et al*, 2007).

In India, *Ae. aegypti* larvae were susceptible to Temephos (Singh *et al*., 2013), but in Malaysia, *Ae. aegypti* larvae showed tolerance to Temephos (Lee and Lime, 1989; Chen *et al*, 2005).

In the present study, larvae at dose of 20mg/l, Methoprene and Diflubenzuron produced (100% & 87.5%) mortality respectively. IE_{50} Methoprene and Diflubenzuron were 0.49 & 0.86mg/l, respectively indicated that larvae were more susceptible to Methoprene than Diflubenzuron by 1.8 folds. The present efficacy of Diflubenzuron disagreed with Nusrat *et al* (2011) and Seccacini *et al* (2008) who found Diflubenzuron was highly effective for adult emergence inhibition and 100% adult emergence inhibition was at 0.1 ppm.

In the present study, with low concentration (0.032mg/l), Methoprene was less effective to reduce adult emergence, which agreed with Nayar *et al.* (2002). The IE_{50}-IE_{95} ranged (0.86- 93.8mg\l) post exposure against Diflubenzuron indicated less effect at larval stages as compared with Methoprene (0.49-10.9mg/l). The effectiveness of Methoprene against larvae in Jazan District agreed with Seccacini *et al.* (2008), Baraga *et al.* (2005), Garg and Donahue, (1989), Sulaiman *et al.* (2004), Silva *et al* (2007) and Chen *et al.* (2008). *Ae. aegypti* larvae exhibited less resistance to Methoprene and to Pyriproxyfen with ratios of 12.7 & 1.4, respectively, but susceptible to Diflubenzuron (Lau *et al*, 2015). In the present study, Methoprene was more effective and can be used as an alternative larvicide in areas with Diflubenzuron low efficacy & high resistance to organophorous.

In the present study, as to mortality 24 hours after exposure to Deltamethrin, Permethrin, Cyfluthrin, Lambda-cyhalothrin, and DDT, the adults from different parts of Jazan were 100% susceptible to Cyfluthrin and with some resistance to Fenitrothion, Deltamethrin, and Permethrin (93.6%, 93.3% & 86.8%, respectively). *Aedes* were resistant to Lambda-cyhalo-thrin (76.5%) and DDT (56.3%) but highly resistant to Bendiocarb (17%). *Ae. aegypti* showed some resistance to Deltamethrin and Permethrin (mortality 93.3%, 86.8%, respectively). This agreed with Jirakanjanakit *et* *al* (2007a) in Thailand who reported the resistance to Permethrin and Deltamethrin.

In the present study, Deltamethrin and Permethrin could result from the cross-resistance with DDT. The use of household aerosol insecticides where the main active ingredient is Permethrin might contribute to the resistance.

The development of high resistance against Bendiocarb (17%) and possibility of resistance against Fenitrothion (93.6%) could be due to mosquito selection for resistance resulting from municipal and agricultural applications (Lines, 1988). Also, Fenitrothion was used as an interdomiciliary residual spray between 1987 and 1994. However, the high resistance to Bendiocarb in the present study was contrasted with Bisset *et al.* (2013) in Costa Rica, where *Ae. aegypti* was susceptible to it

In the present study, *Ae. aegypti* showed resistance to Lambda-cyhalothrin and to DDT, which agreed Sharma *et al* (2004) in India, except in the susceptibility of *Ae. aegypti* to Lambda-cyhalothrin. Conversely, when taking the LC_{50} into consideration. Alghamdi *et al.* (2008) in Jeddah found the larvae of *Ae. aegypti* was susceptible to Lambda-cyhalothrin at a 0.01 ppm. In Jazan region, the mosquito larvae exhibited resistance to DDT as early as 1987 where the insecticide was longer used to control of *Anopheles*.

In the present study, resistance to multiple insecticides (Pyrethroids & Organochlorines) in *Aedes* existed also between Pyrethroids and Organophosphates as reported in South-East Asia (Jirakanjanakit *et al*, 2007a), South America and the Caribbean (Rawlins *et al*, 1998; Marcombe *et al*, 2009). 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 contributed to accelerating such resistance. Generally speaking, all hydrocarbon insecticides: are risky for environment and human health (El-Bahnasawy *et al*, 2014b)

In the present study, 100% mortality of Cyfluthrin against adults of *Ae. aegypti*, may be due to the fact that Cyfluthrin is newly introduced insecticide. Resistance in descending order was Bendiocarb> DDT> Lambda-cyhalothrin> Per-methrin> Delta methrin> Fenitrothion> Cyfluthrin.

Besides, *Ae. aegypti* in Saudi Arabia (Gandhi *et al*, 2016), it was reported in Yemen (Fah-my *et al*, 2015), in Southern Egypt (Shoukry *et al*, 2012) mainly Aswan Governorate (Saleh, 2012) and *Ae. aegypti, Ae. cretinus* were reported in Lebanon (Knio *et al*, 2005). Besides, Al-Qahtani *et al*. (2016) reported that Zika virus is an emerging arbovirus of Flaviviridae family and related to dengue, Chikungunya, West Nile, yellow fever, and Japanese encephalitis virus. Generally, all these zoonotic viral hemorrhagic fevers are transmitted by *Aedes aegyptii* and other *Aedes* species.

Conclusion

Cyfluthrin proved to be the only effective Pyrethroid used in Jazan region against adult *Ae. aegypti.* Methoprene proved to be an effective IGR against local larvae. 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. IGRs should be an alternative when the larvae develop resistance to conventional insecticides.

Undoubtedly, the regional collaboration to control the spreading and prevalence of *Aedes aegypti* is a must.

Conflict of interest: The authors declared that they have no conflict of interest.

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