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The Potency of *Aedes* species in transmitting dengue fever virus with evaluating the susceptibility of vector larval stages to some insecticides.

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ABSTRACT

The present study was planned to use the technique of Polymerase chain reaction (PCR) to detect the virus of dengue fever in mosquito females of *Aedes aegypti* (L.) and *Ae. caspius* (Pallas). The results indicated that all samples tested for virus of dengue were found negative (-) except one sample of *Ae. aegypti* which was positive(+). On the other hand, susceptibility levels of mosquito larvae of field strains of *Ae. aegypti*, which is the vector of dengue fever, to some insecticides currently in use in Jeddah governorate were determined. The LC_{50} values (concentration which kills 50% of mosquito larvae) were taken in consideration. Mosquito larvae of *Ae. aegypti* proved to be more susceptible to Bactilarvae (0.012 ppm) than Safroten (0.020 ppm), Temephos (0.032ppm), Solfac (.039 ppm) and Icon (0.047ppm), respectively.

Key words: Dengue fever, *Aedes aegypti*, *Ae. caspius*,insecticides,PCR

INTRODUCTION

Dengue fever is an acute viral disease of the tropical and sub-tropical regions around the world, especially in urban and semi-urban areas (Halsted *et al*., 2001).A recent estimate shows that more than 50 million people are at risk of dengue virus exposure worldwide. Annually, there are 2 million infections, 500,000 cases of dengue hemorrhagic fever, and 12,000 deaths (Guha-Sapir and Schimme, 2005; WHO, 2005).

In Jeddah city, Saudi Arabia, dengue fever made its first appearance in 1994; and by the end of the year, approximately 300 cases were diagnosed and three dengue serotypes (Den1, Den2, and Den3) of viruses were reported (Fakeeh and Zaki, 2003). In 2006, dengue fever reported cases had risen drastically compared to the earlier recorded numbers. The government of Saudi Arabia has responded to the problem immediately by allocating more than one billion Saudi Riyals (more than two hundred fifty US dollars) to combat the disease and to bring down the number of confirmed cases drastically. The government decision has identified roles and responsibilities among different government agencies and ministries including the Ministry of Municipality and Rural Affairs; Ministry of Health; Ministry of Agriculture, and Ministry of Water and Electricity (Aburas, 2007). On the other hand, dengue fever is a mosquito-borne viral illness that caused by one of the four serotypes of dengue virus belonging to the family Flaviviridae and predominantly transmitted by many members of *Aedes* species. Lanciott (1992) was the first to describe the technique of polymerase chain reaction (PCR) to detect dengue virus in the body of the mosquito vector, and isolated the four dengue virus patterns from mosquito *A. aegypti*. Fouque *et al.* (2004), and David *et al.* (2003) identified three types of mosquitoes, *Ae. Aegypti, Ae. hensilli, Ae.albopictus* as vectors of dengue virus.

Currently there is no single vaccine with an economic value is available against serotype of dengue viruses, and therefore the primary protection against infection with the virus depends on the control of mosquitoes. The spraying of pesticides in the hotbeds of breeding to reduce the source of infection is a modern method which is used globally to reduce the reproduction and multiplication of mosquito vectors of disease and restrict their number in the environment to the extent that they do not spread the disease (WHO, 1990). This study aims to use the new approach to mosquitoes control through the identification of the carrier type of the virus that causes dengue fever with an evaluation of the effectiveness of some insecticides commonly used in control programs in the province of Jeddah against the larvae of the carrier type.

MATERIALS AND METHODS

Molocular studies of dengue virus

Preparation of mosquitoes samples

Light traps of Black-Hole was used to collect samples of adult mosquitoes from various locations of the Jeddah province, samples after collection from the field brought to the laboratory and kept in the low temperature (-86 ° C) to kill insects by freezing while preserving the genetic material. The female mosquito of genus *Aedes* species were isolated by using anatomical microscope and the identification of the species was done by using taxonomic keys (Harbach, 1988; Al-Tubiakh, 1995). Each type of insects has been distributed at each location within the vial tubes and a special code number was given to each sample.

Isolate the DNA of the virus

The viral RNA was extracted from the complex of mosquito sample and 140 ml of it was added to Kit Qiagen (QIA amp-Viral RNA Mini Kit Cat.NO.5294) according to the proposed kit protocol (MWG-Biotech, Germany).

Polymerase chain reaction (PCR)

During Reverse transcription polymerase chain reaction (RT-PCR) two primers (D1, D2) were used to identify the virus that causes dengue fever in addition to the DNA extracted with the rest of the chemicals for interaction, (Table 1). The overall mix has been developed (25 micro liter of reaction mixture) inside the PCR tube, which is specially designed to withstand the heat of polymerization chain reactions. (The device, named Ependroff Master cycler® gradient thermal cycler from Germany.).

Table 1: Diagnostic materials used in the detection of dengue virus in the body of female *Aedes* mosquitoes collected from different locations in Jeddah city.

Materials	Quantity (μ l)
PCR buffer *	5
(dNTP) A mixture of nitrogenous bases	1
D1(5'-TCA ATA TGG TGA AAC GCG CGA GAA ACC G-3')	1
D2 (5'-TTG CAC CAA CAG TCA ATG TCT TCA GGT TC-3')	1
Taq polymerase *	0.5
(25ml) Magnesium chloride solution	4
Water treatment free of RNA ase	7.5
RNA extracted	5
Total	25

* The polymerase enzyme solution and the regulator in the presence of magnesium salts stimulate the prefixes on the link DNA to be doubled and reduce the link prefixes to other sites on the DNA similar to the gene to be isolated.

Amplification of DNA sequence which requires three temperature shifts was done in the thermal cycler. The polymerase chain reaction was done in three steps.

1. In the first step the temperature was raised to 95^{0}C for 15 minutes to denature the double stranded DNA (dsDNA) which unzip the DNA strand.
2. In the second step the temperature is reduced to 55^{0}C for one minute for annealing of the primers to single strand DNA (ssDNA). (High temperature do not allow primers to anneal efficiently). This allows the link between two strings in 3' end of the one primer and 5' end of other primer.
3. The third step is to extend the DNA by raising the temperature to 72^{0}C for two minutes to construct a DNA chain by *Taq* polymerase enzyme (isolated from different thermophilic bacteria) which attaches at each primers site and extend a new DNA strand. After this extension step the temperature is raised to 72^{0}C for ten minutes. This stage is called final extension. Millions of DNA copies will be formed after 45 cycles. At the end of PCR session the out puts of RT-PCR was subjected to electrophoresis on 1.5% Agarose gel. 3 ml (it is not a true volume) of the sample was mixed with 2 ml (it is not a true volume) of Ethidium Bromide dye (Et-Br) after the integration with barcode (marker) DNA, then the sample was loaded on the gel and a current of 450 volts (it is not a true volts) for a period of eighteen hours (it is not a true time) was passed to get a length of 100pairs baseline for comparison with a sample of DNA of the virus and backwater from the tissues of insects by using electrophoresis of nucleic acids and products of PCR samples.

Evaluation of some insecticides against larvae of *Aedes aegypti*

Mosquito strain rearing

Tests were performed on a field strain of *Aedes aegypti* (L.) raised from wild larvae, collected from Jeddah. The larvae were reared until pupation and adult emergence took place for maintaining the stock colony. This strain was maintained at a room temperature of (27 ± 1C$^{\circ}$) and (70 ± 5% R. H.) with a 14: 10 (L: D) photoperiod throughout this study.

Compounds tested

The following insecticides were used:

1. The synthetic pyrethroid **Solfac** (Cyfluthrin 5%).
2. The synthetic pyrethroid **Icon** (Lambda-cyhalothrin 2.5%).
3. The organophosphates **Temephos** (Temephos 10%).
4. The organophosphates **Safroten** (Propelamphos 20%).
5. The bacterial insecticide

Bactilarvae (*Bacillus thuringiensis* 1.2%)

Larval bioassay

Susceptibility tests of *A. aegypti* larvae were conducted according to the procedure recommended by WHO (1981). Treatments were carried out by using early fourth instar larvae to serial concentrations of the tested insecticides for 24 hrs in groups of glass beakers containing 100 ml of distilled water. Five replicates of 20 larvae for each concentration and for control trials were set up. Larval mortalities were record at 24hrs post-treatment (WHO, 2005).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating the LC_{50} and LC_{95}, values which calculated by using the Finney (1971) method. GW BASIC probit1 Statistical software was used.

RESULTS AND DISCUSSION

Molecular studies of dengue virus

Fig. 1 Shows radiological picture of the agarose gel and bundles of DNA dyed textured Eithidium bromide (Et Br). As shown in the figure contains the first slot on the DNA scale. DNA Ladder is a cut or packages of different lengths of DNA known to contain holes from 2 to 9 DNA samples isolated from female mosquitoes *Ae. aegypti* to be detected for their ability to carry the virus that causes dengue fever and take Code numbers of 494 to 502 and contains openings from

10 to 14 DNA samples isolated from female mosquitoes *Ae. caspius*be detected for their ability to carry the virus that causes dengue fever and take code numbers from MP65 to MP69 and contains the slot 15 on DNA isolated from virus positive samples for comparison. It contains the slot 16 of a sample free of DNA solution as a negative control. The results showed that the sample with code number 501 were carriers of the virus as it appears on the gel packs stacked on top of each other was almost identical to the positive control treatment. This confirms that the sample with code number 501 isolated from female *Ae. aegypti*, which was collected from the area of the country to examine the positive polymerase chain, was getting package DNA with a length of 511 base pairs.

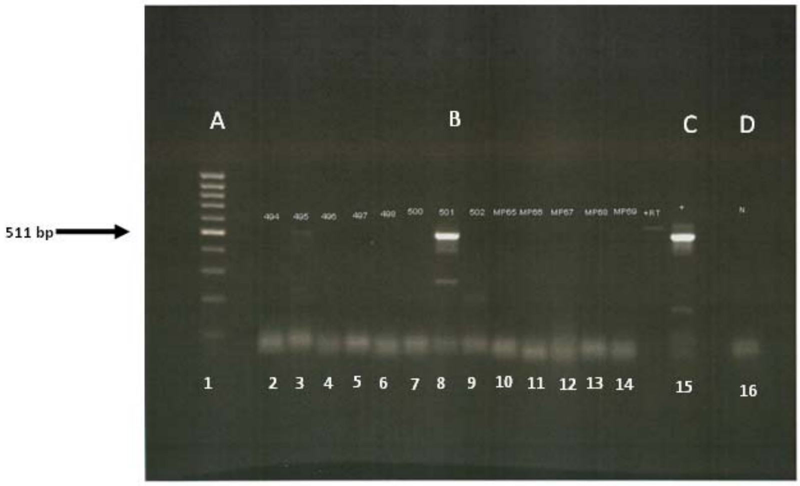

Fig. 1: The radiological image of agarose gel (1.5%) and stained by the bundles of DNA after duplicate serial by polymerization reactions. The length of the virus band is 511 base pairs.

Column A: DNA marker ladder in 100-base pairs. Column B: Tested samples for carrying the virus that causes dengue fever. Column C: Positive control for the virus length of 511 base pairs. Column D: Negative control (solution is free of DNA).

Overall this study showed the ability of female *Ae. Aegypti* in transmitting the virus that causes dengue fever in Jeddah. It was only in one sample which carriers the virus, while the rest of the samples were negative. These results are consistent with many previous studies and demonstrated the ability of *Ae. aegypti* in the transfer of dengue virus using PCR technique in different regions of the world Feres *et al.* 2006; Pinheiro *et al.*, 2005) ; (Urdaneta *et al.*, 2005).

Evaluation of some insecticides against larvae of *Aedes aegypti* mosquitoes of dengue virus in Jeddah City.

Results of susceptibility levels of fourth instar larvae of *A. aegypti* after treatments with different concentrations of the chemical insecticides Solfac, Icon, Temephos and Safroten as well as the bacterial insecticide Bactilarvae were recorded in Table 3. The effective concentrations of the chemical insecticide ranged from 0.01 – 0.12 ppm for the *Ae. aegypti* mosquito larvae. The corresponding larval mortalities were in respect 17-98% (Table 3). Records of susceptibility levels of *A. aegypti* mosquito larvae against the bacterial insecticide Bactilarvae are indicated that the range of effective concentrations of the test biocide was 0.01-0.08 ppm. Results of larval mortalities varied from 38-96%. According to the LC_{50} values, the results confirms that the bacterial insecticide Bactilarvae was more effective against the larvae of *Ae. aegypti* larvae than chemical insecticides, Safroten, Temephos, Solfac and Iconat about 1.6, 2.66, 3.25 and 3.92 Folds, respectively.

Table 1: Larvicidal activity of tested compounds against *A. aegypti.*

Insecticides	Statical parameters: effective Contcen. (ppm)	Larval[a] Mortality (%)	LC_{50} (LC_{95}) (ppm)	95%Confidence limit: LFL[c] LC_{50} (LC_{95})	UFL[a] LC_{50} (LC_{95})	Slope	χ^2 (df=3)[b]
Solfac	0.02 – 0.12	15 - 97	0.039 (0.110)	0.03 (0.09)	0.04 (0.13)	3.8	1.28
Icon	0.02 – 0.12	10-90	0.047 (0.133)	0.04 (0.11)	0.05 (0.15)	3.6	1.10
Temephos	0.01 - 0.10	28-94	0.032 (0.124)	0.03 (0.10)	0.04 (0.15)	2.75	1.24
Safroten	0.01 - 0.10	31 - 95	0.020 (0.090)	0.02 (0.07)	0.03 (0.11)	2.64	2.98
Bactilarvae	0.01 - 0.08	38- 96	0.012 (0.091)	0.008 (0.07)	0.015 (0.12)	1.88	3.11

a: Five replicates, 20 larvae each. (Nil mortality in control samples)
b: Tabulated value of χ 2 = 7.81 when the (df) degrees of freedom=3,The level of significance (0.05) to which is larger than the calculated value of χ 2 and this confirms data homogeneity.
c: LFL – lower fiducial limit.
d: UFL – upper fiducial limit, χ 2 – Chi-square value.

Generally, comparison of larvicidal activity of the tested insecticides against *A. aegypti* larvae indicates that the bacterial insecticide Bactilarvae proved to be the most effective compound followed by the OP Safroten, Temephos, and Solfac while the Icon was the least effective one as shown in Fig, 2. In general, the records indicated that mosquito larvae were more susceptible to the tested bacterial insecticide when compared with the chemical insecticides. The increase in the tolerance level of chemical insecticides may be due to the extensive local application of different chemical insecticides during control measures against present immature and adult mosquito species vectors in Jeddah. However, laboratory and field studies in this respect were carried out by several authors against a wide spectrum of mosquitoes (Saleh and Kassem, 1982; Hemingway and Ranson, 2008; Liu *et al.*, 2004; Fansiri *et al*. 2006; Al-Ghamdi *et al*. 2008; Qusti *et al*. 2010; Al-Ghamdi and Mahyoub, 2010; Saleh and Aly, 1987; Canyon and Hii, 1999; Nazni *et al.*, 2005; Lawler *et al.*, 2007).

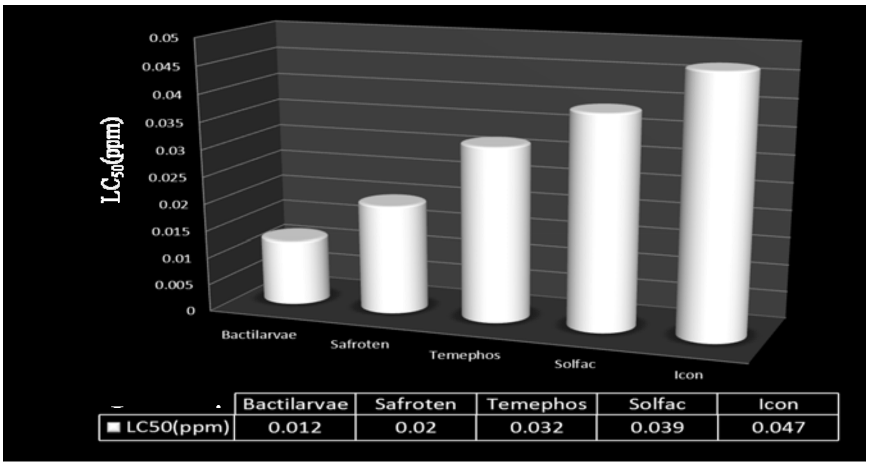

Fig. 2: lethal concentrations for 50% of fourth instar larvae of the mosquito *Ae. aegypti* after 24 hours of treatment with pesticides commonly used in mosquito control programs in the province of Jeddah.

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