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Susceptibility Tests on Insecticides Used to Control Mosquitoes in Jordan

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Abstract

Insecticide susceptibility tests were conducted on five mosquito species collected from two localities in the Jordan Valley (Deir Alla and Ghor as Safi) in 2014. Diagnostic concentration of Temephos (0.25mg/L) was used against the larvae, while diagnostic concentrations of Deltamethrin (0.025%) and Lambdacyhalothrin (0.03g/m2) were used against the adults. The procedures of WHO were followed. A total of 25 larvae were used for adult or larval test for each replicate. All experiments were conducted at 27±2°C. Results showed that all larvae of the tested species in both locations were susceptible to Temephos. The Deir Alla population of *An. superpictus* adults showed a transit susceptibility (96% pooled mortality) to Deltamethrin. The Ghor as Safi populations of *Cx. theirelli* and *Cx. laticinctus* showed resistance to Deltamethrin with 87 and 86% pooled mortality, respectively. *Cx. pipiens* was resistant to Lambdacyhalothrin at the diagnostic doses (78% pooled corrected mortality). The least pooled corrected mortality (76%), among all populations and species, was found in Deir Alla population of *Cx. theirelli*, when treated with Lambdacyhalothrin. Further tests are needed to study the response of a larger number of populations covering different locations in Jordan. A control program should be prepared taking in consideration the resistance to insecticide by alternation with different groups including Bti or juvenile hormones.

Keywords: Mosquitoes, Insecticides, Susceptibility, Resistance, Jordan.

1. Introduction

Mosquitoes are common annoyance insects and are known to be vectors of some important diseases like malaria. Control programs implemented by the personnel of Malaria Control Program (MCP) at the Ministry of Health (MOH) to control mosquito larvae from 1953 to 1966, included the use of Solar Oil and DDT (dichlorodiphenyltrichloroethane) on a weekly basis. From 1967 to 1968, only the Solar Oil was used. From 1969 up to date, Temephos at the concentration (0.5 ppm) was applied weekly with an interruption of spraying for 3 to 4 months a year during the cold months. For controlling adult mosquitoes, Residual House Spraying (RHS), including Indoor Residual Spraying (IRS) with DDT, using a dosage of 2g/m2, was applied since 1959, with two rounds a year until 1983, and then one round a year until 1993. Deltamethrin 2.5% EC was used for wall and space spraying from 1994 up to date by MCP very strictly and in limited areas around diagnosed malaria cases when epidemiologic investigation showed a risk of local transmission.

Research on Jordanian mosquitoes included their ecology, systematics, biology and control. Al-Khalili (1997) surveyed the mosquitos' species in Jordan and provided significant data about their distribution. In addition, he constructed identification keys with original drawings for species in Jordan and surrounding areas. A list of 54 mosquito species occurring in Jordan and the surrounding countries was given. In 1996 and 1997, Al-Khalili *et al.* (2000) conducted a countrywide survey of mosquitos' larvae and raised the number of species known in Jordan from 23 to 28. Their distribution, habitats, collecting dates, species associations and number of larvae examined were provided. Al-Jaran and Katbeh-Bader (2001) studied the biology of the Jordanian population of *Culiseta longiareolata* (Macquart) which is not known to feed on human blood. Khyami-Horani *et al.* (1995) studied the susceptibility of *Culex pipiens molestus* to standard strains of *Bacillus thuringiensis* (Bti -IPS 82, 15000 I.T.U. against *Aedes aegypti* L.) and

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Bacillus sphaericus (Bs-SPH 88, 1700 I.T.U. against *A. aegypti*) in Jordan. Khyami-Horani *et al.* (1996) evaluated the mosquito larvicidal toxicity of endospore-forming bacilli isolated from Jordan. Katbeh-Bader *et al.* (1999) studied the effect of temperature on the susceptibility of *Culiseta longiareolata* (Macquart) to two standard strains of biocontrol bacteria. Khiyami *et al.* (1999) isolated endospore-forming bacilli toxic to *C. longiareolata* in Jordan. They found 10 out of 80 endospore-forming bacilli, isolated from various habitats in Jordan, to be highly toxic to the 4th instar larvae of *C. longiareolata*.

The available literature shows that no data have been published on the susceptibility of the Jordanian mosquitoes to insecticides. Therefore, the aim of this study is to evaluate the susceptibility of five mosquito species to insecticides that have been used to control them for a long time in Jordan.

2. Materials and Methods

Mosquito larvae were collected from two localities in the Jordan Valley: Deir Alla (Abatah site: 31° 03' 53"N, 35°29' 50") and Ghor as Safi (Assal site: 31° 11' 36"N, 31°38"E). Both locations are below the sea level. Susceptibility tests were conducted on a group of the collected larvae soon after the collection, while the other group was reared to adult stage to be used in the susceptibility tests. Mosquito species, insecticides used, their concentrations and localities are provided in Table 1.

The procedures recommended by World Health Organization (WHO) were followed for adult mosquito tests (WHO, 2013), using the diagnostic doses and concentrations. In this procedure, six sheets of clean white paper (12 x 15 cm), rolled into a cylinder shape, were inserted into six holding tubes (one per tube) and fastened into position with a steel spring-wire clip. The tubes were attached to slides. A total of 150 active female mosquitoes were aspirated (in batches) from a mosquito cage into the six holding tubes through the filling hole in the slide to give six replicate samples of 25 mosquitoes per tube (4 treated and 2 control). Once the mosquitoes were transferred, the slide unit was closed and the holding tubes were set in an upright position for one hour. Each of the 4 exposure tubes was lined with a sheet of insecticide-impregnated paper, while the 2 control exposure tubes were lined with oil-impregnated papers; each was fastened into position with a copper spring-wire clip. The empty exposure tubes were attached to the vacant position on the slides and with the slide unit open, the mosquitoes were blown gently into the exposure tubes. Once all the mosquitoes were in the exposure tubes, the slide unit was closed and the holding tubes were detached and set to one side. Mosquitoes were kept in the exposure tubes, which were set in a vertical position with the mesh-screen end uppermost, for a period of 1 hour. Then the mosquitoes were transferred back to the holding tubes. The exposure tubes were detached from the slide units. A pad of a cotton-wool soaked in sugar water was placed on the mesh-screen end of the holding tubes. Mosquitoes were maintained in the holding tubes for 24 hours (the recovery period). At the end of recovery period, the number of dead mosquitoes was counted and recorded. An adult mosquito was considered to be alive if it was able to fly, regardless to the number of legs remaining. Any knocked down mosquitoes, whether or not with lost legs or wings, were considered moribund and were counted as dead. All tests were conducted at 27±2°C.

The procedures recommended by WHO (2005) were followed for larval mosquitoes tests. Batches of 25 third or fourth instar larvae were transferred by means of screen loops small disposable test cups, each containing 249 ml of water and 1 ml of the insecticide solution to give the required concentration. The test containers were held at 27±2°C and, after 24 h of exposure, larval mortality rate was recorded. Dead larvae were those that did not move when probed with a needle. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. If the control mortality was between 5 and 20%, the mortalities of the treated groups were corrected according to Abbott's formula:

$$\text{Mortality (\%)} = \frac{X - Y}{X} 100$$

where X = percentage survival in the untreated control and Y = percentage survival in the treated sample.

Table 1. List of mosquito species, stages, insecticides concentrations and localities in Jordan.

Mosquito species	Stage	Insecticides concentration	Locality
An. sergenti	Larvae	Temephos 0.25mg/L	Ghor as Safi
An. superpictus	Larvae	Temephos 0.25mg/L	Deir-Alla
Cx. laticinctus	Larvae	Temephos 0.25mg/L	Ghor as Safi
Cx. laticinctus	Larvae	Temephos 0.25mg/L	Deir-Alla
An. sergenti	Adult	Deltamethrin 0.025%	Ghor as Safi
An. superpictus	Adult	Deltamethrin 0.025%	Deir-Alla
Cx. laticinctus	Adult	Deltamethrin 0.025%	Ghor as Safi
Cx. theirelli	Adult	Deltamethrin 0.025%	Ghor as Safi
Cx. laticinctus	Adult	Deltamethrin 0.025%	Deir-Alla
Cx. pipiens	Adult	Lambdacyhalothrin 0.03g/m^2	Ghor as Safi
An. superpictus	Adult	Lambdacyhalothrin 0.03g/m^2	Deir-Alla
Cx. theirelli	Adult	Lambdacyhalothrin 0.03g/m^2	Deir-Alla

3. Results and Discussion

Results of susceptibility tests are presented in Table 2 which shows that all larvae of the tested species, in the two locations, were susceptible to Temephos. Their pooled corrected mortality rates ranged between 99-100%. However, the Deir Alla population of *An. superpictus* adults showed a transit susceptibility to

Deltamethrin (96% mortality). The Ghor as Safi populations of *Cx. theirelli* and *Cx. laticinctus* showed a relative resistance to Deltamethrin (87 and 86% mortality, respectively). *Cx. pipiens* was found to be resistant to Lambdacyhalothrin (78% mortality) at the diagnostic doses. The least pooled corrected mortality (76%), among all species, was found in Deir Alla population of *Cx. theirelli* when treated with Lambdacyhalothrin.

The high susceptibility of the larvae to Temephos, despite the fact that it was applied since 1969 till now on a weekly basis, may be due to the interruption of spraying for 3 months a year during the cold months and/or to the migration of the susceptible mosquitoes from the untreated populations in Jordan or in Palestine and mixing with the Jordanian populations. However, this needs further investigation taking into consideration the control programs implemented in Palestine. The status of pesticide resistance in arthropod pests in Palestine was reviewed, including houseflies and mosquitoes. *Cx. pipiens* was found to be resistant to Chlorpyrifos, Cypermethrin and Permethrin between the years 1994 and 1996 (Horowitz et al., 1998).

Mosquitos' resistance to insecticides depends on the type and frequency of the insecticides used. According to WHO (2013), if the observed mortality rate is between 90 and 97%, the presence of resistant genes in the vector population must be confirmed. The confirmation of resistance may be obtained by performing additional bioassay tests using the same insecticide on the same population or on the progeny of any surviving mosquitoes (reared under insectary conditions) and/ or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently showed a mortality rate below 98%, then the resistance is confirmed. Therefore, additional tests are needed on the Dayr Alla population of *An. superpictus* because the corrected mortality rate was 96%.

If the mortality rate is less than 90%, confirmation of the existence of resistant genes in the tested population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of each species was tested (WHO, 2013). In the present tests, *Cx. theirelli* and *Cx. laticinctus* showed resistance to Deltamethrin, as the mortality rates were 87 and 86%, respectively. *Cx. pipiens* and *Cx. theirelli* also showed resistance to Lambdacyhalothrin with 78 and 76% mortalities, respectively. Therefore, further investigation of the mechanisms and distribution of resistance should be undertaken according to (WHO, 2013).

Resistance of mosquitoes to insecticides varies according to the environmental conditions, the species studied and the control programs implemented, which may vary in type and frequency of insecticide use. Therefore, comparing obtained results to test results of mosquitoes that were exposed to different combinations and/ or frequencies of insecticidal applications is irrelevant.

However, when a mosquito species becomes resistant to a certain insecticide in any country, it may become resistant in another country, if exposed to the same insecticide under similar conditions. Results of susceptibility tests can be found in many countries around the world. For example, *C. pipiens pipiens* populations on Cyprus were sampled between 2002 and 2008 to evaluate the insecticidal resistance to Temephos, Chlorpyrifos, and Permethrin and to study susceptibility levels to the *Bacillus thuringiensis* subsp. *israelensis* De Barjac and the juvenile hormone analog, Methoprene. Susceptibility to the three conventional chemical insecticides varied among different collections. Most collections showed moderate or low resistance. The 2004 Akrotiri collection had the highest Temephos resistance ratio, 167-fold at the LC_{95}, although later sampling showed that the population returned to susceptibility after the treatments were stopped (Vasquez *et al*., 2009). Resistance to the organophosphates Temephos and Chlorpyrifos, the carbamate Propoxur, the pyrethroid Permethrin, and the organochloride DDT was investigated in Tunisian populations of *C. pipiens pipiens* collected between 1990 and 1996 (Cheikh *et al*., 1998). Resistance to Temephos was uniformly low and reached 10-folds in the most resistant population. In contrast, resistance to Chlorpyrifos was highly variable and reached the highest level (>10,000-folds) recorded worldwide. The Chlorpyrifos-resistant populations were also highly resistant to Propoxur. Some populations showed high resistance to Permethrin (up to 5,000-folds) and moderate resistance to DDT (≃20- folds). Araújo *et al.* (2013) studied the susceptibility of Brazilian *Aedes aegypti* populations displaying Temephos resistance to *B. t. israelensis.* They found that their data showed a lack of cross-resistance between Temephos and *Bti*, suggesting that *Bti* can be used in an integrated control program to fight *Ae. aegypti* and counteract the Temephos resistance that was found among all populations analyzed.

Table 2. Susceptibility of larvae and adult mosquitoes to various recommended concentrations of insecticides in Jordan in 2014.

Mosquito species	Stage	Insecticides concentration	Locality	Pooled corrected mortality %	Status
An. sergenti	Larvae	Temephos 0.25mg/L	Ghor as Safi	100	Susceptible
An. superpictus	Larvae	Temephos 0.25mg/L	Deir-Alla	100	Susceptible
Cx. laticinctus	Larvae	Temephos 0.25mg/L	Ghor as Safi	99	Susceptible
Cx. laticinctus	Larvae	Temephos 0.25mg/L	Deir-Alla	100	Susceptible
An. sergenti	Adult	Deltamethrin 0.025%	Ghor as Safi	98	Susceptible
An. superpictus	Adult	Deltamethrin 0.025%	Deir-Alla	96	Transit
Cx. laticinctus	Adult	Deltamethrin 0.025%	Ghor as Safi	100	Susceptible
Cx. theirelli	Adult	Deltamethrin 0.025%	Ghor as Safi	87	Resistant
Cx. laticinctus	Adult	Deltamethrin 0.025%	Deir-Alla	86	Resistant
Cx. pipiens	Adult	Lambdacyhalothrin 0.03g/m^2	Ghor as Safi	78	Resistant
An. superpictus	Adult	Lambdacyhalothrin 0.03g/m^2	Deir-Alla	100	Susceptible
Cx. theirelli	Adult	Lambdacyhalothrin 0.03g/m^2	Deir-Alla	76	Resistant

4. Conclusion

All larvae of the tested species were susceptible to Temephos. The Deir Alla population of *An. superpictus* adults showed a transit susceptibility to Deltamethrin. The Ghor as Safi populations of *Cx. theirelli* and *Cx. laticinctus* showed resistance to Deltamethrin. *Cx. pipiens* was found to be resistant to Lambdacyhalothrin at the diagnostic doses. Conducting susceptibility tests on a larger number of Jordanian populations covering diverse habitats is recommended. Alternative control methods to chemicals to which mosquitoes were found resistant should be considered. Insecticides used to control agricultural pests may reach mosquitoes, as non-target pest, when exposed to such insecticides, which accelerates their resistance. Therefore, changing insecticides is recommended when resistance is detected. Establishing susceptible mosquito colonies to be used as reference strains is needed for future tests.

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