

## Original Article

# Laboratory Evaluation of Temephos against *Anopheles stephensi* and *Culex pipiens* Larvae in Iran

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### Abstract

**Background:** Malaria is still a health problem in Iran. There are several vector control activities, including Indoor Residual spraying, using insecticide treated nets and larviciding including Temephos. In addition nuisance mosquitos are prevalent in the urban areas. So that evaluation of this species to larvicide will provide a clue for management of vector control activities.

**Methods:** Two mosquito species were used in this study: *Anopheles stephensi* were collected from Kazeroun and *Culex pipiens* from Tehran, capital of Iran. All the tests were carried out according to the WHO method. All the test kis was provided by WHO.

**Results:** Results showed a  $LC_{50}=0.0523$  and  $LC_{90}=0.3822$  mg/l for *An. stephensi*. The figure for *Cx. pipiens* was 0.1838 and 0.8505 mg/l respectively.

**Conclusion:** monitoring of insecticide resistance to Temephos should be evaluated regularly for management of vector control.

**Keywords:** Temephos, *Anopheles stephensi*, *Culex pipiens*, Evaluation, Efficacy

## Introduction

Mosquito-borne diseases are from the main public health problems around the world, especially in tropical and semi-tropical areas. Among them malaria, dirofilariasis, dengue, west Nile, yellow fever, and some other viral diseases have the main morbidity. Malaria occurs in tropical and some temperate regions of the world with an annual morbidity of 300–500 million cases and 574000 deaths in 2013 (WHO 2014). Iran is going to eliminate the disease by 2025 although local transmission happens in some areas in south and southeast. *Anopheles stephensi* is one of the main malaria vectors is coastal lowlands of southern Iran (Hanafi-Bojd et al. 2011). West Nile and dirofilariasis are diseases which can be transmitted by *Cx. pipiens*, a worldwide distributed mosquito species. Both diseases are potentially transmittable in Iran

(Azari-Hamidian et al. 2009, Ahmadnejad et al. 2011). The first line in malaria control program in Iran is indoor residual spraying to interrupt the transmission cycle, followed by distributing long lasting insecticide impregnated nets (LLINs) and larviciding. The latter is doing by biological larvicides in current years, although Temephos, an organophosphate compound, has been used for years in mosquito larvae control. There are reports of resistance to some insecticides such as DDT, dieldrin and malathion in *An. stephensi*, as well as some indications of resistance to pyrethroids in current years (Davari et al. 2006, Hanafi-Bojd et al. 2012, Vatandoost and Hanafi-Bojd 2012), although current studies have confirmed full susceptibility of this species to malathion (Iranpour et al. 1993, Vatandoost et al. 2005, Hanafi-

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Bojd et al. 2012). By the way, larvicides tests showed resistance/tolerance to fenthion and fenitrothion in this species, but susceptibility to temephos (Vatandoost et al. 2004a, 2005, Vatandoost and Hanafi-Bojd 2005, Hanafi-Bojd et al. 2012, Soltani et al. 2013).

In insecticide resistance management it is necessary to evaluate used insecticides/ larvicides periodically to monitor susceptibility status of the vectors. Considering the extensive use of insecticides in public health sector as well as agriculture and urban pests, physiological and behavioral resistance is a big issue that must be determined in mosquito vector species. Organophosphates are using widely in the agriculture sector, while they are using for larviciding against mosquitoes, it is possible to develop resistance in some vectors. A study on different geographical strains of *An. stephensi* in Iran showed considerable variations in temephos resistance ratios collected from different localities (Soltani et al. 2013).

*Culex pipiens* is known as urban pest nuisance around the world and has a wide distribution in the northern and central parts of Iran (Azari-Hamidian 2007). It can transmit dirofilariasis and has high resistance levels to different insecticides (Maraghi et al. 2006, Azari-Hamidian et al. 2009). This species usually breeds in waste waters which are contaminated with different detergents, insecticides, industrial pollutants, oil compounds etc. Therefore it is possible to develop resistance to insecticides and/or larvicides in this mosquito, as it has been reported in recent years (Vatandoost et al. 2004b).

Temephos, a most widely used organophosphate insecticide, has been included in the list of World Health Organization (WHO) as a suitable and safe mosquito larvicide that can be used even in drinking water for controlling of the most mosquito vectors. The toxicity of this insecticide is low and unlikely to present acute hazard for human (WHO 2006). In 2006 for the first

time in the Middle East, resistance to temephos was confirmed in *An. stephensi* breeding in water storage tanks in the Al-Dhahira region of Oman (Anderasen 2006).

During last three decades temephos has considered as a safe larvicide ( $LC_{50} = 8600$  mg/l) in vector control programs (Pierce et al. 1989). World health organization suggested 1 ppm of this compound for larviciding in drinking water bodies. It is effective for 3 months in this concentration (Bang et al. 1972), and this is confirmed by several studies against different mosquito species (Mulla et al. 2004, Thavara et al. 2004, 2005). Also larval control in natural breeding places such as riversides has achieved by temephos EC 1% (Shililu 2001, Parvez and Al Wahabi 2003). Laboratory tests on this formulation resulted to 15 weeks residual and larvicidal effect against *Aedes aegypti* (Chen et al. 2006).

With due attention to the long history of insecticide/larvicides resistance in *Anopheles stephensi* and *Culex pipiens* in Iran and other countries it is necessary to screen the susceptibility status of these species at regular intervals using WHO standard bioassay tests and to map the spatial and temporal changes in their level of susceptibility/resistance. Therefore this study was aimed to determine the susceptibility of these species to temephos in two sentinel sites in Iran.

## Materials and Methods

### Mosquito collection and rearing

Two mosquito species were used in this study: *An. stephensi* mysorensis collected from Kazeroun County, south of Iran and *Cx. pipiens* collected from Tehran, the capital city of Iran. Collected larvae were transferred to the insectary (Kazeroun Station, School of Public Health, Tehran University of Medical Sciences for *An. stephensi* and Department of Medical Entomology and

Vector Control, School of Public Health, Tehran University of Medical Sciences for *Cx. pipiens*) and fed on fish flakes (Tetra-mine®). The specimens were reared under 28±1 °C temperature and 14:12 L: D photo-period to late 3<sup>rd</sup> early 4<sup>th</sup> instars, the suitable physiological ages for performing tests.

**Susceptibility tests**

Standard WHO method (1981, 2005) was followed for the tests using WHO standard kit and 1.25, 6.25, 31.25 and 156.25 mg/l concentration were used to provide the final concentration of 0.005, 0.025, 0.125 and 0.0625 when added 1 cc of each concentration to 249 cc of tape water. The obtained results were corrected by Abbotts’ formula when mortality rate in control group was 5–20 percent (Abbott 1925) and analyzed by Probit (Finney 1971) to obtain LC<sub>50</sub>, LC<sub>90</sub>

and regression line. For each larvicides concentration 4 replicates of 25 larvae were used and 2 replicated for control. After 24 hrs all larvae without movement on the water surface were considered as dead.

**Results**

Table 1 shows the results of mortality of *An. stephensi* to temephos. We obtained LC<sub>50</sub>= 0.0523 and LC<sub>90</sub>=0.3822 for this species (Tables 1 and 2). Regression line is presented in Fig. 1. It shows Y= 1.9014+ 1.4837X.

For *Cx. pipiens* a mortality rate of 3–99% was obtained in different concentrations (Table 3). LC<sub>50</sub> and LC<sub>90</sub> for this species were calculated as 0.1838 and 0.8505 ppm (Table 4). Regression line is presented in Fig. 2. It shows Y=1.4171+1.9264 X.

**Table 1.** Mortality rate of *Anopheles stephensi* larvae (Kazeroun Strain) against WHO standard concentrations of Temephos, 2014

Concentration (PPM)	Replicates	No. of tested	No. of dead	Mortality rate (%)	Observed probit mortality	Expected probit mortality
0.005	4	100	8	8	3.595	3.487
0.025	4	100	26	26	4.357	4.524
0.125	4	100	77	77	6.555	5.561
0.625	4	100	93	93	6.476	6.599
Control	2	50	0	0	-	-

**Table 2.** Lethal concentration values obtained from regression analysis of Temephos against *Anopheles stephensi* larvae of Kazeroun Strain, 2014

A	b ± SE	LC <sub>50</sub> (ppm) ± 95% C.L.	LC <sub>90</sub> (ppm) ± 95% C.L.	<sup>2</sup> (heterogeneity)	<sup>2</sup> table (df)	p-Value
1.9014	1.4837 ± 0.128	0.0411 0.0523 0.0664	0.2695 0.3822 0.6001	3.906 *	5.991 (2)	0.001

\* No Heterogeneity

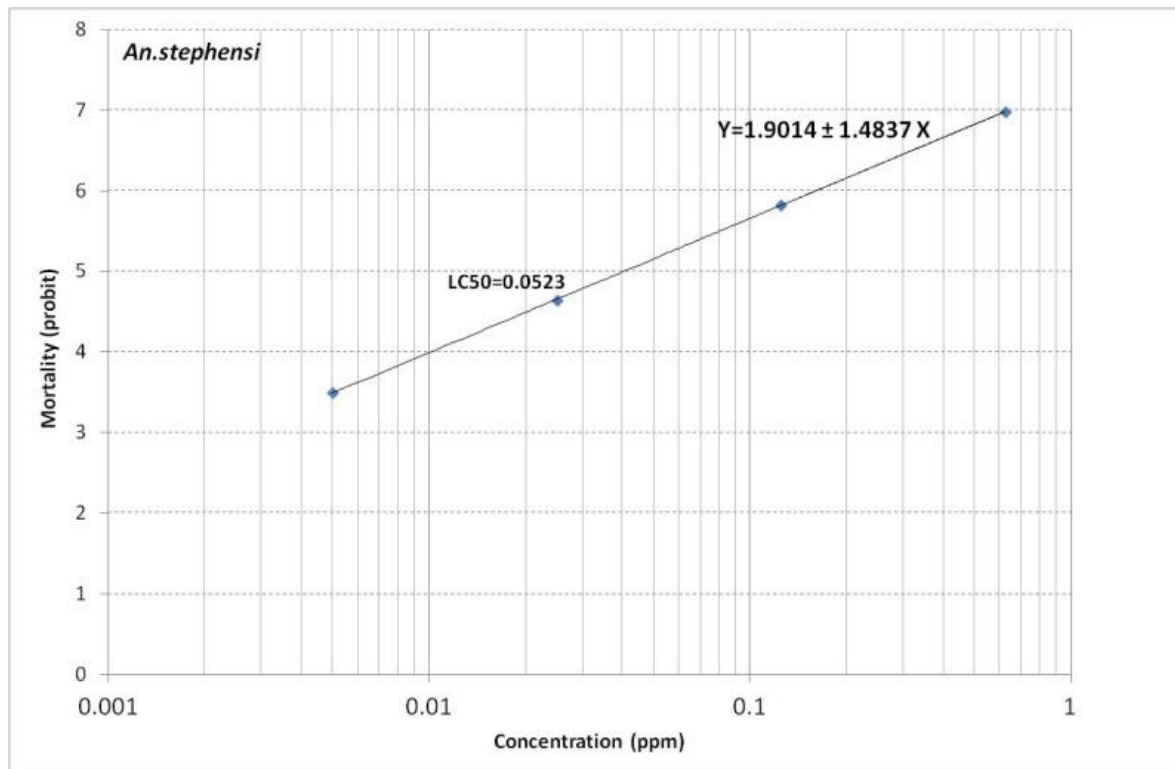
**Table 3.** Mortality rate of *Culex pipiens* larvae (Tehran Strain) against WHO standard concentrations of Temephos, 2014

Concentration (PPM)	Replicates	No. of Tested larvae	No. of mortality	Mortality rate (%)	Observed mortality probit	Expected mortality probit
0.005	4	100	3	3	3.119	1.984
0.025	4	100	8	8	3.595	3.331
0.125	4	100	11	11	3.773	3.677
0.625	4	100	99	99	7.327	6.024
Control	2	50	0	0	-	-

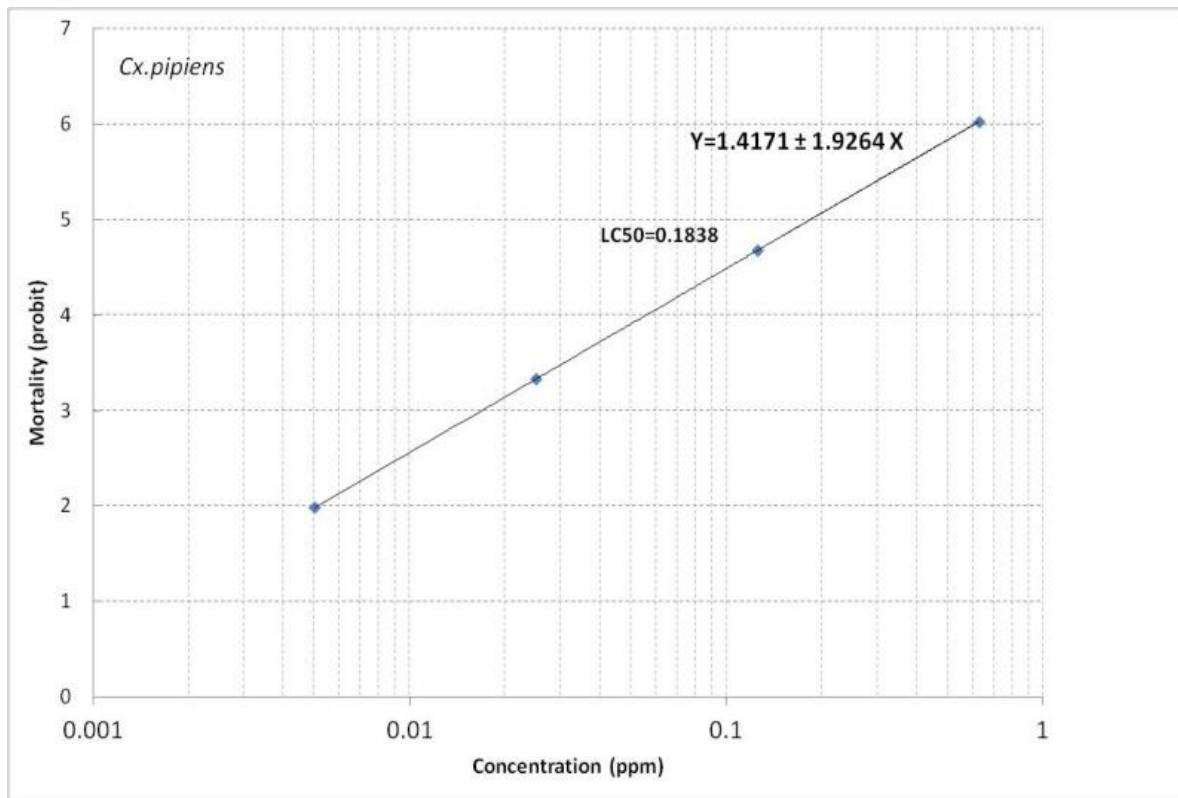
**Table 4.** Lethal concentration values obtained from regression analysis of Temephos against *Culex pipiens* larvae of Tehran Strain, 2014

A	b ± SE	LC <sub>50</sub> (ppm) ± 95% C.L.	LC <sub>90</sub> (ppm) ± 95% C.L.	$\chi^2$ (heterogeneity)	$\chi^2$ table (df)	p-Value
1.4171	1.9264 ± 0.174	0.1493 <b>0.1838</b> 0.2285	0.6186 <b>0.8505</b> 1.3039	11.113 *	5.991 (2)	0.001

\* Heterogeneity



**Fig. 1.** Mortality regression line of Temephos against *Anopheles stephensi* of Kazeroun Strain, 2014



**Fig. 2.** Mortality regression line of Temephos against *Culex pipiens* of Tehran Strain, 2014

## Discussion

Our study showed  $LC_{50}$  value of 0.0523 for *An. stephensi* in Kazeroun area, southern Iran. Previous studies on this species from different localities of southern regions of the country have reported 0.001613 mg/l (Vatandoost and Hanafi-Bojd 2005) and 0.0022–0.0141 mg/l (Soltani et al. 2013). Other studies on diagnostic dose of temephos (0.25 mg/l) found *An. stephensi* was susceptible to this larvicide in Iran (Vatandoost et al. 2004a, Hanafi-Bojd et al. 2012). By the way as it shown in table 1, in concentration of 0.625 mg/l only 93% mortality has occurred. Considering diagnostic dose for this species, Kazeroun strain is resistant to temephos. By the way these findings should be confirmed by more studies with different batches of WHO standard kits for temephos on more larval population collected from the same area.

Resistance of *An. stephensi* to this larvicide has previously reported from Oman Country (Parvez and Al Wahaibi 2003). *Anopheles stephensi* has been reported susceptible to temephos in India with  $LC_{50}$  range of 0.008–0.015 (Tikar et al. 2011, Singh et al. 2014). Study on *An. labranchiae* in Morocco found low resistance to temephos in a sentinel site. They revised the diagnostic dose of temephos for this species (Faraj et al. 2010). A more recent study in Iran showed that the altered enzymes are responsible for temephos resistance in *An. stephensi* (Soltani et al. 2015). Although temephos is not used in national malaria program in last years but the study area has different rice fields and citrus gardens, and farmers use organophosphate insecticides to control pests. This may affect susceptibility

of *An. stephensi* as well. Integrated pest management (IPM) in agriculture sector should be considered as an important priority parallel to integrated vector management (IVM) in health sector to overcome vector/pests resistance to pesticides.

Our results on *Cx. pipiens* also showed  $LC_{50}$  (0.1838 mg/l) was more than diagnostic dose (0.02 mg/l) recommended by WHO for this species. It means resistance to temephos. Other studies on temephos in Cyprus and Tunisia against *Cx. pipiens* reported  $LC_{50}$  ranges of 0.00076–0.310 and 0.0021–0.015, respectively (Ben Cheikh et al. 1993, Vasques et al. 2009). Dose response tests of the technical temephos against *Cx. pipiens* in India showed  $LC_{50}$  of 0.01 mg/l in this species (Badawy et al. 2015). This value for *Culex quinquefasciatus* was 0.0000473 mg/l in another study in that country (Dorta et al. 1993). In Tunisia esterases were found to be contributed to temephos resistance by their increased activity (Ben Cheikh et al. 2008).

Although there is no national program to control this species, but because wastewaters are the main breeding places for *Cx. pipiens* and they are contaminated with different pollutants, insecticides, detergents, etc this species has developed resistance to these compounds to save itself. A longitudinal on *Cx. pipiens* in Martinique showed that resistance ratio for temephos was 8.6 to 42-folds during 1991–1999 comparing 2.9 to 4.6-folds in 1990. They found organophosphate resistance was associated with decreasing of susceptible genotypes at Ester and ace-1 foci, as well as to an allele replacement at the Ester locus (Yebakima et al. 2004).

Our results on  $LC_{50}$  of *Cx. pipiens* were higher than the above mentioned studies in different countries. Therefore, it is necessary to do biochemical and molecular studies to find the reason for resistance to temephos.

In conclusion, it is recommended to do temephos susceptibility tests against both species from the same localities with more

larvae and different batches of WHO standard kits of temephos to confirm their resistance status. The present study shows indications of resistance to this larvicides in *An. stephensi* and *Cx. pipiens* for the first time in Iran.

## Conclusions

The results indicated that the monitoring and evaluation of insecticide resistance should be carried out regularly against mosquito vectors.

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