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## Current Resistant Status of *Anopheles stephensi* Liston to Different Larvicides in Hormozgan Province, Southeastern Iran, 2004

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**Abstract:** In this study the susceptibility status of the field and laboratory strains of *Anopheles stephensi*, the main malaria vector, was determined to different larvicides. Five larvicides, i.e., chlorpyrifos-methyl, *Bacillus thuringiensis*, temephos, fenitrothion and methoprene were tested using WHO standard test method in the laboratory condition, against the field and lab strains of *Anopheles stephensi* Liston of Hormozgan province, southeastern Iran. The  $LC_{50}$  values were calculated from the probit-regression line for each larvicide. Results exhibited the  $LC_{50}$  values for *B. thuringiensis*, chlorpyrifos-methyl, fenitrothion, temephos and methoprene as 0.08483, 0.01115, 0.001131, 0.001613, 0.00073 mg L<sup>-1</sup>, respectively for lab strain. The values of 0.521279, 0.016419, 0.002475, 0.003388 and 0.000825 mg L<sup>-1</sup>, were measured as  $LC_{50}$  against field strain, respectively. From the results it can be concluded that there was significant difference between two strains for *B. thuringiensis*, temephos and fenitrothion at the  $LC_{50}$  level. The field strain was more tolerant than lab strain to the three larvicides. At diagnostic dose as recommended by WHO both strains are susceptible to all larvicides, therefore they can be applied for malaria vector control in the region.

**Key words:** *Anopheles stephensi*, hormozgan, Iran, larvicides, malaria

### INTRODUCTION

Malaria remains a major public health problem in the southern parts of Iran, including Hormozgan province with a population of 1.3 million people where contains about 20% of total malaria cases of the country, annually. *Anopheles stephensi* is considered as a main malaria vector in the Persian Gulf area<sup>[1]</sup> and also in the south of Iran.

Larviciding can be a useful method for malaria control programs, particularly in areas where breeding sites are accessible and relatively limited in number and size. Before implementing a larviciding program, survey should be carried out to assessment the insecticide resistance of target species of the area. Previous studies carried out in Iran have shown that *A. stephensi* is resistant to DDT, dieldrin and malathion at the adult stage<sup>[2-5]</sup>. Resistance to DDT, dieldrin and malathion mainly in the adults of *A. stephensi*, have been widely distributed in Persian-Gulf, Middle-East and Indian-subcontinent areas<sup>[6,7]</sup>, whereas low level of larval resistance was found in Pakistan<sup>[8]</sup>. In south of Iran the larvae of this *Anopheles* showed susceptibility to DDT<sup>[9]</sup>, malathion, temephos and chlorpyrifos in WHO recommended diagnostic dose<sup>[10]</sup>.

The main control measures in the Hormozgan province are the use of lambda-cyhalothrin as a residual spraying as well as chlorpyrifos-methyl and *Bacillus thuringiensis* as larvicides in breeding places. Other anti-malarial measures used in the area include larviciding with fuel oil, temephos, pirimiphos-methyl and chlorpyrifos-methyl; introduction of *Gambusia affinis* and native *Aphanius dispar* larvivorous fish; active and passive case detection; and mass anti-malaria drug distribution. This study was carried out to determine the current susceptibility level of *A. stephensi* to commonly used larvicides in the area.

### MATERIALS AND METHODS

**Study area:** The investigation was carried out over a period of 18 months at Bandar Abbas township, Hormozgan province (25°24'-28°57' N. and 52°41'-59°15' E.), bordered by the Persian Gulf. This province has subtropical weather and is prone to malaria transmission. Mean temperature is ranged from 5 to 45°C in December and July, respectively. Relative humidity is different from 38 to 88%. The annual mean rainfall in the recent ten years is 76.4 mm year<sup>-1</sup>.

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**Larvicides and larval test:** Different concentrations of WHO recommended larvicides including temephos (Chem Service), chlorpyrifos-methyl (Dow AgroSciences), fenitrothion (Sumithomo), methoprene (Babolna Bioenvironmental Center Ltd.), *Bacillus thuringiensis* (O.S.T. Iran) were prepared in appropriate solvents and all the larval tests were carried out according to WHO recommendation test procedure<sup>[11]</sup>. Technical grade larvicides were used to prepare different concentrations employing ethanol, acetone and distilled water as solvent for organophosphate compounds, methoprene and *B. thuringiensis*, respectively. Laboratory and field collected strains of *Anopheles stephensi* were used in the study. Late 3rd and early 4th instar larvae of species were exposed to different concentrations of the larvicides. At most concentrations, 100 larvae representing four replicates of 25 individuals were tested. Two replicates of 25 larvae were used as control in each test. The larvae were fed with fish food and mortality counts were made after 24 h exposure to calculate the  $LC_{50}$  values. Abbott's formula was used to correct the observed mortality<sup>[12]</sup>.

**Statistical analysis:** Probit regression line parameters were calculated on a computer based on Thomas and Sparks<sup>[13]</sup>.

## RESULTS AND DISCUSSION

Results exhibited the  $LC_{50}$  values for *B. thuringiensis*, chlorpyrifos-methyl, fenitrothion, temephos and methoprene as 0.08483, 0.01115, 0.001131, 0.001613, 0.00073  $mg L^{-1}$ , respectively for lab strain (Fig. 1). The values of 0.521279, 0.016419, 0.002475, 0.003388 and 0.000825  $mg L^{-1}$ , were measured as  $LC_{50}$  against field strain, respectively (Fig 2).  $LC_{50}$  values have an increasing process from methoprene to *Bacillus thuringiensis* in both strains, but it is higher for the field strain (Fig. 1 and 2).

Resistance of anopheline malaria vectors to larvicides is reported in different levels, worldwide. *Anopheles stephensi* has an extensive resistance comparing to other species. This anopheles is reported resistant or tolerant to fenitrothion, temephos and fenitrothion in India, fenitrothion and pirimiphos-methyl in Iraq, fenitrothion, temephos, pirimiphos-methyl, chlorfoxim and foxim in Iran and finally fenitrothion in Pakistan<sup>[7,10]</sup>.

Laboratory evaluation of chlorpyrifos-methyl on *A. stephensi* in India showed 0.0019  $mg L^{-1}$  as  $LC_{50}$ <sup>[14]</sup>. Another study on pirimiphos-methyl 50%EC against larvae of *A. stephensi* in India showed 0.023 and 0.045  $mg L^{-1}$  as  $LC_{50}$  and  $LC_{90}$ , respectively<sup>[15]</sup>. The susceptibility tests on effectiveness of temephos

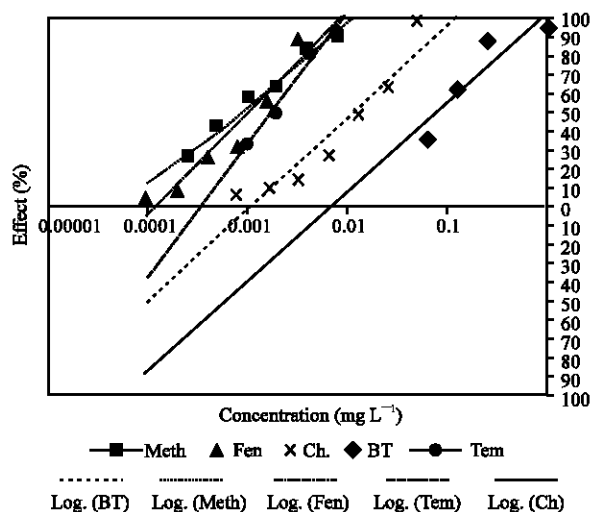


Fig. 1: Regression line of *A. stephensi* (lab strain) exposed to different concentrations of larvicides, Bandar Abbas, southeastern Iran, 2004

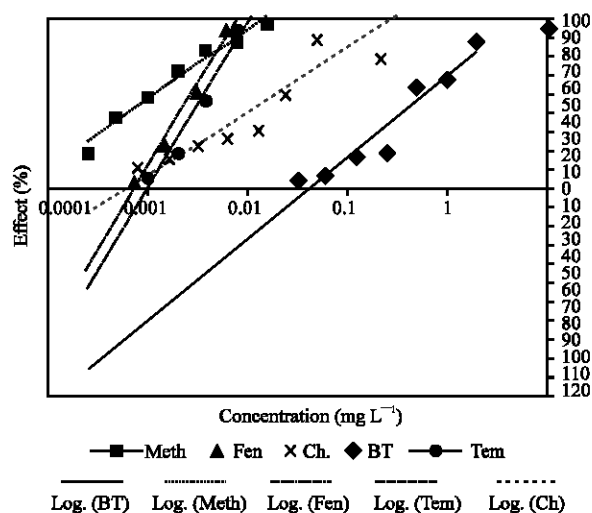


Fig. 2: Regression line of *A. stephensi* (field strain) exposed to different concentrations of larvicides, Bandar Abbas, southeastern Iran, 2004

BT: *B. Thuringiensis*, Meth.: Methoprene, Fen.: fenitrothion, Ch: chlorpyrifos-methyl, Temp.: Temephos

0.125  $mg L^{-1}$  against *A. stephensi* in Oman country, south of Iran, showed 94 and 97% mortality pre and during the intervention program<sup>[16]</sup>. The results of a larval bioassay revealed that *A. stephensi* has more tolerance to deltamethrin than *A. culicifacies* and vice versa for permethrin<sup>[17]</sup>.

Table 1: Laboratory evaluation of five larvicides against larvae of *A. stephensi*, Hormozgan province, Southeastern Iran, 2004

Larvicide	<i>A. stephensi</i> (field strain)	<i>A. stephensi</i> (Lab strain)
Temephos	$3.39 \times 10^{-3}$	$1.61 \times 10^{-3}$
LC50 (mg L <sup>-1</sup> ) (LCL-UCL)	(2.96-3.85 $\times 10^{-3}$ )	(1.31-1.90 $\times 10^{-3}$ )
Chlorpyrifos-methyl	$1.65 \times 10^{-2}$	$1.12 \times 10^{-2}$
LC50 (mg L <sup>-1</sup> ) (LCL-UCL)	(1.26-2.26 $\times 10^{-2}$ )	(0.61-1.68 $\times 10^{-2}$ )
Fenitrothion	$2.48 \times 10^{-3}$	$1.13 \times 10^{-3}$
LC50 (mg L <sup>-1</sup> ) (LCL-UCL)	(2.22-2.7 $\times 10^{-3}$ )	(0.97-1.32 $\times 10^{-3}$ )
Methoprene	$0.83 \times 10^{-3}$	$7.30 \times 10^{-4}$
LC50 (mg L <sup>-1</sup> ) (LCL-UCL)	(0.60-1.09 $\times 10^{-3}$ )	(5.53-9.23 $\times 10^{-4}$ )
<i>Bacillus thuringiensis</i>	$5.21 \times 10^{-1}$	$8.48 \times 10^{-2}$
LC50 (mg L <sup>-1</sup> ) (LCL-UCL)	(4.39-6.25 $\times 10^{-1}$ )	(6.35-10.44 $\times 10^{-2}$ )

We compared the susceptibility of the laboratory strain with that of the field strain. From the results it can be concluded both strains of *A. stephensi* are susceptible to diagnostic dosages recommended by WHO<sup>[11,18]</sup>. There is significant difference between two strains for *B. thuringiensis*, temephos and fenitrothion at the LC<sub>50</sub> level. The field strain was more tolerant than lab strain to three larvicides (Table 1). All tested larvicides can be used in control programs in the area. Although, biannual assessment of common use larvicides is essential and recommended.

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