Short Notes

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Susceptibility status of *Anopheles dthali* and *An. fluviatilis* to commonly used larvicides in an endemic focus of malaria, southern Iran

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Malaria control is increasingly recognised as playing a key role in poverty reduction in high burdened countries. The programme for malaria eradication based on vector control using very large quantities of DDT started in 1949 in the country and resulted in a dramatic reduction in malaria incidence. Vector population recovery, mainly due to resistance to DDT in *Anopheles* populations as well as DDT's lack of selectivity affecting non-target populations including mosquito competitors, predators and pathogens, soon reversed early success[1]. Therefore, other insecticides belonging to organochlorine, organophosphate, carbamate and pyrethroid groups were produced and used in the following years. Extensive use of chemical insecticides against vector mosquitoes for the control of malaria for about four decades, has resulted in development of resistance in vector mosquitoes to these insecticides and hazards to the environment.

The main control measures in Iran are the use of lambdacyhalothrin WP 10% and deltamethrin WP 5% for indoor residual spraying as well as chlorpyrifos-methyl and *Bacillus thuringiensis* as larvicides in breeding places. *Gambusia affinis* and *Aphanius dispar* as larvivorous fish have also been introduced into the breeding sites. Treated bednets using deltamethrin EC 25% is another attempt to control malaria in southern part of the country. In spite of all these vector control activities in Iran, malaria continues to be the most important parasitic and vector-borne disease in the country. *Anopheles fluviatilis* James and *An. dthali* Patton (Diptera : Culicidae) are both considered as the secondary malaria vectors in southern Iran, after *An. stephensi*.

Studies in other countries showed that *An. fluviatilis* is resistant to DDT in Afghanistan, India, Nepal and Pakistan. Also in Pakistan and Saudi Arabia it is reported resistant to Dieldrin[2]. A study on insecticides resistance in India showed that *An. fluviatilis* has developed resistance to HCH and is susceptible to DDT[3]. Investigation carried out in Kazerun, southern Iran, showed that *An. fluviatilis* was susceptible to organochlorine and organophosphorus compounds, mortality rate with 2 and 4% DDT after one-hour exposure was 96–100 and 100%, respectively. The mortality rate with malathion 1.6 and 3.2% after one-hour exposure was 95.8 and 100%, respectively[4]. *An. dthali* is resistant to chlorpyrifos,

fenitrothion, bromofos and some carbamate insecticides in Egypt, and temephos in Jordan[2]. Also there is resistance to DDT in adult *An. dthali* in Iran[2]. This study was carried out to determine the susceptibility levels of *An. fluviatilis* and *An. dthali* larvae to chemical and biolarvicides, using standard method[5].

The investigation was carried out over a period of 18 months at Bandar Abbas county, Hormozgan province (25º 24' – 28º 57' N and 52º 41' – 59º 15' E), bordered by the Persian Gulf. This province has subtropical weather and is prone to malaria transmission. Mean temperature ranges from 5 to 45ºC in December and July, respectively. Relative humidity varies from 38 to 88%. The annual mean rainfall in the last ten years is 76.4 mm/year.

Different concentrations of WHO recommended larvicides including temephos (Chem Service UK), chlorpyrifos-methyl, fenitrothion (Sumitomo Chemical Co. Ltd., Japan), methoprene (Babolna Bioenvironmental Center Ltd., Hungary), *B. thuringiensis* (OST, Iran) were prepared in appropriate solvents and all the larval tests were carried out according to WHO recommended test procedure[5]. Technical grade larvicides were used to prepare different concentrations employing ethanol, acetone and distilled water as solvents for organophosphate compounds, methoprene and *B. thuringiensis*, respectively. Field collected larvae of *An. fluviatilis* and *An. dthali* were used in this study. Late III and early IV instar larvae of each species were exposed to different concentrations of the larvicides. At most concentrations, 100 larvae representing four replicates of 25 larvae each were tested. Two replicates of 25 larvae were used as control in each test. The larvae were fed on fish food, and mortality counts were made after 24 h exposure to calculate the LC_{50} values in organophosphate and *B. thuringiensis* and continued to the time when all larvae were dead or changed to the adults in the case of methoprene test. Abbott's formula was used to correct the observed mortality[6]. Probit regression line parameters were calculated on a computer based on Thomas & Sparks[7].

An. dthali breeds in pebbly margins of rivers, springs, pits around springs with or without vegetation, pools in dried up river beds and palm irrigation canals in Hormozgan. In Bandar Abbas county larvae were also found in mineral and high salinity water sources. Larvae were also found in waters with high salinity. The water temperature of breeding places ranged between 13 and 28°C, with a pH of 6.9–8.0. *An. fluviatilis* is distributed on the mountainous area from the east to west of Hormozgan province. In northern area of Bandar Abbas county, the preferred larval habitats are slow moving water on the margins of rivers, streams with or without vegetation with high dissolved oxygen and pits around springs.

The regression lines from the tests with different larvicides against *An. fluviatilis* and *An. dthali* are presented in Figs. 1 and 2, respectively. The LC_{50} values for *B. thuringiensis* H-14, chlorpyrifos-methyl, fenitrothion, temephos and methoprene were 2.602871, 0.008945, 0.007294, 0.003873 and 0.000665 mg/l, respectively for *An. fluviatilis*. In case of *An. dthali* these values were 0.500830, 0.004845, 0.000805, 0.001610 and 0.000645 mg/l, respectively (Table 1). LC_{50} values were in increasing order from methoprene to *B. thuringiensis* in both the species (Figs. 1 & 2), but were higher in case of *An. fluviatilis* (Table 1).

An. fluviatilis is a main malaria vector in India, Pakistan and Nepal[8]. The sporozoite rate of this species in Iran was reported 3.2% from Kazerun of Fars province, 11% from Behbahan of Khuzistan province and 1.7% in the Chelou area, Hormozgan province, where this species was reported to have a marked tendency to rest indoors[9,10].

An. dthali in Iran has been found in southern parts of the Zagros chain. During epidemiological and entomological studies in the mountainous area of Bandar

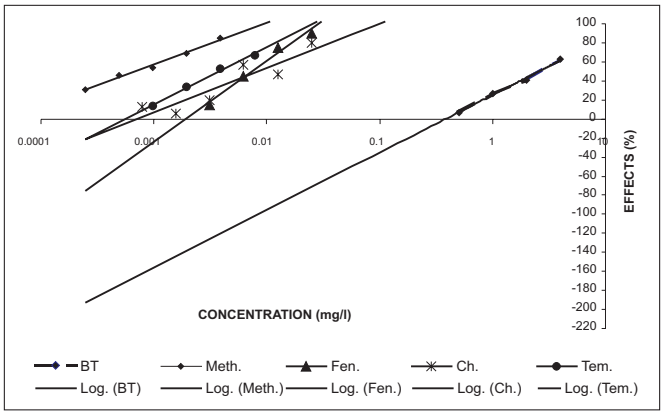

Fig.1: Regression lines of five tested larvicides against *An. fluviatilis* of Bandar Abbas, southern Iran

Abbas, 1.4% of caught specimens were found with infected salivary glands. After that, in the same area, 2.1% collected from the north of Bandar Abbas county were found positive for sporozoites[11]. Studies in another area, southwestern Iran reported 7.7% of dissected *An. dthali* with infected salivary glands[12]. Study on the susceptibility status of *An. stephensi* to different larvicides in the study areas was conducted earlier. The LC_{50} values for *B. thuringiensis*, chlorpyrifos-methyl, fenitrothion, temephos and methoprene were 0.08483, 0.01115, 0.001131, 0.001613 and 0.00073 mg/l, respectively for lab strain of *An. stephensi*. The LC_{50} values against field strain of this *Anopheles* were 0.521279,

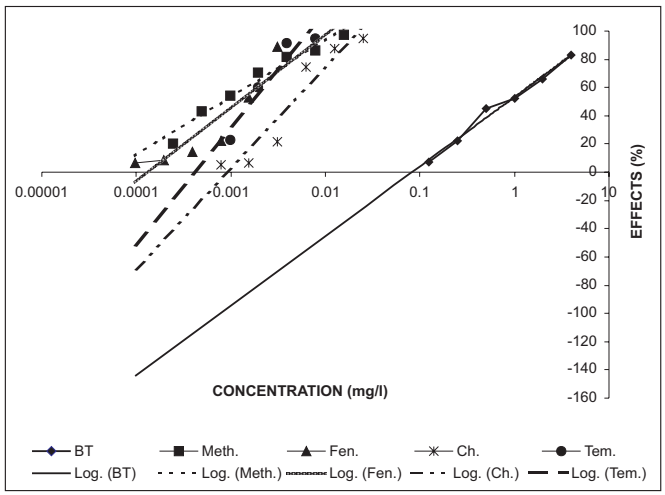

Fig. 2: Regression lines of five tested larvicides against *An. dthali* of Bandar Abbas, southern Iran

Table 1. Laboratory evaluation of five larvicides against larvae of *An. dthali* and *An. fluviatilis*, Hormozgan province, southern Iran

Larvicide	*An. dthali*	*An. fluviatilis*
Temephos		
LC_{50} (mg/l)	0.0016	0.0038
(LCL-UCL)	(0.0013–0.0018)	0.0024–0.0088
χ^2	1.502531	0.229
Chlorpyrifos-methyl		
LC_{50} (mg/l)	0.0048	0.0089
(LCL-UCL)	(0.0042–0.0055)	(0.0059–0.0153)
χ^2	8.408063	3.103640
Fenitrothion		
LC_{50} (mg/l)	0.0012	0.0072
(LCL-UCL)	(0.0008–0.002)	0.0053–0.0097)
χ^2	9.93386	0.205740
Methoprene		
LC_{50} (mg/l)	0.00064	0.00066
(LCL-UCL)	(0.00039–0.00092)	(0.00025–0.00119)
χ^2	8.827783	0.236511
Bacillus thuringiensis		
LC_{50} (mg/l)	0.5	2.6
(LCL-UCL)	(0.08–0.86)	(1.71–5.93)
χ^2	3.228875	0.314793

LCL: Lower confidence limit; UCL: Upper confidence limit.

0.016419, 0.002475, 0.003388 and 0.000825 mg/l, against the above mentioned insecticides[13].

Comparing three organophosphate larvicides against two studied anopheline species showed no significant difference in *An. fluviatilis* ($p > 0.05$). However, there was a significant difference between these larvicides, with a higher LC_{50} for chlorpyrifos-methyl ($p < 0.05$) in *An. dthali* (Fig. 3).

Previous study in the area by diagnostic dosage of four larvicides against *An. fluviatilis* showed 100%

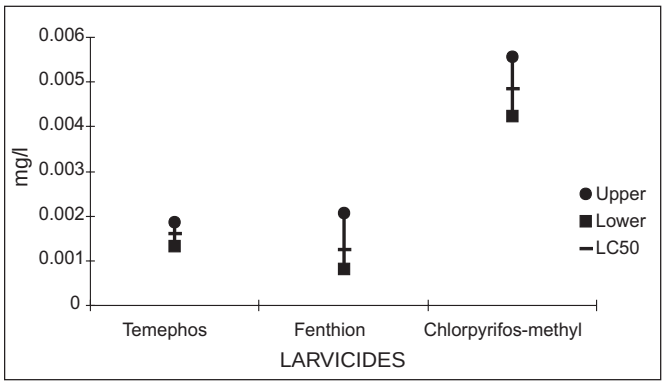

Fig. 3: LC_{50} for *An. dthali* against three organophosphate larvicides in Bandar Abbas, southern Iran

mortality except for fenitrothion with a rate of 80 ± 4%[14]. Reports of susceptibility tests on this species to insecticides in countries neighbouring Iran showed resistance to DDT and dieldrin in Afghanistan and Pakistan, and to dieldrin in Saudi Arabia[2]. A study in India reported that *An. fluviatilis* had developed resistance to HCH[15]. In the current study larvae of *An. fluviatilis* were susceptible to all organophosphate larvicides at WHO recommended diagnostic doses.

Study on susceptibility level of *An. dthali* with the diagnostic dose of fenitrothion in the area has shown mortality of 96 ± 2%[14]. But we observed no resistance or tolerance from this species in the current investigation. *An. dthali* is resistant to chlorpyrifos, fenitrothion, bromofos and some carbamate insecticides in Egypt, and temephos in Jordan[2]. Also there is resistance to DDT in adult *An. dthali* in Iran[2]. Based on the obtained results, both species have been found susceptible to diagnostic doses of the larvicides tested, but it is recommended to periodically evaluate the susceptibility status of the malaria vectors against different larvicides for resistance monitoring.

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