J Vector Borne Dis 45, December 2008, pp. 307–312

Comparative performance of imagicides on *Anopheles stephensi*, main malaria vector in a malarious area, southern Iran

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Abstract

Background & objectives: Jiroft district has subtropical climate and prone to seasonal malaria transmission with annual parasite index (API) 4.2 per 1000 in 2006. *Anopheles stephensi* Liston is a dominant malaria vector. The monitoring of insecticide susceptibility and irritability was conducted using discriminative dose as described by WHO.

Methods: The IV instar larvae were collected from different larval breeding places and transported to the temporary insectary, fed with Bemax® and then 2–3 days-old emerged and sugar-fed adults were used for susceptibility and irritability tests employing WHO methods and kits to organochlorine (OC) and pyrethroid (PY) insecticides.

Results: Mortality rates of field strain of *An. stephensi* were 91.3 ± 0.14 and 90 ± 0.47% to DDT and dieldrin, respectively at one hour exposure time but was susceptible to all pyrethroids tested. The average number of take-offs per min per adult was 2.09 ± 0.13 for DDT, 0.581 ± 0.05 for dieldrin, 1.85 ± 0.08 for permethrin, 1.87 ± 0.21 for lambda-cyhalothrin, 1.53 ± 0.13 for cyfluthrin, and 1.23 ± 0.1 for deltamethrin.

Interpretation & conclusion: Currently, deltamethrin is being used for indoor residual spraying against malaria vectors in the endemic areas of Iran. The findings revealed that the main malaria species is susceptible to all pyrethroids including deltamethrin, permethrin, cyfluthrin and lambda-cyhalothrin but was tolerant to DDT and dieldrin. This report and the finding are coincided with results of previous studies carried out during 1957–61 in the same area. Irritability tests to OC and PY insecticides revealed the moderate level of irritability to DDT compared to pyrethroids and dieldrin. Monitoring for possible cross-resistance between OC and PY insecticides should come into consideration for malaria control programme.

Key words *Anopheles stephensi* – insecticides – Iran – irritability – malaria – susceptibility – tolerance

Introdution

There are several measures for malaria vector control in Iran including larviciding, indoor residual spraying and use of treated bednets. The National Malaria Control Programme in Islamic Repulic, Iran has reached to advanced stage in the last 20 years and only extreme south-east of the country is still in the control stage and characterised as "refractory malaria"[1], which involved three provinces of Sistan and Baluchistan, Hormozgan and tropical areas of Kerman province. Totally, 15,869 malaria cases have been reported in 2006 in the country (Iranian Ministry of Health, unpublished data). However, recent resurgences of malaria in some parts of the country have highlighted the importance of having effective remedial measures in readiness. Because antimalaria operations rely heavily upon residual insecticides and knowledge of anopheline susceptibility status remains important in national control programme[2].

In this part of the country, five anopheline mosquitoes, *Anopheles stephensi, An. dthali, An. fluviatilis, An. superpictus* and *An. culicifacies* (Diptera: Culicidae) are known to be malaria vectors[3]. *An. stephensi* is the main vector responsible for transmission of malaria to human in Persian Gulf area. Sporozoite rates of samples from the southern parts of Iran were reported to be between 0.2 and 1.8%[4]. It is known to be largely endophilic. Resistance to DDT was first recognised in 1957[5] and subsequently to dieldrin in 1960[6], and then to malathion in 1976[7]. After report of malathion resistance in *An. stephensi,* the propoxur was used in 1978[8] and it was used for about 13 years. In recent years, pyrethroids are currently receiving considerable attention as candidate chemical for residual spraying in malaria control programmes. Lambda-cyhalothrin was introduced in malaria control programmes from 1992[9]. From 2003 onwards, deltamethrin was used for residual spraying. However, no resistance has been detected in *An. stephensi* to these two latter insecticides so far. Larval control is now based on chemical control using chlorpyrifos-methyl, biological control using larvivorous fish and also *Bacillus thuringiensis* application in southern Iran[10]. Adult resistance to DDT, dieldrin and malathion was reported in *An. stephensi,* which has been widely distributed in Persian-Gulf, Middle-east and Indian subcontinent causing operational problems for control programmes. In spite of development of DDT resistance in the adults of *An. stephensi,* the larvae of this species remain susceptible to DDT[11,12].

Material & Methods

Study area: Field studies were conducted over a period of 10 months from September 2005 to July 2006 in four villages, Daryacheh, Sephidbaz, Sarghari, Dosari of Jiroft district, Kerman province, southeastern Iran (Longitude: 56º31´–58º45´ Latitude: 28º10´–29º20´). This district has a subtropical climate and is prone to seasonal malaria transmission. The district comprises two regions having different topography; mountainous and plain (Fig. 1.)

The district is a malaria prone area with *Plasmodium vivax* and *P. falciparum* with predominance of the former species[13]. Annual parasite index (API) was 4.2/1000 in 2006. Around 95% cases are Iranian and remaining are refugees from Afghanistan. About 89% of the cases are indigenous in addition to imported, introduced and relapse cases also recorded (Iranian Ministry of Health, unpublished data). The anophelines in this region are—*An. stephensi, An. dthali, An. fluviatilis, An. superpictus, An. culicifacies, An. turkhudi, An. pulcherrimus* and *An. sergenti*[14]. The first five species are the malaria vectors in Iran. This study was undertaken to determine

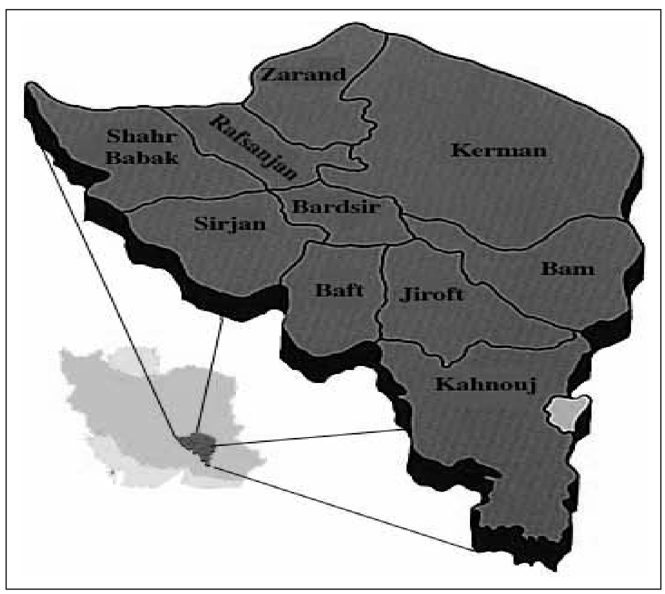

Fig. 1: Map of Kerman province showing study area (Jiroft district)

the susceptibility and irritability levels of field strain of *An. stephensi* (adult) to discriminative dose of some organochlorine (OC), and pyrethroid (PY) insecticides for the first time. The tests were carried out in a temporary laboratory with temperature range of 25–30°C and 60–75% relative humidity.

Susceptibility tests: All susceptibility tests were done using WHO standard methods for adults[15–17]. The IV instar larvae were collected from different larval breeding places of Jiroft district and transported to temporary insectary, fed with Bemax® and then the emerged adults were used for experiments. The tests were carried out on 2–3 days-old and sugar-fed adults. The discriminative dose of insecticide-impregnated papers was provided by WHO and the following insecticides were used: DDT (4%), dieldrin (0.4%), lambda-cyhalothrin (0.05%), permethrin (0.75%), cyfluthrin (0.15%), and deltamethrin (0.05%). The exposure times to OC and PY were 60 min. The mortality was recorded after 24 h recovery period. To monitor the susceptibility status timely, the LT_{50} value of *An. stephensi* employing a log-probit regression line was performed to DDT and dieldrin. Mosquitoes were exposed for different logarithmic times to discriminative dose of insecticide-impregnated papers and subsequently mortality was counted after 24 h recovery period with access to cotton pad-soaked with 10% glucose solution in water. All observed mortalities at different interval times were corrected using Abbott's formula when necessary[15]. The lethal times (LT_{50} and LT_{90}) were determined using log-time and probit mortality regression[16,17].

Irritability test: The irritability levels of *An. stephensi* was determined using the diagnostic dose of DDT and pyrethroid-impregnated papers provided by WHO and the take-off of single mosquito per minute was measured in an exposure chamber at light intensity of eight candle foot according to standard method[17–20]. For each irritability test against one particular insecticide, 30 sugar-fed 2–3 days-old female adults were used. The take-off of each mosquito was observed separately for every minute until 15th minute and the mean and standard error (SE) was calculated for each minute. The ANOVA, Tukey-HSD or Games-Howell test was used for comparisons of take-offs mean among insecticides.

Results

Susceptibility: Results of the susceptibility tests carried out in Jiroft district on *An. stephensi* using OC and PY at discriminating dosages with one hour exposure is given in Table 1. According to WHO criteria, the results indicated that the mortality of field strain of *An. stephensi* were 91.3 ± 0.14 and 90 ± 0.47% respectively to DDT and dieldrin. This species was completely susceptible to lambda-cyhalothrin, permethrin, cyfluthrin and deltamethrin (Fig. 2). The LT_{50} values of *An. stephensi* for DDT (4%) and dieldrin (0.4%) were 19.2 and 28.1 min respectively (Table 2). Comparison of regression lines, a valid method of comparing populations, has proved tolerance of *An. stephensi* enough to detect incipient resistance in the field population. The regression parameters including line formulae are given in Fig. 3.

Irritability: The average number of take-offs per min of *An. stephensi* was 2.09 ± 0.13 for DDT, 0.581 ± 0.05 for dieldrin, 1.85 ± 0.08 for permethrin, 1.87 ± 0.21 for lambda-cyhalothrin, 1.53 ± 0.13 for

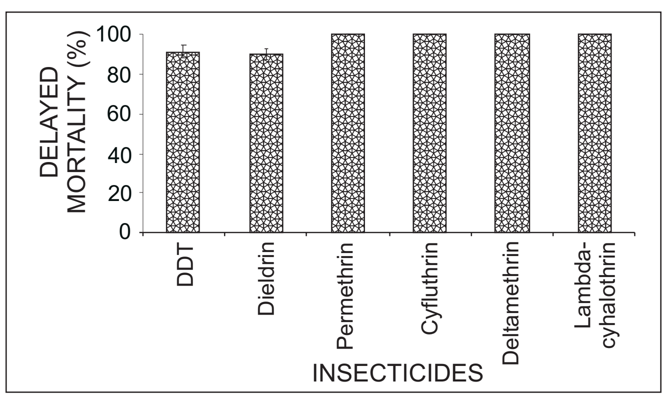

Fig. 2: Regression line of mortality of *An. stephensi* exposed to DDT (4%) impregnated paper using WHO susceptibility kit, Jiroft, southern Iran 2006–07

Table 1. Percent mortality of *An. stephensi* exposed to discriminating dose of insecticides in Jiroft district, Kerman province (2005–06)

Insecticide (%)	Exposure time (min)	Treatment			Control		
		Total	No. dead	Mortality rate (%)	Total	No. dead	Mortality rate (%)
DDT (4)	15	80	18	22.5	25	0	0
	30	80	41	51.3	25	0	0
	60	80	73	91.3	25	0	0
	120	80	80	100	25	0	0
Dieldrin (0.4)	15	80	33	41.3	25	0	0
	30	80	60	75	25	0	0
	60	80	72	90	25	0	0
	120	80	80	100	25	0	0
Permethrin (0.75)	60	100	100	100	25	0	0
Lambda-cyhalothrin (0.05)	60	100	100	100	25	0	0
Deltamethrin (0.05)	60	100	100	100	25	0	0
Cyfluthrin (0.05)	60	100	100	100	25	0	0

Table 2. Comparison of regression lines of mortality of *An. stephensi* exposed to DDT and dieldrin in Jiroft district, Kerman province (2005–06)

Insecticide	a	B ± SE	LT_{50} (95% C.L.)	LT_{90} (95% C.L.)	χ^2 (Heterogeneity)		P-value	Mortality (exposure time 60 min)	Susceptibility level of *An. stephensi* (Jiroft strain)
					Calculated (D.f.)	Table (D.f.)			
Dieldrin	–3.58	2.79 ± 0.34	15.54 (19.18–22.49)	46.04 (55.19–71.47)	1.927 (2)	5.99 (2)	0.05	100	Tolerant
DDT	–5.25	3.62 ± 0.36	24.85 (28.11–31.49)	54.39 (63.44–78.13)	3.053 (2)	5.99 (2)	0.05	91.3	Tolerant

SE—Standard error; LT—Lethal time; C.L.— Confidence limit; D.f—Degrees of freedom

cyfluthrin, 1.23 ± 0.1 for deltamethrin. The statistical analysis showed significant differences between average of take-offs per minute between dieldrin and pyrethroids ($p<0.05$). The mean comparison of take-offs showed the most irritancy to DDT and the least to dieldrin . There was no significant difference in the irritability between DDT and pyrethroids ($p>0.05$).

Discussion

The malaria vector *An. stephensi* is the prevalent endophilic species in southern Iran[18]. The resistance of this species to DDT around the adjacent territories was first reported in Saudi Arabia[19] and Iraq[21,22] in 1955 and 1957 respectively; in Iran in 1957[5] and in India 1965[23]. Subsequently, the resistance was also recognized to dieldrin in 1959[3] in southern Iran. The resistance of *An. stephensi* (wild strain) to DDT and dieldrin as well as its sensitivity to malathion was reconfirmed in southern Iran[24]. The resistance reduction of *An. stephensi* to malathion was well-notified by various researchers[7,25].

The finding of this study revealed the tolerance of *An. stephensi* (field strain) to DDT and dieldrin and its sensitivity to pyrethroids (permetrin, lambda-cyhalothrin, deltamethrin and cyfluthrin). This is the first formal report of tolerance of *An. stephensi* (field strain) to DDT and dieldrin. Similar finding was also reported by Iranian researchers at the same area during 1957[13]. Both OC and OP insecticides were applied as residual spraying in the studied area during 1955–65[26,18]. Nevertheless, the susceptibility tests which carried out during the past decade in various malarious districts also revealed resistance to DDT in southern Iran including Bandar Abbas[27], Kahnooj[28] and, Sistan and Baluchistan[20].

The irritant effect of some insecticides can cause a proportion of insects to leave sprayed surface before acquiring a lethal dose, so the repeated contact is required before mortality occurs. Determination of irritability levels of field strain of *An. stephensi* revealed moderate irritancy to DDT compared to pyrethroids and dieldrin. The irritancy rating to pyrethroids was as follows: permethrin and lambda-cyhalothrin, deltamethrin and cyfluthrin. The irritancy rating from highest to lowest against field strains of *An. stephensi* in neighbouring districts was reported as permethrin and lambda-cyhalothrin at Sistan and Baluchistan[20], permethrin and deltamethrin at Bandar Abbas[27] and permethrin and DDT at Kahnooj[28]. The irritability to insecticides may reduce the effectiveness of the insecticides. Regular monitoring of both physiological and behavioural responses to pyrethroids will be essential in application of pyrethroids in the studied area. Furthermore, the use of pyrethroid insecticides for indoor residual spraying at periodic interval is recommended.

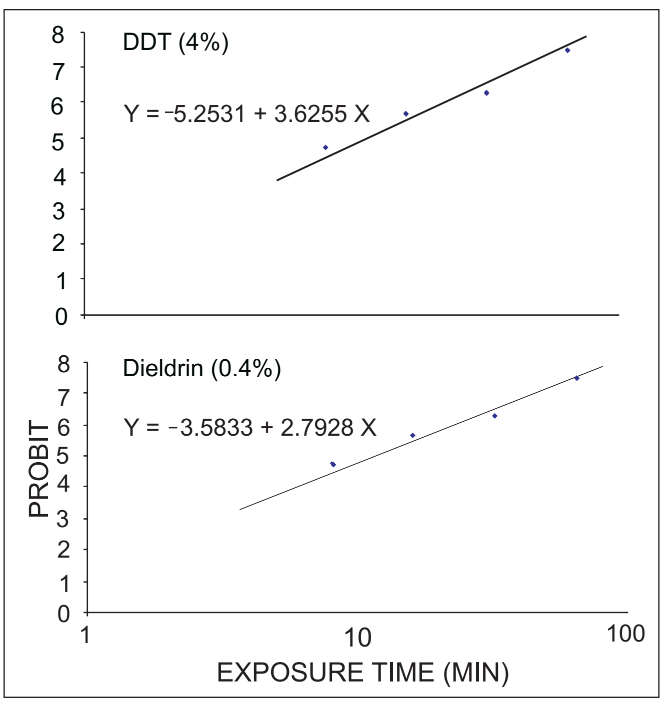

Fig. 3: Regression line of mortality of *An. stephensi* exposed to DDT (4%) and dieldrin (0.4%) impregnated paper using WHO susceptibility kit in Jiroft district, southern Iran (2006–07)

Acknowledgement

This work was financially supported by the Tehran University of Medical Sciences, Research Project No. 5439-27-01-86. The authors are grateful to Dr E. Mohammadi, Head of Jiroft Health Center for providing facilities for implementation of this research. We also wish to thank Mr M.R. Azadavar for his kind field assistance in the study area. The great appreciation goes to the managers of Jiroft and Kerman Province Health Centers for providing the facilities to carry out the field work.

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Received: 4 June 2008 *Accepted in revised form:* 11 September 2008