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Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj district, Kerman province, southeastern Iran

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Background & objectives: Kahnooj district in south of Iran is an endemic area for malaria where *Anopheles stephensi* (Liston) is a main malaria vector and *An. dthali* (Patton) a secondary vector. According to the national strategy plan on monitoring of insecticides resistance, this study was performed on susceptibility and irritability levels of *An. stephensi* and *An. dthali* to different insecticides in the district.

Methods: The susceptibility and irritability levels of field strains of *An. stephensi* and *An. dthali* at the adult and larval stages to discriminative dose of different imagicides was determined as recommended by WHO.

Results: Using discriminative dose and WHO criteria it was found that *An. stephensi* is resistant to DDT and dieldrin with 36.1 ± 2.3 and 62.2 ± 1.95 mortality rates, respectively; but susceptible to other insecticides. *An. dthali* was found to susceptible to all tested insecticides. The larvae of *An. stephensi*, exhibited 100% mortality for temephos and malathion, but 44 ± 4.32 for discriminative dose of fenitrothion. The results of irritability level for DDT and pyrethroids showed that permethrin had the most irritancy effect on *An. stephensi* and *An. dthali*. DDT and deltamethrin showed the least irritancy effect against *An. stephensi* with 0.42 ± 0.08 and 0.77 ± 0.12 take-offs/min/adult, respectively, however, lambdacyhalothrin had the least irritancy effect against *An. dthali* with 0.096 ± 0.02 take-offs/min/adult. The mean number of take-offs/min/adult with permethrin showed significant difference to DDT, lambdacyhalothrin, cyfluthrin and deltamethrin.

Interpretation & conclusion: Pyrethroid insecticides are being used as indoor residual sprays in Iran. Based on our results, the main malaria vectors in the region are still susceptible to pyrethroid insecticides. Therefore, we propose the use of pyrethroids with low irritancy effect in rotation with carbamate insecticides in two interval seasonal peaks of malaria transmission. Biological control including larvivorous fishes, using of local made *Bacillus thuringiensis* and larvicides such as chlorpyrifos-methyl are the main larval control in the region. Result on larval test exhibited the susceptibility of main vectors to some larvicides, although the 100% mortality was not obtained using fenitrothion and this is postulated the use of this insecticide in agriculture pest control. Monitoring and evaluation of insecticides resistance in malaria vectors in the region could provide an essential clue for judicious use of insecticides.

Key words *Anopheles dthali* – *An. stephensi* – insecticides – Iran – malaria

A total of 14,000 malaria cases had been reported in Iran in the year 2004. The disease is a major health problem in southeast of Iran. It is unstable with two seasonal peaks mainly in spring and autumn. Outbreaks usually occur after rainy season. Southeastern part of Iran which includes the provinces of Sistan-Baluchistan, Hormozgan and the tropical areas of Kerman province are characterised by "refractory malaria"[1]. 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.

An. stephensi is one of the main malaria vectors in south of Iran. It is known to be largely endophilic. In this species resistance to DDT was first recognised in 1957[2] and subsequently to dieldrin in 1960[3], and malathion in 1976[4]. After appearance of malathion resistance in *An. stephensi*, propoxur was substituted in 1978 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. Therefore, from 1992 lambdacyhalothrin as pyrethroid was introduced in malaria control programmes. However, no resistance has been detected in *An. stephensi* to these two latter insecticides so far. From 2003 deltamethrin was used for residual spraying. Larval control in south of Iran is now based on chemical control using chlorpyrifos, biological control using larvivorous fish and also *Bacillus thuringiensis*[5].

Resistance to DDT, dieldrin and malathion mainly in the adults of *An. stephensi*, have been widely distributed in Persian-Gulf, Middle-East and Indian subcontinent[6,7] causing operational problems for control programmes. Low level of larval resistance was found in Pakistan[8]. In south of Iran, in spite of development of DDT resistance in the adults of *An. stephensi*, the larvae showed susceptibility to DDT[9]. In Bandar-Abbas, Hormozgan province, adjacent to the Kahnooj district, *An. stephensi* larvae showed susceptibility to malathion, temephos and chlorpyrifos, but resistance to fenitrothion[10].

An. dthali is another vector in southeastern part of Iran. Gland infection of *An. dthali* was reported by Mesghali (unpublished report to WHO 1967) and this was confirmed by Manouchehri *et al*[11]. This species was found to rest in both indoor and outdoor shelters[12]. It is quite susceptible to all insecticides in Iran.

Material & Methods

Study area: Kahnooj district is located in south of Kerman province with surface area of 25,778 km^2, between 56°–59' and 59°–18' E longitude and 26°–29' and 30°–28' N latitude. The area is subtropical and a reservoir of malaria (Fig. l). The district is a ma-

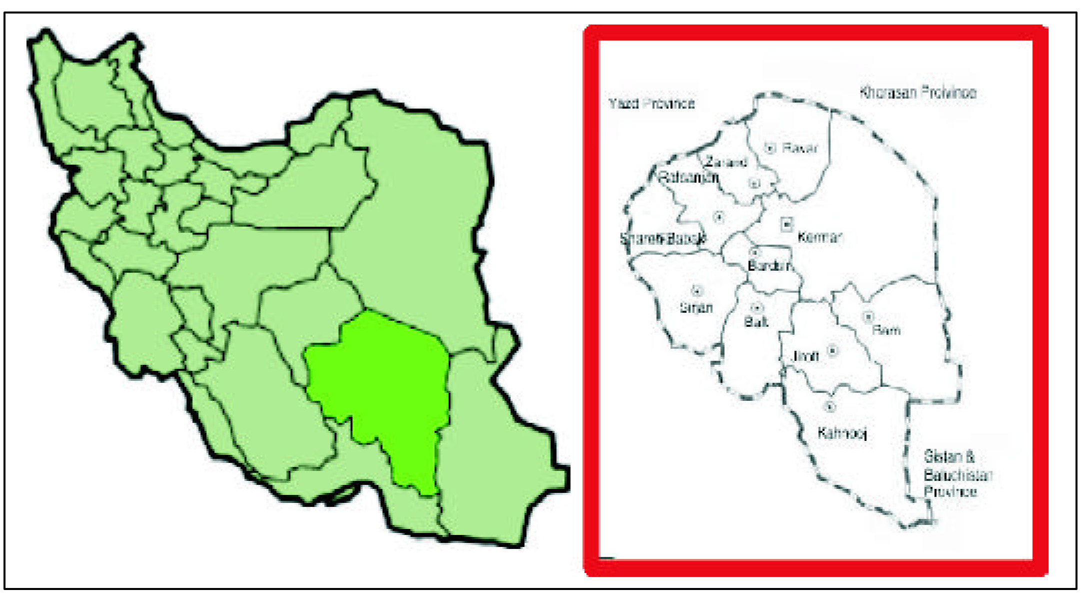

Fig. 1: Map of Kerman province and study area

laria prone area with *Plasmodium vivax* as the dominant species, whereas *P. falciparum* parasite also exists. Annual parasite incidence (API) was 1.2/1000 in 2004. Around 87% cases are Iranian and remaining are refugees from Afghanistan. About 93% of cases are indigenous imported, introduced and relapse cases are also recorded. The anophelines in this region are: *An. stephensi, An. dthali, An. fluviatilis, An. superpictus, An. culicifacies, An. turkhudi, An. sergentii, An. apoci, An. moghulensis* and *An. multicolor.* The first five species are the malaria vectors in Iran.

An investigation was made to determine the susceptibility and irritability levels of Kahnooj field strains of *An. stephensi* (adult & larvae) to discriminative dose of some organochlorine, organophosphate, carbamate and pyrethroid insecticides for the first time. The tests were carried out in a temporary laboratory with a temperature of 25–30°C and 60–75% relative humidity.

All susceptibility tests were done using WHO standard methods for adults and larvae[13]. The tests were carried out on 2–3 days-old, sugar-fed adults which were collected from different larval breeding places of Kahnooj county. After transportation to temporary insectoary, they were fed with Bemax and then emerged adults were tested.

The discriminative dose of impregnated papers which were provided by WHO were as follows: DDT (4%), dieldrin (0.4%), malathion 5%, fenitrothion (1%), propoxur (0.1%), lambdacyhalothrin (0.05%), permethrin (0.75%), cyfluthrin (0.15%), deltamethrin (0.05%) and etofenprox (0.5%). The mortality was scored after 24 h recovery period.

In order to evaluate the susceptibility of *An. stephensi* to different larvicides in Kahnooj county an investigation was made under laboratory conditions at the Kahnooj Health Centre. The larval susceptibility tests were carried out using the WHO standard method on three organophosphorus larvicides. The recommended diagnostic doses of larvicides, were temephos 0.25 mg/l, malathion 3.125 mg/l and fenitrothion 0.125 mg/l. In each test four or five replicates of control and larvicides at the single diagnostic dose were used.

Since there is malaria transmission in the region and *An. stephensi* is the main vector in the region, so that decision was made on using residual insecticides for malaria control for the last decade as the main component of national strategy programme. To monitor the susceptibility status timely the LT_{50} value of *An. stephensi* to different insecticides employing a log-pro bit regression line was performed to all imagicides. Mosquitoes were exposed at different interval times to insecticides and subsequently mortality was counted after for 24 h recovery period with access to cotton pad-soaked with 10% glucose solution in water. All the mortalities at different interval times were corrected using Abbott's formula[14]. The lethal times (LT_{50} and LT_{90}) were determined using log-time and probit-mortality regression model of Finney[15].

The irritability levels of *An. stephensi* against the diagnostic dose of DDT and pyrethroid insecticides were measured in an exposure chamber according to the method described by WHO[16]. As expert committee recommended the light intensity of approximately eight foot candles were used. The field mosquitoes were collected from different larval breeding places of Kahnooj county. In each test, 30 sugar-fed 2–3-day-old adult females were used in 15 min exposure period.

Results

The results of susceptibility tests indicated that the field strain of *An. stephensi* was resistant to DDT and dieldrin. Mortality rate of these insecticides were 36.1$\pm$2.3 and 62.2$\pm$1.95 respectively. This strain was susceptible to malathion, fenitrothion, propoxur, lambdacyhalothrin, permethrin, cyfluthrin, deltamethrin and etofenprox (Table 1). The results of larval susceptibility test showed that the larvae of *An. stephensi* field strain, exhibited 100% mortality after 24 h recovery period using diagnostic doses of temephos and malathion, but 44 $\pm$ 4.32 mortality for discriminative dose of fenitrothion was yielded (Table 2).

Table 1. The mortality of *An. stephensi* females exposed to discriminating dose of insecticides

Insecticides (%)	Replicates	No. mosquitoes tested	No. dead	Mortality (%)	Error bar
DDT (4)	5	116	43	36.1	2.3
Dieldrin (0.4)	4	100	62	62.2	1.95
Malathion (5)	2	49	49	100	0
Fenitrothion (1)	2	47	47	100	0
Propoxur (0.1)	2	47	47	100	0
Lambdacyhalothrin (0.05)	2	48	48	100	0
Deltamethrin (0.05)	2	49	49	100	0
Permethrin (0.75)	4	48	48	100	0
Cyfluthrin (0.15)	4	45	45	100	0
Etofenprox (0.5)	2	43	43	100	0
Control	28	649	28	4.33	0.88

Table 2. The mortality of *An. stephensi* larvae exposed to discriminating dose of larvicides

Larvicides (mg/l)	Replicates	No. larvae	No. dead	Mortality (%)	Error bar
Malathion (3.125)	4	100	100	100	0
Temephos (0.25)	4	100	100	100	0
Fenitrothion (0.125)	4	100	44	44	4.32
Control	9	323	4	1.238	0.53

The LT_{50} values of *An. stephensi* for DDT (4%), dieldrin (0.4%), malathion (5%), propoxur (0.1%), lambdacyhalothrin (0.05%) and deltamethrin (0.05%) were measured as 72.84, 38.72, 9.26, 7.91, 9.43 and 3.14 min, respectively (Table 3). The results of susceptibility tests indicated that the field strain of *An. dthali* exhibited 100% mortality and is susceptible to all the imagicides tested (Table 4). The results of irritability tests showed that permethrin had the most irritancy effect against *An. stephensi* and *An. dthali*. DDT and deltamethrin showed least irritancy effect against *An. stephensi* and lambdacyhalothrin had least irritancy effect against *An. dthali*. The mean number take-offs/min/adult in series of tests with permethrin on *An. stephensi* was 1.54 $\pm$ 0.15, and for DDT, deltamethrin, lambdacyhalothrin, cyf1uthrin and etofenprox were 0.42 $\pm$ 0.08, 0.77 $\pm$ 0.12, 0.78 $\pm$ 0.11, 0.72 $\pm$ 0.11 and 1.19 $\pm$ 0.17, respectively (Fig. 2). The mean of take-offs/min/adult in control cones was 0.036 $\pm$ 0.008. The take-offs/min/adult in series of tests on *An. dthali*, with permethrin was 0.58 $\pm$ 0.06, with DDT 0.42 $\pm$ 0.06, with deltamethrin 0.2l $\pm$ 0.05, lambdacyhalothrin 0.096 $\pm$ 0.02 and, with cyf1uthrin 0.22 $\pm$ 0.03. The mean take-offs/min/adult in control cones was 0.041 $\pm$ 0.013 (Fig 3).

Discussion

The results of susceptibility tests using WHO criteria (98–100% mortality indicating susceptibility and

Table 3. Probit regression line parameters of adults of *An. stephensi* tested with different insecticides, Kahnooj district, Kerman province (2003–04)

Insecticides (%)	A	B ± SE	95% confidence interval		df	χ^2	p-value
			LT_{50}	LT_{95}			
DDT (4)	12.77	6.85 ± 0.87	79.19 72.84 67.81	135.26 112.03 99.20	2	1.33	> 0.05
Dieldrin (0.4)	–2.37	1.46 ± 0.15	53.75 38.72 28.92	548 321.22 140.64	4	1.99	> 0.05
Malathion (5)	–1.61	1.67 ± 0.43	43.69 9.26 4.37	58.67 45.23 18.42	5	42.83	< 0.05
Propoxur (0.1)	–2.11	2.34 ± 0.44	16.10 7.91 4.61	169.6 27.85 14.28	4	16.32	< 0.05
Lamdacyhalothrin (0.05)	–2.23	2.29 ± 0.36	15.03 9.43 5.86	96.77 34.25 20.24	5	15.69	< 0.05
Deltamethrin (0.05)	–0.87	1.74 ± 0.18	3.83 3.14 2.53	26.50 17.02 12.43	4	6.60	> 0.05

Table 4. Mortality of *An. dthali* females exposed to discriminating dose of insecticides

Insecticides (%)	Replicates	No. mosquitoes tested	No. dead	Mortality (%)	Error bar
DDT (4%)	2	45	45	100	0
Dieldrin (0.4)	2	43	43	100	0
Malathion (5)	2	42	42	100	0
Fenitrothion (1)	2	43	43	100	0
Propoxur (0.1)	2	42	42	100	0
Lambdacyhalothrin (0.05)	2	46	46	100	0
Deltamethrin (0.05)	2	41	41	100	0
Permethrin (0.75)	2	44	44	100	0
Cyfluthrin (0.15)	2	43	43	100	0
Etofenprox (0.5)	2	39	39	100	0
Control	8	175	5	2.85	1.2

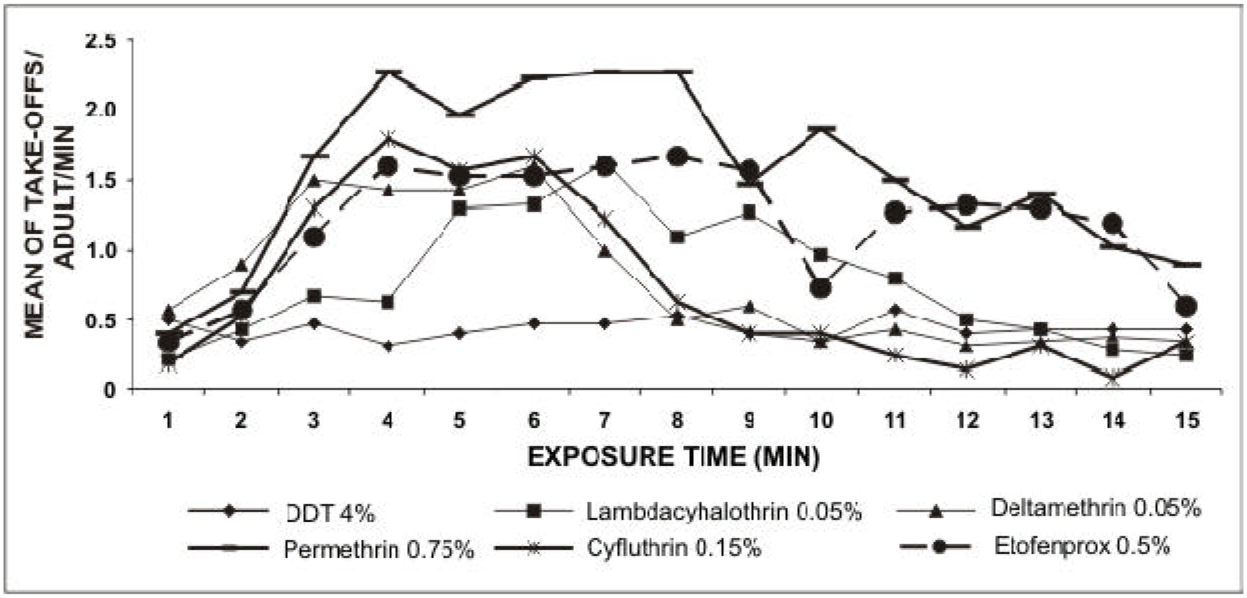

Fig. 2: Irritability level of *An. stephensi* to different insecticide

< 89% mortality indicating resistance), showed that *An. stephensi* is resistant to DDT and dieldrin and susceptible to all other insecticides. However, *An. dthali* is still susceptible to all insecticides. This is attributed to the endophilic and endophagic behaviours of *An. stephensi*, while exophilicity and exophagity of *An. dthali* cause the susceptibility to all insecticides. Using one way analysis of variance (ANOVA) the results showed that there is significant difference among pyrethroids ($p < 0.05$) in irritability level. Due to Levene's test for equality of variances, Games-Howell Post-Hoc was used. The results of this test showed that permethrin had significant difference with DDT, lambdacyhalothrin, cyf1uthrin and deltamethrin in *An. stephensi* whereas significant difference with lambdacyhalothrin, cyfluthrin and deltamethrin is shown in *An. dthali* ($p < 0.05$). The independent sample *t*-test showed significant difference in the mean number of

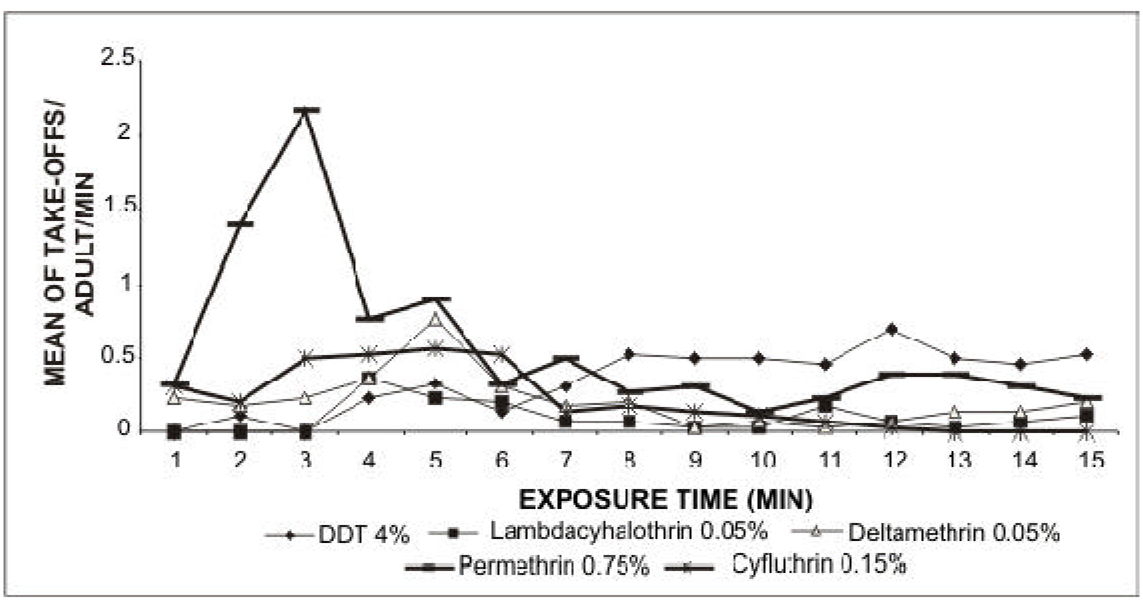

Fig. 3: Irritability level of *An. dthali* to different insecticide

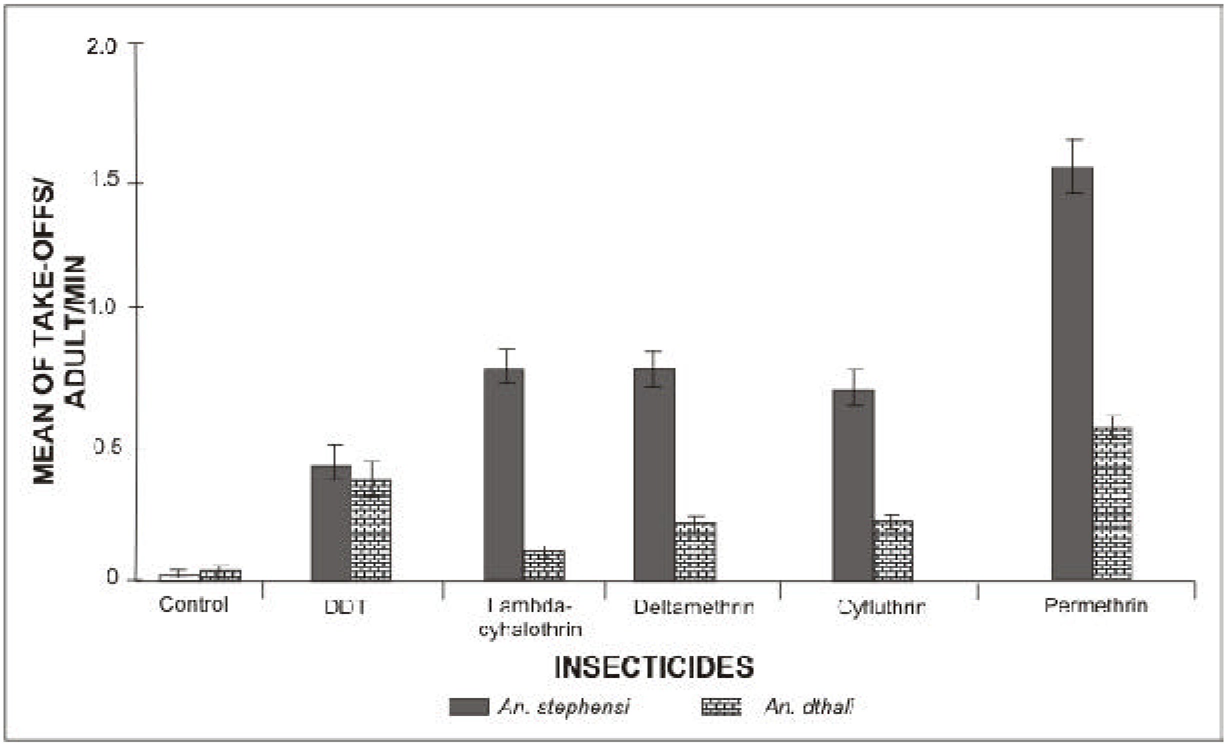

Fig. 4: Comparison of irritability level of *An. stephensi* and *An. dthali*

take-offs/min/adult in pyrethroids between two *Anopheles* species ($p < 0.05$) (Fig 4).

The results showed that irritability level in *An. dthali* was lower than *An. stephensi*. This difference in irritability is related to higher percentage of exophilicity in *An. dthali*. It can be explained that behavioural resistance mechanism is an outcome of the insecticides pressure which appears more rapidly in endophilic than exophilic anopheline species.

Resistance of *An. stephensi* larvae to fenitrothion has been reported from India, Iraq, Iran and Pakistan[7]. Based on previous studies, two genetic factors have a role in resistance of *An. stephensi* to insecticides[17]. In India *An. stephensi* adults were found resistant to DDT, propoxur, malathion, but susceptible to fenthion and deltamethrin[18]. This species was found resistant to DDT and dieldrin, partially resistant to malathion and susceptible to fenitrothion, propoxur and permethrin in the Thar Desert[19]. In Bikaner district, *An. stephensi* was found resistant to DDT and dieldrin and susceptible to fenitrothion and permethrin[20]. In Panaji, Goa, adults of *An. stephensi* were resistant to DDT (4%), dieldrin (0.4%) and malathion (5%). The larvae of this species were also resistant to DDT (2.5 mg/l) and malathion (3.125 mg/l)[21]. Results of the bioassay with two pyrethroids, deltamethrin and permethrin, showed tolerance in *An. stephensi* collected from Mysore city, India[22].

Since resistance to insecticides causing operational problems for control programmes and behavioural resistance mechanism is an outcome of the insecticides pressure it appears more rapidly in endophilic species such as *An. stephensi*, so regular and accurate monitoring of susceptibility/resistance status (regarding baseline data), its mechanisms and study on cross-resistance is essential in evaluating the insecticides.

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. The term repellency (more often excito-repellency) is sometimes related to this phenomenon. Repellency is the prevention of the insect from approaching the insecticide. This irritability would produce heightened activity in the landing mosquitoes and will remain only on the treated surface for a short period of time. The irritability response of vectors was interpreted to have a negative impact on control efforts. Insecticide repellency could prevent vectors from entering human habitations treated with the insecticides. In the long run this is likely to cause reduction in the endophilic mosquitoes and an increase in the exophilic populations. Pyrethroids may repel insects due to air-borne repellency or contact, which raise the possibility of pyrethroid use. In some cases survival of a species in the treated houses is attributed to the reduced intrinsic toxicity of insecticide or occurrence of physiological resistance but this phenomenon might be due to irritancy property of the insecticide. The irritability to insecticides may reduce the effectiveness of residual applications of the insecticides. So careful monitoring of both physiological and behavioural responses to pyrethroids will be essential in evaluating the pyrethroids. Furthermore, our recommendation is periodic interval use of carbamate and pyrethroid insecticides as indoor residual spraying. In addition, as integrated vector management, other control measures such as biological control, using personal protection, using less irritant pyrethroids on impregnated bednets and space spraying in epidemic situation is also recommended.

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