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Insecticide Resistance /Susceptibility Monitoring in *Anopheles pulcherrimus* (*Diptera: Culicidae*) in Ghasreghand District, Sistan and Baluchistan Province, Iran

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ABSTRACT

Two organochlorine, one organophosphate, two carbamate and two pyrethroid insecticides have been studied for their comparative study against field-collected *An.pulcherrimus*. The trail was conducted in Ghasreghand district, Sistan and Baluchistan province, Southeastern Iran using WHO impregnated papers at the diagnostic dose. Results revealed that this species exhibit resistant to 0.4% dieldrin (mortality 86 ± 2.07). Dose response regression line from interval exposure times to 0.4% dieldrin showed that LT50 and LT90 was 14 and 76 minutes, respectively. The efficacles of other insecticides such as 4% DDT, 5% malathion, 0.1% bendiocarb, 0.1% propoxur, 0.25% permethrin and 0.1% lambdacyhalothrin were maximum when mosquitoes exposed 1 hour at diagnostic dose followed by 24 hour recovery period.

INTRODUCTION

Anopheles pulcherrimus theobald has a wide distribution. It is found from Lebanon, Syria, Iraq (2,12,13,17), Iran (23), Pakistan (19), Oman, Saudi Arabia, Syria (21,22), Turkey (10) India (11), Turkmanistan (23), Tajikistan (6), Azerbaijan (4), Uzbekistan (4), Russia (5) and Afghanistan (7). Despite the wide distribution of this species in the geographical area, it has been found to act as a malaria vector in Iraq, Afghanistan and Iran according to 2-Site Immunoradiometric Assay (IRMA)(8).The breeding places of *An.pulcherrimus* has been cited in different localities; slow moving streams, ditches, rice fields, pools, weedy irrigation channels, marshes, and any kind of clean stagnant water are the most favorite site of habitats. In Iran, in Baluchistan province this species is active throughout the year with two peaks of activity, April-May and August-September and it has mainly exophilic habit. WHO listed that this species is resistant to DDT in Afghanistan, Iraq, Saudi Arabia and Syria (22). Dieldrin resistance also reported from Afghanistan and Pakistan. There is no report of susceptibility status of *An.pulcherrimus* in Iran so far. Therefore, an effort has been made to elucidate the current response of *An.pulcherrimus* to conventional insecticides such as DDT, dieldrin, malathion, bendiocarb, propoxur, permethrin and lambdacyhalothrin. This information would be of relevance while planning an effective mosquito control strategy in the Ghasreghand district, Sistan and Baluchistan province in Iran.

MATERIALS AND METHODS

Study Area

For the present study, the district of Ghasreghand in the province of Sistan and Baluchistan were selected because this district has one of the highest API (Annual Parasite Incidence) in the country, i.e., about 30-50 per thousand population. Total population 40,000 live under this malarious area. Rice growing is the main agricultural activities of local people. The average daily temperature in the warmest and coolest months of the year are 34 and 14.5°C, respectively. The average yearly rainfall is about 120 mm. *An.culcicifacies* and *An.pulcherrimus* are the most common anopheline species, where indoor residual spraying, two rounds per year with propoxur and lambdacyhalothrin is being done. Other malaria vectors including; *An.d'thali*, *An.stephensi*, *An.fluviatilis*, and *An.superpictus* are present in this area but in a small number. *An.culicifacies*, however, considered as a main vector (24). This is mostly an endophilic species but a large population also rests outdoors. It is mostly exophagic. There are two peaks of malaria transmission in this region. The first is early spring and the second early autumn. The tow *plasmodium vivax* and *Plasmodium falciparum* are dominant parasites with cyclic changes. About 50% of malaria cases occur under the age of fifteen years old.

Susceptibility Test

Susceptibility to the insecticides of filed-collected mosquitoes was determined by exposing fresh-fed females to the diagnostic doses of insecticide impregnated papers supplied by WHO; i.e., 4% DDT, 0.4% dieldrin, 5% malathion, 0.1% propoxur, 0.1% bendiocarb, 0.25% permethrin and 0.1% lambdacyhalothrin. At each exposure time 8-11 individual replicates of 12-31 adults were used. Due to the knock-down effect of pyrethroids on the adults, the exposure tubes were held at the horizontal position during tests, whereas for the organochlorine, organophosphate and carbamate

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insecticides the exposure tubes were at the vertical positions. Insecticide exposure took place in a room with a temperature of 25 ± 2°C. Exposure time for all the insecticides tested was 1 hr followed by 24 hr holding period. Simultaneously, mosquitoes from the same collections also were exposed to control papers. After exposure, mosquitoes were maintained at 25 ± 2°C and 70-80 % relative humidity with cotton pads for 24 hour before scoring the mortality. If control mortality was within 5-20%, test mortality was corrected by control mortality using Abbott's formula (22). *An.pulcherrimus* also were exposed to the WHO-recommended diagnostic dose of dieldrin for different time intervals (5,10,20,40,80,160 minutes) to plot the dose mortality response. Data collected after a 24 hour recovery period were examined using probit analysis (9), using the probit 79 programme on an IBM computer. Goodness of fit of the points to a straigh line were tested by Chi-square (χ^2) analysis.

RESULTS AND DISCUSSION

Data on dieldrin, DDT, malathion, bendiocarb, propoxur, permethrin and lambdacyhalothrin obtained through exposure to diagnostic dose are tabulated in Table 1 and represented in Fig. 1. The response of 280 mosquitoes of *An.pulcherrimus* population to 0.4% dieldrin papers for 1 hour followed by 24 hour recovery period resulted in the survival of 40 mosquito and mortality was 86 ± 2.07% mortality. Due to appearance of dieldrin resiatnce in this species it was decided to plot the dose mortality regression line. *An.pulcherrimus* were exposed to the WHO impregnated paper for different interval times to obtain 5-95% mortality. This species were exposed at 5,10,20,40,80,160 minutes to 0.4% dieldrin and mortality scored after 24 hour recovery period (Table 2). The results of the study on the efficacy of deildrin and regression line parameters including intercept (a), slope ± standard error (b ± SE), heterogeneity about the regression line with degree of freedom [χ^2 (df)], LT50 ± 95% confidence interval (LT50 ± 95% C.I.), and LT90 ± 95 % confidence interval (LT90 ± 95% C.I.) are presented in Table 3. The LT50 and LT90 values for this species was 14 and 76 minutes, respectively. Although the susceptibility test during the past years have not been performed, it is recommended to carry out such tests during successive years in order to find resistance level of species to certain insecticides. Exposure to 5% malathion for 1 hour did not discriminate susceptible and resistant strains of *An.pulcherrimus*. Out of 192 mosquitoes exposed to this insecticide only 2 individual remained alive after recovery period.

In Iraq DDT resistance in *An.pulcherrimus* was indicated from the tests carried out in 1971 in a locality in Baghdad province (12), where one hour exposure to 4% DDT gave 47.3% mortality. Three years later, tests with the same exposure in the same locality gave 69.6% mortality, and prolonged exposure for two hours on the same concentration gave only 88% mortality. At the same time, this species showed normal susceptibility to malathion. The presence of DDT resistance in Iraq was confirmed (14) in 1978. The species remained susceptible to malathion. One hour exposure to 4% DDT in 1968-1970 yielded 100% mortality, but successive monitoring in 1972 revealed only 96.8% mortality and in 1974 only 89.7% mortality indicating incipient DDT resistance (15). In 1970 this species was susceptible to dieldrin. In Saudi Arabia susceptibility test in 1957-1958 indicating incipient DDT resistance (15). The results showed that *An.pulcherrimus* was susceptible to DDT and resistant to dieldrin.

Table 1. **Mortality of *Anopheles pulcherrimus* to a diagnostic dose of insecticides in Sistan and Baluchistan Province, Iran**

Pesticide	No. tested	No. Dead	Mr (%)	repli cates	Error bar
Dieldrin 0.4%	280	240	86.00	11	± 2.07
DDT 4%	258	257	99.60	11	± 0.40
Malathion 5%	192	190	98.96	8	± 0.73
Bendiocarb 0.1%	187	184	98.70	8	± 0.92
Propoxur 0.1%	190	190	100.00	8	± 0.00
Permethrin 0.25%	204	204	100.00	8	± 0.00
Lambdacyhalothrin 0.1%	187	185	98.94	8	± 0.75
Control	190	6	3.00	8	± 1.24

Each replicate comprise 12-31 individual female mosquitoes

Table 2. **Mortality of *An.pulcherrimus* exposed to dieldrin 0.4% at different interval times**

Exposure time (minutes)	Dead	Alive	Mr (%)
5.0	13	62	17
10.0	25	37	40
20.0	73	27	73
40.0	79	21	79
80.0	92	12	88
160.0	43	2	95

Table 3. **Probit regression line parameters of *An.pulcherrimus* exposed to 0.4% dieldrin**

a	b ± SE	LT50 ± 5%C.I	LT90 ± 95%C.I	X^2 (df)	P
-1.98	1.73 ± 0.16	11.08 14.00 16.80	58.71 76.00 106.10	6.67 (4)	<0.05

Susceptibility of six anopheline mosquito species from nine localities in Punjab province, Pakistan to DDT, dieldrin, malathion, fenitrothion, fenthion and propoxur were determined in 1980 (18). *An.pulcherrimus* was found to be susceptible to DDT. Dieldrin resistance was present in all six species; *An.pulcherrimus, An.annularis, An.culicifacies, An.stephensi, An.subpictus* and

An.nigerrimus. In Russia *An.pulcherrimus* was susceptible to malathion, fenitrothion, propoxur and DDT (20).

Data on carbamate insecticides such as bendiocarb and propoxur are shown in Table 1. Propoxur is being used at the rate of 2 g/m^2 one round a year in Ghasreghand district from 1991-1997. Data on the efficacy of propoxur showed that out of 190 mosquito tested 100% mortality resulted. In the case of bendiocarb, females of this species showed only 98.7 ± 0.92% moratlity. The values for pyrethroid insecticides showed that permethrin and lambdacyhalothrin were quite effective against this species. Permethrin and lambdacyhalothrin yielded 100% and 98.94 ± 0.75% mortality, respectively.

The present status of insecticide resistance/susceptibility monitoring in the region indicates serious need for recognizing the whole system of insecticide susceptibility testing, recording, analysis, reporting and application of the information emerging from such tests, in order to implement most cost-effective vector control. Moreover, regular and accurate monitoring of susceptibility/resistance mechanism monitoring is essential for resistance management, especially where selective application of pesticides is the policy.

Fig. 1. **Mortality of *Anopheles pulcherrimus* to a diagnostic dose of insecticides in Sistan and Baluchistan Province, Iran**

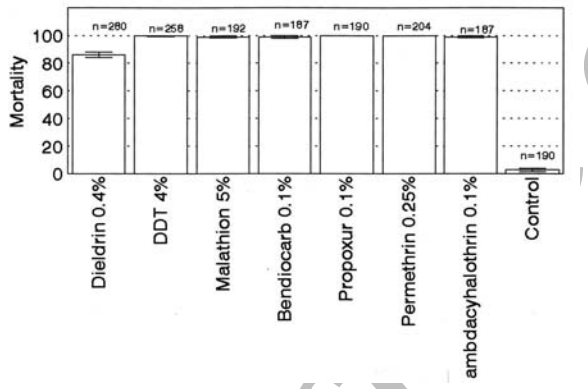
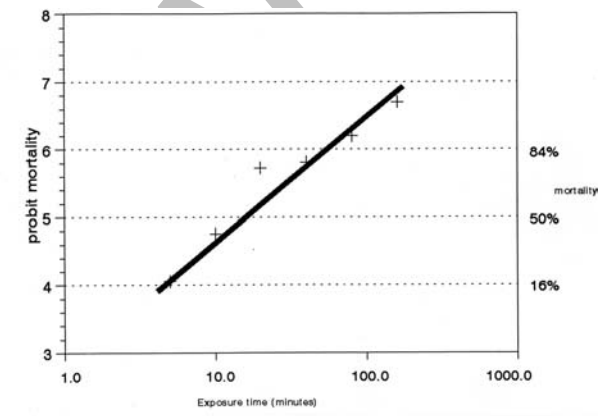


Fig. 2. **Probit regression line parameters of *An.pulcherrimus* exposed to 0.4% dieldrin**



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