STATUS OF INSECTICIDE RESISTANCE IN ANOPHELES CULICIFACIES (DIPTERA: CULICIDAE) IN GHASREGHAND DISTRICT, SISTAN AND BALUCHISTAN PROVINCE, IRAN,(1997)

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Abstract - Anopheles culicifacies s.l. plays an important role in transmission of malaria in Sistan and Baluchistan province, southeastern Iran. Adult susceptibility test on field-collected mosquitoes was conducted in Ghasreghand district. WHO diagnostic test procedures revealed that adult females were resistant to 0.4% dieldrin (mortality 64.5 ± 3.13), tolerant to 0.1% propoxur (mortality 88.5 ± 2.24) and susceptible to 4% DDT (mortality 98.75 ± 0.8), 5% malathion (mortality 100%), 0.1% bendiocarb (mortality 98.86 ± 0.7), 0.25% permethrin (mortality 98.4 ± 0.1), and 0.1% lambdacyhalothrin (mortality 100%). Malathion and lambdacyhalothrin had the highest efficacy against this species when they were exposed at the diagnostic dose for 1 hour followed by a 24 hour recovery period. Dieldrin, DDT and malathion had been used for malaria control as an indoor residual spraying. The implication of these findings in the control programme is discussed.
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Key Words: Anopheles, culicifacies, insecticide, resistance

INTRODUCTION

An. culicifacies as a malaria vector is widely distributed in the area of Asia and Indian subcontinent. Its distribution extends from Ethiopia, Yemen, Oman, United Arab Emirates, Iran and Afghanistan in the west to Pakistan, India, Bangladesh, Myanmar, Thailand, and Sri Lanka in the south. According to locality, four reproductively isolated populations have been found, namely species A,B,C, and D (1). They can be distinguished by new enzymatic and genetic identification methods. Occurrence of species was reported in Ghasreghand by Zaim and Javaherian (1991). In this district, it of a main material vector. This species is mainly endophilic, exophagic and zoophilic.

In the wake of the resurgence of malaria, the problem of vector control has reached a difficult stage due to several ecological, genetical, behavioral and operational reasons. Development of insecticide resistance in vector populations seems to be one of the reasons for control failure. Although more reliance is being laid on environmental and biological control, in the absence of alternative methods one may have to use insecticides to deal with epidemics. Thus it is necessary to assess the susceptibility status of vector species to different insecticides which have been previously used for vector control as well as those that are currently being used.

An. culicifacies which breeds mainly in Sistan and Baluchistan province, (the study area) and is largely responsible for the epidemic of malaria in this region. Therefore, a study was undertaken to reassess the efficacy and resistance susceptibility status of this species to seven insecticides viz., 4% DDT, 0.4% dieldrin, 5% malathion, 0.1% propoxur, 0.1% bendiocarb, 0.25% permethrin and 0.1% lambdacyhalothrin.

MATERIAL AND METHODS

For the present study, the district of Ghasreghand in the province of Sistan and Baluchistan was selected because this district has one of the highest annual parasite incidences (API) in the country, i.e., about 30-50 per thousand population. A total population of 40000 live in this malarious area. Rice growing is the main agricultural activity of the inhabitants. 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. culicifacies* 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 low density. *An. culicifacies*, however, is considered the main vector(2). This is mostly an endophilic species but a large population rests outdoors. It is predominantly exophagic. There are two peaks of

malaria transmission in this region. The first is early spring and the second early autumn. *Plasmodium vivax* and *Plasmodium falciparum* are dominant parasites with cyclic changes. About 50% of malaria cases are under fifteen years of age.

Susceptibility tests

From two villages in Ghasreghand district (Fig. 1) during May-June 1997, all anopheline mosquitoes resting in human dwelling and cattle sheds were collected with an aspirator and a flashlight between 6:00 and 8:00 a.m. Mosquitoes were brought to the laboratory (situated 7-10 Km from the collection sites) within 30 minutes of collection in cloth cages (30 × 30 × 30cm) covered with wet towels. Susceptibility to the insecticides of field-collected mosquitoes was determined by exposing freshfed females to the diagnostic doses of insecticide impregnant 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 individual replicates of 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 insecticides the exposure tubes were at the vertical positions. Insecticides exposure took place in a room with a temperature of 29 ± 2°C Exposure time for all the insecticides tested was 1 hour followed by 24 hours holding period. Simultaneously, mosquitoes from the same collections were exposed to control papers. After exposure, mosquitoes were maintained at 25 ± 3° C and 30-50% relative humidity with cotton pads for 24 hours before scoring the mortality. If control mortality was within 5-20%, test mortality was corrected by control mortality using Abbott's formula(3).

A total number of mosquitoes tested against seven insecticides was 1689. among which 97.76% (n=1651) was *An. culicifacies* and remaining mosquitoes comprised An. pulcherimus (0.18%), An. fluviatilis (1%), and An. stephensi (1.06%) (Fig. 2). After each test the mosquito species were identified and percentages of each species recorded. Due to dominancy of *An. culicifacies* (97.76%) among mosquitoes tested, its mortality was recorded and all the other species were ignored.

RESULTS

The results of the studies on the efficacy of different insecticides against fresh fed and wildcaught females of An.culicifacies are summarized in Table 1 and presented in Figure 3. Adulticidal efficacy of the insecticides were in the following order, lambdacyhalothrin, malathion, bendiocarb, DDT, permethrin, propoxur and dieldrin. Table 1 shows that susceptibility to 0.4% dieldrin has decreased. Exposure of adults for 1 hour to 0.4% dieldrin only yielded 64.5% ± 3.13% mortality.

DISCUSSION

These results contrast with results of earlier studies of Manouchehri and coworkers(5) who showed that the An. culicifacies population is susceptible to dieldrin in Iranshahr, Sistan and Baluchistan province. Oppenoorth and Welling (6) stated that it is not possible to predict whether resistance will develop in any particular situation, since this will depend on the insecticide selection pressure applied as well as unknown characteristics of the insect populations.

Effective management of resistance is dependent on understanding the relevant biology and ecology of An. culicifacies. Movement and dispersal of a vector can have a significant impact on the evolution of resistance to insecticides, because immigration of susceptible individuals can counteract the evolutionary effects of insecticidal selection. Regular monitoring of the frequency of resistance to pyrethroids in field conditions may allow selection of appropriate control startegies. Reversion to susceptibility in a strain of An. stephensi from United Arab Emirates was mentioned (Vatandoost and Townson, in preparation). In this respect Farnhman (7) proposed that as *kdr*, which conferes cross-resistance to DDT and pyrethroids in the house fly, confers a recessive trail, natural dilution of the population on removal of selection pressure would increase the number of heterozygotes which are susceptible to DDT or pyrethroids. Curtis and coworkers(8) pointed out that populations of insects containing a high frequency of alleles for cyclodiene resistance often revert towards susceptibility when the cyclodiene is withdrawal from use and re-exposure to pyrethroids will result in rapid increase in resistance. Migration of insects from populations untreated with a given insecticide would tend to delay the evolution of resistance in a neighbouring treated population. It has therefore been proposed that an insecticide should not be applied uniformly but in alternate sectors of a "grid"(8). It is sometimes erroneously stated or implied that reversions could result merely from the recessiveness of resistance genes. In fact, however reversion could result only from selection against the resistance gene because of associated reduced survival or fecundity (i.e. reduced fitness), or from immigration from more susceptible populations or a combination of the two(8). A stepwise pattern of reduction of

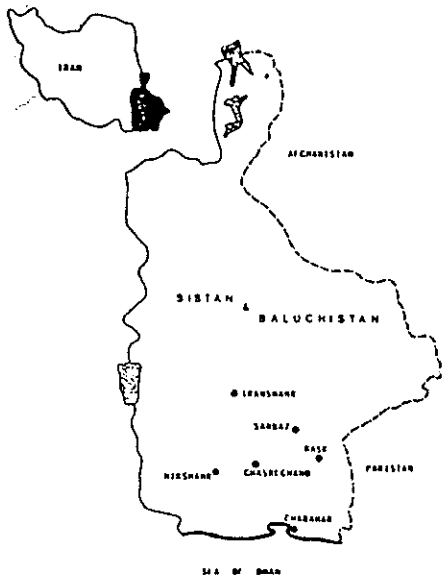

Fig. 1. Map of the study area in the province of Sistan and Baluchistan, Iran, 1997 (after Zaim and Javaherian, 1991)(4)

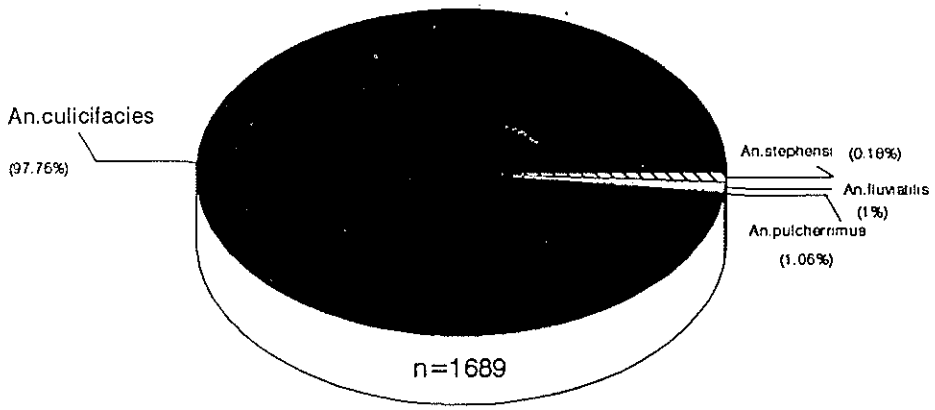

Fig. 2. Percentage of mosquito species collected for bioassay tests from Ghasreghand district, Sistan and Baluchistan province. May-June 1997. Iran

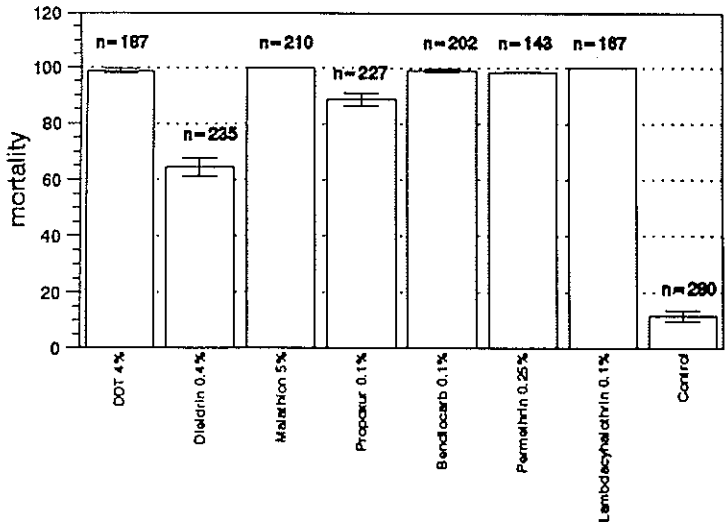

Fig. 3. Mortality of *An.culicifacies* exposed to a diagnostic dose of insecticides in Ghasreghand district, Sistan and Baluchistan province, Iran, May-June, 1997

pyrethroid resistance which is controlled by recessive genes was reported in a German cockroach population(9,10). M.domestica(11) and H.irritans (Kunz). Instability of pyrethroid-resistance after 13 generations of withdrawal of pyrethroid application has been reported in field populations of the German cockroach(10). Mofidi quoted by Curtis and coworkers (8) reported that DDT-resistant populations of An. Stephensi from Iran showed slow reversion towards susceptibility over a period of up to 6 years (approximately 72-generations), when DDT spraying had been withdrawal. Reversion of permethrin-resistance has been reported in Pectinophora gossypiella from Arizona (12). If reversion goes far enough it may be possible to effectively re-introduce the insecticide until re-selection of resistance cause control problems once again, a type of resistance management known as insecticide rotation(13).

Since the beginning of the malaria eradication programme in Iran, this area had been under house spraying with DDT for ten years, dieldrin for three years and malathion for 5 years. In 1964 and 1965, Baluchistan was sprayed with dieldrin, and from 1967 to 1973 with DDT and malathion, two rounds of each insecticide per year(14). Results of susceptibility tests conducted by Roy and coworkers(15) in 1973-78 in Karnataka, India, showed widespread resistance to both DDT and dieldrin. DDT resistance in An. culicifacies was reported by Das and Rajagopalan(16) from a colonized strain(16). DDT and dieldrin resistance on *An. culicifacies* has been reported from Afghanistan, Pakistan and Oman. DDT resistance has also been reported from Iran(17).

Manouchehri and coworkers(5) carried out susceptibility test on *An. culicifacies* in 1973-1974, in Iranshahr(5). They showed that this species was resistant to DDT. In the present study, An. culicifacies from Ghasreghand district exhibits 98.75 ± 0.8% mortality when they exposed at 4% DDT for 1 hour at diagnostic dose.i.e., this species is susceptible to DDT. Among the different classes of insecticide tested, the only cyclodiene insecticide, dieldrin, had leas effect. Although Das and coworkers stated that DDT and dieldrin resistance in *An. culicifacies* did not confer cross-resistance to deltamethrin, but this study can not prove that dieldrin resistance is due to cross-resistance between pyrethroids (permethrin and lambdacyhalothrin) or true physiological resistance(18). Recently Bloomquist(19) stated the mode of action of pyrethroids and new insecticides with different modes of action may confer cross-resistance due to common resistance mechanism. Malathion resistance has been reported from Pakistan in *An.culicifacies*(17).

In 1974 Manouchehri and coworkers(20) reported that a field population of An.stephensi in Iran was resistant to DDT. and malathion resistance in the population from Bandar Abbas, Iran. Manouchehri and coworkers(5,21) reported that *An.culicifacies* is susceptible to malathion in Sistan and Baluchistan province. Although malathion and pirimiphos-methyl (Actellic) had been used for malaria control in Ghasreghand district from 1992-1995 at the rate of 2 g/m^2, there is no evidence of malathion resistance in this population (mortality 100%).

Rajagopal(22) first reported malathion resitance in the population of *An.culicifacies* in Gujarat, India. The nature of malathion resistance in a population of *An.culicifacies* from Maharashtra, India has been investigated by Herath and Davidson(23). They suggested the presence of at least two mechanisms; one involving specific carboxylesterase and the other the least specific mixed function oxidase system. Raghavendra and coworkers(24) conducted a study in Andhra Paradesh and Gujarat states of India on *An. culicifacies*. The results revealed that these populations were resistant to malathion and this resistance was attributed to the extensive use of agricultural pesticides. Subsequently in Haryyana India, upon the diagnostic dose of malathion (5% for 1 hour), 41-47% of the mosquitoes survived. *An. culicifacies* populations in this area comprise two sibling species, A. and B, malathion resistance patterns; species A and 62-66% in species B, Likewise, species A was found to be more susceptible to DDT than species B(25). In another report, malathion resistance was reported from Oman and United Arab Emirates in *An.culicifacies*(17).

Table 1. Mortality of *Anopheles culicifacies* to a diagnostic dose of insecticides in Ghasreghand, Sistan and Baluchistan province, Iran, May-June, 1997

Pesticide	No. tested	No. Dead	Mr %	corrected Mr	Error bar
Dieldrin 0.4%	235	161	68.50	64.50	±3.13
DDT 4%	187	185	98.90	98.75	±0.80
Malathion 5%	210	210	100.0	100.0	± 0.00
Bendiocarb 0.1%	202	200	99.00	98.86	± 0.70
Propoxur 0.1%	227	204	89.87	88.50	± 2.24
Permethin 0.25%	143	141	98.60	98.40	± 0.10
Lambdacyhalothrin 0.1%	167	167	100.00	100.0	± 0.00
Control	280	33	11.80	11.80	± 1.93

Each replicate comprise 12-31 individual female mosquitoes

Propuxur are being. used at the rate of 2 g/m^2, as an indoor residual spraying two rounds per year from 1991-1997 in our study area. In 1996 lamdacyhalothrin (ICON 10% wp) was used with propoxur in this district. In the case of propoxur, females of *An.culicifacies* shows only 88.5±2.24% mortality. There is no indication of cross-resistance between bendiocarb as a carbamate insecticide and propoxur, when bendiocarb yielded 98.86±0.7% mortality against this species. Propoxur resistance has been reported from Oman and United Arab Emirates(17).

Pyrethoid insecticides such as permethrin and lambdacyhalothrin were found to be highly effective against this species. These insecticides caused 98.4 ± 1 and 100% mortality, respectively.

The length of time a field population has been exposed to insecticide selection pressure can influence the stability of resistance. Other factors that can influence stabilty include the size and geographic area occupied by the resistant population, the presence of unexposed segments of the population, and opportunities for movement within the geographical area(10).

Pyrethroids such as deltamethrin, cyfluthrin with lower dermal toxicity and irritant effect can serve as an alternative measure. Newly emerged insecticides with different modes of action such as ethofenprox could be further investigated at large scale in areas where resistance to other insecticides hamper control programmes.

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