Research

Insecticide resistance in *Anopheles sacharovi* Favre in southern Turkey

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We report the resistance to 12 insecticides of specimens of *Anopheles sacharovi*, both in laboratory cultures and those collected in the malarious areas of Adana, Adiyaman, Antalya, Aydin, and Muğla in southern Turkey. Mortality was higher 24 h after exposure than immediately after exposure but was unaffected by temperature (24 °C or 29 °C) or the position of the test kit (horizontal or vertical). In Adana, Adiyaman and Antalya, *A. sacharovi* was susceptible only to malathion and pirimiphos-methyl. In Aydin it was susceptible to both these insecticides as well as to dieldrin, λ-cyhalothrin, and etofenprox; and in Muğla it was susceptible to dieldrin, fenitrothion, λ-cyhalothrin, cyfluthrin and etofenprox, as well as to malathion and pirimiphos-methyl.

Keywords: Turkey; Anopheles; insecticides; drug resistance; comparative study.

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Introduction

Malaria is endemic in southern Anatolia. The number of cases has increased in recent years, particularly in south-east Anatolia where the GAP (Southeast Anatolia Project) irrigation and development project is about to be completed. Under the project, irrigation began three years ago, and the area covered by it is increasing annually.

The principal vector of the malaria parasite in Turkey is *Anopheles sacharovi* Favre; *A. superpictus* Grassi is also a vector (*1*, *2*). The population densities of both species are highest in malarious areas but even here the distribution of *A. superpictus* is rather localized. This species occurs in small populations with lower densities in midsummer and is still susceptible to many insecticides.

In Turkey, *A. sacharovi* has been subjected to continuous field selection pressure by many insecticides that have been used for both agricultural and public health purposes since the introduction of DDT (clofenotane) in 1952. Residuals were introduced throughout the country for malaria control in the following sequence: DDT, γ-hexanechlorocyclohexane (γ-HCH), dieldrin, malathion, pirimiphos-methyl, bendiocarb. DDT and γ-HCH were used during the 1950s and 1960s but resistance developed to them in *A. sacharovi* in 1959 (*3*). Dieldrin resistance was detected in *A. sacharovi* in 1970, and malathion (an organophosphorus insecticide) then replaced the organochlorines (*4–6*). Resistance to malathion was first recorded in 1974 (*5*), and cross-resistance developed to a wide range of organophosphorus and carbamate insecticides, even though they had never been used for house spraying (*7, 8*). The use of malathion was discontinued in 1984 because *A. sacharovi* developed resistance to malathion and because householders objected to its unpleasant odour and the stains it left on walls (*9*). Pirimiphos-methyl replaced malathion in 1984 and was used successfully until 1990 when it was replaced by bendiocarb. *A. sacharovi* was still susceptible to pirimiphos-methyl in 1992 (*10*) and was still so even in 1996 (*11*). In recent years, pirimiphos-methyl, bendiocarb and various residual pyrethroids have been used by the malaria control authorities.

Hemingway et al. (*12*) reported that DDT resistance in *A. sacharovi* was being scattered in the population in 1984 despite the replacement of DDT by malathion for malaria control 13 years previously. They also reported that populations of this species in Çukurova had an altered acetylcholinesterase resistance mechanism, conferring broad-spectrum resistance against organophosphates and carbamates. Specimens of *A. sacharovi* collected in the field in 1989–90 were still resistant to DDT, organophosphates and carbamates, although at lower frequencies than in 1984 (*11, 13*).

The present study compares baseline data on susceptibility under WHO test conditions with results obtained on field samples, in order to evaluate current trends in insecticide resistance in southern Turkey.

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Materials and methods

Study sites

The study was carried out in five malarial areas in southern Turkey, which varied in insecticide usage, geographical features, social infrastructure, agricultural development and tourism characteristics — factors that might have had a bearing on the emergence of insecticide resistance.

- **Adana** is situated in the centre of the Çukurova Plain on the Mediterranean coast, where malaria has long been endemic. Samples of *A. sacharovi* were collected in Karatas-Tuzla, which had a high population density of mosquitos.
- **Antalya** is located 560 km west of Adana. Orchards and irrigated cotton crops are common in this area. Few cases of malaria occur and there is intensive vector control, conducted by private bodies and the National Malaria Service. Most of the specimens of *A. sacharovi* were obtained in the village of Küçük Belkis, which was not sprayed before collecting was carried out.
- **Muğla** is situated 300 km west of Antalya, with which it shares certain characteristics. However, there are more forests in Muğla. Specimens of *A. sacharovi* were obtained in Gökova, Yatağan and Milas.
- **Aydin**, which has relatively few malaria cases, contains much irrigated low country used mainly for cotton and rice crops. Samples were taken in Söke and the surrounding villages of Aydin, where the population density of mosquitos was high. The flow of water was not controlled and consequently many patches of stagnant water provided suitable habitats for mosquito breeding, especially in Söke.
- The GAP Project area covers six provinces including **Adiyaman**, which is about 370 km east of Adana. The irrigation area will be ten times greater than that of Çukurova when the scheme has been completed. At present, only the Harran and Bozova plains, approximately equal in area to Çukurova, are irrigated and have been for three years. Specimens were collected in coastal villages on Lake Gölbaşi.

Study design and methods

The field collection of *A. sacharovi* and susceptibility tests were conducted between late May and September 1998 in Adana and in June and July 1998 in the other provinces, when the populations of the mosquito reached high levels.

Insecticide-impregnated papers prepared in accordance with WHO specifications were purchased from the WHO Collaborating Centre in Malaysia (Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, Minden, Penang). Pirimiphos-methyl papers were freshly prepared by spreading 2 ml of insecticide solution in acetone on 15 cm x 12 cm rectangles of Whatman No.1 filter-paper. Twelve insecticides approved by WHO for vector control were used:

– 4% DDT, 0.4% dieldrin (organochlorines);
– 5% malathion, 1% fenitrothion, 1% pirimiphos-methyl (organophosphates);
– 0.1% bendiocarb, 0.1% propoxur (carbamates);
– 0.025% deltamethrin (former WHO discriminating dose), 0.25% permethrin (former WHO discriminating dose), 0.1% λ-cyhalothrin, 0.05% and 0.1% cyfluthrin, 1% and 2% etofenprox (pyrethroids).

Tests were conducted on female *A. sacharovi* of various ages taken from a laboratory culture of the Adana strain maintained at the Medical Biology Department, Çukurova University, and on field samples in accordance with standard WHO procedures (*14*) but with some modifications. Two WHO test kit positions (vertical and horizontal), two exposure times (1 h and 2 h) and two mean temperatures (24 °C and 29 °C) were used. The temperature and relative humidity in the holding tubes were recorded during exposure and 24 h later. Mortality was noted immediately after exposure (as percentage knockdown) and 24 h later as percentage dead. During the 24-h holding period the test kits were kept in a cardboard box covered with wet towels.

SPSS for MS Windows software was used to calculate *t*-tests.

Results

The mortality rates 24 h after exposure of culture specimens are shown in Table 1. The mortality rates immediately and 24 h after exposure of field specimens are given in Table 2 and Table 3, respectively, for the vertical and horizontal test kit positions.

The tests on both the culture specimens and the field samples showed that the mortality rates 24 h after exposure were higher than those immediately after exposure for most insecticides (P = 0.000). Mortality rates at 29 °C were not significantly higher than those at 24 °C (P = 0.250, Table 1). Mortality after 2 hours of exposure was significantly higher than that after exposure for 1 hour (P = 0.000). Mortality rates for some insecticides were slightly higher for the horizontal test kit position than for the vertical position, but were lower for some others; the differences, however, were not statistically significant (P = 0.858).

On the basis of the initial results the two combinations shown below were investigated:

– vertical test kit position and exposure for 1 h;
– horizontal test kit position and exposure for 2 h.

If 100% mortality occurred with the first combination the second was not used.

By applying the WHO criteria (98–100% mortality indicates susceptibility, <80% mortality suggests resistance and 80–97% mortality requires confirmation of resistance) (*15*), it was found that laboratory culture samples were susceptible to malathion, pirimiphos-methyl and etofenprox; possibly resistant to dieldrin, deltamethrin and λ-cyhalothrin; and resistant to the other insecticides (Table 1).

Field samples from Adana were susceptible only to malathion and pirimiphos-methyl (Table 2 and Table 3). In Adiyaman, *A. sacharovi* was susceptible to malathion, pirimiphos-methyl, λ-cyhalothrin and etofenprox, possibly resistant to propoxur and cyfluthrin, and resistant to the other insecticides; in Antalya it was susceptible to malathion, pirimiphos-methyl and 2% etofenprox (horizontal test kit position and 2-h exposure), and resistant to the other insecticides; in Aydin the species was susceptible to dieldrin, malathion, pirimiphos-methyl, fenitrothion, λ-cyhalothrin and etofenprox, possibly resistant to permethrin and cyfluthrin, and resistant to the other insecticides; in Muğla it was resistant to DDT and bendiocarb, possibly resistant to propoxur, deltamethrin and permethrin, and susceptible to the other insecticides.

Discussion

A. sacharovi demonstrated multiple resistance to all four groups of insecticides. The laboratory culture, established almost 20 years previously, still exhibited resistance to many insecticides, presumably because field specimens had been introduced from time to time during the first 10 years.

Higher mortality occurred at the longer exposure time in all groups but neither test kit position nor temperature had a significant effect. The apparent link between test kit position and knock-down efficacy was possibly attributable to the use of susceptible adults. The increase in mortality associated with the horizontal test kit position was more pronounced in the relatively susceptible Aydin and Muğla populations of *A. sacharovi* than in those of Adiyaman, Adana and Antalya, which had genes for resistance to many insecticides. There is a positive correlation between mortality and temperature for organochlorines and organophosphates (*7, 16*). It has been shown that for organophosphates mortality increases with time of exposure, whereas for carbamates the insecticide concentration is more important (*16*). In Çukurova the mortality rates for *Culex tritaeniorhynchus* and *Aedes caspius* were positively correlated with temperature and time of exposure to the four groups of insecticides (*17*).

In Çukurova, carbamates and pyrethroid insecticides are used in about equal proportions in agriculture but mostly aerosol formulations of pyrethroids are used for mosquito control by private bodies and municipalities (*17*). Insecticide usage in this area has, for many years, selected for resistance

Table 1. **Twenty-four-hour post-exposure percentage mortality of *A. sacharovi* laboratory culture samples for insecticides tested at 65–75% relative humidity[a]**

Insecticide	% Mortality at exposure temperature							
	24 °C Exposure tube position				29 °C Exposure tube position			
	Vertical Exposure time		Horizontal Exposure time		Vertical Exposure time		Horizontal Exposure time	
	1h	2h	1h	2h	1h	2h	1h	2h
DDT 4%	10	40	0	40	25	30	7	30
Dieldrin 0.4%	80	95	98	88	45	90	90	100
Malathion 5%	100	100	100	100	100	100	100	100
Primiphos-methyl 1%	100	100	100	100	100	100	100	100
Fenitrothion 1%	15	41	0	32	0	30	30	18
Bendiocarb 0.1%	5	24	0	18	15	58	0	31
Propoxur 0.1%	73	82	45	86	40	63	53	85
Deltamethrin 0.025%	80	82	88	92	75	83	93	93
Permethrin 0.25%	43	51	23	66	45	67	63	84
λ-cyhalothrin 0.1%	79	94	90	90	75	83	100	95
Cyfluthrin 0.5%	16	32	33	37	40	50	40	77
Cyfluthrin 1%	75	81	63	78	60	77	80	98
Etofenprox 1%	90	95	90	100	85	97	90	100
Etofenprox 2%	100	100	100	100	100	100	97	97
Control[b]								
OC	0	0	5	5	0	0	5	0
OP	0	0	0	0	0	0	0	5
PM	0	0	0	0	0	0	5	0
PY	0	10	0	10	0	10	5	10

[a] Mortality data are means for 3–5 replicates, each comprising 10–20 mosquitos.

[b] OC = organochlorine; OP = organophosphate; PM = primiphos-methyl; PY = pyrethroid.

Table 2. **Results of susceptibility tests (vertical position) on field samples of *A. sacharovi* collected in five areas of southern Turkey**[a]

Insecticide	Locality									
	Adana		Adiyaman		Antalya		Aydin		Muğla	
	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure
DDT 4%	0	0	6	6	3	4	48	67	62	77
Dieldrin 0.4%	0	25	0	55	0	64	45	98	28	100
Malathion 5%	100	100	93	100	95	98	97	98	72	100
Fenitrothion 1%	0	6	4	39	18	55	28	91	8	100
Pirimiphos-methyl 1%	100	100	100	100	100	100	100	100	100	100
Bendiocarb 0.1%	9	4	56	42	53	47	35	32	45	58
Propoxur 0.1%	64	43	93	77	77	71	82	82	70	93
Deltamethrin 0.025%	7	13	82	77	48	51	100	64	96	93
Permethrin 0.25%	3	7	59	56	18	30	76	63	82	85
λ-Cyalothrin 0.1%	23	32	83	94	64	65	100	98	100	100
Cyfluthrin 0.05%	2	19	77	81	41	41	92	90	92	88
Cyfluthrin 0.1%	0	14	88	74	59	64	100	90	100	100
Etofenprox 1%	46	36	85	91	32	42	97	91	100	100
Etofenprox 2%	31	57	100	100	70	77	100	100	100	100
Control[b]										
OC	0	0	0	0	0	10	0	0	0	0
OP	0	2	0	0	0	0	0	2	0	0
PM	0	0	0	8	0	0	0	0	0	0
PY	0	2	0	10	0	0	0	0	0	0

[a] Tests were conducted at 65–75% relative humidity and 28–29 °C with the test tube in the vertical position. The exposure period was 1 h. The mortality rates are means of 4 replicates for insecticides and of 2–3 replicates for controls, each replicate containing 10–20 mosquitos.

[b] OC = Organochlorine; OP = organophosphate; PM = pyrimiphos-methyl; PY = pyrethroid.

(*3, 5, 7, 8, 11–13*). The *kdr*-type gene responsible for pyrethroid resistance was recently detected in specimens of *A. sacharovi* from Adana (*18*). *A. sacharovi* was resistant to malathion in 1984 but is now fully susceptible.

In the Gölbaşi area of Adiyaman the wide range of resistance was attributable to many years of insecticide usage. Although mosquito control efforts with insecticides have not received much emphasis in Gölbaşi recently, since it is a nature reserve, aerosol pyrethroid formulations are being applied by fogging. Intensive insecticide usage against agricultural pests and mosquitos has selected resistance in *A. sacharovi* to a number of compounds, as in Adana and Antalya .

In the Aydin and Muğla areas, mosquitos are susceptible to most of the insecticides but caution is needed in order to avoid development of cross-resistance.

The WHO insecticide discriminating concentrations used in this study were reviewed in 1998 (*15*). The new discriminating values are 0.75% (w/w) permethrin, 0.05% deltamethrin, 0.1% λ-cyhalothrin (for *A. sacharovi* only), 0.5% etofenprox and 0.15% cyfluthrin. The use of these new concentrations may have influenced our results for some insecticides.

A total of 32 agricultural pesticides (1 organochlorine, 15 organophosphates 7 carbamates and 9 pyrethroids) were used in Çukurova between 1992 and 1995 (*17*). They included potential mosquito control insecticides, i.e. chlorpyriphos, fenithrothion, malathion, λ-cyhalothrin, permethrin, deltamethrin, cypermethrin and cyfluthrin. In recent years, municipalities and many other official and private bodies have switched between insecticides, notably pyrethroids as cold aerosols and fogs (*17*). Such intensive use of insecticides for long periods selects resistance in mosquito populations.

The selection of resistance in *A. sacharovi* in Çukurova as a consequence of agricultural pesticide usage has been discussed in relation to 11 agricultural organophosphates and carbamates (*8*) and of 1% propoxur, 0.25% permethrin and 0.025% deltamethrin (*11*), although these insecticides had been used hardly at all for mosquito control. Mouchet (*19*), reviewing resistance to insecticides that had never been used for public health purposes, indicated that resistance in *A. gambiae* in Africa, *A. albimanus* in Central America, *A. culicifacies* and *A. aconitus* in South-east Asia, *A. sacharovi* in Turkey and Greece, and *C. tritaeniorhynchus* in the Western Pacific and South-East Asia Regions was associated with agricultural applications. Such associations were also indicated for *A. nigerrimus* in Sri Lanka (*20*) and *A. albimanus* in Guatemela (*21*) and Mexico (*22*). Clearly, vectors are among the non-target organisms affected by agricultural practices. Initially, the destruction of vector populations is a beneficial side-effect but agricultural

Table 3. **Results of susceptibility tests (horizontal position) on field samples of *A. sacharovi* collected in five areas of southern Turkey**[a]

Insecticide	Locality									
	Adana		Adiyaman		Antalya		Aydin		Muğla	
	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure	Straight after exposure	24 h after exposure
DDT 4%	1	8	22	29	36	33	76	85	100	100
Dieldrin 0.4%	1	55	80	100	22	58	90	92	73	100
Malathion 5%	100	100								
Fenitrothion 1%	7	20	50	85	75	82	100	100	62	90
Pirimiphos-methyl 1%	100	100								
Bendiocarb 0.1%	28	18	80	80	85	72	83	78	88	90
Propoxur 0.1%	57	32	95	100	93	90	97	100	95	98
Deltamethrin 0.025%	31	44	81	93	76	83	97	93	97	100
Permethrin 0.25%	27	35	80	91	46	55	97	97	100	100
λ-cyalothrin 0.1%	46	57	100	100	89	86	100	100	98	100
Cyfluthrin 0.05%	15	35	80	95	32	60	100	100	100	100
Cyfluthrin 0.1%	9	26	75	90	90	87	91	91	98	100
Etofenprox 1%	74	77	100	100	56	78	100	100	100	100
Etofenprox 2%	79	83			95	100	100	100	98	100
Control[b]										
OC	0	0	0	2	10	10	0	0	0	4
OP	0	2	0	2	0	3	0	6	0	0
PM	0	0	0	8	19	7	0	0	0	7
PY	0	2	0	10	0	0	0	8	0	0

[a] Tests were conducted at 65–75% relative humidity and 28–29 °C with the test tube in the horizontal position. The exposure period was 2 h. The mortality rates are means of 4 replicates for insecticides and of 2–3 replicates for controls, each replicate containing 10–20 mosquitos.

[b] OC = Organochlorine; OP = organophosphate; PM = pirimiphos-methyl; PY= pyrethroid.

treatments eventually produce severe selective pressure (*19*). The marked reductions in the numbers of malaria cases and of mosquitos that have occurred in recent years in Adana and Antalya, where the disease is endemic, may reflect the beneficial stage. The influence of the interaction between agricultural and public health insecticide usage on the selection of resistance is still poorly understood, partly, perhaps, because of the lack of information on insecticides in the environment.

Close cooperation is necessary between health, agriculture and ecosystem analysts in order to understand the influence of insecticide usage. The problem of multiple resistance demands specific and periodic monitoring in each locality. The first step is to assess trends in the frequency of the resistance genes by means of susceptibility tests, and to investigate the efficacy of insecticides in the field with bioassays. The most effective insecticides, possibly giving 100% kill, should then be used in rotation. ■

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Résumé

Résistance d'*Anopheles sacharovi* Favre aux insecticides (sud de la Turquie)

Nous étudions ici la résistance à 12 insecticides de spécimens d'*Anopheles sacharovi* provenant d'élevages de laboratoire ou capturés dans les régions impaludées d'Adana, d'Adiyaman, d'Antalya, d'Aydin et de Muğla (sud de la Turquie). On observe une mortalité plus élevée au bout de 24 heures qu'immédiatement après l'exposition, mais elle n'est pas modifiée par la température (24 °C ou 29 °C) ni par la position du nécessaire d'épreuve (horizontale ou verticale). A Adana, Adiyaman et Antalya, *A. sacharovi* n'a été sensible qu'au malathion et au pirimiphos-méthyl. A Aydin, il a été sensible à ces deux insecticides ainsi qu'à la dieldrine, à la lambda-cyhalothrine et à l'étofenprox ; enfin, à Muğla, il a été sensible à la dieldrine, au fénitrothion, à la lambda-cyhalothrine, à la cyfluthrine et à l'étofenprox, ainsi qu'au malathion et au pirimiphos-méthyl.

Resumen

Resistencia de *Anopheles sacharovi* Favre a los insecticidas en el sur de Turquía

Informamos de la resistencia desarrollada contra 12 insecticidas por *Anopheles sacharovi*, tanto en cultivos de laboratorio como entre los ejemplares capturados en las zonas palúdicas de Adana, Adiyaman, Antalya, Aydin y Muğla, en el sur de Turquía. La mortalidad fue mayor al cabo de 24 horas de la exposición que inmediatamente después de la misma, pero no se vio afectada ni por la temperatura (24° C o 29° C) ni por la posición del kit de prueba (horizontal o vertical). En Adana, Adiyaman y Antalya, *A. Sacharovi* sólo fue sensible al malatión y al pirimifós-metilo. En Aydin fue sensible a esos dos insecticidas, así como a la dieldrina, la lambda-cihalotrina, la ciflutrina y el etofenprox; y en Muğla fue sensible a la dieldrina, el fenitrotión, la lambda-cihalotrina, la ciflutrina y el etofenprox, así como al malatión y el pirimifós-metilo.

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