STUDIES ON INSECTICIDE SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* s.l. AND *CULEX QUINQUEFASCIATUS* IN THE AREA OF OUAGADOUGOU, BURKINA FASO (WEST AFRICA)

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ABSTRACT. Tests to evaluate the susceptibility level in *Anopheles gambiae* s.l. and *Culex quinquefasciatus* from Ouagadougou and two nearby villages have been carried out. *Anopheles gambiae* s.l. larvae from Ouagadougou showed complete susceptibility to organophosphates and carbamates, and adults showed low level resistance to DDT. Nine percent survival of adult *An. gambiae* s.l. to one hour exposure of 4% DDT was observed in samples from Zaghtouli village while in those from Koubri village, where dieldrin also was tested, resistance to both organochlorine insecticides was detected. *Culex quinquefasciatus* showed susceptibility to fenthion, malathion, fenitrothion, and chlorpyrifos, whereas incipient resistance to temephos was detected. Electrophoretic analysis showed polymorphism of high and low active esterases (Est-2 + Est-3) confirming the association between OP resistance and enhanced esterase activity in this species.

INTRODUCTION

Within the framework of the Italian Government's cooperative program with Developing Countries, a project of malaria and vector control has been established in Ouagadougou, Burkina Faso (formerly Upper Volta).

Ouagadougou is located in a typical sudan savanna region where malaria transmission occurs at high levels during the rainy season. A number of basins in the urban and neighboring rural areas supply water for agricultural and domestic purposes. The edges of those basins, together with various natural and manmade pools, provide larval breeding places particularly for *Anopheles gambiae* s.l. Entomological studies carried out to assess the malaria vectorial system in this area have shown that *An. gambiae* s.l. is the most common vector in the town and in the surrounding rural areas. Cytotaxonomic identifications showed the presence of two members of the *An. gambiae* complex, *An. gambiae* Giles and *An. arabiensis* Patton, whose seasonal density and distribution have been recently described (Petrarca et al. 1984, Rossi et al. 1984). The other mosquito species of relevant health importance is *Culex quinquefasciatus* Say. This species is widespread in the urban area where it is particularly important as a nuisance.

Recent investigations on insecticide susceptibility of *An. gambiae* s.l. are not available and the history of insecticide treatment for control of vector-borne diseases and crop protection is not fully documented for any part of the country. Among the few records available, are those from a malaria vector control project conducted by residual house spraying in the Bobo Dioulasso region from 1952 to 1961 (Brown and Pal 1973). Spraying operations with DDT at 2.2 g a.i./m^2 and dieldrin at a dosage of 0.5 g a.i./m^2 achieved fairly good results. However, in 1957, *An. gambiae* s.l. resistant to dieldrin was found in the village of Pala (Hamon et al. 1957) while DDT resistance was observed later by Hamon (in Brown and Pal 1973). No information is available on the frequency of spraying nor on the dosages in other parts of Burkina Faso and in the town of Ouagadougou where DDT, gamma-HCH, and diesel oil as larvicide have been used during a period of 20 years from 1950 to 1970. Among organophosphorus (OP) insecticides, only malathion, and recently temephos have been used by local health authorities in some vector control activities. Negligible results have been achieved and no control measures have been taken with any regularity since 1975.

Studies on susceptibility of *Culex quinquefasciatus* to organochlorine (OC) insecticides showed DDT resistance in Bobo Dioulasso (Hamon et al. 1958). In Ouagadougou, complete susceptibility to OP and OC compounds was found by Mouchet et al. (1968).

In view of the relevance for planning malaria and vector control measures in the present Ouagadougou project, the susceptibility status of *An. gambiae* s.l. and *Cx. quinquefasciatus* has been studied during 1983/84 and the results are reported herein.

MATERIALS AND METHODS

Insecticide susceptibility methods utilized were those recommended by the World Health Organization (1981a, 1981b). Field collected larvae of *An. gambiae* s.l. and *Cx. quinquefasciatus* from Ouagadougou urban area were tested. A laboratory susceptible strain of *Cx. pipiens* Linn. from Italy, maintained in Istituto Superiore di

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Sanità since 1982, was tested as a reference strain.

Larval test solutions and impregnated papers were supplied by WHO (except pyrimiphos-methyl). Twenty larvae of third to early fourth instar were placed in 300-ml glass beakers containing 250 ml of tap water (pH 7.4±0.2). For treatments, serial dilutions of each insecticide were made to obtain the appropriate range of concentrations for testing. Each time, 5–6 concentrations of an insecticide were applied to each of four beakers (replicates) receiving a concentration while four beakers were left untreated as controls. The complete test was repeated four times for each OP insecticide on different occasions. The control mortality observed was usually below 5%. When its value was between 5% and 20% the percentage mortalities of the samples tested were corrected by Abbott's formula (Abbott 1925). The mortality values, at least 4 between 0% and 100%, obtained after 24 hr exposure to a range of insecticide concentrations were analyzed by a log-probit regression program on a Tetronik computer model 4051.

Recent blood-fed females of *An. gambiae* s.l. were used for adult susceptibility tests. The mosquitoes were collected on different occasions from indoor resting places in the town as well as in two villages, Zaghtouli and Koubri, 19 and 27 km from Ouagadougou, respectively. The specimens were tested immediately after collection, having been kept during the transport to the laboratory in paper cups. Sufficient relative humidity was assured by placing small pieces of cotton wool impregnated with water on the cups. Further tests with DDT were carried out on laboratory strains of *An. arabiensis* and *An. gambiae* from Koubri using 3–5 day-old unfed females from the F_2 generation. For adult susceptibility tests, batches of 15–20 females were exposed for one hour to paper impregnated with 1% fenitrothion, 5% malathion, 0.02% pirimiphos-methyl (prepared following WHO instructions), 0.1% bendiocarb, 0.1% propoxur, 4% DDT or 0.4% dieldrin. The exposure was extended to 2 hr when survival was observed after 1 hr. The adult mortality in a test was adjusted against any mortality (included between 5 and 20%) in the controls (Abbott 1925). Both adult and larval susceptibility tests were carried out on various occasions in January–February, during the dry season (23–27°C, 20–25% RH) and in August–September, during the rainy season (24–32°C, 70–85% RH). The laboratory relative humidity in the dry season during the adult tests ranged from 40 to 55%. Data for each insecticide were pooled since they were found statistically homogeneous despite seasonal variability in environmental conditions.

Esterase patterns of *Cx. quinquefasciatus* adults have been examined by means of horizontal starch gel electrophoresis. Poulik's buffer system plus 2mM EDTA was employed for gels and electrodes. After 5 hr at about 8 V/cm, the gels were sliced and incubated with 25 ml of 0.1 M Tris-maleate buffer (pH 6.4) plus 0.5% + naphthylacetate in acetone, then stained with 50 mg Fast Blue RR salt dissolved in 25 ml of 0.1 M Tris-maleate buffer (Villani et al. 1983).

RESULTS AND DISCUSSION

Anopheles gambiae s.l. Larvae of *An. gambiae* s.l. from the urban area of Ouagadougou were fully susceptible to the OP compounds tested (Table 1). Complete susceptibility to the OP and carbamate insecticides was also observed in the adult samples tested (Table 2).

Results of the OC tests are reported in Table 3. Adults exposed for 1 hr to 4% DDT showed 11.5% survival in the samples from the town of Ouagadougou and 9% in those from the village of Zaghtouli. Remarkable resistance to DDT and dieldrin was observed in the samples from the village of Koubri: 2 hr exposure to 4% DDT caused 64% mortality, and 2 hr exposure to 0.4% dieldrin gave 20% mortality. The observed resistance to DDT might be explained by selection pressure due to the agricultural usage of this insecticide especially in the Koubri area where rice had been and still is

Table 1. Susceptibility to organophosphorus insecticides of field-collected larvae of *Anopheles gambiae* s.l. from Ouagadougou, Burkina Faso.

Insecticide	24-hr lethal concentration (mg/liter)				
	LC_{50}	95% conf. limits	LC_{50}	95% conf. limits	slope
Chlorpyrifos	0.0014	0.0012–0.0017	0.0069	0.0050–0.0097	3.5
Fenitrothion	0.0159	0.0145–0.0174	0.0310	0.0267–0.0359	2.4
Fenthion	0.0059	0.0054–0.0064	0.0108	0.0094–0.0124	1.5
Malathion	0.0518	0.0443–0.0619	0.1024	0.0860–0.1238	1.7
Temephos	0.0038	0.0031–0.0047	0.0214	0.0134–0.0344	3.5

Table 2. Susceptibility to various insecticides of field-collected adult *Anopheles gambiae* s.l. from urban areas of Ouagadougou, Burkina Faso (Figures in parentheses are numbers of mosquitoes tested).

Insecticide	Concentration (%)	Exposure time (hr)	Mortality rate (%)
Fenitrothion	1.0	1	(204) 100
Malathion	5.0	1	(282) 100
Pirimiphos-methyl	0.02	1	(296) 100
Bendiocarb	0.1	1	(262) 100
Propoxur	0.1	1	(285) 100

Table 4. Organochlorine susceptibility of laboratory-colonized *Anopheles gambiae* s.l. from Koubri, Village of Ouagadougou, Burkina Faso. (Figures in parentheses are numbers of mosquitoes tested).

Insecticide	Concentration (%)	Exposure time (hr)	Mortality rate (%)
		An. arabiensis	
DDT	4	1	(120) 100
		An. gambiae	
DDT	4	1	(180) 70
DDT	4	2	(104) 100

intensively cultivated. It is possible that selection acted particularly against *An. gambiae* s.s., which is more frequent than *An. arabiensis* in that zone, as shown from the cytotaxonomic identification of samples collected during 1984 rainy season (Petrarca et al. 1984). While the relative frequency of the two species in Ouagadougou and Zaghtouli was about 50%, in Koubri about 90% of the specimens were identified as *An. gambiae* s.s. More than 85% of *An. gambiae* s.s. was represented by the Mopti chromosomal form, which is apparently associated with permanent breeding places like those occurring in rice fields (Touré et al. 1983, Coluzzi et al. 1985). A different impact of DDT on the two members of the *gambiae* complex in that area has been tested using two laboratory strains of the F_3 generation which originated from indoor resting samples collected in Koubri. A difference was actually observed, since the insecticide tests showed complete susceptibility of *An. arabiensis*, while *An. gambiae* s.s. showed a considerable level of resistance (70% mortality after 1 hr exposure to 4% DDT; see Table 4.). The epidemiological implications of the observed differential response to the residual insecticide should be taken into account.

Culex quinquefasciatus. Results of larval susceptibility tests on *Cx. quinquefasciatus* from the urban area of Ouagadougou, reported in Table 5, were compared with those from the susceptible reference strain. Data from Mouchet et al. (1968) referring to a population of *Cx. quinquefasciatus* collected in the same sampling area, are also reported. The LC_{50} values of the Ouagadougou population were always higher than those of the reference *Cx. pipiens* susceptible strain (see R/S values). A low level of resistance for chlorpyrifos (2.45x) and a moderate one for temephos (7.71x) was detected. Moreover, an increase of resistance level to temephos (3.3x) was also observed comparing the present data with the above mentioned ones obtained by Mouchet et al. (1968).

It has been shown that enhanced levels of esterase activity are often associated with organophosphorus insecticide resistance in members of *Cx. pipiens* complex (Curtis and Pasteur 1981, Villani et al. 1983, Georghiou et al. 1980). Therefore, electrophoretic analysis of *Cx. quinquefasciatus* was carried out to evaluate such a correlation and add further information on the susceptibility level of the populations studied.

The results showed the same esterase pattern already described by Villani et al. (1983) in populations of *Cx. quinquefasciatus* from different geographical areas. A mixture of individuals characterized by esterases with low and enhanced activity was observed. Two highly active bands coded by Est-2 and Est-3 loci were

Table 3. Organochlorine susceptibility of field-collected adult *Anopheles gambiae* s.l. from Ouagadougou and two nearby villages (Figures in parentheses are numbers of mosquitoes tested).

Location	Insecticide	Concentration (%)	Exposure time (hr)	Mortality (%)
Ouagadougou	DDT	4.0	1	(352) 88.5
Ouagadougou	DDT	4.0	2	(265) 100.0
Zaghtouli	DDT	4.0	1	(220) 91.0
Koubri	DDT	4.0	1	(202) 50.0
Koubri	DDT	4.0	2	(237) 64.0
Koubri	dieldrin	0.4	1	(280) 2.0
Koubri	dieldrin	0.4	2	(205) 20.0

Table 5. Susceptibility values (24-hr lethal concentrations in mg/liter) to organophosphorus insecticides of field-collected larvae of *Culex quinquefasciatus* from Ouagadougou (Burkina Faso), compared to a susceptible strain of *Cx. pipiens* (Italy) and *Cx. quinquefasciatus* from Ouagadougou[a].

Insecticide	*Cx. quinquefasciatus*			*Cx. pipiens*, Ref. strain			*Cx. quinquefasciatus*[a]		
	LC_{50}[b]	LC_{90}[b]	Slope	LC_{50}	LC_{90}[b]	R/S[c]	LC_{50}[b]	LC_{90}[b]	R/S_d
Chlorpyrifos	0.0008 (0.0007–0.0009)[e]	0.0021 (0.0015–0.0029)	2.2	0.0003	0.0010	2.45	0.0007	0.0009	1.10
Fenitrothion	0.0095 (0.0082–0.0111)	0.0336 (0.0233–0.0485)	2.8	0.0052	0.0166	1.85	0.0110	0.0190	0.86
Fenthion	0.0034 (0.0031–0.0037)	0.0074 (0.0064–0.0086)	1.7	0.0024	0.0058	1.41	0.0028	0.0037	1.21
Malathion	0.0621 (0.0545–0.0707)	0.1368 (0.1025–0.1852)	1.9	0.0421	0.1320	1.47	0.0630	0.0930	0.98
Temephos	0.0015 (0.0013–0.0017)	0.0046 (0.0037–0.0056)	2.3	0.0002	0.00052	7.71	0.0005	0.0008	3.30

[a] From Mouchet et al. 1968.
[b] Lethal concentrations in mg/liter after 24-h.
[c] LC_{50} of *Cx. quinquefasciatus*/LC_{50} of *Cx. pipiens*.
[d] LC_{50} of *Cx. quinquefasciatus*/LC_{50} reported by Mouchet et al. (1968).
[e] 95% confidence limits (in parentheses).

present in 16 out of 53 specimens tested, i.e., 30.2%. Assuming that high activity levels of esterases are associated with OP insecticide resistance, the polymorphic esterase levels found agree well with the bioassay data, particularly in the case of temephos.

CONCLUSIONS

The results of susceptibility tests on *An. gambiae* s.l. showed the presence of low level DDT resistance in the urban area of Ouagadougou and in the nearby village of Zaghtouli, whereas evidence of DDT and dieldrin resistance was detected in the village of Koubri.

The observed resistance to chlorinated compounds makes the use of DDT inadvisable in the latter village, while it can still be used for indoor spraying in Ouagadougou and Zaghtouli. The complete susceptibility to OP and carbamate insecticides offers the possibility for a wide choice of compounds for larviciding and adulticiding operations.

The population of *Cx. quinquefasciatus*, as shown by the bioassays and the electrophoretic results, is polymorphic for the esterase genes and shows decreased susceptibility to OP compounds, particularly to temephos. Therefore, an accurate survey of the susceptibility status is needed to evaluate possible future changes, which may be of great importance in vector control activities.

ACKNOWLEDGMENTS

The authors are grateful to Prof. M. Coluzzi, Istituto di Parassitologia, Università di Roma "La Sapienza," Italy, Dr R. Subra, WHO Onchocerciasis Control Program, Ouagadougou, Burkina Faso, Dr C. F. Curtis, Department of Entomology, London School of Hygiene and Tropical Medicine, London, U.K., for reviewing the manuscript and furnishing useful suggestions. The authors are especially grateful to Mr. G. Pierdominici, Istituto Superiore di Sanità, Rome, Italy, for the technical assistance and to Mr. G. Petrangeli, Istituto di Parassitologia Università di Roma "La Sapienza," for kindly providing the laboratory strains of *An. arabiensis* and *An. gambiae* s.s.

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