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Very high DDT-resistant population of *Anopheles pharoensis* Theobald (Diptera: Culicidae) from Gorgora, northern Ethiopia

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Summary:

Standard WHO insecticide bioassay tests were carried out in Gorgora, northern Ethiopia to evaluate the susceptibility status of *Anopheles pharoensis* Theobald for the insecticides DDT, malathion, permethrin and deltamethrin. The mortality and when appropriate knockdown effect of the insecticides were observed. The results indicated that this species was resistant to DDT. A high mortality was obtained after exposure to permethrin and deltamethrin but below 97 % which is the limit for susceptibility according to WHO. A prolonged knockdown time was noted for DDT and the two pyrethroids. *An. pharoensis* was found to be susceptible to malathion.

KEY WORDS : *Anopheles pharoensis*, insecticide resistance, DDT, pyrethroid, malathion, Ethiopia.

Résumé : Très haut niveau de résistance au DDT d'une population d'*Anopheles pharoensis* Theobald (Diptera : Culicidae) dans le nord de l'Éthiopie

*L'efficacité du DDT, du malathion, de la perméthrine et de la deltaméthrine vis-à-vis d'*Anopheles pharoensis *Theobald a été évaluée selon les tests standardisés de l'OMS. La mortalité et l'effet paralysant éventuel ont été relevés pour chaque insecticide. Les résultats font ressortir que cette espèce est résistante au DDT. Avec la perméthrine et la deltaméthrine, les pourcentages de mortalité observés sont élevés, mais en dessous du seuil de 97 % qui est la limite de sensibilité définie par l'OMS. Avec le DDT et les deux pyréthrinoïdes, les temps d'immobilisation sont augmentés.* An. pharoensis *reste sensible au malathion.*

MOTS CLÉS : Anopheles pharoensis, *résistance, insecticide, DDT, pyréthrinoïde, malathion, Éthiopie.*

In Ethiopia, *Anopheles pharoensis* Theobald is regarded as an auxiliary vector of malaria based on salivary gland sporozoite infections and evidence on its strong anthropophilic behavior (Gebre-Mariam, 1988; Abose *et al.*, 1998). In a countrywide entomological survey between 1984 and 1988, it was noted that this species was the second abundant mosquito next to *An. gambiae* s.l., and it was also found to feed mostly outdoors and rest indoors (Tulu, 1993). Such behavior of malaria vectors including *An. arabiensis*, a major malaria vector, was the basis to utilize indoor residual sprayings with DDT to control them and also minimize the burden of malaria. This practice has been in place for several years in the past. In spite of the long history of vector control in the country, little is known about the DDT resistance status of *An. pharoensis* and there is now a need to evaluate its resistance status since the strategy adopted by the Federal Ministry of Health of Ethiopia is to use DDT and malathion for indoor residual spraying and pyrethroids for impregnation of mosquito nets (MOH, 2006)

The present field study was conducted in Gorgora, in northern Ethiopia in October and November 2005 to assess the susceptibility status of this species to DDT, malathion, permethrin and deltamethrin.

MATERIAL AND METHODS

Study site

Gorgora is situated at 1,800 m altitude, in the northern tip of Lake Tana, the source of the Blue Nile. It is 808 kms northwest of Addis Ababa and 60 kms southwest from the historical town of Gondar. This area is part of the Dembia plain that was known in the past as one of the malarious areas where the disease claimed many lives in the late 1950's (Fontaine *et al.*, 1961). Malaria still causes immense morbidity and mortality. Until recently DDT indoor sprays remained the mainstay of vector control. However, the insecticide susceptibility status of vector mosquitoes prevailing in the area was not known.

Biossays

Bioassays were conducted on adult females of *An. pharoensis* following the guidelines of WHO (1998). Immature forms (larvae and pupae) were sampled from different breeding sites and reared to adults in big cages.

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Insecticide	Number tested	% mortality	KT_{50} (minute)	KT_{90} (minute)
4 % DDT	162	6.2	No Kd	No Kd
0.75 % permethrin	108	94.4	19.4 (17.5-21.3)	26.6 (24,3-30.1)
0.05 % deltamethrin	108	94.4	22.8 (16.5-28.4)	31.9 (26.8-46.4)
5 % malathion	106	98.1	NA	NA

No Kd: only seven mosquitoes were knocked down after 80 minutes.
NA = not applicable.
95 % confidence interval is shown in parenthesis.

Table I. – Results of WHO diagnostic tests with *Anopheles pharoensis* from Gorgora, northern Ethiopia.

Tests were carried out on 2-3 days old, non blood fed females. Each test was done by exposing 18 mosquitoes to WHO insecticide impregnated papers containing discriminating doses of 4 % DDT, 0.75 % permethrin, 0.05 % deltamethrin and 5 % malathion for one hour. In the case of DDT, permethrin and deltamethrin knocked down mosquitoes were recorded at intervals of five minutes to determine the KT_{50} and KT_{90}, the time which takes to knockdown 50 % and 90 % of the test population, respectively. When there was no complete knockdown during the exposure time, additional observation was made for another 20 minutes after transferring the mosquitoes into holding tubes. Mortality was checked after 24-hours. Replicates of six to 10 were carried out. In all the tests there was no mortality in control mosquitoes. The temperature remained between 22 and 24° C.

A colony of F_1 generation was maintained from females surviving to DDT exposure and their progenies were tested against DDT and permethrin.

RESULTS

An. pharoensis was found to be highly resistant to DDT as 94 % of the test population survived to insecticide exposure (Table I). The number of mosquitoes knocked down during the one-hour exposure time and after 20 minutes was very low. On the other hand, a high mortality was observed with females exposed to permethrin and deltamethrin, although there was substantial increase on the KT_{50} and KT_{90}. The other insecticide, malathion was found to be highly toxic to mosquitoes.

53 adult females from the F_1 generation of the DDT resistant females were tested against DDT and all of them were alive at the end of the test suggesting the presence of physiological resistance (Table II). Similarly, nine F_1 mosquitoes were tested against permethrin and 77.8 % were killed by this insecticide.

Insecticide concentration	Number tested	% mortality
4 % DDT	53	0.0
0.75 % permethrin	9	77.8

Table II. - Results of WHO diagnostic tests with F_1 generation of *An. pharoensis* raised from DDT surviving individuals.

DISCUSSION

Survival of more than 90 % of the population and the small number of knocked down mosquitoes at 80 minutes were an indication of resistance of *An. pharoensis* to DDT, according to WHO (1998) criteria. The 100 % survival of the progenies from DDT resistant females provides additional evidence for the existence of resistance probably conferred by metabolic or molecular mechanisms.

Previously, Wozam & Seulu (1994) reported a high DDT resistance (52.5 % survival) in *An. pharoensis* from Ziway, central Ethiopia (Rift Valley) and recommended malathion for indoor residual sprayings in replacement of DDT in the area. The exceptionally high DDT resistance in *An. pharoensis* in the present study strengthens the urgent need to shift to other insecticides such as malathion for indoor residual spraying in addition to the current use of insecticide treated nets in Ethiopia.

According to WHO criteria, mortality between 80 and 97 % suggests the presence of resistance that need to be confirmed. Thus, we cannot exclude that some *An. pharoensis* from our study were also resistant to pyrethroids since the mortality was around 95 %. Although there are no published data available on KT_{50} and KT_{90} for *An. pharoensis*, we observed a significant increase of these values (2-3 fold) compared to susceptible reference strain of other species such as *An. gambiae* (Chandre *et al.*, 2000; Etang *et al.*, 2003). The possible cross resistance between DDT and pyrethroids, the absence of knockdown effect of DDT, and the increase of KT for pyrethroids strongly suggested that at least a target site mutation (*kdr*) was involved in resistance. Although this requires further evaluation, we still recommend the use of the two pyrethroids for impregnation of mosquito nets provided the major vector, *An. arabiensis*, remains susceptible to these insecticides.

DDT resistance could have arisen due either to adult exposure to treated surfaces or to larval exposure to runoff DDT or other similar agricultural pesticides into breeding habitats. This species prefers permanent and shaded breeding sites including irrigation canals, rice fields, swampy areas and lake shores (Gillies & De Meillon, 1968) where insecticides could persist for a prolonged time acting as selective forces. However, there is no indication that DDT has been in use in Gorgora for purposes other than indoor sprayings. In the future, we envisage to investigate the molecular and metabolic mechanisms of resistance to identify the genes and enzymes that are responsible for resistance in *An. pharoensis*.

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