

Research Article

Review of Temephos Discriminating Concentration for Monitoring the Susceptibility of *Anopheles labranchiae* (Falleroni, 1926), Malaria Vector in Morocco

C. Faraj,¹ E. Adlaoui,¹ M. Elkohli,¹ T. Herrak,² B. Ameur,² and F. Chandre³

¹Laboratoire d'Entomologie Médicale, Institut National d'Hygiène, 27 Avenue Ibn Batouta, Agdal, Rabat 10090, Morocco

²Service de lutte Antivectorielle, Direction de l'Epidémiologie et de Lutte contre les Maladies, Agdal, Rabat 10080, Morocco

³Institut de Recherche pour le Développement (IRD), UR016, Caractérisation et Contrôle des Populations de Vecteurs, Montpellier Cedex 5, France

Correspondence should be addressed to C. Faraj, chafikaf@gmail.com

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In Morocco, the resistance monitoring of *Anopheles labranchiae* larvae to temephos is done using discriminating concentration of 0.125 mg, which is half of the WHO recommended dose for *Anopheles*. However, this dosage seemed to be too high to allow an early detection of the resistance and its revision was found necessary. The present study was carried out during May-June 2008 and 2009 in nine provinces from the north-west of the country. The aim was to determine the lethal concentrations LC100 of temephos for the most susceptible populations and to define the discriminating dosage as the double of this value. The bioassays were conducted according to WHO standard operating protocol to establish the dose-mortality relationship and deduct the LC50 and LC95. The results of this study indicated that the LC100 obtained on the most susceptible populations was close to 0.05 mg/L. Therefore, the temephos discriminating dosage for susceptibility monitoring of *An. labranchiae* larvae in Morocco was set to be 0.1 mg/L.

1. Introduction

In Morocco, last autochthonous malaria case was registered in 2004. Since then a vector control program was established to prevent a possible return of malaria transmission. It is mainly based on the larval control of the main vector: *Anopheles labranchiae* (Falleroni 1926) [1]. This control includes integrated management using environmental methods and larvivorous fish (*Gambusia holbrooki*) as biological control. Insecticides are used as the last option but they take an important place in the National Malaria Control Programme (NMCP).

Larval treatments started in the 1950's using DDT [2]. In 1978, an organophosphate insecticide, the temephos (Abate 500 EC) was introduced, and since then, it has been the only insecticide used for *An. labranchiae* larval control.

The monitoring of *An. labranchiae* susceptibility to insecticides used in larval and adult control is an essential

component of the NMCP. This activity has started with the launch of the programme following WHO protocol, using discriminating dosage [3]. The first susceptibility tests of *Anopheles labranchiae* to temephos were carried out before its introduction for larval control using concentrations supplied by WHO (0.005, 0.025, 0.125, 0.625 mg/L). Results of these tests, carried out on natural populations, showed that the lowest dosage involving regularly 100% mortality was 0.125 mg/L. This concentration was considered as a specific discriminating dose for Moroccan *An. labranchiae* and was used in routine resistance monitoring. Although this dose represents half of the diagnostic concentration recommended by WHO (0.25 mg/L) [4] for *Anopheles*, this is higher than the operational dosage of temephos that is still effective for larval control. Indeed, operational treatments are made using a dosage of 50 g/ha, corresponding to a concentration of 0.05 to 0.1 mg/L for breeding sites, respectively, of about 10 and 5 cm of depth. The diagnostic dose of

0.125 mg/L seems to be too high to allow an early detection of the resistance, and so its revision was found necessary.

The present study was carried out to determine a more accurate diagnostic dose, for the larvae of *An. labranchiae* from Morocco, if possible lower than the operational dose to early detect a significant reduction of insecticide susceptibility among field populations. This study will also serve as reference data which could be applied in countries around Mediterranean sea, particularly Algeria, Tunisia, Italy, and France where *An. labranchiae* is present and could constitute a risk for malaria transmission [5–8].

2. Material and Methods

2.1. Study Areas. *An. labranchiae* is an eurygamic species. It is almost impossible to rear in insectarium and hence difficult to have a susceptible reference strain to estimate its baseline susceptibility. So, we attempted to search for a wild population as susceptible as possible, in regions where the selection pressure by insecticide treatment was low. Pressure of organophosphate (OP) used in public health is low as temephos is the first and the only OP used in Anopheles larvae control, and DDT was the only insecticide used in adult control. However, pressure of insecticides used in agriculture could be important because *An. labranchiae* is abundant in the agricultural zones particularly in the north western of the country [9] where the main culture practices are wheat, corn, sugar cane, and rice cultivation. To mitigate this way, larvae were collected in sites as far as possible from cultivation areas. Hence, the study was carried out in nine provinces (Figure 1).

2.2. Mosquitoes. Because Morocco is in a phase of prevention of malaria reintroduction, the vector *An. labranchiae* is submitted to regular controls. Consequently, its density is low and it was not easy to find many positive breeding sites in sufficient density to realize bioassays. We were then forced to realize tests in sites where density was allowable, generally far from villages. Larvae were collected, using standard dipping method, in different biotopes (swamps, rivers, rice fields) during May-June 2008 and 2009 (Table 1). Specimens were identified morphologically [10].

2.3. Bioassays. Bioassays were carried out following WHO standard procedures to establish the dose-mortality relationship and to calculate lethal concentrations LC50 and LC95 (concentrations involving, resp., the death of 50% and 95% of the tested population) [11]. Tests were carried out on the third and fourth instar larvae. Ranges of 5 to 6 concentrations of temephos and control were prepared to determine the LC50 and LC95 for each population. For each dilution, 3 to 4 replicates were done, with 15 to 25 larvae each. Larvae were placed in 99 mL of water; 1 mL of adequate concentration of temephos was then added. After 24 hours of exposition at ambient temperature (21-22°C) without feeding, alive and dead larvae were counted. When it was possible, 2 tests were conducted on the same population in the same experimental conditions in 2 different days. Tests involving less than four

sets with mortalities different from 0 and 100% or with mortality in control higher than 20% were not considered [12].

Results analysis was made using log-probit analysis software (WinDL version 2.0) developed by CIRAD-CA/MABIS [13]. It allows calculating LC50, LC95, and their confidence intervals.

Diagnostic concentration was calculated as the double of the observed LC100 of the most susceptible populations.

3. Results

A total of more than 7,000 larvae were tested for 18 different tests conducted in 12 villages. Among these bioassays, only 12 were valid. Among the six tests considered as invalid, mortality in control was superior to 20% in 2 tests, and there were 4 tests involving less than four sets with mortalities different from 0 and 100%.

Results of bioassays are presented in Table 2.

The LC95 varies from 0.036 to 0.105 mg/L among the different populations, representing a ratio less than 3 folds. The most susceptible populations were collected, respectively, in Benslimane and Meknes. The least susceptible populations were collected in Khemisset and Larache.

The lowest concentration involving 100% of mortality on the natural populations is of 0.0625 mg/L. It was obtained on Ben Slimane and Boucharen populations. As the highest tested concentration producing a mortality less than 100% is 0.025 mg/L, the real LC100 should be situated between 0.025 mg/L and 0.0625 mg/L.

Concentration of 0.125 mg/L did not involve 100% mortality in the population of Sbih (Sidi Kacem) suggesting the emergence of a beginning of resistance.

4. Discussion

The standardized bioassays on larvae or adults using a discriminating dosage of insecticides are largely employed for the resistance monitoring of mosquito populations targeted by vector control programmes [14]. This method has the advantage of being simply used, fast, and inexpensive, as well as of giving reproducible results and requiring only a small number of specimens compared to the conventional bioassays.

The discriminating dosage corresponds to the theoretical LC100 of susceptible individuals obtained by extrapolating the regression curve (probit mortality/log dosage) to a mortality rate of 99.9% [15]. In practice and to keep a safety margin, this dosage corresponds to twice the LC99.9% or preferentially twice the lowest dosage giving 100% of mortality of a fully susceptible population, since the LC99.9% has a low statistical value when the slope of probit line is low or the population is heterogeneous.

The temephos discriminating dosage, established by WHO, for the genus *Anopheles* is 0.25 mg/L. Afterward, it was stated locally for a limited number of species [16]. For *An. Hyrcanus*, this dose was set at 0.025 mg/L and it was 0.625 mg/L for *An. sacharov* that belongs to the same

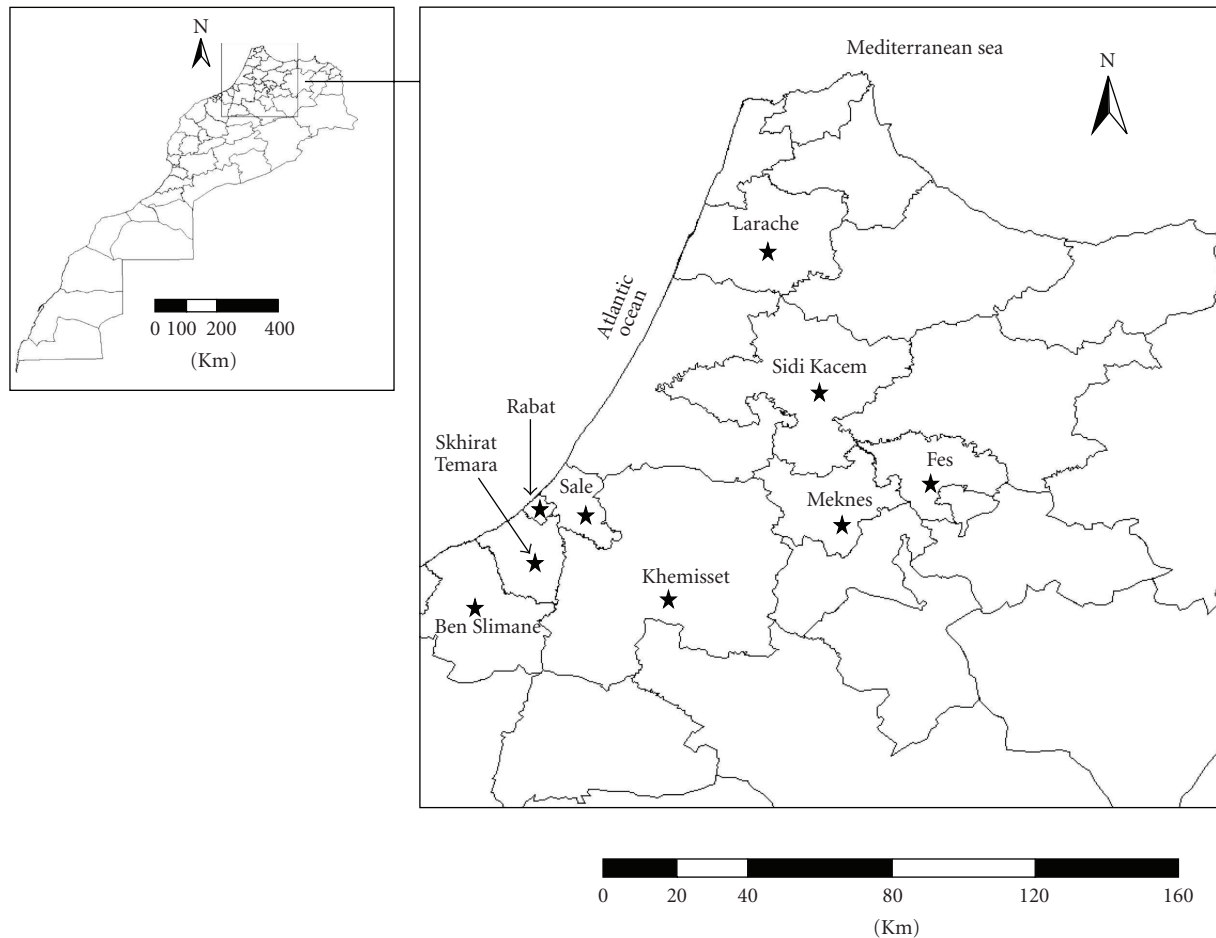


FIGURE 1: Map of Morocco showing provinces where *An. labranchiae* populations were sampled.

TABLE 1: Details of larval breeding sites which were sampled.

| Population | Province | Geographical coordinates | | Breeding site | Type of breeding site |
|-------------------|----------------|--------------------------|----------|---------------|-----------------------|
| | | Longitude | Latitude | | |
| Sidi Allal Msader | Khemisset | 06°02' W | 33°44' N | River | Permanent |
| Beggara | Larache | 06°06' W | 35°10' N | Swamps | Permanent |
| Boucharen | Larache | 06°04' W | 35°07' N | Rice field | Temporary |
| Ain Aghbal | Meknes | 05°27' W | 33°40' N | River | Permanent |
| Ben Slimane | Ben Slimane | 07°04' W | 33°38' N | Daya | Temporary |
| Skhirat | Skhirat-Temara | 07°05' W | 33°50' N | Swamps | Permanent |
| Ain Elouali | Fes | 004°54' W | 34°07' N | River | Permanent |
| Rabat | Rabat | 06°46' W | 33°58' N | Swamp | Temporary |
| Sbih | Sidi Kacem | 05°26' W | 34°24' N | Swamps | Permanent |
| Sehoul | Sale | 06°35' W | 33°51' N | Swamps | Temporary |

complex as *An. labranchiae*. In Morocco, before the first use of temephos, 0.025 mg/L did not involve systematically 100% mortality on populations having never been in contact with this product, and so they were expected to be fully susceptible. The discriminating dosage would have been around or higher than 0.05 mg/L. As a compromise the concentration of 0.125 mg/L was chosen and was probably superior to the real discriminating dosage.

Bioassays carried out by resistance monitoring sentinel sites of Khemisset, Meknes, and Tetouan between 2004 and 2008 showed that several populations used to have a LC100 sometimes equal to 0.025 mg/L or slightly higher. Accordingly, the LC100 of the most susceptible populations in Morocco was found to be 0.05 mg/L. This dose allows for determining the diagnostic dosage of temephos for *An. labranchiae* to be 0.1 mg/L, which is finally very close

TABLE 2: Susceptibility of *An. labranchiae* larvae to temephos in mg/L.

| Province | Population | LC50 (CI) | LC95 (CI) | LC100 |
|----------------|-------------------|------------------------|------------------------|--------|
| Khemisset | Sidi Allal Msader | 0.0198 (0.0052–0.0947) | 0.1058 (0.0407–0.4210) | >0.05 |
| | | 0.0196 (0.0016–0.0295) | 0.0676 (0.0413–0.3013) | >0.05 |
| Sale | Shoul | 0.0098 (0.0074–0.0121) | 0.0853 (0.0571–0.1632) | >0.05 |
| | | 0.0094 (0.0078–0.0112) | 0.0693 (0.0500–0.1090) | >0.05 |
| Larache | Boucharen | 0.0079 (0.0061–0.0101) | 0.0586 (0.0391–0.1088) | 0.0625 |
| | Beggara | 0.0186 (0.0127–0.0262) | 0.1002 (0.0622–0.2301) | 0.1250 |
| Ben Slimane | Ben Slimane | 0.0084 (0.0072–0.0098) | 0.0362 (0.0288–0.0491) | 0.0625 |
| | | 0.0208 (0.0186–0.0233) | 0.0537 (0.0447–0.0692) | 0.1250 |
| Skhirat-Temara | Skhirat | 0.0067 (0.0055–0.0080) | 0.0485 (0.0366–0.0703) | 0.1250 |
| Rabat | Rabat | 0.0188 (0.0073–0.0343) | 0.0688 (0.0368–0.9785) | 0.1250 |
| Sidi Kacem | Sbih | 0.0301 (0.0223–0.0399) | 0.0967 (0.0663–0.1961) | 0.2500 |
| Meknes | Ain Aghbal | 0.0178 (0.0160–0.0197) | 0.0371 (0.0314–0.0480) | 0.1250 |

LC50/LC95: lethal concentrations for 50% and 95% of larvae with confidence intervals (CI) at 5% level.

LC100: observed concentration involving 100% of mortality.

to the diagnostic concentration previously used by the national control programme and which could be higher than operational dose.

Results of this study show that, with the exception of Sidi Kacem population where a low resistance was suspected, all tested populations are still susceptible to temephos. Although temephos is used in vector control in Morocco within the framework of a resistance management programme which privileges the environmental control and the use of larvivorous fishes as biological control, it is essential that the NMCP have replacement insecticides before the emergence of *Anopheles labranchiae* resistance to temephos and the failure of the present vector control program. Attention must be steered towards the use of microbial control agents and insect growth inhibitors taking into account cost effectiveness, feasibility, and respect of the nontarget fauna and the environment.

The adoption of this discriminating dosage will allow simplifying the procedure for monitoring the susceptibility of *An. labranchiae* to temephos in the different provinces of Morocco.

Conflict of Interests

The authors declare that they have no conflict of interests.

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