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# Susceptibility of a malaria vector *Anopheles gambiae* Sl (Diptera: Culicidae) to WHO recommended insecticides in Togo (West Africa)

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**Abstract**
Malaria vector control is a very important concern in Togo and is mostly based on the use of insecticide-treated bednets. In this study we assessed countrywide susceptibility tests in the malaria vector *Anopheles gambiae* from Togo to four classes WHO recommended insecticides. WHO bioassays were performed on female of *Anopheles gambiae s.l.* emerged from larvae collected from 6 sentinel sites in Togo. The insecticides used were organochlorine (DDT 4%), pyrethroids (0.05% deltamethrin, 0.75% permethrin and 0.05% λ-cyhalothrin), organophosphate (5% malathion) and carbamate (0.4% carbosulfan). Bioassays showed phenotypic resistance particularly to DDT (mortality rate < 1% in Kovié) and pyrethroids (mortality rates are 54% and 1.78% in Dapaong for deltamethrin and permethrin respectively). *Anopheles* population was also resistant to carbosulfan in Kara (58.33%), Tchamba (31.58%), Kovié (26.36%) and Akodesséwa (40%) and susceptible to malathion in all sites (> 97%). This study showed resistance in *An. gambiae s.l.* particularly to DDT and pyrethroids. These data can be used for a good decision making in malaria vectors control in Togo as recommended the World Health Organization.

**Keywords:** Susceptibility, *Anopheles gambiae s.l.*, WHO recommended insecticides, Togo

## 1. Introduction
Malaria vectors control relies essentially on the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) throughout sub-Saharan Africa as recommended by the World Health Organization (WHO) [1]. Pyrethroïds are the only class of insecticide approved for use on LLINs because of their relatively low human toxicity, excito-repellent properties, rapid rate of knock-down and killing effects [2]. Malaria control strategies mainly depend on the pyrethroïds. But pyrethroïds are facing a problem of resistance raised among *Anopheles* populations and this situation can jeopardize the mosquito control efforts [3]. However, these strategies can be well done through the knowledge of vectors distributions, biology and changing trends on susceptibility status to insecticides currently used in public health. An increasing of household LLIN ownership from 3% in 2000 to 56% in 2012 has been recently reported in the WHO Africa region through the mass distribution [1]. In Togo, an evaluation was made in 2012 concerning the 2011 LLIN for universal coverage and the results showed that the possession of at least one bed net and/or LLIN increased from 41.3% to 96.7%; household possession of at least one campaign LLIN was 93.3% [4]. Ahadji-Dabla *et al.* [5] reported pyrethroids resistance in *Anopheles gambiae s.l* in the southern part of the country. The emergence of pyrethroids resistance is due to their use in agriculture, mainly in cotton-growing, rice cultivation and vegetable farming areas [6-9]. Increased insecticide detoxification (metabolic resistance) and target site insensitivity are the two major mechanisms exhibited by the anopheline populations [10]. The target site insensitivity is a result of point mutations in the voltage gated sodium channel gene which was named knockdown resistance [10, 11].
The dramatic increase in insecticide resistance in malaria-endemic countries leads the WHO and Roll Back Malaria partnership to launch the Global Plan for Insecticide Resistance Management (GPIRM). According to the GPIRM, every country should have a strategy, even though there are other immediate priorities in a resource-constrained environment. Each strategy should be implemented, including updating vector control plan on basis of new information by considering geographic areas with different histories of insecticide use in both the public health as well as agricultural sector [12].

Studies carried out from 2002 to 2004 in the six sentinel sites of the National Malaria Control Programme (NMCP) revealed that unfed Anopheline populations were fully susceptible to deltamethrin in the Central, Kara and Savannah regions whereas in Coastal and Highland regions there was reduction in susceptibility [13]. The present study aimed to present the actual insecticide susceptibility profile of *An. gambiae s.l.* in the NMCP sentinel sites across the country.

## 2. Material and Methods

### 2.1. Study area

The study was carried out in 6 selected sentinel sites from different ecological zones of Togo in 2009 (Fig. 1). Togo is a costal country located in West Africa, with a population of ~7 million inhabitants. The country has 2 tropical climates: the Sudanian in the north characterized by one rainy season and one dry season; the subequatorial characterized by two dry seasons (from December to March and from August to September) and two rainy seasons (from April to July and from October to November). According to Ern [14], Togo covers five ecological zones: the plains zone of the north (zone I), the mountains zone of the north (zone II), the central plains zone (zone III), the Mounts Togo meridional zone (zone IV) and the southern coastal zone (zone V). The geographic coordinates of the sites where the larvae were collected are presented in Table 1.

**Table 1:** Geographic coordinates of the study sites

| Study sites | Main agriculture practices | Ecological zones | Period of collection | Geographic coordinates | |
|---|---|---|---|---|---|
| Dapaong | Cereals, tubers | I | Oct, Nov 2009 | N 10°51'39.0" | 0°12'52.5" E |
| Kara | Cereals | II | Oct, Nov 2009 | N 09°38'121" | 001°07'58.8" E |
| Tchamba | Cereals, tubers | III | Oct, Nov 2009 | N 09°2'86.0" | 001°25'17.0" E |
| Kpalimé | Cereals, cocoa, coffee | IV | Oct, Nov 2009 | N 07°52'57.0" | 0°43'27.5" E |
| Kovié | Rice, vegetables | V | Oct, Nov 2009 | N 06°20'87.7" | 001°7'24.8" E |
| Akodesséwa | Vegetables | V | Oct, Nov 2009 | N06°9'23.6" | 001°16'02.4" E |

### 2.2. Insecticide bio-assays

Larvae of *An. gambiae s.l.* were collected from each breeding site using the "dipping method". All collected larvae were identified morphologically to species using the keys of Gillies & Coetzee [15], and reared in insectary to adult. The emerged adults were fed with 10% glucose solution. The susceptibility tests were carried out using unfed female of three to five days old. In areas where larvae were not abundant, only few number of mosquitoes were used for the test. Laboratory reared Kisumu strain of *An. gambiae* s.s. was used as reference for each of the susceptibility test. The insecticide bio-assay were performed following the WHO standard protocol [16].

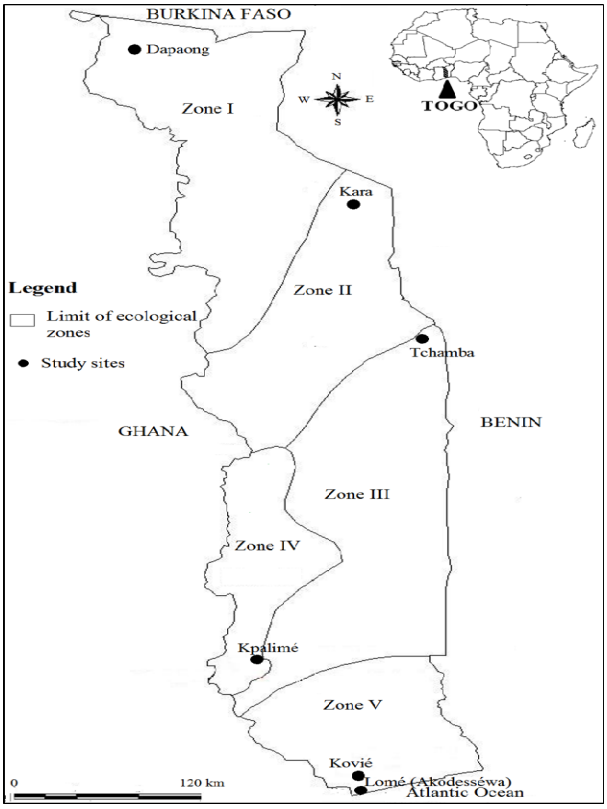


**Fig 1:** Map of Togo showing the different ecological zones and the study sites

For each treatment, five test tubes were used: one untreated paper as a control and four treated papers. An average of 25 female mosquitoes were exposed to each WHO insecticide treated paper in holding tubes for 60 min under laboratory conditions (26 ± 2 °C and 75-80% relative humidity). During this exposure time, the number of mosquitoes knocked down was recorded every 10 min. The insecticides used for the susceptibility test were 4% DDT, 0.05% deltamethrin, 0.75%

permethrin, 0.05% λ-cyhalothrin, 5% malathion and 0.4% carbosulfan. The number of dead mosquitoes was recorded after 24 hours during which they were held in observation tubes and supplied with 10% glucose solution.

### 2.3. Statistical analyses

The $KDT_{50}$ and $KDT_{90}$ of tested mosquitoes were estimated using probit analysis [17] provided from PoloPlus version 1.0 (LeOra Software). The $KDT_{50}$ recorded from field-collected mosquitoes was compared with that of the *An. gambiae* Kisumu reference susceptible strain by estimating the $KDT_{50}$ ratios (RR). When mortality rate in control was between 5% and 20%, Abbott's formula was used to correct the observed mortality in adult susceptibility tests [18]. The resistance/susceptibility status of the tested populations was determined for each insecticide according to WHO criteria [16].

## 3. Results

A total of 3223 *An. gambiae s.l.* from 6 sentinel sites of NMCP were tested against the six insecticides of which 494, 485, 534, 503, 603 and 604 were exposed to DDT, deltamethrin, permethrin, λ-cyhalothrin, malathion and carbosulfan, respectively. The knock down times and the mortality rates of *Anopheles* populations 24 h post-exposure to DDT, deltamethrin, permethrin, λ-cyhalothrin are shown in Tables 2–5, respectively. *Anopheles gambiae s.l.* was found to be highly resistant to DDT in all the sentinel sites. Resistance to all the pyrethroids used in this study was observed; the highest mortality (82%) was obtained in Kara at the north (Zone II) with deltamethrin. Figure 2 shows the mortality rates while *Anopheles* were exposed to carbosulfan (Fig. 2A) and malathion (Fig. 2B). Reduced susceptibility to carbosulfan was recorded in four of the six sentinel sites (Akodesséwa, Kovié, Tchamba and Kara); highest mortality rates were only recorded in Dapaong (82.47%) and in Kpalimé (96.77%). Full susceptibility to malathion was recorded in almost all the sites with mortality rates ranging from 97% to 100%.

High knock down times ($KDT_{50}$ and $KDT_{90}$) were recorded with pyrethroids. Confidence intervals (95% CI) were even large for permethrin and in some cases for deltamethrin and λ-cyhalothrin. The lowest $KDT_{50}$ ratios (RR) were recorded with deltamethrin but they were all superior to 2 in all the sites. No mosquito was knocked down with DDT during the whole tests, the KDT were not determined.

**Table 2:** Knock-down time and mortality rates of *Anopheles* mosquitoes exposed to 4% DDT for a period of 60 min

| Study sites | N | $KDT_{50}$ (min) | CI (95%) | $KDT_{90}$ (min) | CI (95%) | $KDT_{50}$ ratio (RR) | Mortality (%) |
|---|---|---|---|---|---|---|---|
| Dapaong | 95 | ND | - | ND | - | ND | 17.89 |
| Kara | 24 | ND | - | ND | - | ND | 16.66 |
| Tchamba | 63 | ND | - | ND | - | ND | 34.92 |
| Kpalimé | 119 | ND | - | ND | - | ND | 4.2 |
| Kovié | 109 | ND | - | ND | - | ND | 0.91 |
| Akodesséwa | 84 | ND | - | ND | - | ND | 1.19 |
| Kisumu | 105 | 17.28 | [13.71-20.85] | 31.48 | [25.67-43.58] | 1 | 100 |

**Table 3:** Knock-down time and mortality rates of *Anopheles* mosquitoes exposed to 0.05% deltamethrin for a period of 60 min

| Study sites | N | $KDT_{50}$ (min) | CI (95%) | $KDT_{90}$ (min) | CI (95%) | $KDT_{50}$ ratio (RR) | Mortality (%) |
|---|---|---|---|---|---|---|---|
| Dapaong | 100 | 38.8 | [33.07-48.32] | 91.27 | [66.85-170.36] | 2.48 | 54 |
| Kara | 28 | 55.5 | [49.35-67.31] | 97.42 | [76.89-162.09] | 3.55 | 82.14 |
| Tchamba | 55 | 48.26 | [44.43-53.41] | 89.63 | [75.74-117.32] | 3.09 | 54.54 |
| Kpalimé | 93 | 53.53 | [43.63-86.66] | 102.12 | [70.55-439.61] | 3.42 | 54.83 |
| Kovié | 108 | 53.78 | [50.66-57.94] | 93.31 | [81.88-112.85] | 3.44 | 55.55 |
| Akodesséwa | 101 | 44.86 | [42.91-46.98] | 68.63 | [63.59-75.96] | 2.87 | 56.45 |
| Kisumu | 103 | 15.63 | [13.27-18.01] | 27.86 | [23.78-34.87] | 1 | 100 |

## 4. Discussion

The level of resistance of *An. gambiae s.l.* to DDT and pyrethroids was very high at almost all study sites. This situation is not surprising considering how these insecticides are used in Togo in agriculture for crop protection and in households for vector control.

Excessive use of agricultural pesticide and household use of insecticide has been implicated in the development of resistance in *An. gambiae* in many West African countries [6, 19-21]. According to Corbel *et al.* [20], the rapid expansion of urban agriculture is one of the major factors that contributes to a large distribution of pyrethroid resistance in *An. gambiae s.l.* Additionally, Yadouleton *et al.* [8] reported that urban farming in Benin has enormously contributed to the emergence of resistance in *Anopheles* populations.

**Table 4:** Knock-down time and mortality rates of *Anopheles* mosquitoes exposed to 0.75% permethrin for a period of 60 min

| Study sites | N | $KDT_{50}$ (min) | CI (95%) | $KDT_{90}$ (min) | CI (95%) | $KDT_{50}$ ratio (RR) | Mortality (%) |
|---|---|---|---|---|---|---|---|
| Dapaong | 112 | ND | - | ND | - | ND | 1.78 |
| Kara | 29 | 200.2 | [95.26-5287.7] | 1367.8 | [315.04-12334] | 10.74 | 27.58 |
| Tchamba | 57 | 93.28 | [67.85-167.96] | 38.42 | [259.78-2310.27] | 5 | 42.1 |
| Kpalimé | 135 | 177.89 | [117.68-431.7] | 675.47 | [313.37-3619.6] | 9.55 | 22.96 |
| Kovié | 103 | 82.65 | [55.43-269.49] | 376.23 | [155.02-7951.05] | 4.43 | 48.54 |
| Akodesséwa | 98 | ND | - | ND | - | ND | 11.22 |
| Kisumu | 100 | 18.63 | [12.38-24.93] | 33.12 | [24.77-63.57] | 1 | 100 |

**Table 5:** Knock-down time and mortality rates of *Anopheles* mosquitoes exposed to 0.05% λ-cyhalothrin for a period of 60 min

| Study sites | N | $KDT_{50}$ (min) | CI (95%) | $KDT_{90}$ (min) | CI (95%) | $KDT_{50}$ ratio (RR) | Mortality (%) |
|---|---|---|---|---|---|---|---|
| Dapaong | 111 | 104.54 | [76.53-196.1] | 374.31 | [187.86-1251.65] | 5.78 | 22.52 |
| Kara | 16 | - | - | - | - | - | 9.67 |
| Tchamba | 57 | 68.30 | [58.28-90.25] | 164.22 | [115.23-328.62] | 3.78 | 36.84 |
| Kpalimé | 128 | 133.72 | [98.61-232.41] | 512.62 | [279.25-1591.97] | 7.40 | 40.62 |
| Kovié | 107 | - | - | - | - | - | 14.95 |
| Akodesséwa | 84 | - | - | - | - | - | 22.61 |
| Kisumu | 96 | 18.07 | [14.08-22.06] | 33.67 | [27.15-47.89] | 1 | 100 |

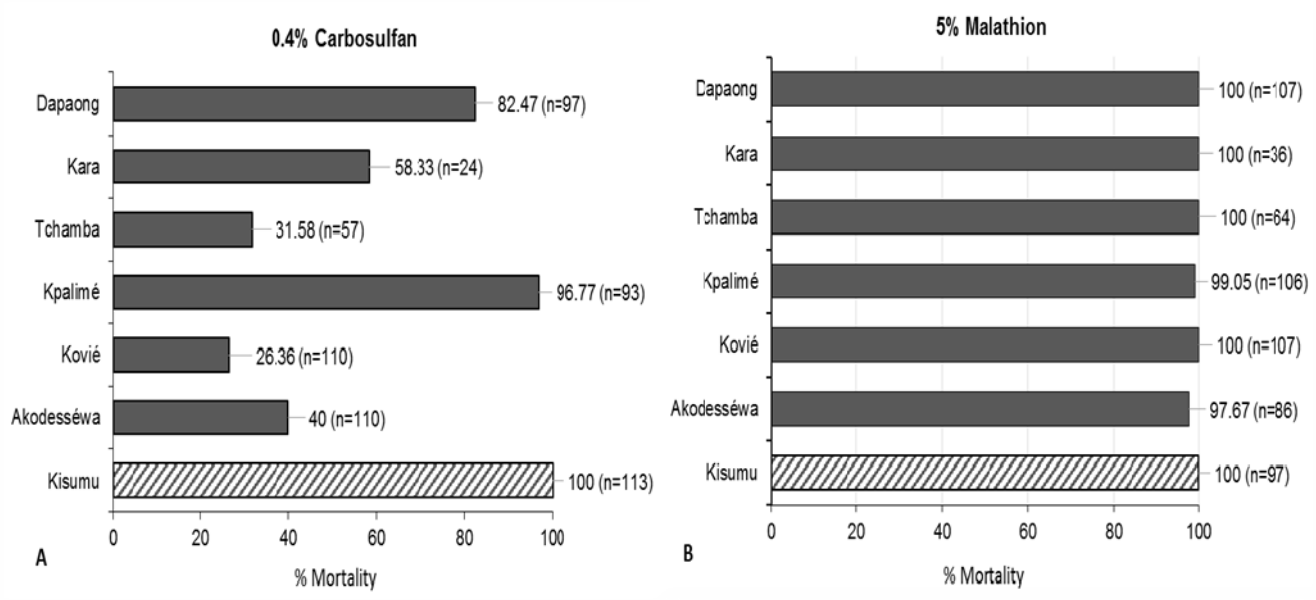


**Fig 2:** Susceptibility of *An. gambiae s.l.* to Carbosulfan (A) and Malathion (B) through the sentinel sites in Togo

Insecticide resistance is widespread and is now reported in nearly two thirds of countries with ongoing malaria transmission. The significant increase in insecticide-based malaria vector control in the past decade has resulted in increasing resistance among malaria vectors because of the selection pressure placed on resistance genes [12]. In 2002, a countrywide survey showed resistance among *Anopheles* populations in Togo [13] and in 2009, Ahadji-Dabla *et al.* [5] reported DDT and pyrethroids resistance in the southern part of the country.

To our knowledge, this study is the first one showing a large scale insecticide resistance detected at a high level. A nationwide survey made in Benin, a neighboring country showed that resistance to permethrin and DDT was widely distributed in *An. gambiae s.l* [22]. Malathion performed better than pyrethroids and DDT in all the sites. This is an accordance with the results reported by Kudom *et al.* [23] in Ghana (West Africa). A nationwide insecticide susceptibility tests carried out in 13 sentinel sites in Zimbabwe (Southern Africa) also showed 100% mortality in *Anopheles gambiae* populations exposed to malathion [24].

In Togo, the malaria vectors are primarily controlled by the use of LLINs. According to the World Health Organization, resistance is not an immediate threat so vector control is still effective. This action is in two points: 1) Monitoring action that is to a) conduct frequent susceptibility tests to confirm that there is no resistance emerging, b) monitor closely the quality and coverage of vector control interventions, which could be responsible for the increase in malaria cases (if there is an increase in confirmed malaria cases); c) check and if necessary reinforce epidemiological surveillance and 2) Vector control action. Concerning this second action, it is important to ensure system for timely replacement of worn-out nets and assure the quality and extent of LLIN coverage [12].

In this study, only susceptibility tests were done to confirm the existence of resistance in *An. gambiae s.l.* There is a need to conduct frequent and extensive susceptibility tests to monitor any increase in resistance and check whether metabolic resistance is present using bio-chemical and molecular testing methods as recommended by the WHO [12].

## 5. Conclusion

The results found in this study showed resistance in *An. gambiae s.l* particularly to DDT and pyrethroids considering the low mortality rates and the high knockdown time ratios recorded. Only malathion exhibited high mortality rates. These data can be used by the NMCP for a good decision making in malaria vectors control as recommended the World Health Organization.

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