SUSCEPTIBILITY STATUS OF MALARIA VECTOR ANOPHELES GAMBIAE IN SETTLEMENT VILLAGES OF SOUTHWESTERN ETHIOPIA

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ABSTRACT

Long-lasting insecticidal treated nets (LLINs) and indoor residual spraying (IRS) are the main vector control interventions in Ethiopia for malaria control. This study evaluates the susceptibility status of principal malaria vector *Anopheles gambiae* sensulato against the most widely used insecticides in the study areas. Mosquito larvae were collected by using a standard dipper from breeding sites, including rain water collection pools, road potholes, hoof prints around swamps at Bena and Kusaye villages from May up to June 2018 in Darimu district. Insecticide susceptibility bioassays were performed according to standard guidelines. The findings revealed that *A. gambiae* were found to be resistant to malathion (5%), deltamethrin (0.05%), and DDT (4%) but susceptible to propoxur (0.1%) and bendiocarb (0.1%). Knocked down time at 50 min (KDT50) and at 90 min (KDT90) values of DDT were very high—59.37 (57.37–73.46) and 110.59 (96.25–135.09), while (KDT50) and (KDT90) values of malathion was from 77.42 (69.32–90.48) to 125.58 (108.15–154.78), respectively. Since the current study was conducted in the newly formed resettlement area, almost similar trends of resistance to DDT, malathion and deltamethrin were observed.

Key words: Anopheles gambiae, susceptibility, malathion, DDT, propoxur, cypermethrin, bioassays, KDT50, KDT90, toxicity

In Ethiopia the main strategies employed by the National Malaria Control Program have been the use of insecticide treated mosquito nets (ITNs); indoor residual spraying (IRS); accurate diagnosis and prompt treatment with artemisinin-based combination therapies (ACTs); and intermittent preventive treatment for pregnant women (IPTp) (Anonymous, 2019). The malaria vector control tools highly rely on indoor residual spraying (IRS), LLINs distribution long-lasting insecticidal nets (LLINs), which uses insecticides from four chemical classes (Anonymous, 2017; WHO, 2019). Insecticide resistance in the most important malaria vector *Anopheles gambiae* L., against insecticides including DDT (organochlorine), malathion (organophosphate), bendiocarb and propoxur (carbamates) and alpha-cypermethrin, cyfuthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin (pyrethroids) is documented in different malaria endemic areas of Ethiopia (Messenger et al., 2017; Tesfahuneygn and Gebreegziabher, 2018).

Insecticides used for agricultural purposes played essential role in the development of resistance in malaria vector and most insecticides used were of the same chemical classes such as DDT and pyrethroids as these have similar modes of action and thus select for resistance in mosquitoes (Toe et al., 2014; Nkya et al., 2014). DDT spraying was discontinued in 2009 and replaced by deltamethrin. Furthermore, in 2012 Ethiopia switched from deltamethrin to bendiocarb in response to the observed resistance (WHO, 2012; 2019; Toe et al., 2014). Currently distributions of LLINs impregnated with pyrethroids together with annual rotation of bendicarb, propoxur, and primiphose methyl are widely employed (WHO, 2012; 2019). In the study site of the present study, like other parts of the country both IRS and LLIN impregnated with insecticides have been widely distributed by malaria vector control program. On the study site there is no documented information so far on the insecticide resistance, in particular in the resettlement area of Bena and Kusaye villages Southwest Ethiopia. This study investigates the susceptibility status of local malaria vector *An. gambiae* L. toward most widely used insecticides.

MATERIALS AND METHODS

The study was conducted from May up to June
2018 in Darimu district of the Southwestern part of Ethiopia, located 700 km away from Addis Abeba in Ilu Aba Bor administrative zones. The study area has two rainy seasons, June to September—the main rainy season and March to May—the small rainy season and receives between 1,300 and 1,800 mm of annual rainfall and has a mean annual temperature of 19°C. The district has a total of 42 kebeles (Smallest administrative unit in Ethiopia) among them Bena and Kusaye villages are resettlement sites since 2005. According to the clinical records from the health center malaria is common in both villages. Hence malaria prevention and intervention program are being implemented. This includes annual Indoor Residual Spray (IRS), Distribution of LongLasting Insecticides Treated nets (LLINs) and prompt treatment of malaria cases using Artemisinin based Combination Therapy (ACT) (Coartem) as the first line of treatments.

Mosquito larvae were collected using a standard dipper from breeding sites, including rain water collection pools, road potholes, hoof prints around swamps at Bena and Kusaye villages. Emerged pupae were sucked from the larval containers using a plastic pipette and placed in plastic cups inside the mosquito cages to prevent emerging adult mosquitoes from escaping. Emerged adult mosquitoes were identified morphologically using identification keys[12, 13]. (Gillies and Coetzee, 1987; Gillies and de Meillon, 1968).

Insecticide susceptibility bioassays were performed according to standard guidelines (WHO, 2016), at the Mettu University laboratory with discriminating dosages of five insecticides: DDT 4%, malathion 5.0%; deltamethrin 0.05%; bendiocarb 0.1 % and propoxur 0.1%, replicated five times, along with one untreated control, of 25 mosquitoes/ tube. Two to five-day old female mosquitoes were used for these bioassays. Exposed mosquitoes were monitored at time intervals of 10, 15, 20, 30, 40, 50 and 60 min, respectively, and the numbers knocked-down recorded. After exposure time, all mosquitoes were transferred to the holding tubes and provided with 10% glucose solution through a cotton wool. The mortality rates were determined at 24 hr post exposure. In each bioassay, a control experiment using papers impregnated only with insecticide carrier oil was performed in the same way as in treatment experiments. These tests were conducted in the laboratory under 25±2°C and 70-80%RH. Dead and surviving mosquitoes at the end of an experiment were kept in separate Eppendorf tubes suitably labeled (WHO, 2016).

Abbreviations used include: ACT: Artemisinin based Combination Therapy; Dichloro-Diphenyl-Trichloroethane (DDT); KT50: Down time at 50 min; KT90: Knock Down time at 90 min; WHO: World Health organization; FMOH: Federal Ministry of Health; LLIN: Long Lasting Insecticidal Net; PMI: President Malaria Initiative.

The 24 hr mortality rate (%) was established by counting the number killed at the end of the holding period (24 hr) divided by the total number of mosquito exposed times 100. Mortality range 98-100% indicates susceptible mosquito population; 90-97% suggests possible resistance that needs to be confirmed and below 90% indicates existence of resistance. The adult mosquito mortalities in control experiments were less than 5%, therefore no correction made by Abbott’s formula. Probit analysis was done using SPSS version 16 to estimate the 50% knockdown time (K\textsubscript{DT50}) and the 90% knockdown time (K\textsubscript{DT90}), which is the time taken to knockdown 50% and 90% of the exposed mosquitoes, as well as their 95% confidence interval. In analysis, number of mosquitoes knocked down was considered as response frequency. Total number of mosquitoes used/ test was considered as total number observed, insecticides were considered as covariates and time was considered as a factor.

RESULTS AND DISCUSSION

The knockdown effect of the evaluated insecticides against An. gambiae collected from Bena and Kusaye villages after 1 hr exposure are given in Fig. 1; 100% knockdown with propoxur, and bendiocarb was observed, and for deltamethrin it was 98%; while for DDT and malathion, it was 38% and 25% respectively. The mortality after 24 hr exposure, was 100% with propoxur and bendiocarb, while for deltamethrin, malathion and DDT it was 80, 75 and 38% respectively. Probit analysis for knock down activity time duration 60 min indicated that the least K\textsubscript{DT50} and K\textsubscript{DT90} values of 25.8 and 28.37 min was for malathion (Table 1; Fig. 2).

Local mosquitoes from Bena and kusaye villages were exposed to the insecticides recommended by World Health Organization Pesticide Scheme (WHOPES) using World Health Organization (WHO) tube bioassay. It was observed that bendiocarb (0.1%) and propoxur (0.1%) were the most effective; after 1 hr exposure 100% mortality was seen. Similar
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Fig. 1. Knockdown of female *An. gambiae* with insecticides

![Graph showing knockdown of female An. gambiae with insecticides](Image)

**Table 1. Knockdown rate for insecticides (1 hr exposure)**

<table>
<thead>
<tr>
<th>Insecticide (Conc.)</th>
<th>Slope (SE)*</th>
<th>KDT&lt;sub&gt;50&lt;/sub&gt; (95% CI)</th>
<th>KDT&lt;sub&gt;90&lt;/sub&gt; (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bendicarb (0.1%)</td>
<td>6.52 (0.47)</td>
<td>25.84 (0.22-37.02)</td>
<td>28.37 (19.63-48.96)</td>
</tr>
<tr>
<td>Malathion (5%)</td>
<td>2.97 (0.65)</td>
<td>77.42 (69.32-90.48)</td>
<td>125.58 (108.15-154.78)</td>
</tr>
<tr>
<td>Propoxur (0.1%)</td>
<td>3.23 (0.03)</td>
<td>26.41 (23.51-29.70)</td>
<td>57.20 (55.53-88.24)</td>
</tr>
<tr>
<td>DDT (4%)</td>
<td>3.68 (0.58)</td>
<td>59.37 (57.37-73.46)</td>
<td>110.59 (96.25-135.09)</td>
</tr>
<tr>
<td>Deltamethrin (0.05%)</td>
<td>5.30 (0.42)</td>
<td>16.23 (8.26-23.21)</td>
<td>(19.63-48.96)</td>
</tr>
</tbody>
</table>

*K<sub>D</sub>*<sup>50</sup> knocked down time of 50% individuals; K<sub>D</sub>*<sup>90</sup>, knocked down time of 90% individuals and 95% Confidence Interval (CI), respectively.

findings were reported by Nazaire et al. (2014) and Nnko et al. (2017). Additionally, PMI (Anonymous, 2017) confirmed that currently both insecticides can be considered as candidate insecticides for vector control activities in Ethiopia. DDT, malathion and deltamethrin gave <90% knockdown, comparable with study conducted in Southwest Cameroon (Boussougou-Sambe et al. (2017)). Thus, 24 hr exposure showed resistance as per the WHO standards to deltamethrin, malathion and DDT, as had been shown in Nigeria to deltamethrin (Umar et al., 2014; Awolola Samson et al., 2014), and to malathion (Kristan et al., 2003; Sharp et al., 2007).

Deltamethrin resistance of Anopheline mosquitoes in the recent years may be due to its impregnation on long lasting insecticidal net (LLIN) (Anonymous, 2016; 2017). All these activities might have increased the selection in malaria vector to this class of insecticides. This is in agreement with (Ranson et al., 2011; Oyewole et al., 2018). Additionally, pyrethroids based aerosols and coils are used for control of mosquitoes.
and domestic pests and it might contribute to the development of resistance as reported by Kristan et al. (2003). The resistance pattern of the local mosquito species toward Malathion as observed in this study can be attributed to the agricultural activities in the area where malathion (5%) are used extensively for pest control specifically on chat (Chat edulis) and maize (Zea mays) plantation. Previous studies also associated resistance to malathion (5%) with the intensity of agricultural practices (Kristan et al., 2003; Ranson et al., 2011). Furthermore, K_{DT50} and K_{DT90} values of DDT were very high- from59.37 (57.37-73.46) to 110.59 (96.25-135.09) while K_{DT50} and K_{DT90} values of malathion was 77.42 (69.32-90.48) to 125.58 (108.15-154.78) which means 59.37 min is needed for knocked down 50% of An. gambiae and 110.59 min are required for 90% knock down of An. gambiae using DDT while 77.42 and 125.58 min were required for 50% and 90% knock down. Study conducted in Nigeria showed that the K_{DT50} and K_{DT90} values for DDT were very high, ranging between 189.56 (CI: 94.97-272.66) and 234.06 (CI: 104.70-356.80), respectively (Oyewole, 2018).

Since the current study was conducted in newly formed resettlement area, almost similar trends of resistance status of local malaria vector An. gambiae L. to DDT, malathion and deltamethrin were observed, and propoxur and bendiocarb are still better.

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