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Larvicidal activities of Biostop Moustiques®, a botanical insecticide on field collected malaria mosquito *Anopheles gambiae* sensu lato in Togo

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ABSTRACT

The larvicidal activity of a botanical insecticide, Biostop Moustiques® (BM), was studied on field collected larvae of *Anopheles gambiae* s.l at the concentrations of 1, 5, 10 and 20 mL/L of water. The samples were made based on three different localities of Togo: Lomé, Kovié and Kolokopé. BM had high mortality rates at 20 mL/L in all instars larvae. Specifically, significant high mortality rates were recorded in L3 and L4 from field collected strain at a very low concentration (1 mL/L), compared to laboratory strain. In general, this study showed that the botanical insecticide tested has almost the same larvicidal effect no matter the strains of *Anopheles gambiae* used.

Keywords: Biostop Moustiques®, Larvicidal activity, *Anopheles gambiae*, Togo.

INTRODUCTION

Malaria is a very serious public health concern worldwide. WHO estimates at 207 million the number of malaria cases worldwide in 2012 with 627 000 deaths. Most cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) were children under 5 years of age (WHO, 2013). In Togo, it was reported that in 2009, 19% of people hospitalized for malaria cases died (PNLP, 2009).

In West Africa, especially in Togo, *Anopheles gambiae* is the major vector of malaria transmission (Ahadji-Dabla et al., 2014). The control of this vector is done through different strategies including the use of insecticide treated nets (ITNs), indoor residual spraying (IRS) and in some specific settings, larval control. In Togo, malaria prevention interventions are specifically done through the distribution of ITNs but this strategy is facing vector resistant problem. *Anopheles gambiae* has developed genetic and metabolic resistant to Pyrethroids which are the most common insecticides used to impregnate ITNs (Ketoh et al., 2009; Ahadji-Dabla et al., 2014). Another drawback with the use of these chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment (Omena et al., 2007). Plant products have been used traditionally in many parts of the world against the vectors and the species of insects. Some of them are still being used extensively throughout rural communities in Benin (Abagli and Alavo, 2011), Tanzania (Kweka

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et al., 2011), Côte d'Ivoire (Azokou et al., 2013). Plant extracts and phytochemicals derived from plants can act as larvicides, insect growth regulators, and repellents (Prajapati et al., 2005; Prabhu et al., 2011; Alagarmalai et al., 2012; Azokou et al., 2013). In this study, we investigate the larvicidal activities of Biostop Moustiques® (BM), a botanical insecticide against field collected *Anopheles gambiae* s.l compared with the laboratory strains.

MATERIALS AND METHODS

Sites of larvae collection

The field larvae of *An. gambiae* s.l were collected in Lomé, Kovié and Kolokopé (Figure 1), Lomé and Kovié being part of the Southern coastal region (Région Maritime) with an annual rainfall of about 1,000 mm; the mean temperature is 28 ºC. Kovié is a rural setting located in an area where irrigated rice-growing is the main agricultural activity. Mosquitoes were sampled near the Zio River in a rice field (N 06°20'87''; 01°7'24'' E). Lomé, the capital city of Togo is the urban setting located on the Gulf of Guinea (06° 07'30''N; 01° 13'34''E) and covers an area of 333km^2. In the outskirts of Lomé, insecticides are essentially used for market gardening (Mondédji, 2010).The climate conditions in Lomé are similar to those in Kovié. Kolokopé (07°47'59'N – 01°18'00E) is a cotton growing area with an approximate production of 1000 t per year. To protect their farms, farmers use a lot of insecticides to spray the field. The village is located in the "Région des plateau" and at 200 km from Lomé. It is characterized by a long rainy season from March to October and a dry season from November to February. The annual rainfall is estimated at 1300-1500 mm per year.

Insecticide and tested organisms

The insecticide used in this study was Biostop Moustiques® (BM), 100% natural oil from coconut and other foodstuffs. Its production method has been developed through researches in France since 1992 and was used as cutaneous insect repellent (Sorge et al., 2011). This biocide was provided by courtesy INVESTEKGROUP Company, Lomé (Togo).

Field collected *An. gambiae* s.l and two laboratory strains of *An. gambiae* s.s were used for the experiments. In the field, the larvae were collected using dipping method, conditioned in a cool box and brought to the laboratory for rearing. The laboratory strains constituted of Kisumu, a susceptible strain, and Acerkis, a resistant strain. Both larvae were reared under laboratory conditions (27 ± 2°C; 70-75% relative humidity), under 12L (Light): 12D (Dark) photoperiod cycles. The larvae were fed with TetraMin® Baby Fish food (from Tetra, Avignon, France). Second, third and fourth instars larvae were used for the larvicidal bioassay.

Larvicidal bioassay

The larvicidal activity of BM was assessed according to the WHO standard protocol with slight modification (WHO, 2005). Ten larvae of second (L2), third (L3) and fourth (L4) instars were separately introduced into plastic cups containing 100 mL of BM solutions at the concentration of 0, 1, 5, 10 and 20 mL/L in water containing 10 mg TetraMin® Baby fish food. Six replicates were made for each concentration and the whole experiment was independently repeated three times. The controls were not treated with BM. Mortality rates were recorded after 24 h of exposure. Larvae were considered as dead if they failed to move after probing with a needle on the siphon or cervical region.

Data analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). Data were analyzed using one-way analysis of variance (one-way ANOVA) and a Tukey's multiple comparison was used as a post test. A *p*-value < 0.05 was considered statistically significant. Data were expressed as means ± standard deviations (SD).

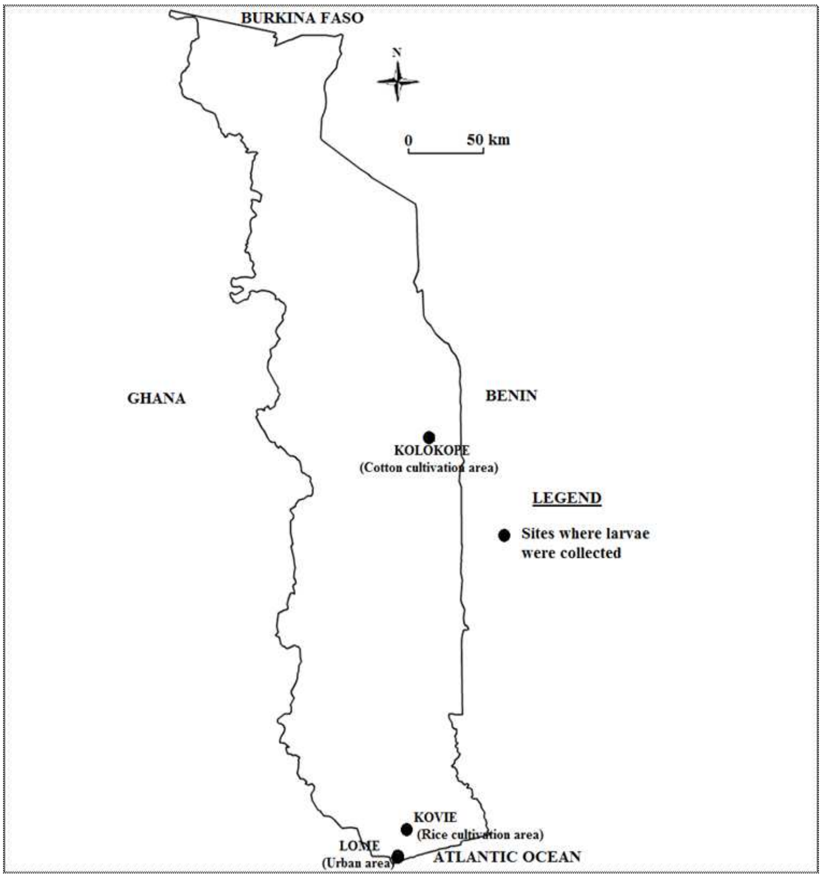

Figure 1: Map of Togo showing the sites where the larvae were collected.

RESULTS

Larvicidal activity of Biostop Moustiques® on laboratory strains. In second instars larvae (L2) (Fig.2), the lowest concentration of 5 mL/L induced 22.2 ± 2.6% and 13.0% ± 0.0 mortality in Kisumu and Acerkis strains, respectively. At 10 mL/L, 52.8 ± 1.9% and 23.9 ± 1.0% mortality were recorded in Kisumu and Acerkis strains, respectively. At these two concentrations, mortalities were significantly higher in Kisumu than in Acerkis ($F = 2.26.10^3$, df = 9, $p < 0.001$). At 20 mL/L, 100% mortality was observed in Kisumu strain and no significant difference was recorded in Acerkis strain (p = 0.181).

In third instars larvae (L3) (Figure 3), 11.7 ± 1.7% and 7.2 ± 1.0% mortality were respectively observed in Kisumu and Acerkis strains at 5 mL/L ($F = 3.5. 10^3$, df = 9, p = 0.009). At 10 mL/L, 73.3 ± 2.9% and 53.9 ± 1.9% mortality were observed in Kisumu and Acerkis strains, respectively ($F = 3.5. 10^3$, df = 9, $p < 0.001$). At 20 mL/L, 100% mortality was induced in both strains.

In fourth instars larvae (L4), no difference was observed between strains at each concentration (F = 247.35, df = 9, p = 1.0) (Figure 4). The highest effects were elicited at 20 mL/L and were 91.0 ± 6.5% and 91.0 ± 1.7% mortality in Kisumu and Acerkis strains, respectively.

Larvicidal activity of Biostop Moustiques® on field collected strains

The larvicidal activity of BM on field collected larvae was dose-dependent regardless the locality. In L2, the mortality rates were 8.89 ± 0.96, 35.56 ± 0.96 and 31.6% at 5 mL/L and 46.67 ± 1.67, 82.22 ± 1.67 and 100% at 20 mL/L in Lomé, Kovié and Kolokopé respectively (Figure 2). In L3, they were 16.67 ± 5.77, 96.67 ± 5.77 and 20% at 5mL/L and 93.89 ± 1.92, 100 and 91.11 ± 0.96% at 20 mL/L in Lomé, Kovié and Kolokopé respectively (Figure 3). In L4, the mortality rates were 93.33 ± 11.55, 90 ± 10 and 100% at 5 mL/L and 100% at 20 mL/L in Lomé, Kovié and Kolokopé respectively (Figure 4).

When compared to laboratory strains, field collected fourth instars larvae of *An. gambiae* were highly susceptible to BM at all concentrations ($p < 0.05$). At 20 mL/L, mortality rates in L2 (Kolokopé) and L3 (Kovié) were comparable to that of Kisumu and Acerkis ($p > 0.05$). Considering both strains, mortality rates were superior to 90% at 20 mL/L in L3 and L4.

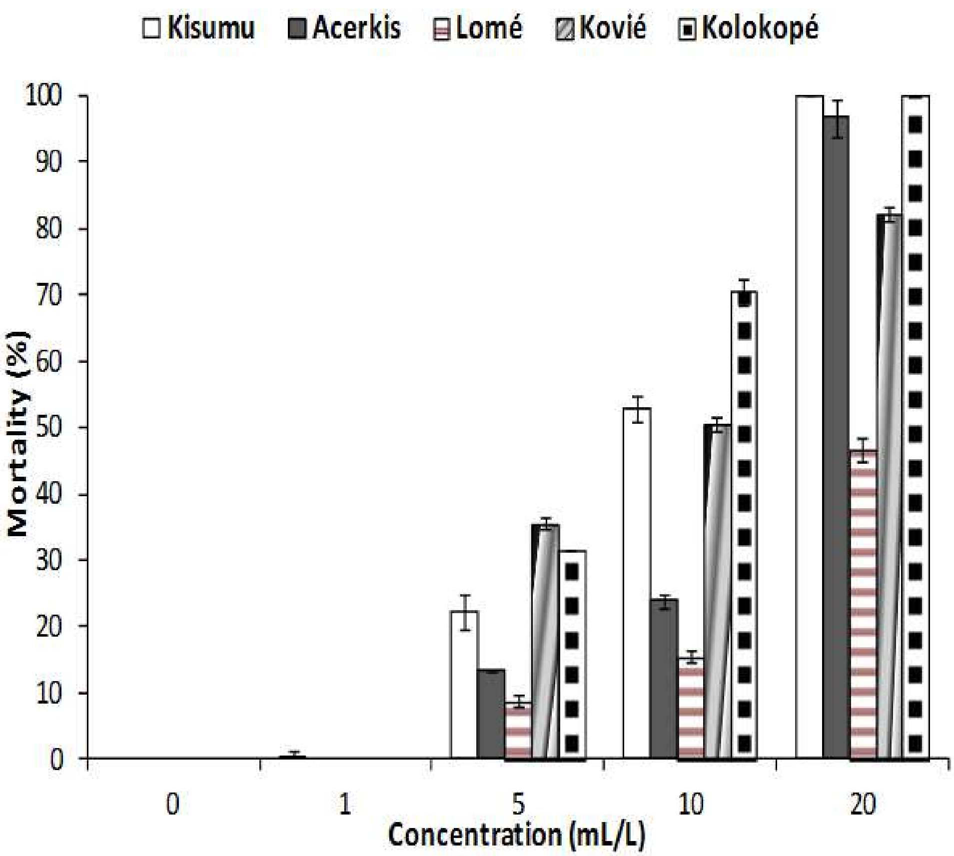

Figure 2: Larvicidal activities of Biostop Moustiques® on different strains of second instars larvae.

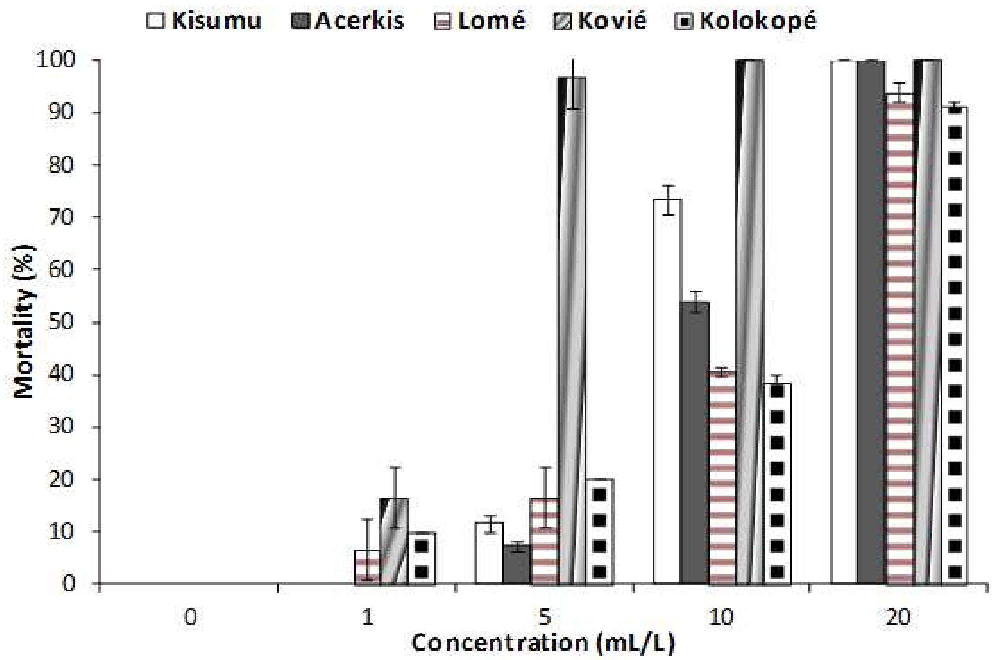

Figure 3: Larvicidal activities of Biostop Moustiques® on different strains of third instars larvae.

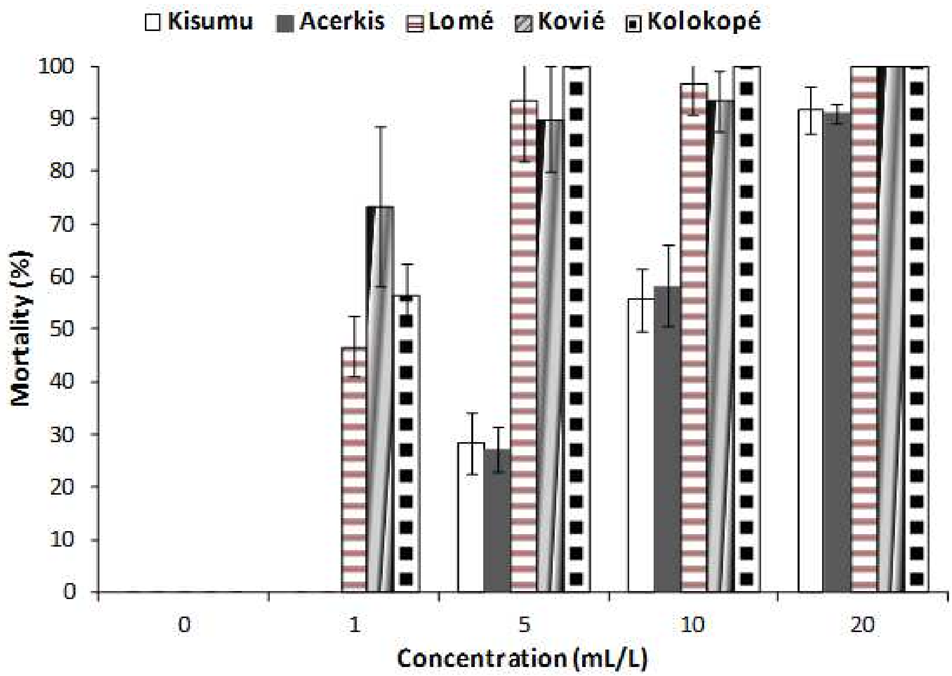

Figure 4: Larvicidal activities of Biostop Moustiques® on different strains of fourth instars larvae.

DISCUSSION

In this study, the BM oil showed a larvicidal effect against different stages of *Anopheles gambiae* from three localities. According to Ahadji-Dabla et al. (2014), this wild population of *Anopheles gambiae* has developed some genetic resistance against synthetic insecticides such as pyrethroids. BM exhibits an interesting larvicidal activity with the same effect especially on L3 L4 at 20 mL/L both in laboratory and field collected strains of the malaria vector *An. gambiae*. At concentration < 20 mL/L, it induces different effect on the two strains. BM presents different toxicity profiles in L2 and L3, with a higher toxicity at low concentrations in some of field collected strains (Kolokopé in L2 and Kovié in L3). Besides, observation of the dead larvae showed some warping and necrosis of tissues. The insecticidal activity of different plant extracts could have important implications in mosquito larvae control. However, further researches are needed to determine the effectiveness of biocide on natural breeding sites of *An. gambiae*. Nevertheless, BM derived from coconut oil can be supposed to have non-toxic effect on human being and then need to be considered in search for alternatives to conventional insecticides.

It was demonstrated that plants extracts contain secondary metabolites that have insecticidal, antifeedant or repellent activity in insects of agricultural and medical importance (Rahuman et al., 2008; Shivakumar and Kataria, 2011; Prabhu et al., 2011). However, extracts from certain plants such as *Persea americana* or *Azadirachta indica* can elicit cell and tissue lysis and impairment of hormonal secretion in mosquitoes (Koua et al., 1998; Seye et al., 2006).

Some studies seem to indicate a low toxicity of plant extracts on non-target species. It has been demonstrated that some plant extracts exhibit larvicidal properties but an absence of toxicity to vertebrates such as fishes or mice (Promsiri et al., 2006; Shivakumar and Kataria, 2011).

Conclusion

In this study, the larvicidal activity of Biostop Moustiques® was evaluated on field collected larvae *An. gambiae*. The results showed the efficacy of this insecticide even at low concentrations. It can be considered like one of the alternatives of synthetic insecticides and be successfully used against malaria vectors in Togo.

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REFERENCES

Abagli AZ, Alavo TBC. 2011. Essential oil from bush mint, *Hyptis suaveolens*, is as effective as DEET for personal protection against mosquito bites. *Open Entomol. J.*, **5**: 45–8.

Ahadji-Dabla KM, Ketoh GK, Nyamador WS, Apétogbo GY, Glitho IA. 2014. Susceptibility to DDT and pyrethroids, and detection of knockdown resistance mutation in *Anopheles gambiae sensu lato* in Southern Togo. *Int. J. Biol. Chem. Sci.*, **8**(1): 314-323.

Alagarmalai J, Selvaraj P, Kuppusamy E. 2012. Biological activities of *Solanum pseudocapsicum* (Solanaceae) against, cotton bollworm, *Helicoverpa armigera* Hübner and armyworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Asian Pac. J. Trop. Biomed.*, **5**: 1–5.

Azokou A, Koné MW, Koudou BG, Tra Bi HF. 2013. Larvicidal potential of some plants from West Africa against *Culex quinquefasciatus* (Say) and *Anopheles gambiae* Giles (Diptera: Culicidae) *J. Vector Borne Dis.*, **50**: 103–110

Ketoh GK, Ahadji-Dabla KM, Dery D, Chabi J, Glitho IA, Brengues C, Chandre F, Corbel V, Hougard J M, Fontenille D.

2009. Seasonal distribution and insecticide resistance in *Anopheles gambiae* s.l in Lomé. Poster, 5th MIM Conference.

Koua HK, Han SH, d'Almeida M-A. 1998. Histopathology of *Anopheles gambiae* s.l Giles, 1902 (Diptera, Culicidae) subjected to the larvicidal activity of the aqueous extract of *Persea americana* Miller, 1768 (Lauraceae). *Bull. Soc. Pathol. Exot.*, **91**: 252-256.

Kweka EJ, Nyindo M, Mosha F, Silva AG. 2011. Insecticidal activity of the essential oil from fruits and seeds of *Schinus terebinthifolia* Raddi against African malaria vectors. *Parasit. Vectors*, **4***:*129.

Mondédji AD. 2010. Potentiel d'utilisation d'extraits de feuilles de neem (*Azadirachta indica* A. Juss) et de *Carica papaya* L. dans le contrôle des insectes ravageurs du chou (*Brassica oleracea* L.) en zones urbaines et périurbaines au sud du Togo. Thèse de doctorat, Université de Lomé, Togo, p. 193.

Omena MC, de Navarro DMAF, Paula de JE, Luna JS, Ferreira de Lima MR, Sant'Ana AEG. 2007. Larvicidal activities against *Aedes aegypti* of some Brazilian medicinal plants. *Biores. Technol.*, **98**: 2549–2556.

Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. 2011. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac. J. Trop. Biomed.*, **1**: 124-129.

PNLP. 2009. Programme National de Lutte contre le Paludisme Ministère de la Santé du Togo. Rapport d'activités, 60p.

Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS. 2005. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Biores. Technol.*, **96**: 1749–1757.

Promsiri S, Naksathit A, Kruatrachue M, Thavaara U. 2006. Evaluation of larvicidal activivty of medicinal plants extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on non-target fish. *Insect Sci.*, **13**: 179-188.

Rahuman AA, Venkatesan P, Gopalakrishnan G. 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol. Res.*, **103**: 1383–1390.

Seye F, Ndione RD, Ndiaye M. 2006. Etude comparative de deux produits de neem (huile et poudre) sur les stades préimaginaux du moustique *Culex quinquefasciatus* (Diptera: Culicidae). *Afrique Sci.*, **2**: 212-225.

Shivakumar MS, Kataria R. 2011. Comparative efficacy of Azadirachtin on larval population of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae) in Gujaret, India. *Int. J. Pharm. BioSci.*, **2**: 41-47.

Sorge F, Tamburro M, de Pertat, T, les membres du Groupe de pédiatrie tropicale. 2011. Usages et effets des insectifuges cutanés chez les nourrissons lors de l'épidémie de chikungunya à La Réunion en 2005-2006 : étude INR 2009. *BEH* 6/22 février 2011.

WHO. 2005. *Guidelines for Laboratory and Field Testing of Mosquito Larvicides.* WHO/CDS/WHOPES/GCDPP/13.

WHO. 2013. *Test Procedures for Insecticide Resistance Monitoring in Malaria Vectors Mosquitoes*. World Health Organization: Geneva; 40.