Cameroon Journal of Biological and Biochemical Sciences, 2025, 33, 52-63.
DOI: https://doi.org/10.63342/cjbbs2025.33.02

CAMEROON JOURNAL OF BIOLOGICAL AND BIOCHEMICAL SCIENCES
Published by the Cameroon Biosciences Society (CBS)

Research Article

Status of domestic anti-mosquito control tools against resistant *Anopheles gambiae* s.l. and *Culex* mosquitoes from the city of Kribi, South Cameroon

***EKOKO WOLFGANG E.**[*1,2], **DOUMBE-BELISSE P.L.**[2], **MANDENG S.E.** [2,3], **TOTO J.C.**[2], **BAHEBEGUE SAMUEL**[2] and **AWONO-AMBENE H.P.**[2]*

[1] Higher Teacher Training College, Department of Biology, University of Bamenda, P.O. Box 39, Bambili, Cameroon
[2] Institut de Recherche de Yaoundé (IRY), Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), P.O Box 288 Yaoundé, Cameroun
[3] Faculty of Sciences, University of Yaoundé I, P.O. Box 337, Yaoundé, Cameroon

** Corresponding author: ewolfgang388@gmail.com*

Received: 21 Jan 2025, Reviewed: 15 Mar 2025, Revised: 09 Apr 2025, Accepted: 14 Apr 2025, Published: 08 May 2025

ABSTRACT

Communities living in the tropics use insecticide products to prevent or control mosquito nuisance in general. In Cameroon, these products are used at home without evidence of their efficacy against targeted mosquito populations. In this paper, we present a sample of anti-mosquito tools used by households in the city of Kribi, and their efficacy versus local *Anopheles* and *Culex* mosquitoes. The study protocol included: i) a survey in 500 randomly selected households using a questionnaire; ii) mosquito larvae and pupae collections in breeding sites and rearing; iii) WHO cone and susceptibility bioassays were conducted, respectively, using reared *An. gambiae* s.l. and *Culex* mosquitoes; and iv) molecular identification of members of the *An. gambiae* complex via PCR. The household survey revealed that long-lasting insecticidal nets (LLINs) (89%), insecticide aerosols (51.5%), or insecticide coils (35.3%) were the most used, as anti-mosquito tools. The aerosols provided optimal efficacy against *An. gambiae* s.l. and *Culex* mosquitoes with 98.6% and 100% mortality, respectively. Coils showed minimal efficacy (78.7%) to no efficacy (27.1%), respectively, against *Anopheles* and *Culex* mosquitoes, and LLINs showed no efficacy (25.6%) against *Anopheles* mosquitoes (P<<0,005). *An. gambiae* and *Culex* mosquitoes equally showed resistance to deltamethrin (81.7% and 78.5%, respectively) and permethrin (34.3% and 27.1 respectively). Bioassays conducted with the insecticide bendiocarb demonstrated that *An. gambiae* s.l. was fully susceptible, achieving a mortality rate of 100%. These findings highlight the challenges of individual anti-mosquito control measures in Kribi thus, there is a need for an integrated mosquito control approach involving effective household anti-mosquito tools to complement the use of LLINs, in the context of increasing insecticide resistance in natural mosquito populations.

Key words: Efficacy, anti-mosquito tools, insecticide resistance, mosquitoes, Kribi.

RÉSUMÉ

Les communautés vivant sous les tropiques utilisent des produits insecticides pour prévenir ou contrôler la nuisance des moustiques en général. Au Cameroun, ces produits sont utilisés à domicile sans que leur efficacité contre les populations de moustiques ciblées ne soit démontrée. Dans cet article, nous présentons un échantillon d'outils anti-moustiques utilisés par les ménages dans la ville de Kribi, et leur efficacité contre les moustiques locaux Anopheles et Culex. Le protocole de l'étude comprenait : i) une enquête dans 500 ménages sélectionnés au hasard à l'aide d'un questionnaire ; ii) des collectes de larves et de nymphes de moustiques dans des sites de reproduction et d'élevage ; iii) des essais biologiques de cône et de sensibilité de l'OMS ont été menés, respectivement, en utilisant des moustiques *An. gambiae* s.l. et Culex élevés ; et iv) l'identification moléculaire des membres du complexe *An. gambiae* via la PCR. L'enquête auprès des ménages a révélé que les moustiquaires imprégnées d'insecticide de longue durée (MIILD) (89%), les aérosols d'insecticide (51,5%) ou les serpentins d'insecticide (35,3%) étaient les outils anti-moustiques les plus utilisés. Les aérosols ont fourni une efficacité optimale contre *An. gambiae* s.l. et les moustiques Culex avec 98.6% et 100% de mortalité respectivement. Les serpentins ont montré une efficacité minimale (78,7 %) à nulle (27,1 %), respectivement, contre les moustiques Anopheles et Culex, et les MILD n'ont montré aucune efficacité (25,6 %) contre les moustiques Anopheles (P<<0,005). Les moustiques *An. gambiae* et Culex ont également montré une résistance à la deltaméthrine (81,7 % et 78,5 %, respectivement) et à la perméthrine (34,3 % et 27,1 respectivement). Les bioessais réalisés avec l'insecticide bendiocarbe ont montré qu'*An. gambiae* s.l. était totalement sensible, atteignant un taux de mortalité de 100 %. Ces résultats soulignent les défis des mesures individuelles de lutte contre les moustiques à Kribi. Ainsi, il y a un besoin pour une approche intégrée de lutte contre les moustiques impliquant des outils efficaces de lutte contre les moustiques dans les ménages pour compléter l'utilisation des MILD, dans le contexte d'une résistance croissante aux insecticides dans les populations naturelles de moustiques.

Mots clés : Efficacité, outils anti-moustiques, résistance aux insecticides, moustiques, Kribi.

1. BACKGROUND

Vector-borne diseases (VBDs) are infections caused by pathogens that are transmitted by arthropods such as mosquitoes, triatomine bugs, blackflies, tsetse flies, sand flies, lice, and ticks. They exert a huge burden of morbidity and mortality worldwide, particularly affecting the poorest of the poor populations living in the tropics and subtropics (WHO 2017). Perhaps the best-known VBD are mosquito-borne diseases, principally malaria, which is a major cause of morbidity and mortality, particularly in Sub-Saharan Africa (SSA), with approximately half the world's population predicted to be at risk of malaria (WHO, 2023). The principal method by which mosquito-borne diseases are controlled is through vector control, which has a long and distinguished history. Vector control is the major pillar of mosquito prevention, and its main target is to limit or prevent human-vector contact. It should be noted that the fight against nocturnal mosquitoes such as *Culex* Spp & *Mansonia* Spp has benefited from the intensive and extensive control of the malaria vector, *Anopheles* Spp.

Prevention and control mechanisms with respect to addressing the vector population largely depends on combining different methods such as chemical control (use of insecticides, repellents, larvicides), biological control (regulate Anopheles populations naturally via predation, parasitism, and competition), genetic control (which covers all methods by which a mechanism for pest or vector control is introduced into a wild population through mating), bio-pesticides (plant-derived substances to protect against insect vectors), environmental management by educating the populations on the need for environmental and personal hygiene especially in keeping the immediate surrounding clean with proper drainage system, and above all instigating a change in behaviour of the population at risk (Jamison *et al.* 2006). The direct output of a vector control activity must be an important reduction of the vectorial capacity. An integrated vector control program would incorporate the collection of local information about vector distribution and behavior to identify one or more control techniques that would be effective, affordable, and acceptable to local communities. The two broadly applicable measures for vector control are long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS).

The community-wide use of LLINs has been reported to reduce the vector population significantly, and when used by a majority of the target population (80%), it provides protection to everybody in the community, including those who do not themselves sleep under nets (Lengeler, 1998). As the LLINs shorten the mean mosquito life span, very few mosquitoes can survive long enough for the sporogonic cycle to be completed, thus reducing the transmission (Phillips-Howard *et al.* 2003). As the LLINs also inhibit mosquito feeding, the reproductive potential of highly anthropophilic vectors is also reduced. LLIN is the most technologically advanced form of treated net available today, which maintains efficacy without re-treatment for about 3–5 years, and represents an important innovation that could facilitate sustainable scale-up of mosquito prevention. LLINs act in three different ways; firstly, through provision of personal protection, by acting as a physical barrier between mosquitoes and the person sleeping under the net, secondly by reducing indoor biting by a combination of increased mosquito mortality, which is caused by the insecticide on the net and the reduction of mosquito house entry caused by the nets- excito-repellent properties (Lines *et al.* 1987; Lindsay *et al.* 1991). These properties combined lead to good protection and an even bigger reduction in transmission, producing a community effect where high population coverage is achieved (Maxwell *et al.* 2002; Hawley *et al.* 2003; Killeen *et al.* 2007; Le Menach *et al.* 2007). IRS is the application of a long-lasting, residual insecticide to potential mosquito resting surfaces such as internal walls, eaves, and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide. It is a powerful intervention to rapidly reduce adult mosquito vector density and longevity and, therefore, reduce vector-borne disease transmission. Also, the efficacy and persistence of residual insecticides vary with the type of surfaces sprayed (mud, wood, palm leaves, cement brick, etc), public acceptance and strong financial support. In these circumstances, the IRS can reduce the vector life span, vector population, and the number of humans bitten. Unfortunately, the availability of low-risk and cost-effective insecticides is diminishing due to increasing mosquito resistance and little development of new compounds over the past 20 years. The considerable resources required for IRS combined with the potential for the development of insecticide resistance and the possible environmental hazards, necessitate a strict justification for its use as a vector control measure.

Alongside these control tools, there has been an increased use of domestic chemical control products in Cameroon in different contexts such as human habitations, working sites, resting, and relaxing places, etc. These products are commonly referred to as household insecticides (Zaim & Jambulingam, 2004). Among these; insecticide aerosol sprays and coils are the major products the public has been using as supplementing protection measures outside bet net (Wang, 1993). Unfortunately, the effectiveness of these supplementing tools is difficult to address. Also, very little data are available on the bio-efficacy of different household products in both laboratory and field conditions.

Given that these products are already widely used, they would therefore be expected to have community acceptance if they were introduced as a formal tool against mosquito control in an integrated vector management (IVM) strategy to address residual malaria transmission and certain VBDs. Thus, such information could help to better design and monitor vector control programs. The current study, therefore, presents a sample of anti-mosquito tools used by households in the city of Kribi and their efficacy against local *Anopheles* and *Culex* mosquitoes, as well as their insecticide susceptibility status.

2. METHODS

2.1. Study area

The study was conducted in the city of Kribi (2°56' 6.00" N; 9°54' 36.00"E) (figure 1) from August 2016 to April 2017. Kribi is a city of about 80,957 inhabitants situated along the coast of the Atlantic Ocean in the Gulf of Guinea at 270 km West of Yaoundé, the capital city of Cameroon. Kribi is divided into three administrative units: Kribi 1, Kribi 2, and Lokoundje. It is a strategic point for maritime traffic in the Gulf of Guinea with its new seaport, and it is also the end point of the Chad-Cameroon petroleum pipeline. The city has a maritime equatorial climate with an average rainfall of about 2900 mm and humidity of about 80%. This high rainfall and humidity are kept constant throughout the year, making the city an area favourable for the breeding and development of mosquitoes. Four seasons can be distinguished: a long rainy season from mid-August to mid-November and a small rainy season from April to June; a long dry season from December to mid-March and a short dry season from June to mid-August. The mean temperature is about 25°C thus, the town of Kribi is a favourable site for mosquito proliferation, and malaria transmission is perennial, with a 40.6% morbidity rate. The main activities of the inhabitants are fishing, tourism, and trade.

2.2. Anti-mosquito tools survey

A survey was conducted in 500 randomly selected households from neighborhoods of the town of Kribi using a questionnaire to determine the different kinds of anti-mosquito products used for personal protection against mosquito bites by residents. Twenty questions were provided in this survey, including information on the head of household or its representative, household composition, and use and perception of household insecticide tools. We also gained information on the frequency at which residents purchase household insecticide products. All information collected was through personal contact from heads of households by the entomology team from OCEAC (Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale).

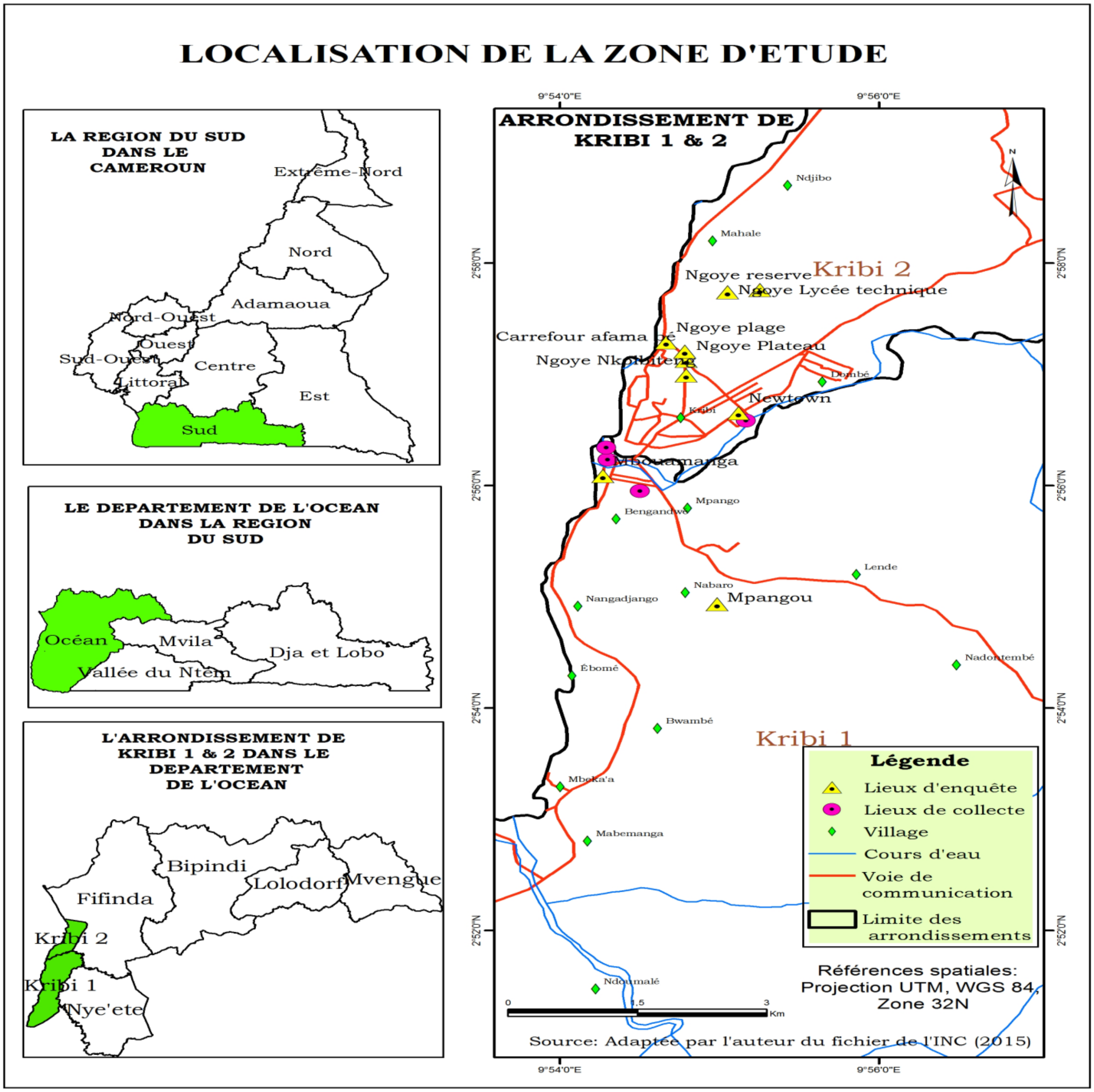

Figure 1: Localization of study site.

2.3. Mosquito larvae collections

Following an assessment of potential breeding sites in surrounding human habitat, mosquito larvae and pupae were collected via the dipping method (Service, 1993) in various aquatic habitats, i.e., pools, abandoned canoes, channel drains, puddles, and lowlands. In general, collected larvae from at least 30 breeding sites were pooled according to species and transported to the laboratory for rearing and to further perform bioassays.

2.4. Insecticide susceptibility tests

Bioassays were performed using WHO test kits and standard procedures (WHO, 2016) to monitor the susceptibility of 2-5-days-old wild F0 *An. gambiae* s.l and *Culex* populations to deltamethrin 0.05%, permethrin 0.75%, and 0.1% bendiocarb insecticides. Bioassays were carried out at 25 ± 2 °C and 75 ± 10 % RH.

2.5. Bioefficacy tests

Three categories of insecticide personal protection tools (coils, aerosols & LLINs) were evaluated. A representative tool from each category was tested via three standards WHO methods:

- WHO cone bioassay on five domestic used net samples (just one year of use) collected from the local population and replaced by new ones.
- Test cages as cubic plastic polystyrene boxes of dimension 270 mm L x 210 mm W x 175 mm H and volume 6 L with a well-fitted lid for the bioassays with aerosols and coils.

2.5. Bioassays for LLINs

2.5.1. Insecticide aerosol and coil testing

The two most represented commercial formulations of each tool were tested. These aerosols and coils were purchased from a local supermarket. The spray dose and flaming coil dose were determined by extrapolating the recommended dose of spraying in the air in a standard room for 10 seconds and the recommended dose of flaming in a standard room for eight to nine hours as recommended by producers (WHO, 2016). For the aerosols, the boxes were sprayed for one second while for coils, the coil was lit and allowed to burn for a few seconds. once the tip was able to smoulder, the flame was extinguished, and the flaming coil fitted to its metal holder was placed in the box for 50 seconds, then removed. 2-5 days non-blood fed F0 female mosquitoes (field and susceptible strain) previously held for 1h in holding cups were immediately inserted into the sprayed and smoked boxes, respectively for 3 minutes. After this time, cups were removed and observed for 1h in order to record knocked-down mosquitoes. The mosquitoes held in the cups were then provided with a 10% sugar solution, and mortality rates were recorded 24h post-exposure. This bioassay was conducted with four replicates of 25 mosquitoes alongside controls. Tests were carried out in separate rooms for the aerosols and coils to avoid contamination at temperatures ranging between 25-29°C and relative humidity of 68-80%.

2.5.2. Mosquito identification

Before bioassay tests, adult mosquitoes were morphologically segregated into Anophelines and Culicines. The Anophelines were identified as *An. gambiae* s.l. (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987) and a sub-sample of control, dead and alive mosquitoes from bioassay tests were then subjected to DNA extractions and further identified molecularly to species by PCR-RFLP (Collins *et al.* 1987).

2.6. Data analysis

Data processing and analysis were performed in Excel software. Efficacy of the anti-mosquito tools was estimated by means of mosquito knock-down rates 60 min post-exposure (KD 60min) and mortality rates 24h post-exposure to determine if they met the WHO efficacy requirements (WHO, 2016). The susceptibility status of the mosquito populations was also interpreted according to WHO (2016) procedures. The variations of KD 60min and mortality rates in the mosquito populations were analysed using the Pearson's Chi-square test of independence, with R software Version 2.15.2 (R Development Core Team, 2005).

3. RESULTS

3.1. Variety of anti-mosquito tools used for personal protection

The household survey (Figure 2) revealed that respondents protected themselves from mosquito bites mainly by using LLINs (89%), insecticide aerosols (51.5%), or insecticide coils (35.3%). Some respondents used the anti-mosquito tools singly or in combinations. A variety of other methods, including vaporizers, burning of incense, body repellants, etc., represented about 2% of the total anti-mosquito tools surveyed.

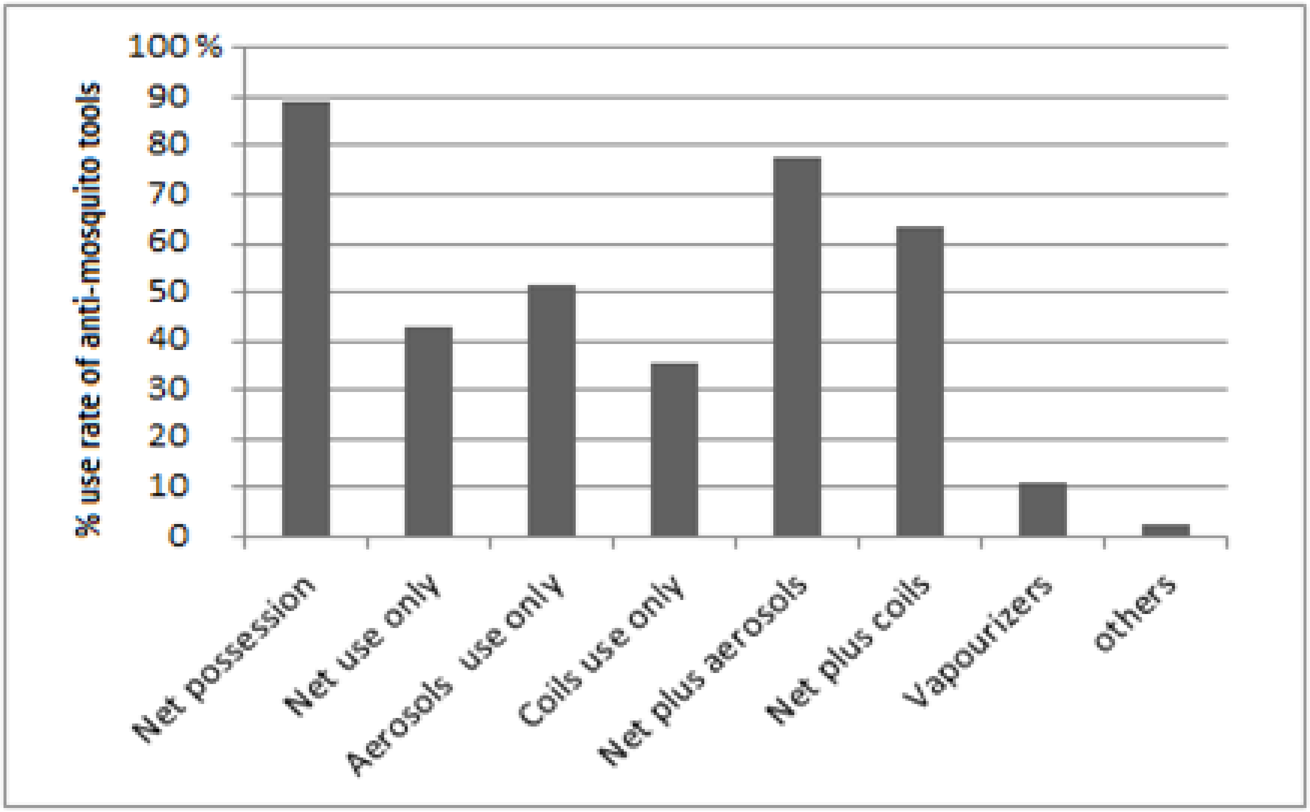

Figure 2: Anti-mosquito interventions used for personal protection in Kribi

3.2. Chemical composition of anti-mosquito products used for personal protection

Table 1: Active ingredients and formulations of anti-mosquito tools surveyed in Kribi.

Treatment code	Active ingredients	Formulation	Recommended usage dose
A1*	Permethrine 0,26% + Tetramethrine 0,20% + Peperonyl-butoxyde 0,2%	Aerosol	1 second discharge rate/m^3
A2*	Tetramethrine 0,25% + Cypermethrine 0,14%	Aerosol	1 second discharge rate/m^3
A3	Cyfluthrine 0,025% + Transfluthrine 0,04%	Aerosol	1 second discharge rate/m^3
A4	Transfluthrin 0,20% + D-phenothrine 0,046%	Aerosol	1 second discharge rate/m^3
A5	D-phenothrine 0,1% + Imiprothrine 0,04%	Aerosol	1 second discharge rate/m^3
C1*	Permethrine 0,26% + Tetramethrine 0,20%	Coil	12g for 8 hours of combustion
C2*	D-Allethrine 0,30%	Coil	12g for 9 hours of combustion
C3	D-trans-Alléthrine 0,26%	Coil	12g for 8 hours of combustion
C4	D-Allethrine 0,30%	Coil	12g for 9 hours of combustion
C5	Tetramethrin 0.25%	Coil	12g for 8 hours of combustion
N1*	Deltamethrin	LLIN	55mg/m^2
N2*	Permethrin	LLIN	700mg/m^2
N3*	Alpha-cypermethrin	LLIN	200mg/m^2

Codes marked with asterisk (*) are the products tested in the current study.

Table 1 summarizes the varieties of mosquito tools, their active ingredient as well as their formulations. A total of five varieties of aerosols, five varieties of coils, and three brands of LLINs were recorded in the current study. All of the aerosol formulations contained two synthetic pyrethroids, except for one (A1*), where a synergist (PBO) was recorded in addition. The coils contained just one synthetic pyrethroid as well as the LLINs. In total, 7 types of synthetic pyrethroids were recorded for aerosols, 3 types for coils, and 3 types recorded for LLINs.

Mosquito coils, priced at just 50 FCFA each, are the most affordable insecticidal products and are primarily sold in small shops and neighborhood stores. They are typically purchased daily. In contrast, aerosols, which cost between 800 and 1500 FCFA per unit, are generally purchased weekly and are more commonly available in supermarkets and small to large shops. Meanwhile, all the LLINs surveyed in the current study originated from the free mass distribution campaign conducted in 2015/2016 by the Cameroonian government.

3.3. Insecticide susceptibility status

A total of 534 wild *An. gambiae* s.l females aged 2-4 days were tested to assess their susceptibility profile to insecticide (255, 110, and 169 exposed to deltamethrin 0.05%, permethrin 0.75%, and bendiocarb 0.1%, respectively) and 204 female *Culex* exposed to deltamethrin 0.05%; N=105 and permethrin 0.75%; N=99. The Kisumu susceptible strain was used as a control. The data presented in this study indicate that pyrethroid resistance is well-established in both *An. gambiae* s.l. and *Culex* populations in Kribi, while *An. gambiae* s.l. remains susceptible to bendiocarb (Figure 3).

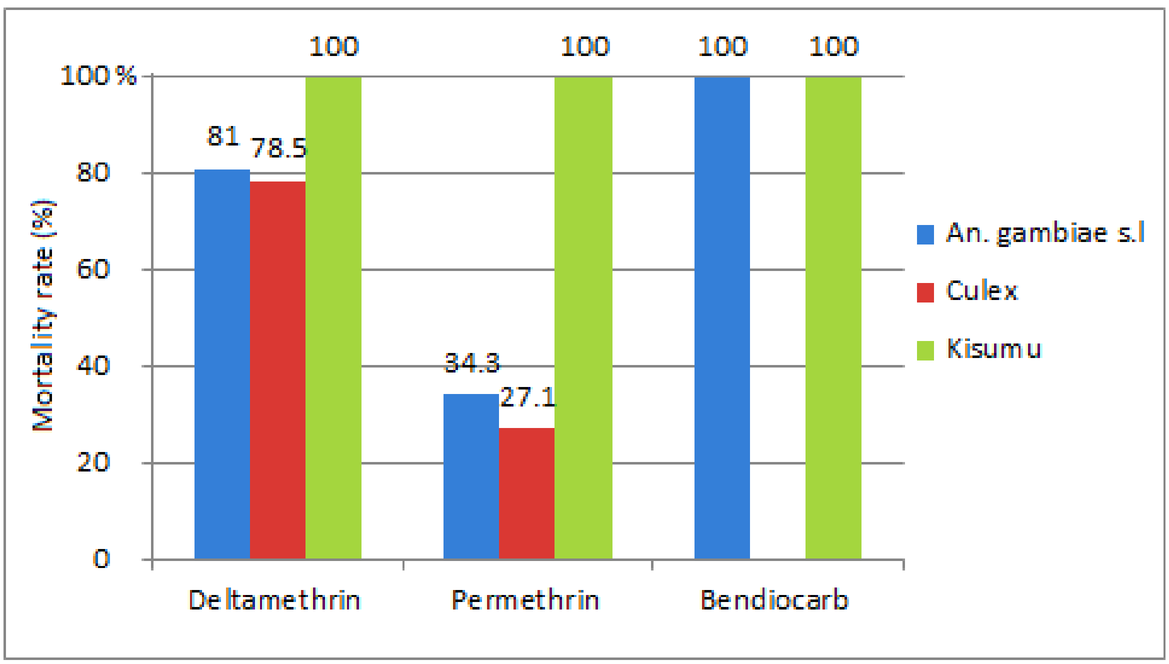

Figure 3: Mortality rates of mosquito populations exposed to insecticides in Kribi.

3.4. Bio-efficacy of the anti-mosquito tools used in the town of Kribi

A total of 1022 wild female *An. gambiae* s.l mosquitoes were used for the bio-efficacy bioassays; including 222 exposed to aerosols, 268 to coils, 514 to bed nets; 800 kisumu susceptible strain used as control (150, 150, 500 respectively for aerosols, coils and bed nets) and 117 *Culex* (62 to aerosols and 55 to coils). Figure 4 displays the average knockdown and mortality rates associated with the anti-mosquito tools. Only aerosols met the conditions for optimal efficacy both in *An. gambiae* and *Culex* populations, while coils presented minimal efficacy and the bed nets no efficacy. There was a significant difference (P =0,00023) between knockdown and mortality rates of bed nets versus aerosols and coils, the difference was again far greater (P =0,0001) between the field strain exposed to bed nets and the susceptible Kisumu strain.

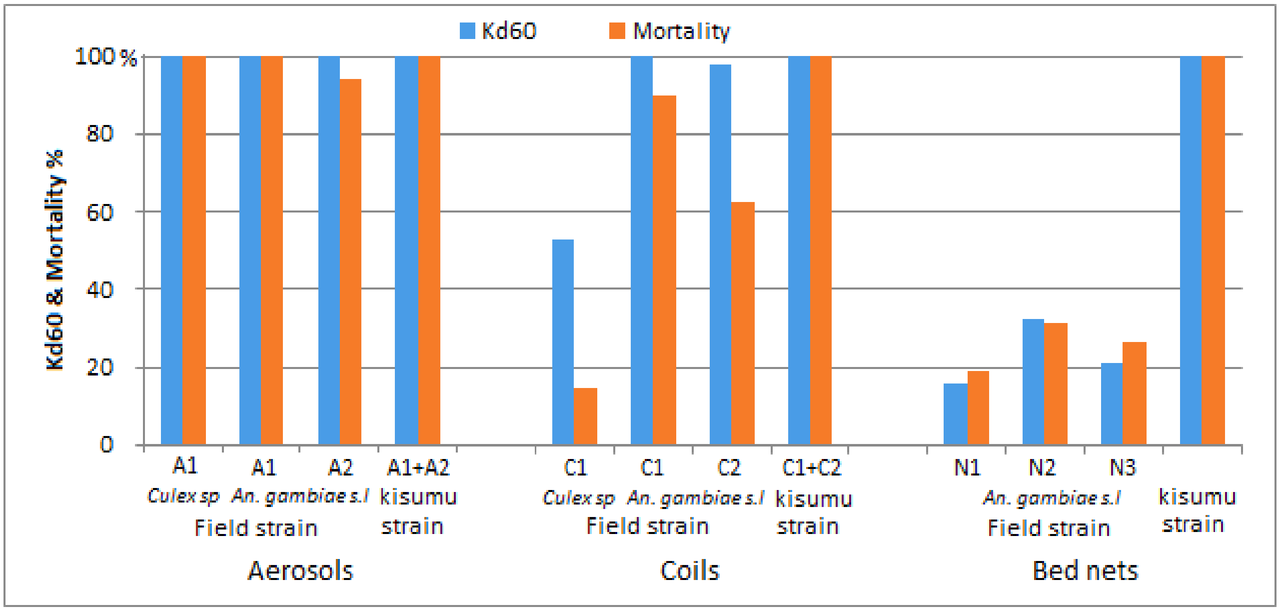

Figure 3: Knockdown and mortality rates after exposure of *An. gambiae* s.l and Culex populations to the different anti-mosquito tools used in Kribi. The codes A1 & A2 are the aerosols tested; A1+A2 is the control using the laboratory colony. The same applies to C1 & C2, which represent the coils tested, and N1, N2, and N3, which are the nets tested.

3.5. Anopheline composition

A sub-sample of 200 *An. gambiae* s.l specimens selected randomly after the bio-assays were molecularly identified to species. Two members of the *An. gambiae* complex were identified as *An. coluzii* (71%) and *An. gambiae* (29%).

4. DISCUSSION

Vector control has been the principal method of preventing VBDs for over 100 years and remains highly effective when comprehensively applied and sustained. It remains, for several diseases, the only control tool currently available. Surveillance systems and monitoring and evaluation need significant strengthening to allow programs to target interventions, track progress against programmatic indicators, and make adjustments as needed. The results in the current study reveal that LLINs, insecticide aerosols, and insecticide coils are the major personal mosquito control tools used by the inhabitants in Kribi. This is in agreement with some previous studies carried out in other parts of Cameroon (Desfontaines *et al*. 1990; Manga *et al*. 1995; Ndo *et al*. 2011).

Our evaluation of the effectiveness of these control tools found that they were effective against laboratory mosquitoes (Kisumu strain) but did not perform very well against field mosquitoes. Insecticide aerosols induced total knockdown of the mosquitoes (both laboratory and field strains) and greater than 98% mortality rates, suggesting good efficacy of the aerosol products. Our results are similar to those recorded in Thailand (Lee & Khadri 1997; Khadri *et al*. 2009). However, product A1* induced total mortality of mosquitoes at 24 hours post-exposure compared to product A2*. Indeed, product A1* contains Peperonyl butoxide (PBO), which is a synergist and its action is to improve the effectiveness of the insecticide (Nwane *et al*. 2009).

On the other hand, the mortality rates obtained after exposure of the field mosquito populations to the insecticide coils differ from those recorded with insecticide aerosols. Our evaluation recorded 100% mortality of laboratory mosquitoes and approximately 79% mortality in field mosquitoes. This would be explained by the composition of the active ingredient in the insecticide coils. The insecticide coils are composed of just one compound, D-Allethrin, a type I pyrethroid, which is mainly a repellent. However, field studies on the efficacy of mosquito coils containing D-allethrin have reported good protection (> 90%) from biting mosquitoes (Yap *et al*. 1990; Yap *et al*. 1996).

Concerning the LLINs, less than 50% knockdown and mortality rates of field mosquitoes were recorded for the 5 LLINs tested, regardless of the brand, suggesting no efficacy of these nets. The laboratory Kisumu strain gave satisfactory results with 100% mortality and knock down rates recorded when exposed to the LLIN samples; this would mean that the LLINs are effective, but lose their efficacy on wild population strains. This might be because compliance with the manufacturer's recommendations on how the nets should be handled, washed, and dried is not respected by the local inhabitants, even though the role in the loss of biological efficacy is not well elucidated. The maintenance of the bio-efficacy of the LLINs might also be influenced by the type of house-building materials, as poor house material facilitates dirtiness of the nets (Ogoma *et al*. 2012). However, we cannot attribute the poor bio-efficacy performance of the LLINs solely to the above-mentioned factors because pyrethroid resistance seen in some mosquito populations might reduce the efficacy of these nets as well as the mosquito coils. Some studies have demonstrated a loss of the efficacy of LLINs in areas of strong pyrethroid resistance through experimental hut trials (Dabiré *et al*. 2006; N'Guessan *et al*. 2007; Irish *et al*. 2008; Asidi *et al*. 2012), although other studies have reported that LLINs remain efficient in pyrethroid resistance areas (Etang *et al*. 2007; Nwane *et al*. 2009). The efficacy of LLINs against field mosquitoes varies according to the resistance level of the local mosquito populations, as reported in *An. arabiensis* from South-Western Ethiopia (Yewhalaw *et al*. 2012), and *An. gambiae* from Uganda (Okia *et al*. 2013). Many of the studies on the bio-efficacy of LLINs have found at least one type of brand effective against the field strain (Chareonviriyaphap *et al*. 2002; Norris 2011; Soleimani-Ahmadi *et al*. 2012; Bobanga *et al*. 2013). Thus, the situation observed in the current study, where none of the LLINs met WHO targets against the field mosquitoes, represents a real problem for vector control. However, reports of LLINs failure are increasing. For example, in Kenya, pyrethroid-resistant mosquitoes were found resting inside holed LLINs and, when tested via cone bioassays, these LLINs were also found to be ineffective at killing the local vectors (Ochomo *et al*. 2013). This study demonstrates the occurrence of resistance to pyrethroid insecticides (deltamethrin & permethrin) in populations of *An. gambiae* s.l and *Culex* in Kribi. The existence of resistance to this class of insecticides in populations of *An. gambiae* and *Culex* in urban Kribi is worrisome and an indication of the threatened sustainability of vector control programmes utilizing any of these insecticides. Since pyrethroid insecticides have been commonly used in the study sites and LLINs were distributed two times across the city at universal coverage in 2011 and 2015, field populations of *An. gambiae* s.l. and *Culex* might be exposed to different patterns of selection pressure from one locality to another and from one year to another. Also, several other factors might influence the status of insecticide resistance, including the number of genes interacting to produce the phenotype of resistance, the dominance relationship of the alleles, as well as the size and proportion of the population affected by insecticide treatments, as suggested by Chareonviriyaphap *et al*. (2002). The 100% mortality rates recorded after exposure to Bendiocarb (4%) reflect the susceptibility of *An. gambiae* s.l. and *Culex* populations to the carbamate class of insecticide in Kribi. This result suggests that this insecticide class can be used as alternatives to pyrethroids in Kribi but the major challenge arising from these results is that the choice of carbamate may not be a suitable alternative in the event of switching to another class of insecticide because resistance to this class of insecticide which is commonly used in IRS, has been reported in certain countries across Africa (Padonou *et al*. 2011).

Molecular analyses of the *Anopheles gambiae* complex revealed the dominance of the *An. coluzzii* species (71.43%) over *An. gambiae* (28.57%). These results are compatible with many previous studies conducted in Cameroon, indicating the high prevalence of *An. coluzzii* in the coastal zone. Also, we highlight the absence of the species *An. melas* in the current study area, unlike Bigoga *et al*. (2007), who found 14.4% of *An. melas* in the coastal zone of South-West Cameroon and Mbida *et al*. (2016), who found 4% of this species on the Manoka island in the littoral region of Cameroon.

5. CONCLUSIONS

In summary, this study observed a high possession rate of LLINs among households, but usage rates were far below average and provided no efficacy against field mosquitoes, leading to increased use of alternative domestic insecticide tools. This situation might have implications for the nation's vector control program because people might lose interest in the use of LLINs when they fail to protect users from bites of resistant mosquitoes. In order to have a good alternative vector control strategy to tackle mosquito nuisance in general, we suggest that vector control components should be broadened to include the evaluation, monitoring, and regulation of all anti-mosquito tools and thus introduce them as a formal tool of vector control alongside LLINs in an integrated vector management strategy.

AUTHORS' CONTRIBUTIONS. WEE and AHP conceived and designed the study protocol. WEE, AHP, DPLV, MSE, and BS carried out field and laboratory assays. WEE, AHP, and DPLV analyzed and interpreted data, and drafted the manuscript. MSE, BS, and TJC critically reviewed the manuscript. All authors read and approved the final manuscript

ACKNOWLEDGMENTS. The authors of this manuscript thank the local administration and populations of the study sites. We are grateful for their contribution to the realization of this work.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE. Ethical clearance for the study was requested from the National Ethics Committee of Cameroon and approved through the authorization number 102/CNE/SE/09. Informed consent was obtained from heads of households or their representatives and community leaders before access to houses.

CONFLICT OF INTEREST. The authors declare no conflict of interest

REFERENCES

Asidi A, N'Guessan R, Akogbeto M, Curtis C, Rowland M (2012). Loss of household protection from use of insecticide-treated nets against pyrethroid-resistant mosquitoes, Benin. *Emerg Infect Dis* 18(7), 1101–1106. doi: 10.3201/eid1807.120218.

Bigoga J D, Manga L, Titanji V P K, Coetzee M & Leke R G F (2007). Malaria vectors and transmission dynamics in coastal south-western Cameroon. *Malar J* 6, 5. https://doi.org/10.1186/1475-2875-6-5.

Bobanga T, Ayieko W, Zanga M, Umesumbu S, Landela A, Fataki O, Mandoko A S, Tshibamba J, Nyabola L (2013). Field efficacy and acceptability of PermaNet(R) 3.0 and OlysetNet(R) in Kinshasa, Democratic Republic of the Congo. *Journal of Vector Borne Diseases* 50(3), 206-214.

Chareonviriyaphap T, Rongnoparut P, Juntarumporn P (2002). Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, malaria vector in Thailand. *J Vector Ecol* 27, 222–9. PMID:12546458.

Collins F H, Mendez M A, Razmussen M O, Mehaffey P C, Besansky N J, Finnerty V A (1987). Ribosomal RNA gene probe differentiates member species of *Anopheles gambiae* complex. *Am J Trop Med Hyg* 37, 37–41.

Dabiré R K, Diabate A, Baldet T, Pare-Toe L, Guiguemde R T, Ouedraogo J B, Skovmand O (2006). Personal protection of long-lasting insecticide-treated nets in areas of *Anopheles gambiae* s.s. resistance to pyrethroids. *Malar J* 5, 12. https://doi.org/10.1186/1475-2875-5-12.

Desfontaines M, Gelas H, Cabon H, Goghomu A, Kouka-Bemba D, Carnevale P (1990). Evaluation des pratiques et des coûts de lutte antivectorielle à l'échelon familial en Afrique Centrale, Ia Ville de Douala (Cameroun). *Ann Soc Belg Med Trop* 70, 137–44.

Etang J, Chouaibou M, Toto JC, Faye O, Manga L, Same-Ekobo A, Awono Ambene P & Simard F (2007). A preliminary test of the protective efficacy of permethrin-treated bed nets in an area of *Anopheles gambiae* metabolic resistance to pyrethroids in north Cameroon. *Trans RoySoc Trop Med Hyg* 881–884. https://doi.org/10.1016/j.trstmh.2007.05.012.

Fanello C, Santolamazza F, & Della Torré A (2002). Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology 16*, 461-464. https://doi.org/10.1046/j.1365-2915.2002.00393.

Gillies M T & De Meillon B A (1968). The Anophelinae of Africa south of the Sahara *(Ethiopian Zoogeographical Region)*. 2nd Edn. *South African Institute for Medical Research Johannesburg* 54, 131-132.

Gillies M T & Coetzee M (1987). A supplement to the Anophelinae of Africa South of the Sahara *(Afro-tropical region)*. *South African Institute for Medical Research Johannesburg* 55, 78-143.

Hawley W A, Phillips-Howard P A, Kuile F O T, Terlouw D J, Vulule J M (2003). Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in Western Kenya. *Am J Trop Med Hyg* 68, 121-7.

Irish S, N'Guessan R, Boko P, Metonnou C, Odjo A, Akogbeto M, Rowland M (2008). Loss of protection with insecticide-treated nets against pyrethroid-resistant *Culex quinquefasciatus* mosquitoes once nets become holed: an experimental hut study. *Parasites Vectors* 1, 17. https://doi.org/10.1186/1756-3305-1-17.

Jamison Dean T (2006). Disease control priorities in developing countries. 2nd edition. *New York: Oxford University Press* eISBN: 0-8213-6180-5, 417- 463. ncbi.nlm.nih.gov.

Khadri M S, Kwok K L, Noor M I & Lee H L (2009). Efficacy of commercial household insecticide aerosol sprays against *Aedes aegypti* (linn.) Under simulated field conditions. *Southeast Asian J Trop Med Public Health* 40,6.

Killeen G F, Tami A, Kihonda J, Okumu F O, Kotas M E, Grundmann H, Kasigudi N, Ngonyani H, Mayagaya V, Nathan R, Abdulla S, Charlwood J D, Smith T A & Lengeler C (2007). Cost-sharing strategies combining targeted public subsidies with private-sector delivery achieve high bednet coverage and reduced malaria transmission in Kilombero Valley, southern Tanzania. *BMC Infect Dis* 7,121. https://doi.org/10.1186/1471-2334-7-121.

Le Menach A S, Takala F E, McKenzie A, Perisse A, Harris A, Flahault & Smith D L (2007). An elaborated feeding cycle model for reductions in vectorial capacity of night biting mosquitoes by insecticide-treated nets. *Malar J* 6, 10. https://doi.org/10.1186/1475-2875-6-10.

Lee H L & Khadri M S. (1997). Effect of household aerosol on mosquitoes - preliminary observations. *Trop Biomed* Vol. 14, No. 1/2, 117-119.

Lengeler C (1998). Insecticide-treated bed nets and curtains for malaria control. *The Cochrane Library* Issue 3, Oxford, UK, Update Software.

Lindsay S W, Adiamah J H, Miller J E & Armstrong J R M (1991). Pyrethroid-treated bed net effects on mosquitoes of the *Anopheles gambiae* complex in the Gambia. *Medical and Veterinary Entomology* 5, 477–483. https://doi.org/10.1111/j.1365-2915.1991.tb00576.

Lines J D, Myamba J & Curtis C F (1987). Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Med Vet Entomol* 1(1), 37-51. https://doi.org/10.1111/j.1365-2915.1987.tb00321.

Manga L, Robert V, Carnevale P (1995). Efficacité des serpentins et des diffuseurs en plaque dans la protection contre les vecteurs du paludisme au Cameroun. *Cah Santé : Cahiers d'Etudes et de Recherches Francophones*, 5 (2), 85-88. ISSN 1157-5999.

Maxwell C A, Msuya E, Sudi M, Njunwa K J, Carneiro (2002). Effect of community-wide use of insecticide-treated nets for 3–4 years on malarial morbidity in Tanzania. *Trop. Med. Int. Health* 7, 1003–8. https://doi.org/10.1046/j.1365-3156.2002.00966.

Mbida A M, Etang J, Ntonga P, Talipouo A, Awono-Ambene P, Oke-Agbo F, Eboumbou C, Akogbéto M, Osse R, Lehman G, Ekoko W, Binyang J, Tagne D, Tchoffo R, Dongmo A & Mimpfoundi R (2016). Preliminary investigation on aggressive culicidae fauna and malaria transmission in two wetlands of the Wouri river estuary, Littoral-Cameroon. *J. Entomol. Zool. Stud. JEZS* **105,** 105–110.

Ndo C, Menze-Djantion B, Antonio- Nkondjio C (2011). Awareness, attitudes and prevention of malaria in the cities of Douala and Yaounde (Cameroon). *Parasites Vectors* 4, 181. https://doi.org/10.1186/1756-3305-4-181.

N'Guessan R, Corbel V, Akogbeto M, Rowland M (2007). Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerg Infect Dis* 13, 199-206. doi: 10.3201/eid1302.060631. PMID: 17479880; PMCID: PMC2725864.

Norris D E (2011). Efficacy of long-lasting insecticidal nets in use in Macha, Zambia, against the local *Anopheles arabiensis* population. *Malar J* 10, 254.

Norris L C, Wang C H (1993). Application of dosage technology on insecticides for household use. *The Bulletin of Environmental Health* 1, 23-37.

Nwane P, Etang J, Chouaibou M, Toto JC, Kerah-Hinzoumbé C, Mimpfoundi R, Awono-Ambene HP, Simard F (2009). Trends in DDT and pyrethroid resistance in *Anopheles gambiae* s.s. populations from urban and agro-industrial settings in southern Cameroon. *Biology Molecular Cellular Infect Diseases* 9,163. https://doi.org/10.1186/1471-2334-9-163.

Ochomo E O, Bayoh N M, Walker E D, Abongo B O, Ombok M O, Ouma C, Githeko A K, Vulule J, Yan G, Gimnig J E (2013). The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance. *Malar J* 12, 368. https://doi.org/10.1186/1475-2875-12-368.

Ogoma B S, Moore S J & Maia F M (2012). A systematic review of mosquito coils and passive emanators: defining recommendations for spatial repellency testing methodologies. *Parasites and Vectors* 5, 287.

Okia M, Ndyomugyenyi R, Kirunda J, Byaruhanga A, Adibaku S, Lwamafa D K, Kironde F (2013). Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of *Anopheles gambiae* s.s. from different malaria transmission zones in Uganda. *Parasit Vectors* 6, 130. https://doi.org/10.1186/1756-3305-6-130.

Padonou G G, Sezonlin M, Gbedjissi G L, Ayi I, Azondekon R, Djenontin A, Bangana S, Oussou O, Yadouleton A, Boakye D, Akogbeto M (2011). Biology *of Anopheles gambiae* and insecticide resistance: Entomological study for a large-scale of indoor residual spraying in South East Benin. *J Parasitol Vector Biol*, **3**(4), 59-68. https://doi.org/10.5897/JPVB2024.0454.

Phillips-Howard P A, Nahlan B L, Kolczak M S, Hightower A W, Ter Kuile F O (2003). Efficacy of Permethrin-Treated Bed Nets in the Prevention of Mortality in Young Children in an Area of High Perennial Malaria Transmission in Western Kenya. *The American Journal of Tropical Medicine and Hygiene* 68 (Suppl 4), 23-29.

Service M W (1993). Mosquito ecology, field sampling methods vector biology and control. 2nd ed. Liverpool. *Liverpool School of Tropical Medicine*. https://doi.org/10.1007/978-94-011-1554-4_5.

Soleimani-Ahmadi M, Vatandoost H, Shaeghi M, Raeisi A, Abedi F, Eshraghian M R, Madani A, Safari R, Oshaghi M A, Abtahi M, Hajjaran H (2012). Field evaluation of permethrin long-lasting insecticide-treated nets (Olyset (R)) for malaria control in an endemic area, southeast of Iran. *Acta tropica* 123, 146-153. https://doi.org/10.1016/j.actatropica.2012.04.004.

WHO (2005). Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. http://www.who.int/publications/i/item/9789241505277.

WHO (2016). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. https://apps.who.int/iris/bitstream/10665/250677/1/9789241511575-eng.pdf.

WHO (2023). World malaria report 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023.

WHO (2017). World Health Organization Global Vector Control Response 2017. https://mesamalaria.org/resource-hub/global-vector-control-response-2017-2030.

Yap H H, Lee C Y, Chong N L, Yahaya A M, Baba R, Awang A H (1996). Performance of mosquito coils containing transfluthrin against *Culex quinquefasciatus* (Say) in an urban squatter environment. *Trop Biomed* I 3. Vol. 13 No. 1, 101-103.

Yap H H, Tan H l, Yahaya A M, Baba R, Loh P Y, Chong N L (1990). Field efficacy of mosquito coil formulations containing o-allethrin and o-transallethrin against indoor mosquitoes, especially *Culex quinquefasciatus* Say. *Southeast Asian J Trop Med Public Health* 2l, 558-563.

Yewhalaw D, Asale A, Tushune K, Getachew Y, Duchateau L, Speybroeck N (2012). Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid-resistant *Anopheles arabiensis* from South-Western Ethiopia. *Parasit Vectors* 5, 159. 21(4):558-563. PMID: 2098916.

Zaim M, Jambulingam P (2004). Global insecticide use for vector-borne disease control. WHO/CDS/ WHOPES /GCDPP /2004. 9.