



Evaluation Of the Level of Sensitivity of Anopheles to Insecticides Used in Public Health in The Town of Kindu

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ABSTRACT

Vector control is one of the national malaria control strategies in the DRC, which is mainly based on the use of insecticides using the mass distribution of long-lasting insecticide-treated nets and intra-home spraying of insecticide. The general objective of this study is to evaluate the level of sensitivity of anopheles to insecticides used in public health. We carried out a descriptive study that consisted in describing the bio-ecological parameters of anopheles gites and determining their behaviour towards conventional insecticides. After carrying out quality control on the consistency of the data collected, they were entered into Excel 2010 and the analyses were carried out on the Statistical Package for the Social Sciences

23 (SPSS 23) software. The gambiae sl Anopheles tested showed different behaviours depending on the site and type of insecticides, and the Deltamethrin test showed a mortality rate of 99% in Basoko and 52% in RVA. After pre-exposure to the PBO, mortality was 98% at RVA. The permethrin test recorded a mortality rate of 79% at BASOKO and 31% at RVA. An increase in the sensitivity of Anopheles gambiae sl was observed after pre-exposure to 5% PBO. This resistance is largely of metabolic origin linked to mono oxygenase P450. In view of these results, it is imperative to consider new control strategies to control the emergence of this resistance.

Keywords: Anopheles, Sensibility, Insecticide, usual

INTRODUCTION

Malaria is a parasitic disease caused by Plasmodiums and transmitted by mosquitoes of the genus Anopheles. The Anopheles mosquito is the world's most dangerous arthropod and the vector of malaria, which continues to be the main cause of morbidity and mortality in sub-Saharan Africa. According to WHO estimates, 216 million cases of malaria were recorded in 2016, an increase of 5 million compared with the previous year (ANONYMOUS, 2017). The number of associated deaths reached 445,000, almost the same as in 2015, and the fight against malaria is currently stagnating (ANONYMOUS, 2017).

There are more than 3,000 species of Anopheles recorded worldwide, but only around 60 transmit malaria. The African region is the most affected, with 90% of malaria cases and 92% of deaths recorded. These regions have favourable ecological conditions for the development of anopheles, the main species of which are Anopheles gambiae, Anopheles funestus, Anopheles nili and Anopheles moucheti. Malaria is a parasitic disease caused by Plasmodiums and transmitted by mosquitoes of the genus Anopheles.

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There are more than 3,000 species of Anopheles recorded worldwide, but only around 60 transmit malaria. The African region is the most affected, with 90% of malaria cases and 92% of deaths recorded. These regions have favourable ecological conditions for the development of anopheles, the main species of which are Anopheles gambiae, Anopheles funestus, Anopheles nili and Anopheles moucheti. Anopheles gambiae, Anopheles funestus, Anopheles nili and Anopheles moucheti all belong to species complexes and can have highly variable vectorial capacities and behaviour. The Democratic Republic of Congo and Nigeria rank first with 35% of the world's malaria prevalence (ANONYMOUS,2015). Malaria remains one of the causes of mortality in 3 vulnerable groups, namely children under five, pregnant women and the elderly (ANONYMOUS,2014). The DRC, a developing country, faces the dual challenge of economic growth and population health. The rural environment, which accounts for around 70% of the total population, and its essentially agricultural economy, need men in good health to promote

it. However, rural people are exposed to a range of vector-borne diseases, including malaria. Malaria remains endemic and is the leading cause of morbidity and mortality in the DRC (ANONYMOUS, 2016).

In 2016, 15.397.717 cases of malaria were recorded, with 33.997 deaths in the DRC (ANONYMOUS,2016). The most common vectors are *Anopheles gambiae* (92%), *Anopheles funestus*, *Anopheles nili*, *Anopheles moucheti* and *Anopheles paludis* (ANONYMOUS, et al, 2016). In these conditions, vector control seems essential. Vector control requires the control and identification of vector species, in particular their biology, ecology and aetiology, as well as their sensitivity to insecticides (ANONYMOUS, et al, 2016).

The entomological component of the two main prevention interventions implemented by the NMCPs, namely the widespread use of long-lasting insecticide-treated mosquito nets (LLINs) and indoor insecticide spraying (IRS), as a vector control strategy (ANONYMOUS, *et al*, 2013) makes it possible to distinguish three malaria zones:

- Stable malaria zone: anopheline transmission is high with more than 100 infecting bites per man per year, which allows the acquisition of premunity after 5 years;
- Unstable malaria zone: transmission is seasonal and moderate, with a few infecting bites per man per year, which does not allow the acquisition of premunity and the disease rages in the form of an epidemic.
- Intermediate zone between the above two.

CARNEVALE (CARNEVALE et al, 2009) has mapped the epidemiology of malaria in sub-Saharan Africa. He divided the continent into six epidemiological facies based on the biogeography of the region concerned:

- Equatorial facies (forests and post-forest savannas);
- Tropical facies (humid savannas);
- Sahelian facies (dry savannas and steppes);
- Desert facies (Sahelo-Saharan steppes, deserts of the Horn of Africa);
- Southern facies (plateaux of southern Africa) and
- Mountain facies (mountains between 1000 metres and 2000 metres (MOUCHET et al, 1993).

The DRC has three epidemiological facies: the equatorial and tropical facies, where 97% of the population live; this is the zone with stable malaria. The mountainous facies, where 3% of the population lives, is the unstable malaria zone (ANONYMOUS, 2017). In Kindu, malaria transmission is very heterogeneous depending on the environment.

The prevalence of malaria is high in peripheral areas and low in urban areas (DIBOULO *et al*, 2016). ASIDI and colleagues have justified this situation by the disruption of the ecosystem by human activities. Unfortunately, the deployment of these two interventions coincided with the emergence of vector resistance to insecticides in certain regions (N'GUESSAN *et al*, 2010). Despite the many Mosquitos net impregnated with long-lasting insecticide distribution campaigns organised in the DRC in general and in Kindu in particular, malaria transmission still persists. In 2018, the 18 Health Zones of the Kindu Provincial Division reported 463272 cases of malaria with 1032 deaths (ANONYMOUS, 2016).

These high morbidity and mortality rates can be explained by several factors:

- Poor household use of Mosquito net impregnated with long-lasting insecticide;
- The ineffectiveness of insecticides used to impregnate mosquito nets;
- Mosquito resistance to insecticides used to impregnate nets.

Hence the need for an entomological assessment of the behaviour of malaria vectors in relation to the usual insecticides in Kindu. The main synthetic insecticides belong to 5 chemical families:

- Organochlorines
- organophosphates
- carbamates
- pyrethroids and
- benzylureas.

The detection of insecticide resistance should therefore be a component of all national malaria control efforts, to ensure that the most effective vector control methods are implemented. The choice of insecticide for indoor spraying should always be a decision made on the basis of recent local data regarding the susceptibility of target vectors (ANONYMOUS,2015).

The general objective of this study is to assess the level of sensitivity of Anopheles to the usual insecticides used in public health in order to help identify the most effective insecticides in the Basoko and RVA sites in the town of Kindu. Seven species of Anopheles were surveyed and identified in Kindu: *An. Gambiae* sl, *An. funestus*, *An. paludis*, *An. hancocki*, *An. coustani*, *An. Brunnipes* and *An. nili*. A single vector species, *An. Gambiae* sl is responsible for the transmission of malaria; it represents 93.27% of the anopheline fauna (ASIDI *et al*, 1992).

MATERIALS AND METHODS

Setting And Materials:

The study took place at two sites in Kindu (Basoko and Carrière RVA) from 22 January to 23 March 2019. We selected the sites on the basis of a reasoned choice, taking into account the observations of ASIDI and Giovan Francesco (ASIDI *et al*. 2016). All bodies of water likely to serve as larval breeding sites were selected and surveyed during this study.

Surveys of Anopheles larval breeding sites were carried out beforehand at the 2 sites to identify the different types of breeding sites. These sites were categorised into several types according to WHO:

- Small puddles, gutters, ditches, tyre tracks where we examined the surface of the water;
- Marshes, ponds and lakes, where larvae were generally found in the vegetation on the banks, or sometimes far from the bank if there were floating plants;
- Watercourses examined at bank level, where there is vegetation and the flow is slower;
- Special places, such as the well or water reservoirs, where the entire water surface was examined (ANONYMOUS,2014).

The biological material for our study consisted of larval breeding sites and adult anopheles from larvae and pupae collected in the Basoko and RVA breeding sites in Kindu.

The statistical units consisted of larval and anopheline breeding sites.

Methods

Nature of the Study:

This descriptive study involved describing the bioecological parameters of *Anopheles* breeding sites and determining their behaviour in relation to common insecticides.

At each site, potential *Anopheles* breeding sites were identified and characterised by following different routes. The physico-chemical characteristics of the breeding sites were recorded using a multiparameter probe to determine the quality of the water in the breeding sites (temperature (°C), conductivity (µs/cm), turbidity (ppm), pH). These parameters were measured by placing the Hanna Combo multiparametric probe at a depth of 10 cm from each deposit to determine the water quality of the deposits. The type of shelter was determined to characterise them as temporary or permanent.

The vegetation cover allowed us to determine the nature of the sites (sunny or shady). Sites without plant cover were considered sunny and those with plant cover shady.

Larvae were collected using the Dipping method to estimate larval density using the Carron scale, and also for rearing in order to obtain adults for the test. Certain quantitative parameters of the breeding sites were described and presented in the form of tables. The mosquito mortality rate was calculated by summing the number of dead mosquitoes from all exposure tests to the different insecticides and expressing this number as a percentage of the total number of mosquitoes exposed to the insecticides:

$$\text{Mortality rate} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of mosquitoes exposed}} \times 100$$

An in-depth analysis of mosquito resistance was also carried out using probit log analysis software, following the procedure proposed by WHO 2013 (ANONYMOUS, 2016). This software was used to determine the Kdt50 and Kdt95. Samples of larvae and pupae were collected for adult rearing for susceptibility testing. Larvae and pupae were collected from the sites. Two teams of six people were formed. Two entomological technicians, a doctor, a biologist and two community relays, who took it in turns to visit the targeted sites.

To collect the larvae and nymphs correctly, the catcher approaches the nest slowly to avoid any disturbance likely to cause them to dive. He should position himself against the shade to prevent the larvae and nymphs escaping towards the bottom of the nest. The larvae and pupae were collected using a 500 ml plastic dipper with a handle whose colour made it easy to see the larvae and pupae. This dipper was gently dipped into the water at an angle of about 45°, until one edge was just below the surface.

If the larvae and pupae were disturbed/disturbed, a wait of a minute or two was observed for them to rise to the surface and continue sampling. These collected larvae were poured into trays bearing the number of the site and fitted with lids so that they could be nested together to avoid shaking during transport, following the protocol of Williams and Pinto in 2012. (NARDINI *et al*, 2017)

PRESENTATION OF RESULTS

These results relate to a total of 81 larval sites, characterised by 5 types of water collection.

Table I: Types of gites per site

TYPES OF GITES	SITES		(n=81)
	Basoko	RVA	Total
Drain	1 (1,2%)	0 (0%)	1 (1,2%)
Market garden water collection	28 (34,6%)	0 (0%)	28 (34,6%)
Stagnant rainwater	9 (11,1%)	31 (38,3%)	40 (49,4%)
Presence of marshes	7 (8,6%)	1 (1,2%)	8 (9,9%)
Well	1 (1,2%)	3 (3,7%)	4 (4,9%)
TOTAL	46 (56,8%)	35 (43,2%)	81 (100%)

Table 1 shows that the distribution of breeding sites varies according to the site. The number of larval sites colonised by Anopheles in Basoko and RVA was 56.8% and 43.2% respectively. At RVA, Anopheles breeding sites were created by stagnant water (31 sites or 38.3%). However, in Basoko, 28 sites (34.6%) were identified and characterised in market garden collections.

Table II: Physical and physico-chemical parameters at the Basoko and RVA sites

	BASOKO			RVA		
Parameters physico-chemical	Average	Standard deviation	Minimum Maximum	Average	Standard deviation	Minimum Maximum
			Min : 25,2			Min: 26,2
Temperature (°C)	28,3	2,3		31,9	3,5	
			Max: 32,3			Max: 39,2
			Min: 7,1			Min: 6,57
pH	8,1	0,5		7,8	0,6	
			Max: 9,2			Max: 10,1
			Min: 176			Min: 97
Turbidity (ppm)	694,6	427,4		292,2	324,6	
			Max: 2000			Max: 1870
			Min: 353			Min: 189
Conductivity (µs/cm)	1389,2	851,3		589,9	643,9	
			Max: 3999			Max: 3697
			Min: 2			Min: 2
Depth (cm)	17,3	30,9		16,8	15,5	
			Max: 150			Max: 70

Table II shows that the water in all the hatching sites had comparable pH values, which were slightly basic. Temperature was higher at the RVA (31.95±3.57). On the other hand, the Basoko site was characterised by high turbidity (694.6±427.4 ppm) and conductivity (1389.2±851.3 µs/cm) values. However, there was no difference in depth between the different sites.

Table III. Mortality of *Anopheles gambiae* sl 24 hours after 60 minutes of exposure to insecticides.

Sites	Insecticides	N	Kdt ₅₀ (min)	Kdt ₉₅ (min)	Mortality 24h (%)	Status*
Basoko	Deltamethrin 0,05 %	100	35.7 (33.7-37.7)	74.1 (66.4-86.2)	52	R
	Deltamethrin 0,05 % + PBO 5%	100	30.1 (28.3-31.8)	46 (42.3-52.1)	98	S
	Permethrin 0,75 %	100	55.2 (52.7-58.6)	97.6 (85.9-118.2)	31	R
	Permethrin 0,75 % + PBO 5 %	100	40.6 (39.3-41.9)	58.3 (55.1-62.7)	100	S
	Bendiocarb 0,1 %	100	23.45 (22.1-24.8)	35.4 (32.6-39.7)	100	S
	Malathion 5 %	100	26.7 (25.3-28.2)	39.6 (36.7-44.1)	100	S
	DDT 4 %	100	158.7(100.7-827.9) No effect	440.8(193.9-9169.5) No effect	16	R
RVA	Deltamethrin 0,05 %	100	31.0 (29.3-32.7)	49.1 (46.2-53.0)	99	S
	Permethrin 0,75 %	100	47.1 (45.6-48.7)	66.0 (62.9-70.1)	79	R
	Permethrin 0,75 % + PBO 5 %	100	42.1 (40.4-43.7)	61.9 (58.7-66.3)	98	S
	Bendiocarb 0,1 %	100	21.0 (19.8-22.2)	30.7 (28.8-33.4)	100	S
	DDT 4 %	100	69.3 (64.4-95.6) No effect	79.6(70.6-130.2) No effect	10	R
	Malathion 5 %	100	31.7 (29.8-33.6)	45.9 (36.7-44.1)	100	S

*Status: R= resistant (≤ 90) RP= probable resistance S= sensitive (98-100 %)

The Kdt₅₀ and Kdt₉₅ were highly variable depending on the type of insecticide and the test site. Bendiocarb and Malathion were the most effective insecticides at both sites (Basoko and RVA). *Anopheles gambiae* sl tested with these insecticides were all knocked out (100%). The Kdt₅₀ and Kdt₉₅ were too early at the RVA, respectively at 21.0 minutes with 95% CI (19.8-22.2) and 30.7 (28.8-33.4) minutes. However, at Basoko, the Kdt₅₀ and Kdt₉₅ were slightly late, at 23.45 (22.1-24.8) minutes and 30.7 (28.8-33.4) minutes respectively.

Similarly for Malathion, the Kdt₅₀ and Kdt₉₅ were reached too early at the RVA (31.7 (29.8-33.6) minutes and 45.9 (36.7-44.1) minutes) compared with the Basoko site (26.7 (25.3-28.2) minutes). No effect of Kdt₅₀ and Kdt₉₅ observed in anopheles tested with DDT in the sites. Deltamethrin was effective at the RVA. These anopheles were stunned with Kdt₅₀ and Kdt₉₅, respectively at 31.0 (29.3-32.7) minutes and 49.1 (46.2-53.0) minutes. In Basoko, however, Kdt₉₅ was not reached. The anopheles tested against Bendiocarb and Malathion were sensitive, with 100% mortality at both sites. These anophelines were highly resistant to permethrin and DDT at both sites, with low mortality. The mortality rate of anopheles exposed to Deltamethrin was higher at the RVA (99%).

DISCUSSION

The main aim of this work was to determine the level of sensitivity of *Anopheles* to insecticides used in public health, in order to help improve the management of *Anopheles* resistance to these insecticides at the Basoko and RVA sites in the town of Kindu.

Characteristics of Larval Breeding Sites

According to Mouchet et al, the different *Anopheles* species exploit a wide variety of water collections as breeding sites, in particular the residual pools of sunny stagnant surfaces, pools with upright vegetation, brackish water, etc. (GIMONNEAU *et al*, 2017).

This study showed that the majority of larval breeding sites observed in the two sites of Basoko and RVA consisted of stagnant rainwater (49.4%) and collections of water from market gardens (34.6%). The majority of these sites were sunny (85.2%). This difference could be justified by the anthropogenic activities carried out at each site and also the nature of the soil, which is sandy at Basoko and silty-clayey at the RVA.

The same observation was made in Kinshasa by ASIDI and colleagues and METELO and colleagues in Bandundu-ville (MATUBI *et al*, 2015). During the rainy season, in most urban areas, the numerous ruts and small collections of water created by land depressions, human activities and poor urbanisation constitute excellent larval breeding grounds (MATUBI *et al*, 2015).

Physical and Physico-Chemical Parameters

Anopheles. The mean temperature values at Basoko were $28.3 \pm 2.3^{\circ}\text{C}$ (min: 25.2°C ; max: 32.3°C) and at RVA $31.9 \pm 3.5^{\circ}\text{C}$ (min: 26.2°C ; max: 39.2°C).

This difference may be due to the fact that 60% of the sites in the RVA were created by collections of market garden water in which the water is renewed daily by watering the flower beds. In Basoko, on the other hand, the stagnant rainwater (88.5%) was very exposed to the sun. These observations differ from those recorded in Morocco by OUALILALAMI and colleagues, where the average temperature was 23.7°C (min: 20.4°C ; max: 31.4°C), a difference that may be justified by the climate (ELION *et al*, 2010). All the larval sites surveyed had a slightly basic pH. These results corroborate those found by METELO and colleagues in Bandundu-ville (MATUBI *et al* 2015).

In this study, it was observed that turbidity and conductivity were high in the two sites surveyed, with an average of 494.9 ± 376 ppm and 989.6 ± 747.6 $\mu\text{S}/\text{cm}$ respectively. Despite the turbid quality of these larval sites, larval production was at a maximum.

These results are in line with those found by Metelo1 *et al* in Bandundu-ville (MATUBI *et al*, 2015). This is justified by the vectorial capacity of *Anopheles gambiae* to adapt in these water collections. It was observed that the average turbidity and conductivity values at the RVA were double those at Basoko. These observations can be justified by poor waste management, which is thought to be the source of suspended matter and ions.

CONCLUSION

The studies carried out in Kindu at the Basoko and RVA sites revealed the following:

- 5 types of sites were characterised (collections of market-garden water, stagnant rainwater, marshes, wells and gutters). In Basoko, *Anopheles* larvae breeding sites were created by stagnant water, while in the RVA they were created by market garden collections.

- Most of the larval sites surveyed were sunny and temporary.
- The hatching sites had comparable pH values, which were slightly basic, and were characterised by high turbidity and conductivity values.
- *Anopheles gambiae* showed cross-resistance to permethrin and DDT, as well as sensitivity to Bendiocarb and Malathion. This demonstrates the total involvement of oxidases in resistance (KOFFI *et al*, 2013).

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