



## THE INTENSITY OF MALARIA TRANSMISSION AND EFFICACY OF ALPHACYPERMETHRIN AS AN INDOOR RESIDUAL INSECTICIDE AGAINST MALARIA VECTORS IN KADAWA, KANO.

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### ABSTRACT

*Malaria is currently affecting more people in the World than any other disease and no single measure of control through the use of drugs seems effective. Malaria is said to kill one African (Whether child or adult every 15 second) and roughly 300,000 Nigerian children annually. A cross-sectional study was carried out to assess the impact of alphacypermethrin as an indoor residual insecticide against malaria vectors and transmission in Kadawa irrigation area, Kano. A total of 1897 mosquitoes were collected indoors using WHO standard techniques in the selected areas between December 2010 and June 2011. Specimens were identified using morphological keys and PCR-based assays. ELISA tests were used to detect *P. falciparum* sporozoite infection. Of the 1897 *Anopheles* mosquitoes collected, *An. funestus* group, the predominant vector species, represented 63.6% while *An. gambiae* s.l. has 18.5% while other *Anopheles* and *Culiseta* 17.9%. The control group (Alpha cypermethrin treated group) catch 1332 (85.6%) predominated over the intervention group (Non-alpha cypermethrin treated group) 224 (14.4%) with an extreme significant difference ( $P < 0.0001$ ). The result of PCR-based test identified 22% of the mosquitoes of the *Anopheles gambiae* complex collected as *An. gambiae* s.s., 96% and *An. arabiensis* as 4%. The overall sporozoite rates for *An. funestus* and *An. gambiae* s.s. were statistically significant in both groups. Infection rates in both intervention and control group were also statistically significant. The result of the study showed that, the indoor residual spray of alphacypermethrin has seen to have assisted in reduction of malaria transmission and malaria vectors in the study area from 15.9% in control group to 4.1% in intervention group and 78.8% in control group to 21.2% in intervention group respectively.*

**Key words:** Malaria; Alpha cypermethrin; ELISA ; PCR.

### INTRODUCTION

Malaria is a life - threatening parasitic disease transmitted by *Anopheles* mosquitoes. Malaria remains a major cause of morbidity and mortality in tropical areas despite better knowledge of the pathogenesis and management of severe malaria, the development of resistance to the drugs by the parasites and to the conventional insecticides by the anopheline vectors justifies continues research on the disease (WHO, 2000). The disease affects more than 200 million people in the world over. It is the major problem of the developing countries especially those in the tropical Africa where 90% of the reported death have been attributed to malaria infection (May *et al*, 1999, WHO, 2001). More than 40% of the world population is at risk of the disease (Snow *et al*, 2005).

It is widespread in tropical and sub-tropical regions, including parts of the Americas (22 countries), Asia, and Africa. Each year, there are more than 250million cases of malaria (Phillips, 2010), killing between one and three million people, the majority who are young children in sub-Saharan Africa (Snow *et al.*, 2005), ninety percent of Malaria related deaths occur in sub-Saharan Africa. Malaria is commonly associated with poverty and can indeed be a cause of poverty (Fong *et al.*, 1971). Malaria is said to kill one African (Whether child or adult every 15 second and roughly 300,000 Nigerian children annually (Salako, 2002). Malaria is naturally transmitted by the bite of a female *Anopheles* mosquito. Mosquito (from the Spanish or Portuguese word for little fly (Ralph, 2008) is a common insect in the family *Culicidae* (from the Latin *Culex* meaning midge or gnat) (Anonymous, 2009).

The *Anopheles gambiae* complex consists of at least seven morphologically indistinguishable species of mosquitoes in the genus *Anopheles*. The complex was recognised in the 1960s and includes the most important vectors of malaria in sub-Saharan Africa, particularly of the most dangerous malaria parasite, *Plasmodium falciparum*. It is one of the most efficient malaria vectors known (Walter Reed Army Institute of Research, 2007). Vector control program in the world; mostly rely on the usage of natural and synthetic chemical molecules, which have potential to kill the target insects (WHO, 2003).

Primary malaria prevention on a large scale depends on two vector control interventions: indoor residual spraying (IRS) and insecticide-treated mosquito nets (ITNs). Historically, IRS has reduced malaria transmission in many settings in the world, but the health effects of IRS have never been properly quantified. This is important, and will help compare IRS with other vector control intervention. IRS with DDT has been criticized because of supposed environmental damage and physiological resistance of insecticide by the malaria vector. Malathion 25% WP is used in areas with DDT resistance. The disadvantage of organophosphorous compounds (e.g. malathion) is that unlike their use in agriculture where a farmer uses the organophosphorous compound for crop protection only once or twice a year, the spray squads engaged in spraying residual insecticide in the human dwellings work with these compounds for periods extending up to 6 or 7 months. This long exposure results in acute toxic symptoms and if not control properly may lead to mortality. These compounds are also toxic to domestic pets. Pyrethroids are new insecticides introduced for vector borne diseases. The cost of these insecticides is much higher than the cost DDT and Malathion (Mabaso *et al.*, 2004).

Alpha-cypermethrin is a synthetic pyrethroid insecticide which is man-made chemicals similar in structure to the naturally occurring pyrethrins. Alpha-cypermethrin kills insects by affecting the salt balance (sodium channels) on nerve cells. It has a broad spectrum of activity against insects with the main toxic effect on the nervous system (Pascual and Peris, 1992).

The study is aimed at assessing the impact of alphacypermethrin as an indoor residual insecticide against malaria vectors at Kadawa irrigation site, an area of high mosquito density. The present research work reports results of PCR and ELISA studies on *Anopheles* populations and their relative contribution to malaria transmission in the study area.

## MATERIALS AND METHODS

### Mosquitoes Collection and Morphological Identification

Adult Anophelines resting and biting indoors were collected monthly using pyrethrum spray collection (Mobil: 3% tetramethrin, 2% allethrin, 0.44% essential oils, and 92.56% solvents and propellants) (WHO, 2003) from December 2010 to June 2011, in 40 (forty) randomly selected houses. The collection was made between 6:00am to 9:00am on each day of collection. Mosquito's samples were preserved individually in an effendorf tube each and morphologically identified using appropriate keys of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

### PCR Identification of *Anopheles gambiae* Sibling Species

Based on morphological identification, 76 mosquitoes of the *Anopheles gambiae* s.l. were selected at random from a pool of equal representation of mosquito vectors from the seven months. The DNA used for the PCR identification of the sibling species was extracted from mosquito leg and wings of each of the sample following the procedure of Scott *et al.*, (1993). A thermal cycler (Hybaid, Ashford, U.K.) was used to give initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 second and extension at 72°C for 30 seconds, with a final extension at 72°C for 8 minutes. The amplified DNA was separated on a 2.5%-agarose gel, stained with 1% ethidium bromide and visualized on a ultra-violet transilluminator (Scott *et al.*, 1993).

### ELISA Detection of *Plasmodium falciparum* Circumsporozoite Antigen

ELISA test of *Plasmodium falciparum* sporozoites were carried out on a total of 136 identified *An. gambiae* s.s., *An. arabiensis* and *An. funestus* group from which samples for the PCR were drawn to quantify and give a precise identification of sporozoite species present. The head and thorax of each of the mosquitoes was crushed in phosphate buffer solution (PBS) of pH 7.4 and tested for circumsporozoite antigen using a double antibody ELISA (Burkot *et al.*, 1984). The results were first recorded visually and then photometrically at 405 nm using an ELISA plate reader 30 minutes after substrate had been added (Burkot *et al.*, 1984).

## RESULTS

### Mosquito Species Composition

A total of 1897 adult mosquitoes were collected from both intervention (Alpha cypermethrin treated group) and control (Non-alpha cypermethrin treated group) groups within a period of seven month and 1206 (63.6%)

of these were identified as *Anopheles funestus*, 350 (18.5%) as *Anopheles gambiae s.l.* while 247 (12.9%) as other species of Anopheline mosquitoes. The remaining 94 (5.0%) mosquitoes were culicines. The overall number of *Anopheles funestus* caught was significantly

higher (P value is 0.0001) than the number of *Anopheles gambiae* collected. The control group catch 1332 (85.6%) predominated over the intervention group 224 (14.4%) with an extreme significant difference (P value is 0.0001)

**Table 1: Relative abundance of the Anopheline mosquitoes within *Anopheles gambiae* complex and *Anopheles funestus* group in control and intervention communities.**

	Intervention group	Percentage (%)	Control group	Percentage (%)	Total (%)
<i>An. gambiae</i>	180	51.4	170	48.6	350 (22.5)
<i>An. funestus</i>	44	3.6	1162	96.4	1206 (77.5)
<b>Total</b>	<b>224</b>	<b>14.4</b>	<b>1332</b>	<b>85.6</b>	<b>1556</b>

**Seasonal variation in the relative abundance of mosquitoes in the study communities**

The number of the mosquitoes species identified in the sampled communities varied according to the season of the year (Table 2). The overall number of mosquitoes collected in the wet season 1364 (71.9%) was not significantly different when compared statistically with that of dry season 533 (28.1%) (P value is 0.5742).

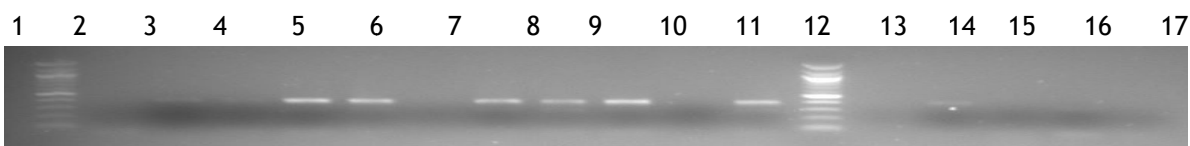
**Molecular characterization of sibling's species within *Anopheles gambiae* complex**

Polymerase chain reaction assays: The *Anopheles gambiae* species specific PCR assay

was employed to distinguish the cryptic species present within the *Anopheles gambiae* group. Out of the 350 identified as *Anopheles gambiae s.l.* a total of 76 were randomly selected representing all the seven months and study areas. Of the total *Anopheles gambiae* analyse, 72 (96%) were identified as *Anopheles gambiae s.s.* while 3 (4%) were *Anopheles arabiensis* (Table 3). The results obtained shows no significant statistical difference between the populations of *Anopheles gambiae s.s.* and *Anopheles arabiensis* (P > 0.1151). However, 2.6% of the species could not be identified.

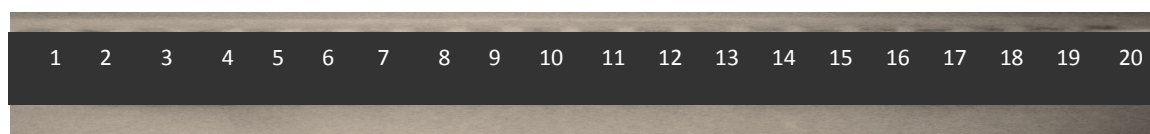
**Table 2: Seasonal variation in the relative abundance of mosquitoes in the study communities**

	Intervention group	Percentage (%)	Control group	Percentage (%)	Total (%)
Dry season (Dec-March)	118	22.1	415	77.9	533 (28.1)
Wet season (April-Jun)	285	20.9	1079	79.1	1364 (71.9)
<b>Total</b>	<b>403</b>	<b>21.2</b>	<b>1494</b>	<b>78.8</b>	<b>1897 (100)</b>



**Figure 1: PCR identification of one sibling species within *Anopheles gambiae* Using Lane 1 and 17 as markers**

Lane 1 is a Kb DNA ladder (standard); Lane 2, 7 and 11 shows no amplification; Lane 3-6, 8-10, 12-14 are *An. gambiae s.s.*; Lane 15 is a positive control of *An. gambiae s.s.*; Lane 16 is a Negative control and Lane 17 is a Positive control of *An. arabiensis* (Nigerian Institute of Medical Research Yaba, Lagos, 2011)



**Figure 2: PCR identification of one Sibling's species within *Anopheles gambiae* Using Lane 1 and 20 as markers**

Lane 1 is a Kb DNA ladder (standard) while Lane 2-20 is an *An. gambiae s.s.* (Nigerian Institute of Medical Research Yaba, Lagos, 2011).

Table 3: Sibling species composition of female *Anopheles gambiae* complex identified in the study area.

Species	Numbers identified	Percentage (%)	Intervention group (%)	Control group (%)	Total (%)
<i>An. Gambiae</i>	72	96	38 (50.7)	34 (45.3)	72 (96)
<i>An arabiensis</i>	3	4	0 (0.0)	3 (100)	3 (4)
<b>Total</b>	75	100	38	37	75 (100)

Table 4: Number of mosquitoes tested and percentage found carrying *Plasmodium falciparum* circumsporozoite antigens in both intervention and control groups

	Intervention Group		Control Group		Total	
	No. tested	No. positive (%)	No. tested	No. positive (%)	No. tested	No. positive (%)
<i>An. gambiae</i>	66	2 (3.0)	45	7 (15.6)	111	9 (8.1)
<i>An arabiensis</i>	0	4 (0)	3	0 (0.0)	3	0 (0.0)
<i>An. funestus</i> group	7	1 (14.3)	15	3 (20.0)	22	4 (18.2)
<b>Total</b>	73	3 (4.1)	63	10 (15.9)	136	13 (9.6)

#### *Plasmodium falciparum* sporozoite rates

Out of the 1803 *Anopheles* mosquitoes collected, 136 were processed for *P. falciparum* circumsporozoite protein ELISA antigen test. The selection was based on the having representativeness of the caught mosquitoes from different areas in the study area and different months. Thirteen (9.6%) of the 136 *Anopheles* mosquitoes tested for *Plasmodium falciparum* circumsporozoite antigen (CSA) were positive. The sporozoite rate was 8.1% for *Anopheles gambiae* s.s., 18.2% for *Anopheles funestus* group and 0% for *Anopheles arabiensis* (Table 4). The Overall sporozoite rates *Anopheles gambiae* s.s. and *Anopheles funestus* group were statistically significant in both the study groups (P value is 0.0203). The percentage of mosquitoes found to be carrying *Plasmodium falciparum* circumsporozoite antigen fell or decline from 15.9% in the control group to 4.1% in the intervention group. The intervention group rates were however significantly lower than the control group rates (P value is 0.0203).

#### DISCUSSION

In the present study a total of 1817 indoor resting knock down mosquito were collected from both control and intervention group throughout the study period. 403 (21.2%) of the total mosquitoes collected were from intervention group and 1494 (78.8%) were from control group (Table 2). This shows that the indoor residual spray of alpha cypermethrin in the study area play insignificant role in reducing vector densities, when the total

number of mosquitoes collected from intervention group were compared statistically with that of control group by Fishers exact test the result shows no significant different (p value is 0.5742). The results of the present study do not correlate with the findings of Lein *et al*, (2008) and Rowland *et al*, (2000) which shows that Alpha cypermethrin appears highly suited for the control of malaria in Pakistan, Democratic Republic of Sao Tome and Principe and South Asia. It is highly toxic to vectors, requires only one treatment per year, and was appreciated by the community.

The result for vector density in intervention group shows that out of 403 (21.2%) of mosquitoes collected 362 (19.8%) of the collected mosquito were *Anopheles* as against 1441(76%) out of 1494 (78.8%) of the collected mosquito from control group which indicates a high density and risk of becoming infected with malaria.

Out of 1803 *Anopheles* mosquitoes collected 1556 (86.3%) fall into 2 major groups of *Anopheles* mosquito: *Anopheles gambiae* (22.5%) and *Anopheles funestus*(77.5%) (Table1). This result is in concordance with many reports from different part of the world. Oyewole *et al*, (2005) shows that *Anopheles gambiae* and *Anopheles funestus* group constituted the largest proportion of malaria vector in Nigeria.

Large number of *Anopheles funestus* collected in the control group could be due to the location of the village and its proximity to the slow moving irrigation channel. (Table 1).

The seasonal variation in mosquito abundance indicates its dependence on the rain. Seasonal variations in population, distribution and composition of Anopheline mosquitoes were observed in all the study communities. The wet season collection was almost three times higher than that of the dry season.

The PCR identification study results confirmed that *Anopheles gambiae* s.s. constituting 96% (n=77) is the predominant vector of malaria in the study area. This is in concordance with Oyewole *et al*, (2005) who reported that *Anopheles gambiae* s.s. and *Anopheles arabiensis* occur in sympatric and in the same vicinity.

The present study showed that sporozoite rates in *Anopheles gambiae* s.s and *Anopheles funestus* were statistically significant (P value is 0.203) (Table 4), this is not in line with the findings of Haridi, (1972); Ameneshewa *et al*, (1996) and Fettene *et al.*, (2004) which show that sporozoite rates in *Anopheles gambiae* s.s, *Anopheles arabiensis* and *Anopheles funestus* were statistically insignificant. The finding of natural sporozoite rates in the salivary gland of *Anopheles gambiae* s.s (8.1%) and *Anopheles funestus* (18.2%) all of which were caught indoors by pyrethrum spray collection emphasizes their epidemiological importance in malaria transmission in the study area.

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The 0% sporozoite rate recorded for *Anopheles arabiensis* showed that it plays no tangible role in malaria transmission in the study area. Lower sporozoite rates observed in intervention group (4.1%) indicates low intensity of malaria transmission in the area (Table 4).

## CONCLUSION

Based on the finding of the present study, it could be deduced that:

The finding of natural sporozoite rates in the salivary gland of *Anopheles gambiae* s.s (8.1%) and *Anopheles funestus* (18.2%) all of which were caught indoors by pyrethrum spray collection emphasizes their epidemiological importance in malaria transmission in the study area.

Although no much reduction in malaria parasite vector density was recorded in the study area, the indoor residual spray of alpha cypermethrin was seen to have assisted in reduction of malaria transmission and malaria vectors in the study area from 15.9% in control group to 4.1% in intervention group and 78.8% in control group to 21.2% in intervention group respectively.

Members of *Anopheles funestus* group are the major malaria vectors in Kadawa irrigation area of Kano state, Nigeria.

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