Malawi

Entomological Monitoring 2017

Final Report

Anopheles species abundance, sporozoite infections and insecticide resistance

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MAC Communicable Diseases Action Centre

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Abbreviations

AMF	Against Malaria Foundation
CU	Concern Universal
ELISA	Enzyme-linked Immunosorbent Assay
GPS	Geographical Positioning System
ITNs	Insecticide-treated Bednets
LLINs	Long-lasting Insecticidal Nets
MIS	Malaria Indicator Survey
NMCP	National Malaria Control Programme
PBO	Piperonyl butoxide
PCR	Polymerase Chain Reaction
UP	United Purpose

Monitoring *Anopheles* vector species abundance, *Plasmodium falciparum* sporozoite infection rates and insecticide resistance status in five districts in Malawi

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Summary

Monitoring the impact of malaria vector control is a critical component to an effective malaria program implementation. Entomological monitoring was carried out in five districts of Chikwawa, Balaka, Ntcheu, Nkhotakota and Karonga in Malawi. Between January and December 2017 live collections to assess insecticide resistance status. Beginning in June, *Anopheles* species abundance were measured monthly. Mosquitoes were sampled in 15 houses at each of the two study villages in each district using three sampling methods, CDC Light Traps (CDC-LTs), Pyrethrum Spray Catches (PSCs) and Window Exit Traps (WETs).

1. A total of 14,613 female mosquitoes were collected across the five monitoring districts. Of these, 38.9% were *An. gambiae s.l* and 36.2% *An. funestus s.l.* However, the distribution of the two species was not uniform as 95% of *An. gambiae s.l.* were collected from Karonga District alone. In the remaining districts, *An. funestus s.l.* was the most common anopheline mosquito collected.
2. *P. falciparum* sporozoite infection rates were estimated at 2.6% across the study sites.
3. High sporozoites infection rates were found in Chikwawa (4.1%; n=31) where both *An. funestus s.s* and *An. arabiensis* had high infections.
4. Despite low *Anopheles* density, high sporozoite infection rates were found in Balaka (3.5%) and Ntcheu (3.2%) Districts followed by Nkhotakota (2.3%).
5. The two study villages in Karonga District where *An. arabiensis* was the predominant *Anopheles* vector recorded the lowest sporozoite infection rates (0.1%).

6. *An. funestus* was the most important vector with infection rates of 3.5%, 4.2%, 3.2% and 2.4% in Balaka, Chikwawa, Nkhotakota and Ntcheu Districts respectively

7. CDC-LTs yielded more mosquitoes (28.8 mosquitoes / trap) followed by PSCs (23.6 mosquitoes / collection) and WETs yielded the least. However, CDC-LTs were biased towards capturing more unfed female *Anopheles* compared to PSCs that largely caught bloodfed *Anopheles*.

8. *An. arabiensis* population in Karonga District was susceptible to alphacypermethrin but showed moderate resistance to permethrin. Further, this population showed suspected resistance to malathion (97.7%)

9. *An. funestus* populations in Nkhotakota and Chikwawa Districts were resistant to both alphacypermethrin (<50% mortality) and permethrin (<10% mortality) when the fixed dose WHO tube assays were used. However, separate CDC bottle assays revealed intense resistance (>600 fold) to both insecticides in the two districts.

10. Similarly, *An. funestus* populations were highly resistant to bendiocarb in Nkhotakota (21.4% mortality) and Chikwawa (4.9% mortality) based on WHO tube assays.

11. Encouragingly, both in Chikwawa and in Nkhotakota Districts, *An. funestus* was fully susceptible (100% mortality) to the two organophosphates (malathion and pirimiphos methyl) tested and chlorfenapyr.

An. funestus s.l. and *An. gambiae s.l.* were widely distributed across Malawi. High levels of *Pf* sporozoite infections and intense pyrethroid insecticide resistance were detected which warrant continued monitoring in order to assess impact of existing and new malaria control interventions in the country.

Introduction

Despite significant achievements made to control malaria the disease remains highly endemic in Malawi. In the past 5 years, Malawi has been implementing the National Strategic Plan (MSP) 2011-2015 which aims for universal coverage of malaria control interventions to reduce the disease burden in the country. Of the major malaria control interventions listed in the MSP, the use of long-lasting insecticidal nets (LLINs) has been the cornerstone approach and consequently they have been associated with significant reductions in malaria morbidity and mortality (MIS 2010, 2012, 2014).

In Malawi, LLINs are distributed nationwide every three years and routinely in all health facilities through antenatal clinics. The last nationwide net campaign was carried out between 2014 and 2016 targeting one net for every two people. In 2016, nets were distributed in 19 districts covering all sleeping spaces with the aim of increasing net coverage and use among households.

In addition to the NMCP, United Purpose (UP) (formerly Concern Universal) distributed two types of piperonyl butoxide (PBO) nets (PermaNet 3.0 and Olyset Plus) in Balaka and Ntcheu Districts with funding from Against Malaria Foundation (AMF). PBO nets in principle have increased effectiveness against pyrethroid resistant mosquitoes, particularly where the underlying mechanism of resistance is oxidase based. The rest of the country was covered with mono-treated nets.

Furthermore, within the effective duration of the previous MSP, indoor residual spraying (IRS) was also implemented in a few localized districts along the lakeshore and low-lying districts in the country. However, the program was later discontinued due to emergence of pyrtheroid insecticide resistance and lack of viable alternatives such as availability of a long-lasting non-pyrethroid insecticide for use in IRS.

The scaling up of malaria vector interventions, however, has also seen development and spread of insecticide resistance among *Anopheles* populations in the country (Mzilahowa et al., 2016) and across sub-Saharan Africa. Also of note, there have been reports of shifts in vector species composition and biting behavior (Bayoh et al. 2010, Lyimo and Ferguson 2009, Yohannes and Boelee 2012).

Such changes in vector species population dynamics, insecticide resistance patterns and biting behavior also pose a challenge to vector monitoring programs. In particular, mosquito sampling methods / tools could be biased towards certain strains that are predominantly endophilic. Deployment of such tools might under report or miss important information on outdoor biting or resting populations. It is important that several sampling tools are trialed out in the field in order to determine their suitability to sampling a representative *Anopheles* population in the study area.

It is imperative therefore that a robust program is put in place to monitor *Anopheles* vector populations and their susceptibility to commonly used public health insecticides in the country. Three sampling methods, CDC light traps (CDC-LTs), pyrethrum spray catches (PSCs) and window exit traps (WETs) were used to sample malaria vector populations in five districts. The main aim of the activity was fourfold, 1) to assess *Anopheles* species abundance, 2) to measure impact of PBO nets on vector populations, 3) to evaluate mosquito sampling methods and 4) to determine the intensity of pyrethroid insecticides resistance in *Anopheles* vector populations across study sites.

Methods

Study sites:

Monitoring *Anopheles* malaria vectors was carried out routinely in 3 historical districts of Karonga, Nkhotakota and Chikwawa. Additional data were collected from Ntcheu and Balaka Districts where United Purpose (UP) (formerly Concern Universal) distributed PBO nets. Field data were collected between June and September, 2017 (Figure 1). Data prior to this period were included in the previous report submitted to CDC.

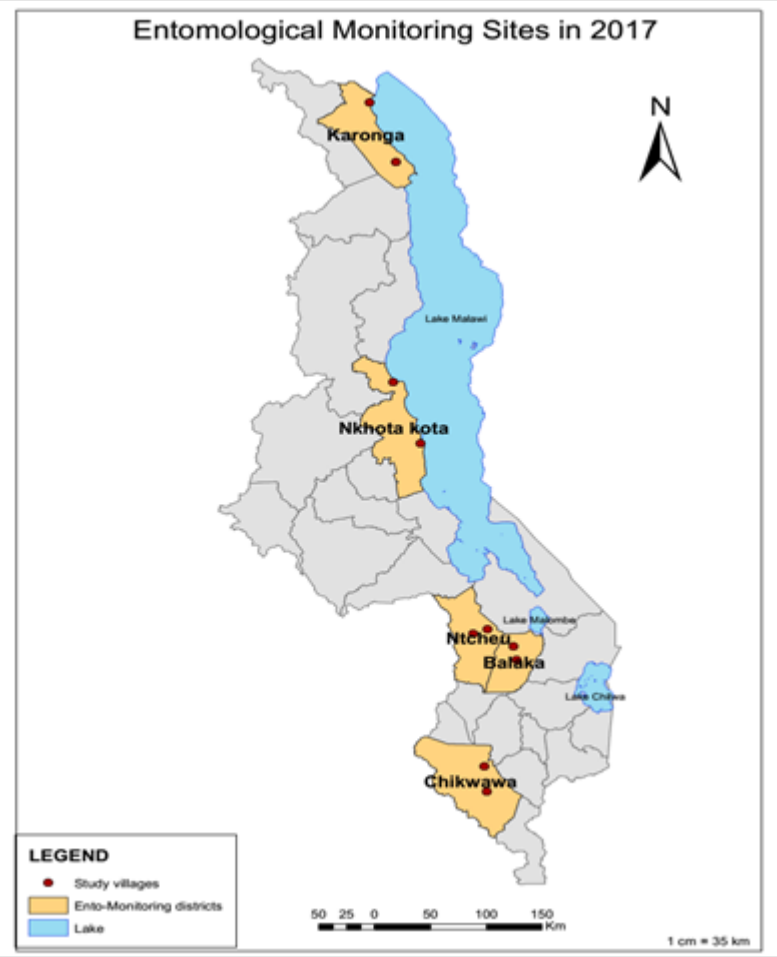

Figure 1: Map of Malawi showing entomological monitoring villages in the five districts across the country

Mosquito collection:

In Karonga, Nkhotakota and Chikwawa Districts data collection was carried out in 2 villages in each district. Mosquito sampling was carried out in 15 houses at each village monthly. Three sampling methods, CDC-LTs, PSCs and WETs were used to sample mosquitoes.

UP with funding from AMF distributed nets in Ntcheu and Balaka District outside of the government programme. Three health facilities in Ntcheu received Olyset (3,098), Olyset plus (14,774), PermaNet 2.0 (3,098) and PermaNet 3.0 (14,984) nets. While in Balaka 2 health facilities received Olyset (2,719), Olyset plus (10,000), PermaNet 2.0 (2,719) and PermaNet 3.0 (9,979) nets. In each district, one village with conventional and another with PBO nets was selected for entomological monitoring. Mosquito sampling was carried out in 15 households in each village as described above. However, many households in the selected study villages had a mixture of different nets types or brands.

Sequence of sampling:

Each of the 15 houses at each site were subjected to all 3 sampling methods. On Day 1 of field visit, CDC-LTs and WETs were deployed in two different bedrooms of each house. PSCs were

carried out early in the morning on Day 2 in the same houses. In order to check whether there was interference between the collection methods, 15 separate houses at each village in Karonga, Nkhotakota and Chikwawa were sampled using PSCs alone.

Mosquito processing:

In the field, specimens were transferred into sample bottles containing 95% alcohol and transported to the lab at MAC in Blantyre for further processing. All mosquito specimens were counted and morphologically identified to genus level using morphological identification keys (Gillies, M. T., and M. Coetzee, 1987). *Anopheles* mosquitoes were further identified to individual sibling species within the *An. funestus s.l.* and *An. gambiae s.l.* species by conventional polymerase chain reaction (PCR). The presence of *Plasmodium falciparum* sporozoites within the head and thorax parts of *Anopheles* mosquitoes was detected by enzyme-linked immunosorbent assays (ELISA).

Live collections:

Live bloodfed female *Anopheles* (F_0) resting inside people's homes were collected from Chikhwawa, Nkhotakota, Karonga, Ntcheu and Balaka using Prokopack aspirators. The collected samples were allowed to lay eggs, reared and resultant adults (F_1) tested for insecticide resistance. In addition, *Anopheles* larvae (F_1) were collected from their breeding habitats and reared to adults and subsequently tested. All mosquito rearing activities were carried out at the MAC Insectary in Blantyre.

Susceptibility assays:

WHO tube and CDC bottle bioassays were used to assess mortality and knockdown (KD) effect of different *Anopheles* species exposed to selected types of insecticides; alphacypermethrin, permethrin, deltamethrin, chlorfenapyr, bendiocarb, malathion and pirimiphos-methyl at their respective diagnostic doses and time intervals of 10, 20, 30, 40, 50 and 60 minutes with final mortality recorded at 24 hours post-exposure (W.H.O. 2013). For susceptibility testing, the report has incorporated data from 2017 to 2018.

Results

Vector abundance

Species abundance and distribution

A total of 17,045 mosquitoes including both males and females were collected in 5 districts during the period under review. A total of 14,613 female mosquitoes were sampled and the results are summarized by species and sentinel site in Table 1. *An. gambiae* (38.9%; n=5,698) and *An. funestus* (36.2%; n=5,296) were the most abundant while *Culex spp.* and *Mansonia spp.* constituted 16.2% (n=2,360) and 8.6% (n=1,253) of the total collections, respectively. *An. coustani* was very rare. More mosquitoes were collected from Nkhotakota (6,582; 45.04%) followed by Karonga (6,377; 43.64%), Chikwawa (1,334; 9.13%), Balaka (185; 1.27%) and Ntcheu (135; 0.92%) [Table 1]. Of the two *Anopheles* malaria vectors sampled, *An. funestus* was predominant across the five study districts with the exception of Karonga District where *An. gambiae* was the most common (Figure 2). Results of *Anopheles* density (mean per house) are shown in Table 2. The three districts of Karonga, Nkhotakota and Chikwawa had a high number of *Anopheles* per house estimated at 45.4, 37.0 and 8.0 respectively during the collection period. *Anopheles* density was low (<1 mosquito/ house) for Balaka and Ntcheu Districts.

Table 1: Mosquito species abundance in the five entomological monitoring districts of Balaka, Chikwawa, Karonga, Nkhotakota and Ntcheu. Data are pooled from the 3 collections methods.

	District					
Species	**Balaka n(%)**	**Chikwawa n (%)**	**Karonga n (%)**	**Nkhotakota n (%)**	**Ntcheu n (%)**	**Total n (%)**
An. coustani	3 (50)	1 (16.67)	0 (0)	0 (0)	2 (33.33)	6 (100)
An. funestus	62 (1.2)	741 (13.99)	36 (0.68)	4,377 (82.65)	80 (1.51)	5,296 (100)
An. gambiae	2 (0.04)	219 (3.84)	5,408 (94.91)	62 (1.09)	7 (0.12)	5,698 (100)
Culex sp	114 (4.83)	290 (12.29)	809 (34.28)	1,101 (46.65)	46 (1.95)	2, 360 (100)
Mansonia sp	4 (0.32)	82 (6.62)	124 (9.90)	1,042 (83.16)	0 (0)	1,253 (100)
Total	**185 (1.27)**	**1,334 (9.13)**	**6,377 (43.64)**	**6,582 (45.04)**	**135 (0.92)**	**14, 613 (100)**

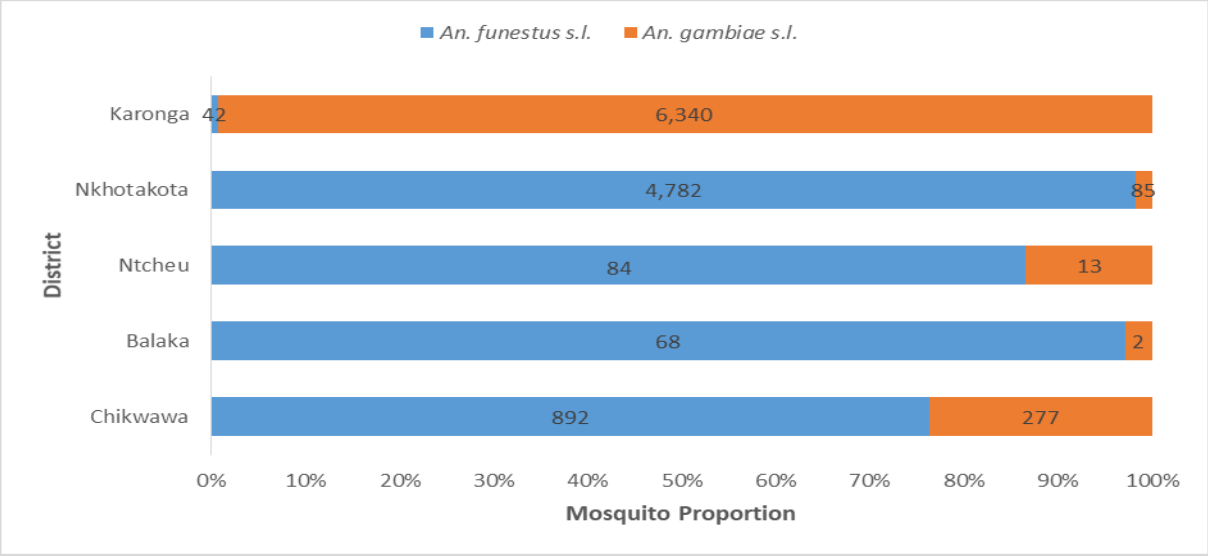

Figure 2: *Anopheles* species abundance (red is *An. gambiae s.l.* and blue is *An. funestus s.l.*) in the five monitoring districts pooled over the two sampling villages in each district. Total numbers are indicated inside the bars

Table 2: Estimated mean density of female *Anopheles* mosquitoes per house across the fives study districts and pooled by collection method and during the sampling period.

Species	District				
	Balaka	**Chikwawa**	**Karonga**	**Nkhotakota**	**Ntcheu**
An. funestus	0.5	6.2	0.3	36.5	0.7
An. gambiae	0.2	1.8	45.1	0.5	0.1
Total	0.7	8	45.4	37.0	0.8

Plasmodium falciparum **sporozoite infections in** ***Anopheles*** **salivary glands**

A sub-sample of 3,397 *Anopheles* mosquitoes from the five study districts was processed and analyzed for *Plasmodium falciparum* sporozoite infections using ELISA and the results are shown in Table 3. Overall, *P. falciparum* infection rates were estimated at 2.6%. However, there were differences in infection rates between study areas and species. High sporozoites infection rates were found in Chikwawa (4.1%; n=31) where both *An. funestus* and *An. arabiensis* had high infections. Despite low *Anopheles* density, high sporozoite infection rates were observed in Balaka (3.5%) and Ntcheu (3.2%) Districts followed by Nkhotakota (2.3%). The two study

villages in Karonga District where *An. arabiensis* was the predominant *Anopheles* vector recorded the lowest sporozoite infection rates (0.1%). In all the districts except Karonga, *An. funestus* was the most important vector with infection rates of 3.5%, 4.2%, 3.2% and 2.4% in Balaka, Chikwawa, Nkhotakota and Ntcheu respectively.

Table 3: *Plasmodium falciparum* sporozoite infecton rates (%) detected in the thorax+head mosquito parts of *An. funestus* and *An. arabiensis* in the five study districts

District	Species	No. tested positive	Number tested	Infection rate (%)
Balaka	**Total**	**4**	**114**	**3.5**
	An. funestus	4	110	3.6
	An. arabiensis		4	0
Chikwawa	**Total**	**31**	**757**	**4.1**
	An. funestus	26	626	4.2
	An. arabiensis	5	131	3.8
Karonga	**Total**	**1**	**841**	**0.1**
	An. funestus		33	0
	An. arabiensis	1	808	0.1
Nkhotatota	**Total**	**51**	**1598**	**3.2**
	An. funestus	51	1581	3.2
	An. arabiensis		17	0
Ntheu	**Total**	**2**	**87**	**2.3**
	An. funestus	2	82	2.4
	An. arabiensis		5	0
Overall		**89**	**3397**	**2.6**

Performance of collection methods

CDC-LTs, WETs and PSCs and *Anopheles* abundance

Table 4 shows the three mosquito sampling methods (CDC-LTs, PSCs and WETs) and their corresponding mean catches. There were differences in the mean catch of *Anopheles* mosquitoes by the three methods. Overall CDC-LTs mean catch (28.8 mosquitoes/trap night) was highest followed by PSCs (23.6 mosquitoes/collection). The WET mean catch (3.7 mosquitoes/trap night) was the lowest. Performance of the three techniques by district is shown in Figure 3. CDC-LTs performed much better in Nkhotakota, Ntcheu and Chikwawa, while PSCs performed better in Karonga and Balaka. WETs collected the lowest number of mosquitoes in all the study

sites. Although data are not shown here, CDC-LTs yielded more of *An. funestus s.l.* while PSCs were biased towards *An. gambiae s.l.* But the former was only apparent in Karonga district.

Table 4: Mean catch of *Anopheles* mosquitoes sampled by CDC-LTs, PSCs and WETs combined across all the study sites

Collection method	Mean *Anopheles* catches (95% CI)
CDC-LTs	28.8 (22.5 – 35.1)
WETs	3.7 (2.1 – 5.3)
PSCs	23.6 (2.2 – 35.1)

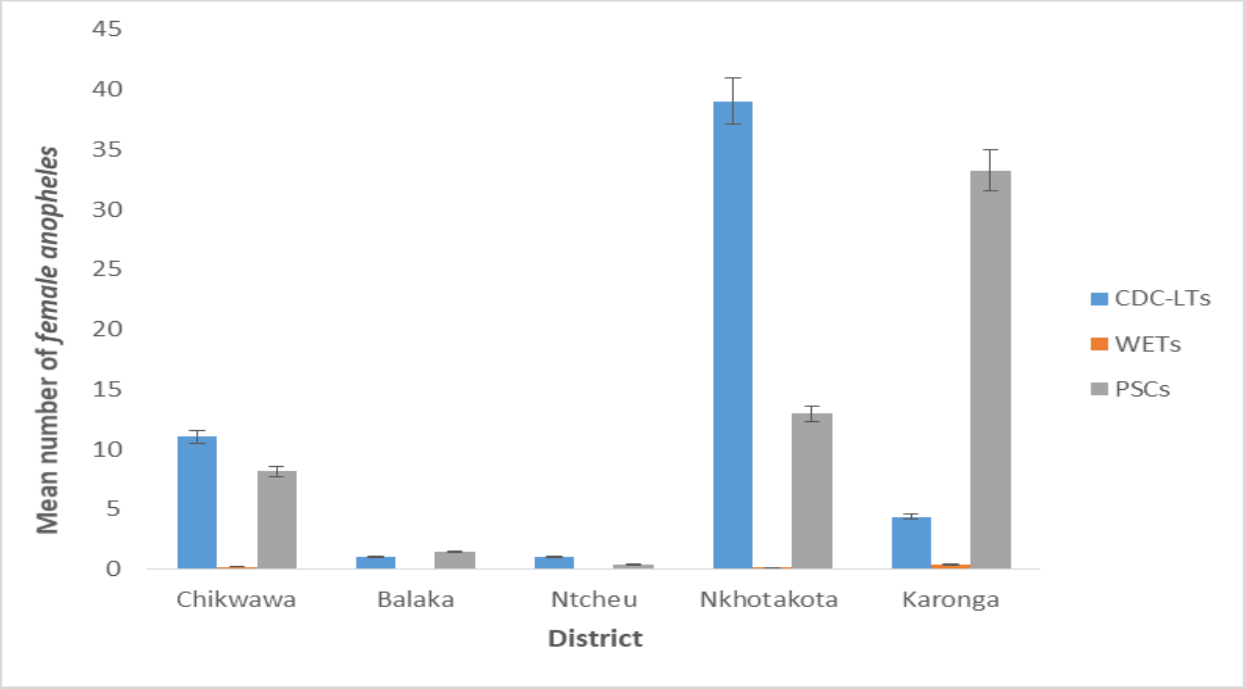

Figure 3: Mean number of female *Anopheles* mosquitoes sampled by CDC-LTs, PSCs and WETs across the five study districts and their 95% confidence levels.

CDC-LTs, WETs and PSCs versus *Anopheles* gonotrophic status

Table 5 summarizes the different female *Anopheles* mosquitoes sampled by CDC-LTs, WETs and PSCs whose gonotrophic status (blood feeding status) was ascertained. Out of 10,968 female *Anopheles* mosquitoes caught, 2,644 were bloodfed, 1,328 were gravid, 571 were half-gravid, 6,321 were unfed and 104 individual female mosquito abdomens could not be identified. There were differences in the proportion of bloodfed, half-gravid, gravid and unfed female *Anopheles* mosquitoes sampled by the three techniques ($\chi 2 = 45.7$, $P<0.0001$) (Figure 4).

Overall, CDC-LTs collected more unfed mosquitoes (91.3%; n=5,243) while PSCs collected more bloodfed mosquitoes (44.7%; n=2,253) and WETs collected more unfed mosquitoes (54.2%; n=45). Figure 5 illustrates the distribution of gonotrophic status of female *Anopheles* mosquitoes in individual sampling districts. Similar trends were observed across the districts except in cases where WETs did not sample any female *Anopheles* mosquito.

Table 5: Proportion of *An. funestus s.l.* and *An. gambiae s.l.* and their gonotrophic status

	Gonotrophic status				
Species	**Bloodfed**	**Gravid**	**Half-gravid**	**Unfed**	**Total n (%)**
An. funestus s.l.	473 (8.9)	506 (9.6)	294 (5.6)	3,996 (75.8)	5,269
An. gambiae s.l.	2,171 (38.8)	822 (14.8)	277 (4.9)	2,325 (41.6)	5,595
Total	**2,664**	**1,328**	**571**	**6,321**	**10,864**

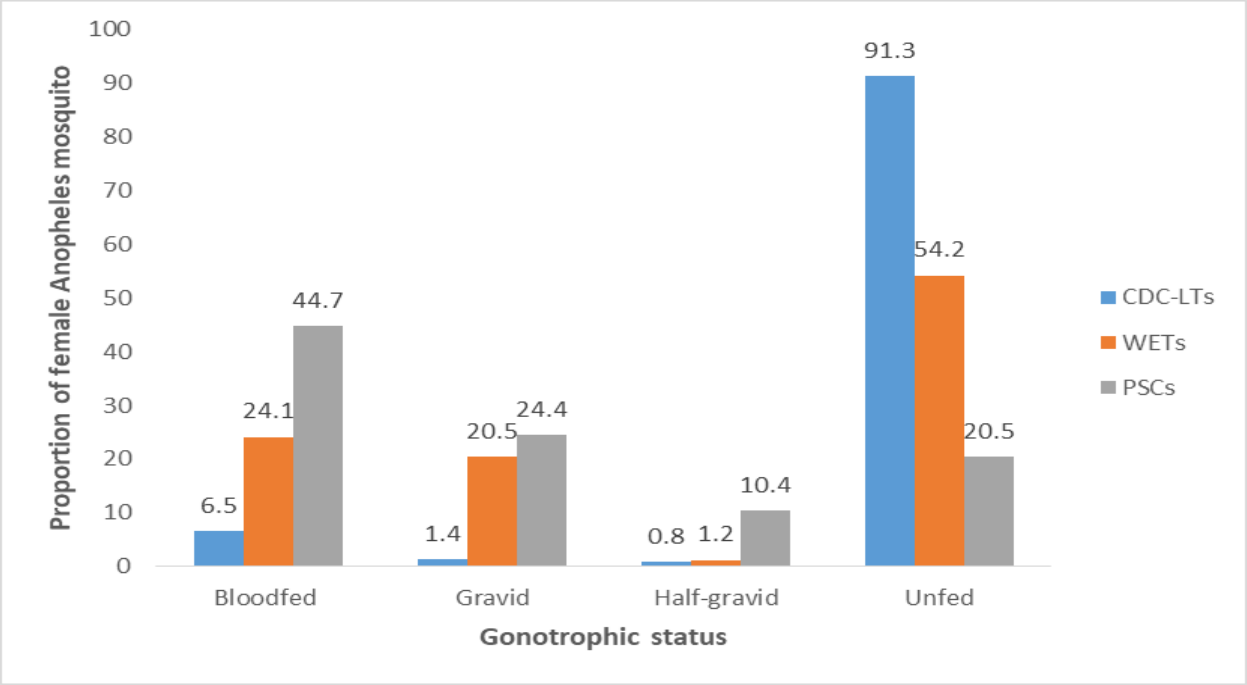

Figure 4: Gonotrophic status of female *Anopheles* mosquitoes sampled by three techniques, CDC-LTs, WETs and PSCs pooled across study sites.

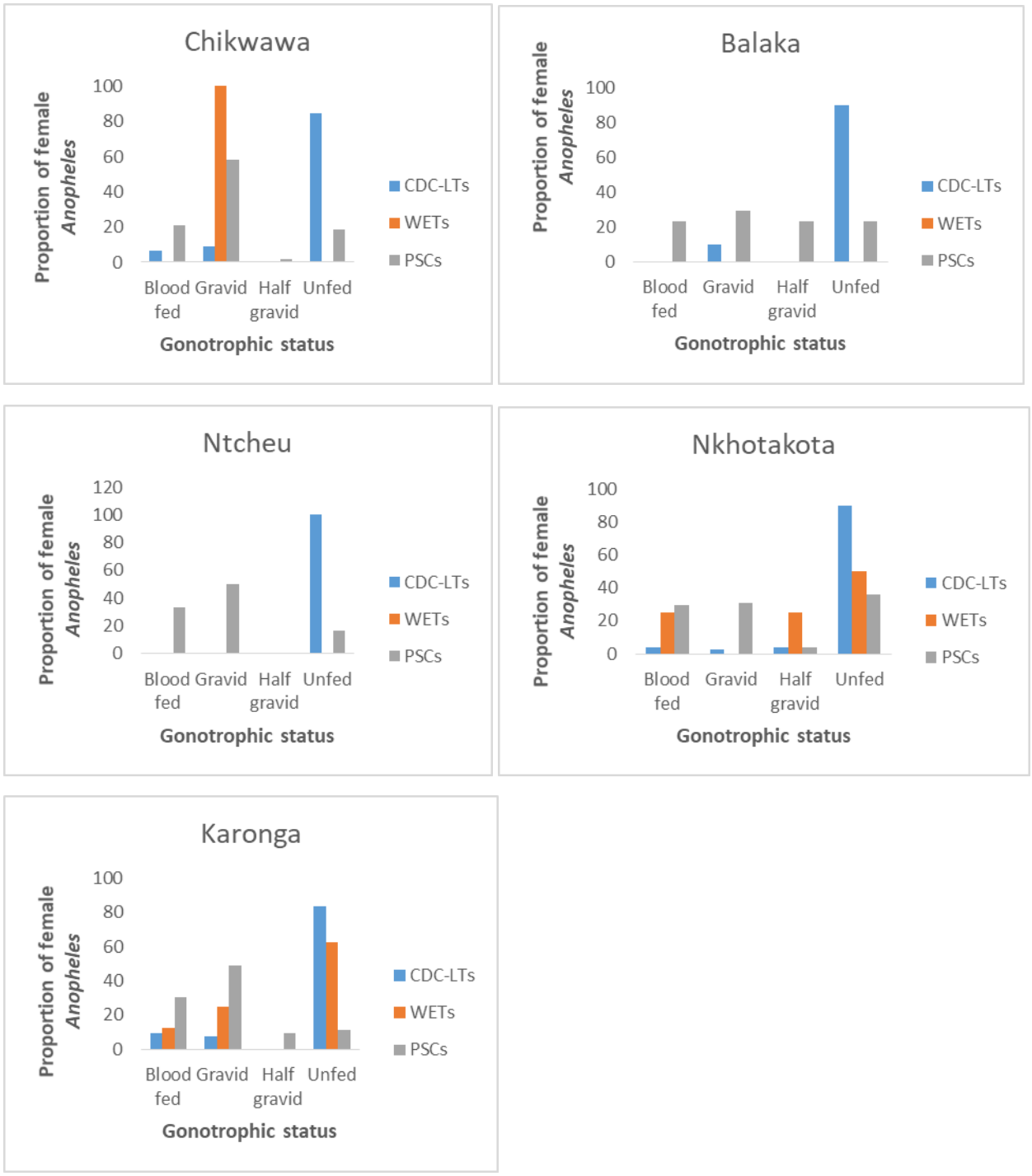

Figure 5: Gonotrophic status of female *Anopheles* mosquitoes sampled by CDC-LTs, WETs and PSCs in different sampling districts.

Insecticide resistance status

Results of *Anopheles* exposure to fixed-dose WHO insecticide test papers are presented in Figure 6. For Karonga, where *An. arabiensis* predominated, mosquitoes were susceptible (100% mortality, n=24) to alphacypermethrin but showed moderate resistance to permethrin (65.7% mortality, n=67). Mortality of *An. arabiensis* to malathion was 97.7% (n=86) which would be classified as "suspected resistance" according to WHO guidelines and therefore follow up tests are required. In Nkhotakota and Chikwawa where *An. funestus* was the predominant vector, high levels of resistance to both alphacypermethrin (49.6%, n=202) and permethrin (7.5%, n=53) in the former and 27.8% mortality (n=79) and 0.0% mortality (n=25) in the latter were detected. Similarly, *An. funestus* showed high resistance to bendiocarb in Nkhotakota (21.4% mortality, n=86) and in Chikwawa (4.9% mortality, n=103). However, *An. funestus* was fully susceptible to the organophosphates, malathion (100% mortality, n=106) and pirimiphos-methyl (100% mortality, n=223) in Nkhotakota. Similarly, this species was fully susceptible to malathion (100% mortality, n=16) and pirimiphos-methyl (100% mortality, n=128) in Chikwawa district.

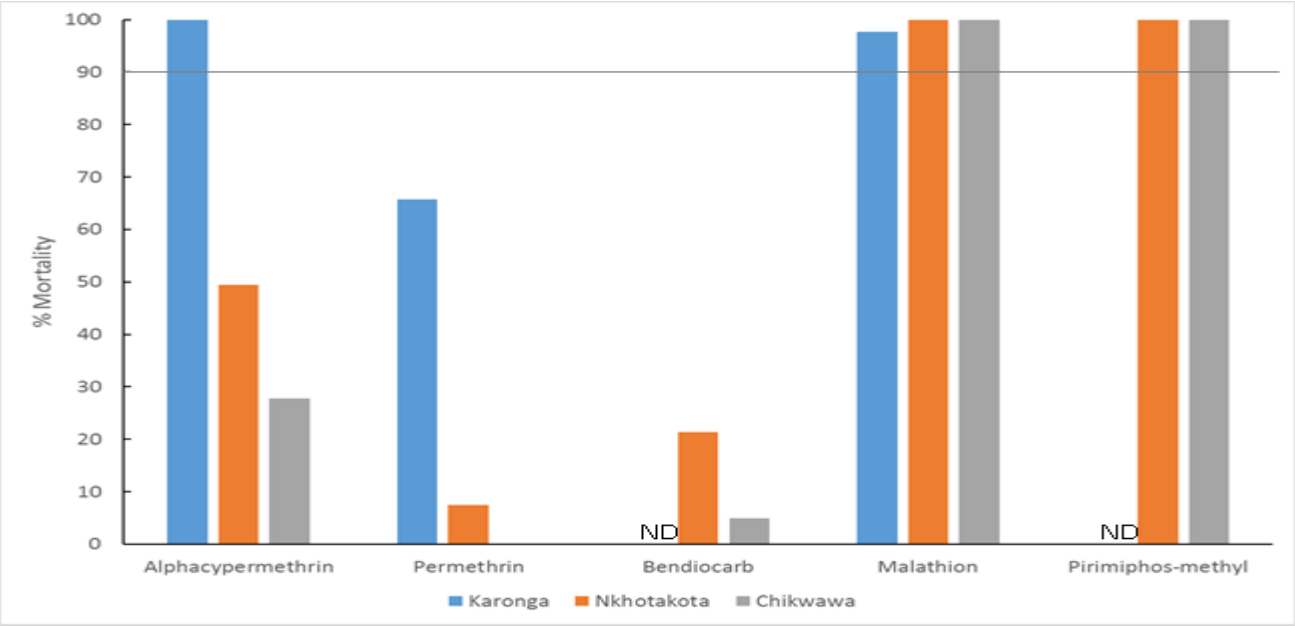

Figure 6. Mortality of mosquitoes collected from Karonga (*An. arabiensis*), Nkhotakota (*An. funestus*) or Chikwawa (*An. funestus*) in WHO resistance assays. ND=Not Done. The solid line is the WHO cut-off point/ threshold below which a population is considered resistant.

CDC bottle bioassays were used to estimate the LC50s in *An. funestus* population to three pyrethroid insecticides (permethrin, alphacypermethrin, deltamethrin) and to chlorfenapyr (a pyrrole). The results are shown in Figs. 7 and 8 for Chikwawa and Nkhotakota Districts. In Chikwawa, *An. funestus* showed high mortality (>98.0%) to permethrin at the 10X dose (n=75). But mortality rates of <90.0% were recorded to concentrations below 7.5X (n=72) indicating high insecticide resistance. Alphacypermethrin (92.9% mortality, n=141) and deltamethrin (81.1% mortality, n=127) (both Type II pyrethroids) completely failed to kill *An. funestus* at 10x the normal dose again indicating very intense resistance to these insecticides. Encouragingly, this species was completely susceptible to Chlorfenapyr at very low doses of 0.5X (100% mortality, n=131) and 1X (100% mortality, n=136) tested. Similar trends were observed in Nkhotakota district where *An. funestus* populations were highly resistant (<90.0% mortality) to the three pyrethroids, permethrin (85.1% mortality, n=67), alphacypermethrin (86.4% mortality, n=132) and deltamethrin (61.5% mortality, n=39) even at high concentrations (10X) and susceptible (100% mortality, n=41) to Chlorfenapyr at very low doses (<1X).

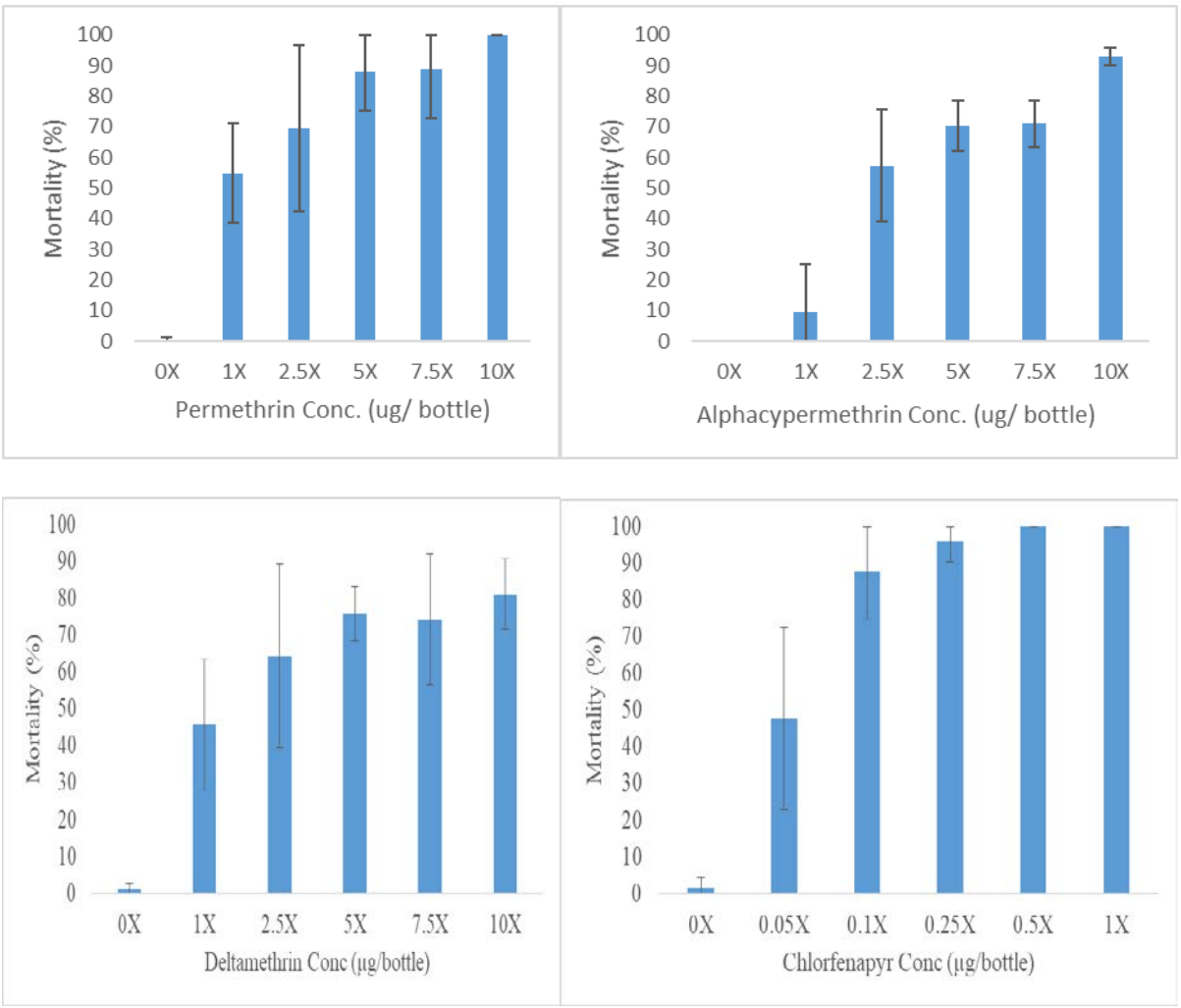

Figure 7: *An. funestus* mortality (%) to varying concentrations of Permethrin (1X=21.5 µg/bottle), Alphacypermethrin (1X=12.5 µg/bottle), Deltamethrin (1X=12.5 µg/ml) and Chlorfenapyr (1X=100 µg/ml) from Chikwawa District using the CDC bottle assay

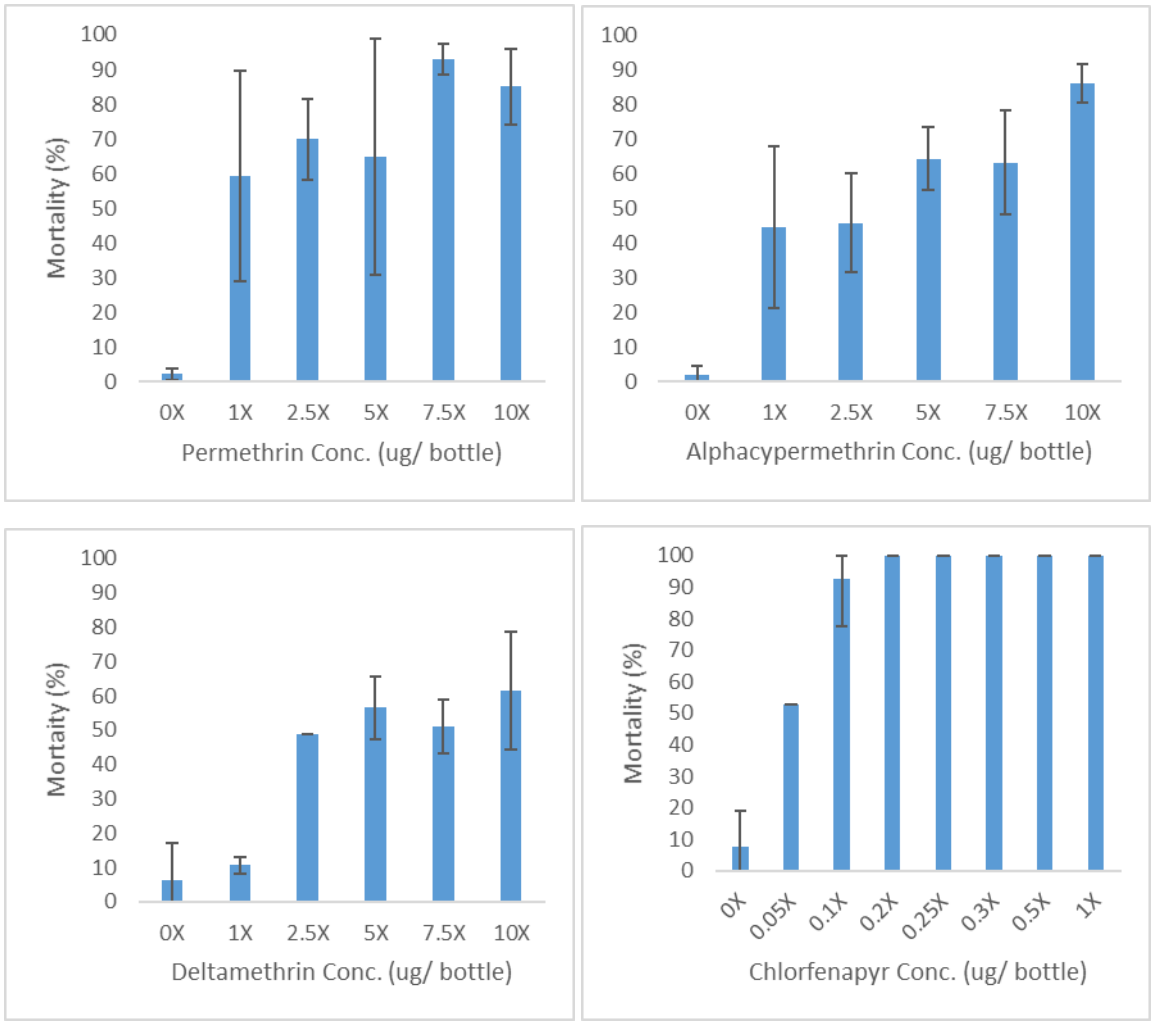

Figure 8: *An. funestus* mortality (%) to varying concentrations of Permethrin (1X=21.5 µg/ml), Alphacypermethrin (1X=12.5 µg/ml), Deltamethrin (1X=12.5 µg/ml) and Chlorfenapyr (1X=100 µg/ml) from Nkhotakota District using the CDC bottle assay

Figure 9 shows results of exposing *An. arabiensis* collected from Karonga to permethrin, alphacypermethrin and deltamethrin at insecticide concentrations of 0X, 0.05X, 0.01X, 0.25X and 1X. At 1X, *An. arabiensis* showed variable responses. It was resistant to permethrin (86.3% mortality, n=95) and alphacypermthrin (96.3% mortality, n=94) but showed susceptibility to deltamethrin (100% mortality, n=42). Furthermore, this species showed complete susceptibility to Chlorfenapyr at 0.5X (100% mortality, n=45) and 1X (100% mortality, n=44).

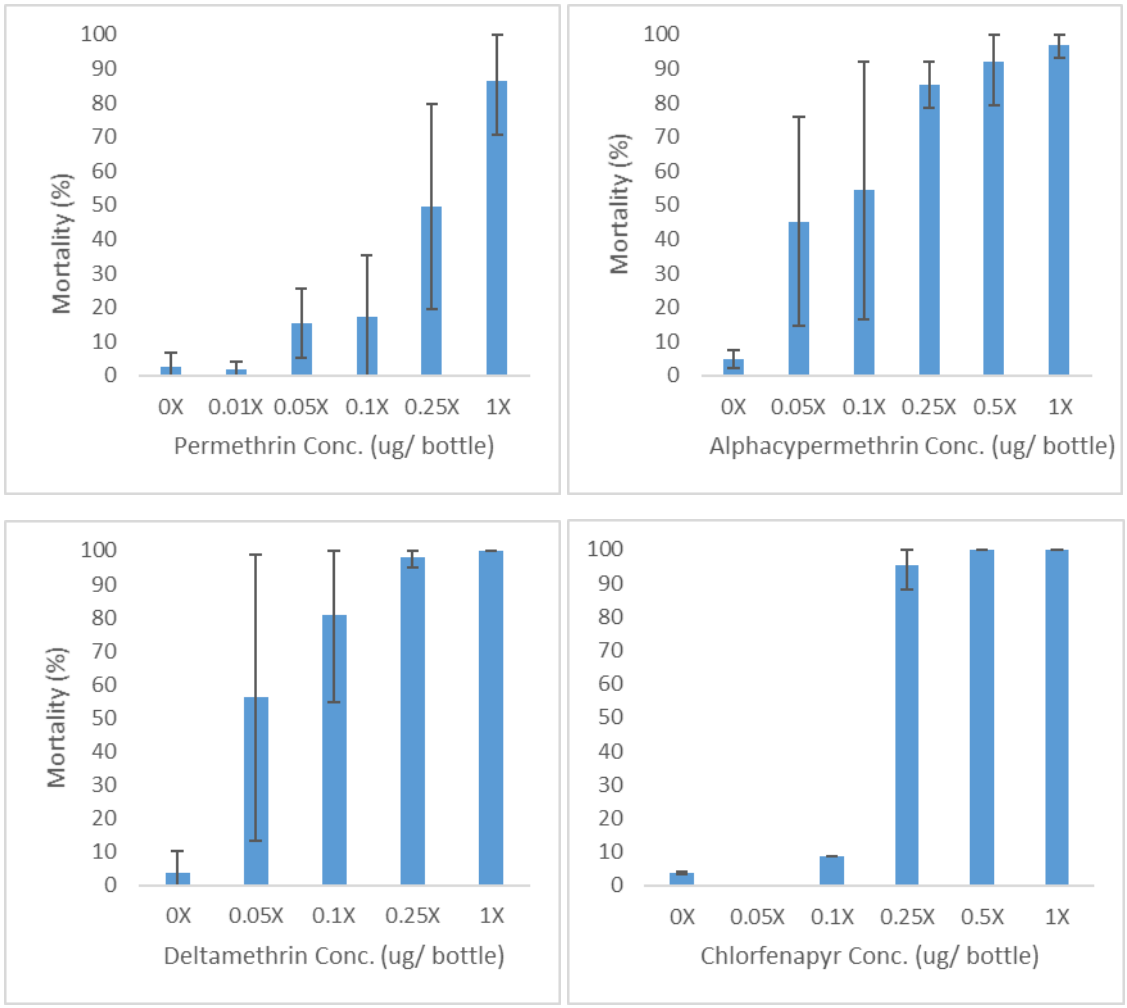

Figure 9: *An. arabiensis* mortality (%) to varying concentrations of Permethrin (1X=21.5 µg/ml), Alphacypermethrin (1X=12.5 µg/ml), Deltamethrin (1X=12.5 µg/ml) and Chlorfenapyr (1X=100 µg/ml) from Karonga District using the CDC bottle assay

Resistance ratios were calculated for permethrin, alphacypermethrin and deltamethrin and the results are shown in Fig. 10. Overall, permethrin showed lower RR compared to the Type II pyrethroids (alphacypermethrin and deltamethrin) and were site specific being higher in Chikwawa and Nkhotakota and low in Karonga probably reflecting the *Anopheles* species differences. *An. funestus* showed high resistance ratios to alphacypermethrin in Nkhotakota (RR=886) and Chikwawa (RR=950). Similarly, high resistance ratios were estimated to deltamethrin in Nkhotakota (>1400) and Chikwawa (692). Lower resistance ratios were estimated for permethrin both in Nkhotakota (RR=111) and Chikwawa (RR=145) although this was largely due to the relatively high LC50 of *An. gambiae* Kisumu strain to permethrin. On the

other hand, *An. arabiensis* showed low resistance ratios to permethrin (RR=26), alphacypermethrin (RR=23) and deltamethrin (RR=12).

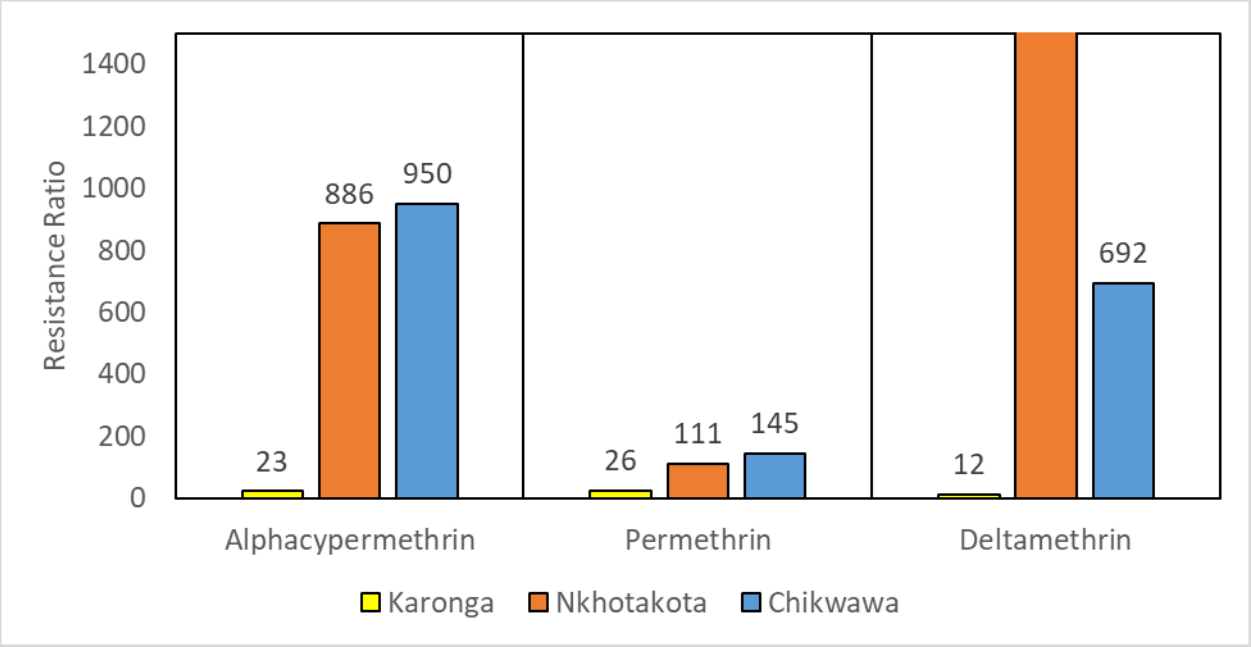

Figure 10. Estimates of alphacypermethrin, permethrin and deltamethrin resistance ratios (RR) for *An. funestus* from Chikwawa and Nkhotakota and *An. arabiensis* from Karonga District.

Discussion

The entomological monitoring activities carried out in 5 districts of Chikwawa, Balaka, Ntcheu, Nkhotakota and Karonga showed that *An. gambiae s.l.* and *An. funestus s.l.* remain the major malaria vectors in Malawi. The latter species was predominant in all study sites except Karonga District in the north where *An. arabiensis* was the predominant *Anopheles* mosquito species. The wide geographical range exhibited by *An. funestus* across multiple sites in the country and the common occurrence of *An. arabiensis* in Karonga District reported here confirms previous entomological monitoring findings. *An. funestus* and *An. arabiensis* naturally exhibit differences in their habitat preference. The former is usually associated with larger and more permanent water bodies while the latter is associated with small shallow temporary water bodies that are common during the wet season and rice paddies (Gimnig et al., 2001). Both study

villages in Karonga District were near formal rice irrigation schemes which could partly explain the finding.

High sporozoite infection rates were detected in Chikwawa (4.1%), Balaka (3.5%), Ntcheu (3.2%) and Nkhotakota (2.3%) and very low in Karonga (0.1%). The presence of *An. funestus* in the four high infection rates districts indicates high malaria transmission in those study districts. This species has previously been found to be an important malaria vector in Malawi (Mzilahowa et al., 2012). However, the high sporozoite infection rates detected in Balaka and Ntcheu District could also be due to the low number of mosquitoes analyzed. Similarly, the low sporozoite infection rates reported for Karonga District could be due to the predominance of *An. arabiensis* in the study areas. This species is capable of transmitting malaria but often feeds on non-human hosts and is therefore a less efficient vector than *An. funestus* which frequently feeds on humans. Previous malaria indicator surveys (MIS) have reported high malaria interventions coverage / use (ITNs) and low malaria parasite prevalence in the northern region (MIS 2010, 2012, 2014) hence the low sporozoite infections found in Karonga District could also be a reflection of the impact of malaria interventions.

CDC-LTs and PSCs were more productive at sampling *Anopheles* mosquitoes compared to WETs which consistently yielded fewer mosquitoes across the study sites. The poor performance by WETs could mean fewer mosquitoes were exiting the houses or there were other exit routes other than the particular windows where traps were fixed through which mosquitoes were exiting.

CDC-LTs performed better in sampling mosquitoes in three districts and where *An. funestus s.l.* was the main vector. These findings confirmed anecdotal results/ reports from other field studies carried out in Malawi. However, PSCs sampled more *An. gambiae s.l.* in Karonga District where this species was quite prevalent. Fontenille *et al* (1997) reported that *An. gambiae* was less attracted to light therefore they were less likely to be captured by CDC-LT.

This study showed that *An. arabiensis* was susceptible to alphacypermethrin and moderately resistant to permethrin in Karonga District. But high levels of phenotypic resistance were detected in *An. funestus* populations from Nkhotakota and Chikwawa to alphacypermethrin and permethrin (both pyrethroids) and bendiocarb (a carbamate). Use of these insecticides in these areas would exacerbate the resistance situation in this population of mosquitoes. However,

An. funestus populations were fully susceptible to the organophosphates (malathion and pirimiphos-methyl). These findings are consistent with previous reports on the status of pyrethroid insecticide resistance in the country (Mzilahowa et al., 2016). Furthermore, the two *Anopheles* vector populations found in the study area were also completely susceptible to chlorfenapyr, a pyrrole chemical compound.

This study has shown intense pyrethroid insecticide resistance especially in *An. funestus*. Comparable data on intensity of pyrethroid resistance from neighboring countries are scarce. However, work carried out in Burkina Faso showed that the strength of pyrethroid resistance had increased by more than 1,000 fold in *An. gambiae* populations in a study correlating levels of resistance and efficacy of bednets (Toe KH et al., 2014). Further, the resistance ratios shown in the present study are much higher than previously reported in Malawi (Riveron et al., 2015). Using the WHO tube assay, Riveron and others reported a resistance ratio of 18.6 fold in *An. funestus* to permethrin 0.75% in Chikwawa District, southern Malawi. Operationally, the impact of pyrethroid resistance particularly in *An. funestus* is unclear but previous studies have demonstrated very low mortality of this species when exposed to unused, unwashed LLINs in standard WHO cone bioassays suggesting that pyrethroid resistance may be seriously undermining the effectiveness of this intervention. However, a study in Machinga district found a significantly lower incidence of infection among users of LLINs compared to non-users of LLINs suggesting that nets still offer some protection (Lindblade et al., 2015). However, the impact of a community effect which is believed to be mediated by the insecticide could not be measured and this mode of action of LLINs may not be operating in settings with such high pyrethroid resistance. A multi-country study employing a similar study design found no correlation between the frequency of pyrethroid resistance as measured in WHO tube assays and incidence of malaria infection. However, a recent study of PBO nets in western Tanzania demonstrated a significant reduction in the prevalence of *Plasmodium falciparum* infection suggesting that observational studies of LLIN effectiveness may underestimate the impact of pyrethroid resistance. No clear effect of PBO nets was observed in Balaka or Ntcheu districts. However, the mosquito numbers were generally low and therefore, the monitoring had limited power to detect a difference. Furthermore, net coverage in these areas was lower than expected and many residents continued to use standard pyrethroid-only nets which may have further masked the effect of pyrethroid resistance and PBO.

Conclusions/ Recommendations

An. funestus and *An. arabiensis* are highly abundant and prevalent across a wide geographical range in Malawi although the latter was much more common in areas near rice irrigation schemes in Karonga District. The two *Anopheles* mosquitoes continue to be important malaria vectors as shown by high sporozoite infections in their salivary glands. The importance of these two vectors in malaria transmission requires further and detailed investigation.

CDC Light Traps and PSCs can be used singly or in combination to sample malaria vectors in the country and depending on study objectives.

The high levels of pyrethroid and carbamate resistance in *An. funestus* populations reported here is concerning and would suggest alternative insecticides are necessary to maximize the impact of vector control. Furthermore, the continued monitoring for insecticide resistance and sporozoite infections is recommended to assess the impact of malaria interventions as indoor residual spraying is re-introduced into Malawi PBO nets will be included as part of the 2018 mass LLIN distribution campaign.

Operationally, this report recommends the following actions for the continued monitoring:

- Moving monitoring sites from Balaka and Ntcheu Districts to different areas due to the low mosquito densities and the need to monitor the impact of IRS and the distribution of PBO nets. Potential areas for continued monitoring would be Nkhata Bay to the north and Salima to the south of Nkhotakota District which will be sprayed in 2018.
- Dropping window exit traps (WETs) after 1 year of their use as they have shown to be less productive using the current design.
- Evaluating PBO or other next generation long-lasting insecticidal nets (LLINs) and other new insecticides
- Since PBO nets are likely to be distributed in Malawi in 2018, PBO pre-exposures for some of the bioassays would be recommended to effectively monitor their impact on mosquito populations and resistance trends.

References

1. MIS: **Malaria Indicator Survey (MIS) 2014**. In. National Malaria Control Programme, Lilongwe, Malawi and ICF International, Calverton, Maryland, USA; 2015.
2. **Bayoh, M. N., D. K. Mathias, M. R. Odiere, F. M. Mutuku, L. Kamau, J. E. Gimnig, J.M. Vulule, W. A. Hawley, M. J. Hamel, and E. D. Walker. 2010**. Anopheles gambiae: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. Malaria Journal 9: 62.
3. **Lyimo, I. N., and H. M. Ferguson. 2009**. Ecological and evolutionary determinants of host species choice in mosquito vectors. Trends Parasitol. 25: 189 Ð196.
4. **Yohannes, M., and E. Boelee. 2012**. Early biting rhythm in the afro-tropical vector of malaria, Anopheles arabiensis, and challenges for its control in Ethiopia. Med. Vet. Entomol. 26: 103Ð105.
5. **Gillies, M. T., and M. Coetzee. 1987**. A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical region). South African Institute for Medical Research, Johannesburg.
6. **Fontenille D., Lochouarn L., Diatta M., Sokhna C., Dia I., Diagne N., Lemasson J.J., Ba K., Tall A., Rogier C., and J. F. Trape. 1997.** Entomological study of the transmission of seasonal malaria in Senegal and the bionomics of Anopheles gambiae and A. arabiensis. Trans R Soc Trop Med Hyg, 91:647-652.
7. MIS: **Malaria Indicator Survey (MIS) 2010**. In. National Malaria Control Programme, Lilongwe, Malawi and ICF International Calverton, Maryland, USA; 2010.
8. MIS: **Malaria Indicator Survey (MIS) 2012**. In. National Malaria Control Programme, Lilongwe, Malawi and ICF International, Calverton, Maryland, USA; 2013.
9. WHO 2013. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva, Switzerland.
10. Mzilahowa T, Hastings IM, Molyneux ME, McCall PJ: **Entomological indices of malaria transmission in Chikhwawa district, Southern Malawi**. *Malaria journal* 2012, **11**(380):1.
11. Mzilahowa T, Chiumia M, Mbewe RB, Uzalili VT, Luka-Banda M, Kutengule A, Mathanga DP, Ali D, Chiphwanya J, Zoya J *et al*: **Increasing insecticide resistance in Anopheles**

funestus and Anopheles arabiensis in Malawi, 2011-2015. *Malaria journal* 2016, **15**(1):563.

12. Riveron JM, M. Chiumia, BD. Menze, KG. Barnes, H. Irving, SS. Ibrahim, GD. Weedall, T. Mzilahowa and CS. Wondji. 2015. **Rise of multiple insecticide resistance in Anopheles funestus in Malawi: a major concern for malaria vector control**. Malar J (2015) 14:344
13. Lindblade, KA, D. Mwandama, T. Mzilahowa, L. Steinhardt, J. Gimnig, M. Shah, A. Bauleni, J. Wong, R. Wiegand, P. Howell, J. Zoya, J. Chiphwanya, DP. Mathanga. 2015. **A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi**. Malar J **14**: 31.
14. Toe, K. H., CM Jones, S. N'Fale, HM. Ismail, RK. Dabire, H. Ranson 2014. **Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso**. Emerg Infect Dis **20**(10): 1691-6
15. Gimnig, J. E., M. Ombok, et al. (2001). **Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya**. J Med Entomol **38**(2): 282-8.

Appendix 1

Table 1A: Summary of number of mosquito samples (N) tested against various insecticides (alphacypermethrin, deltamenthrin, permethrin and chlorfenapyr), their respective doses and source or location of the specimens

District	Species	Insecticide	Intensity	Dose	N
Karonga	*An. arabiensis*	Alphacypermethrin	0X	0	105
Karonga	*An. arabiensis*	Alphacypermethrin	0.05X	0.625	73
Karonga	*An. arabiensis*	Alphacypermethrin	0.1X	1.25	70
Karonga	*An. arabiensis*	Alphacypermethrin	0.25X	3.125	89
Karonga	*An. arabiensis*	Alphacypermethrin	0.5X	6.25	100
Karonga	*An. arabiensis*	Alphacypermethrin	1X	12.5	94
Karonga	*An. arabiensis*	Permethrin	0X	0	118
Karonga	*An. arabiensis*	Permethrin	0.01X	0.215	102
Karonga	*An. arabiensis*	Permethrin	0.05X	1.075	98
Karonga	*An. arabiensis*	Permethrin	0.1X	2.15	93
Karonga	*An. arabiensis*	Permethrin	0.25X	5.375	97
Karonga	*An. arabiensis*	Permethrin	1X	21.5	95
Karonga	*An. arabiensis*	Deltamethrin	0X	0	83
Karonga	*An. arabiensis*	Deltamethrin	0.05X	0.625	57
Karonga	*An. arabiensis*	Deltamethrin	0.1X	1.25	57
Karonga	*An. arabiensis*	Deltamethrin	0.25X	3.125	52
Karonga	*An. arabiensis*	Deltamethrin	1X	12.5	42
Karonga	*An. arabiensis*	Chlorfenapyr	0X	0	53
Karonga	*An. arabiensis*	Chlorfenapyr	0.05X	5	28
Karonga	*An. arabiensis*	Chlorfenapyr	0.1X	10	23
Karonga	*An. arabiensis*	Chlorfenapyr	0.25X	25	43
Karonga	*An. arabiensis*	Chlorfenapyr	0.5X	50	49
Karonga	*An. arabiensis*	Chlorfenapyr	1X	100	44
Nkhotakota	*An. funestus*	Alphacypermethrin	0X	0	191
Nkhotakota	*An. funestus*	Alphacypermethrin	1X	12.5	201
Nkhotakota	*An. funestus*	Alphacypermethrin	2.5X	31.25	96
Nkhotakota	*An. funestus*	Alphacypermethrin	5X	62.5	169
Nkhotakota	*An. funestus*	Alphacypermethrin	7.5X	93.75	101
Nkhotakota	*An. funestus*	Alphacypermethrin	10X	125	132
Nkhotakota	*An. funestus*	Permethrin	0X	0	174
Nkhotakota	*An. funestus*	Permethrin	1X	21.5	191
Nkhotakota	*An. funestus*	Permethrin	2.5X	53.75	103
Nkhotakota	*An. funestus*	Permethrin	5X	107.5	68

District	Species	Insecticide	Intensity	Dose	N
Nkhotakota	*An. funestus*	Permethrin	7.5X	161.25	87
Nkhotakota	*An. funestus*	Permethrin	10X	215	67
Nkhotakota	*An. funestus*	Deltamethrin	0X	0	47
Nkhotakota	*An. funestus*	Deltamethrin	1X	12.5	56
Nkhotakota	*An. funestus*	Deltamethrin	2.5X	31.25	39
Nkhotakota	*An. funestus*	Deltamethrin	5X	62.5	46
Nkhotakota	*An. funestus*	Deltamethrin	7.5X	93.75	51
Nkhotakota	*An. funestus*	Deltamethrin	10X	125	39
Nkhotakota	*An. funestus*	Chlorfenapyr	0X	0	52
Nkhotakota	*An. funestus*	Chlorfenapyr	0.05X	5	19
Nkhotakota	*An. funestus*	Chlorfenapyr	0.1X	10	40
Nkhotakota	*An. funestus*	Chlorfenapyr	0.2X	20	26
Nkhotakota	*An. funestus*	Chlorfenapyr	0.25X	25	15
Nkhotakota	*An. funestus*	Chlorfenapyr	0.3X	30	25
Nkhotakota	*An. funestus*	Chlorfenapyr	0.5X	50	39
Nkhotakota	*An. funestus*	Chlorfenapyr	1X	100	41
Chikwawa	*An. funestus*	Alphacypermethrin	0X	0	148
Chikwawa	*An. funestus*	Alphacypermethrin	1X	12.5	125
Chikwawa	*An. funestus*	Alphacypermethrin	2.5X	31.25	143
Chikwawa	*An. funestus*	Alphacypermethrin	5X	62.5	125
Chikwawa	*An. funestus*	Alphacypermethrin	7.5X	93.75	128
Chikwawa	*An. funestus*	Alphacypermethrin	10X	125	141
Chikwawa	*An. funestus*	Permethrin	0X	0	209
Chikwawa	*An. funestus*	Permethrin	1X	21.5	166
Chikwawa	*An. funestus*	Permethrin	2.5X	53.75	69
Chikwawa	*An. funestus*	Permethrin	5X	107.5	74
Chikwawa	*An. funestus*	Permethrin	7.5X	161.25	72
Chikwawa	*An. funestus*	Permethrin	10X	215	75
Chikwawa	*An. funestus*	Deltamethrin	0X	0	167
Chikwawa	*An. funestus*	Deltamethrin	1X	12.5	120
Chikwawa	*An. funestus*	Deltamethrin	2.5X	31.25	149
Chikwawa	*An. funestus*	Deltamethrin	5X	62.5	145
Chikwawa	*An. funestus*	Deltamethrin	7.5X	93.75	113
Chikwawa	*An. funestus*	Deltamethrin	10X	125	127
Chikwawa	*An. funestus*	Chlorfenapyr	0X	0	133
Chikwawa	*An. funestus*	Chlorfenapyr	0.05X	5	122
Chikwawa	*An. funestus*	Chlorfenapyr	0.1X	10	138
Chikwawa	*An. funestus*	Chlorfenapyr	0.25X	25	123
Chikwawa	*An. funestus*	Chlorfenapyr	0.5X	50	131
Chikwawa	*An. funestus*	Chlorfenapyr	1X	100	136

District	Species	Insecticide	Intensity	Dose	N
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0X	0	190
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.0005X	0.00625	52
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.001X	0.0125	47
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.0025X	0.03125	49
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.005X	0.0625	77
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.01X	0.125	99
Kisumu Strain	*An. gambiae*	Alphacypermethrin	0.05X	0.625	104
Kisumu Strain	*An. gambiae*	Permethrin	0X	0	196
Kisumu Strain	*An. gambiae*	Permethrin	0.0001X	0.00215	91
Kisumu Strain	*An. gambiae*	Permethrin	0.0025X	0.05375	90
Kisumu Strain	*An. gambiae*	Permethrin	0.005X	0.1075	150
Kisumu Strain	*An. gambiae*	Permethrin	0.01X	0.215	144
Kisumu Strain	*An. gambiae*	Permethrin	0.05X	1.075	51
Kisumu Strain	*An. gambiae*	Permethrin	0.1X	2.15	28
Kisumu Strain	*An. gambiae*	0X	Deltamethrin	0	92
Kisumu Strain	*An. gambiae*	0.0001X	Deltamethrin	0.00125	96
Kisumu Strain	*An. gambiae*	0.0005X	Deltamethrin	0.00625	93
Kisumu Strain	*An. gambiae*	0.001X	Deltamethrin	0.0125	91
Kisumu Strain	*An. gambiae*	0.0025X	Deltamethrin	0.03125	89
Kisumu Strain	*An. gambiae*	0.005X	Deltamethrin	0.0625	89
Kisumu Strain	*An. gambiae*	0.01X	Deltamethrin	0.125	93
Kisumu Strain	*An. gambiae*	Chlorfenapyr	0X	0	27
Kisumu Strain	*An. gambiae*	Chlorfenapyr	0.125X	12.5	24
Kisumu Strain	*An. gambiae*	Chlorfenapyr	0.25X	25	22
Kisumu Strain	*An. gambiae*	Chlorfenapyr	0.5X	50	22
Kisumu Strain	*An. gambiae*	Chlorfenapyr	1X	100	25
Kisumu Strain	*An. gambiae*	Chlorfenapyr	2X	200	22