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PMI VECTORLINK MALAWI ANNUAL ENTOMOLOGICAL MONITORING REPORT

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Acronyms

Ace-1	Acetylcholinesterase 1
b/p/h	bites/person/hour
b/p/n	bites/person/night
CDC	Centers for Disease Control and Prevention
EIR	Entomological Inoculation Rate
ELISA	Enzyme-Linked Immunosorbent Assay
$\mathbf{F_0}$	Filial generation 0
$\mathbf{F_1}$	Filial generation 1
FF	Fludora Fusion
HBR	Human Biting Rate
HLC	Human Landing Catch
ib/p/m	infective bites/person/month
IG2	Interceptor G2
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
LT	Light Trap
m/h/d	mosquitoes per house per day
m/t/n	mosquitoes per trap per night
MAC	Malaria Alert Centre
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
s.l.	sensu lato
SOP	Standard Operating Procedure
SR	Sporozoite Rate
s.s.	sensu stricto

SS	SumiShield
USAID	United States Agency for International Development
WHO	World Health Organization
WVI	World Vision International

EXECUTIVE SUMMARY

In Malawi, more than 95% of the country is malaria endemic Ninety-five percent of malaria infection and deaths are due to *Plasmodium falciparum* (*Pf*), the most severe form of the four human malaria parasites. Malaria is the leading cause of morbidity in children under 5 and pregnant women in Malawi and is responsible for approximately 7 million cases and 36% of outpatient visits across all ages (US/PMI MOP, 2022). Insecticide-treated nets have been widely used as a major vector control intervention in the country, and indoor residual spraying (IRS) is done in selected high-burden designated areas. The U.S. President's Malaria Initiative (PMI) VectorLink Project in Malawi and World Vision International (WVI) in collaboration with the Malaria Alert Centre (MAC) conducted longitudinal monitoring from July 2021 to June 2022 in 17 sentinel sites in eight districts to assess malaria vector bionomics and susceptibility of the prime malaria vectors to insecticides in use for public health.

Vector Bionomics: A total of 34,869 female *Anopheles* mosquitoes were collected from 17 sentinel sites in all eight monitoring districts from July 2021 to June 2022. Of these, 20,168 (57.9%) were collected using pyrethrum spray catches (PSCs), 11,972 (34.3%) using Centers for Disease Control and Prevention Traps (CDC-LTs), and 2,729 (7.8%) using human landing catches (HLCs).

Overall, 49.8% (n=17,364) of the *Anopheles* collected were morphologically identified as *An. funestus* s.l., 46.8% (n=16,316) were *An. gambiae* s.l., and 3.4% (n=1,169) were *An. coustani.* However, species composition varied by sentinel site: *An. funestus* s.l. was predominant in Sanga and Kande sites (Nkhata Bay District); Ngalauka, Chimkwende, and Vwawa sites (Nkhotakota District); Nyalubwe and Kachokolo sites (Kasungu District); Chilungo site (Salima District); and Kabota site (Balaka District). *An. gambiae* s.l. was predominant in Mwenimambwe and Mwakanyamale sites (Karonga District); Ntwana and Nyamphota sites (Chikwawa District); Chimdikiti site (Balaka District); and Maluwa and Piyasi sites (Mangochi District). Species composition also varied based on indoor or outdoor location. Overall, the highest number of *An. funestus* s.l. (n=1087, 69.1%) were collected indoors, while the highest numbers of *An. gambiae* s.l. (n=759, 68.1%) and *An. coustani* (n=883, 78.5%) were collected outdoors. Furthermore, 346 other female *Anopheles* and 6,321 mosquitoes of other genera were collected during the monitoring period using the three methods. Among *Anopheles* mosquitoes collected were *An. pharoensis* (n=202), *An. tenebrosus* (n=67), *An. pretoriensis* (n=60), *An. maculipalpis* (n=9), and *An. squamosus* (n=8). Other mosquito genera collected were: 3,773 *Culex*, 2,548 *Mansonia,* and 40 *Aedes.*

A total of 2,585 female *An. gambiae* s.l. were randomly sampled and identified to species level by polymerase chain reaction (PCR). Of these, 2,579 (99.8%) were identified as *An. arabiensis* and 6 (0.2%) as *An. gambiae* s.s. *An. arabiensis* was the predominant member of the *An. gambiae* complex, occurring in all the eight districts, while *An. gambiae* s.s. was found in three districts: Chikwawa (n=1, 0.9%), Karonga (n=1, 0.8%), and Salima (n=3, 2.1%). A total of 565 (99.8%) *An. funestus* s.l. were identified by PCR as *An. funestus* s.s. and 1 (0.2%) was identified as *An. leesoni* from Salima District. *An. funestus* s.s. was the predominant member of *An. funestus* s.l. and occurred in all eight districts. *An. leesoni* was detected for the first time this year.

Human Biting Rate (HBR) and Location: HBR was measured in six districts: Nkhata Bay, Nkhotakota, Salima, Kasungu, Mangochi, and Balaka. Overall, from the HLC collection, *An. funestus* s.l. HBRs for were 4.6 bites per person per night (b/p/n) indoors and 2.5 n/p/n outdoors. The HBRs for *An. gambiae* s.l. were 1.5 b/p/n indoors and 3.19 b/p/n outdoors. The HBRs for *An. coustani* were 1.39 b/p/n indoors and 5.16 b/p/n outdoors. The highest outdoor human biting activity of *An. funestus* s.l.., 5.3 b/p/n, was observed in March. Both the highest indoor and outdoor biting activity of *An. gambiae* s.l. were observed in March, 4.1 b/p/n and 8.8 b/p/n respectively. The highest indoor and outdoor human biting activity of *An. coustani* was observed in March (4.0 b/p/n) and (10.6 b/p/n), respectively. The highest indoor biting activity of *An. funestus* s.l. was recorded indoors in June (30.2 b/p/n) and September (16.8 b/p/n) in Nkhotakota District, and in March (10.3 b/p/n) in Salima District. The highest biting activity of *An. gambiae* s.l. was observed in Nkhata Bay District in March (6.3 b/p/n), indoors, and in Kasungu District (11.9 b/p/n), outdoors. The highest indoor and outdoor biting activity of *An. coustani* was observed in March in Nkhata Bay (14.2 b/p/n) and June in Nkhotakota (61.7 b/p/n) districts, respectively.

Overall indoor HBR from CDC-LT collection was 1.4 b/p/n for *An. funestus* s.l. and 6.8 b/p/n for *An. gambiae* s.l. The highest HBR for *An. funestus* s.l. was 55.8 b/p/n in Nkhotakota in June and for *An. gambiae* s.l. 19.5 b/p/n in Karonga in September. High *An. funestus* s.l. biting rates were recorded in June in Nkhotakota District (55.8 b/p/n) and in March and April in Nkhata Bay District (28.6 b/p/n and 32.9 b/p/n respectively). In the remaining six districts, *An. funestus* s.l. biting activity was very low (<7 b/p/n). The highest *An. gambiae* s.l. biting activity was recorded in September (19.5 b/p/n) and in March (18.0 b/p/n) in Karonga District. In the remaining seven districts, *An. gambiae* s.l. biting activity was very low (<8 b/p/n).

The highest mean indoor resting density (IRD) of *An. funestus* s.l. from PSCs was observed at Ngalauka site in Nkhotakota District with 26.3 mosquitoes/house/day (m/h/d) in July 2021. The mean IRD of *An. funestus* s.l. was very low throughout the collection period in the remaining seven districts with a mean density of <6.0 m/h/d. The highest mean IRD of *An. gambiae* s.l. was recorded in September 2021 at Mwenimambwe site (Karonga) with a mean catch of 407.3 m/h/d. The IRD of *An. gambiae* s.l. remained low throughout the monitoring period in the other 16 sentinel sites (<8.0 m/h/d).

Mwenimambwe site in Karonga District recorded the highest density of *An. gambiae* s.l. in March 2022, with a mean density of 34.1 mosquitoes per trap per night (m/t/n), followed by Chimkwende (Nkhotakota District), which recorded *An. gambiae* s.l. density of 17.5 m/t/n. In the remaining 15 sentinel sites, in both IRS and non-IRS districts, *An. gambiae* s.l. density was very low throughout the collection period at <5 m/t/n. The highest mean density of *An. funestus* s.l. from CDC-LTs was observed in June 2022at Ngalauka and Vwawa sites (Nkhotakota IRS District), with mean densities of 71.3 m/t/n and 67.0 m/t/n, respectively. The density of this species was also high in Nkhata Bay (IRS district) in March 2022 at Sanga site, with 56.4 m/t/n. The mean number of *An. funestus* s.l. remained low (<9.0 m/t/n) throughout the sampling period in the IRS districts of Mangochi (Piyasi and Nyalubwe sites) and Balaka (Chimdikiti and Kabota sites) and in non-IRS districts of Karonga (Mwenimambwe and Mwakanyamale sites), Chikwawa (Ntwana and Nyamphota), Kasungu (Kachokolo and Nyalubwe), and Salima (Chilungo and Cholokoto).

An. funestus s.l. and *An. gambiae* s.l. exhibited a similar preference for biting location, indoors and outdoors. However, *An. funestus* s.l. predominantly fed indoors, while *An. gambiae* s.l. predominantly fed outdoors. The biting activity of both *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk in all the six districts with the greatest proportion (86% *An. funestus*. s.l. and 62% *An. gambiae* s.l.) occurring when people were asleep. Lower levels of morning/daytime biting for both species were observed from 5:00 am until 11:00 am, when people were awake. Overall, in the four districts, 82% and 95% of people entered their houses to sleep between 9:00 and 10:00 pm and between 10:00 and 11:00 pm, respectively; 94% awoke between 5:00 and 6:00 am.

Infection Detection: A total of 8,557 *An. funestus* s.l. collected using PSCs, and CDC-LTs, HLCs in the eight districts were screened for *Pf* infection with an overall sporozoite infection rate (SR) of 1.0%. A total of 4,098 *An. gambiae* s.l. from all three collection methods were also tested for *Pf* infection, and the overall SR was 0.3%.

The estimated risk of malaria transmission for the 12 months (annual entomological inoculation rate (EIR)) was highest in Nkhotakota District, at 94.5 infective bites/person/year (ib/p/yr.)), all from *An. funestus* s.l. The second highest EIR was recorded in Nkhata Bay District (27.4 ib/p/yr.; all. from *An. funestus* s.l.) followed by Kasungu (22.0 ib/p/y, all from *An. funestus* s.l.), Karonga (14.3 ib/p/yr.; all from *An. gambiae* s.l.), and Chikwawa (4.7 ib/p/yr.; all from *An. gambiae* s.l..).

In the two IRS districts supported by WVI and MAC with funding from the Global Fund (Balaka and Mangochi) entomological monitoring started in November and data was collected for eight months. Entomological monitoring is continuing in these districts. The estimated risk of malaria transmission for the eight months (ib/p/8 months EIR) was low for both Balaka (*An. funestus* s.l. = 1.2 ib/p/8 months) and Mangochi (no infective bites from either species over the eight months). NB: Low numbers of samples processed from these two districts might have affected the results.

In the IRS districts of Nkhotakota and Nkhata Bay, the estimated risk of malaria transmission over a 12-month period was 94.5 ib/p/yr. in Nkhotakota and 29.4 ib/p/yr. in Nkhata Bay. However, in Nkhotakota, a relatively high (25.5 ib/p/3 months) EIR was observed before spraying (July–September); it greatly declined soon after spraying (October–May) to 5.3 ib/p/8 months before rapidly rising in June (63.8 ib/p/month), nine months after spraying. In Nkhata Bay, a higher EIR, 18.8 ib/p/3 months, was recorded before spraying (July–September) than after spraying (November–June), when the EIR was 10 ib/p/8 months.

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. The highest estimated risk of malaria transmission over a 12-month period was observed in Kasungu, a piperonyl butoxide (PBO) net distribution district (22.0 ib/p/yr.), followed by Karonga (14.3 ib/p/yr.), where all three types of nets (PBO, Interceptor G2 (IG2), and Royal Guard) were distributed. The EIRs were lower in Chikwawa (4.7 ib/p/yr.), where IG2 nets were distributed; and in Salima (0.5 ib/p/yr.), where Royal Guard nets were distributed.

Parity Rate: Overall, higher proportions of parous females were recorded among *An. funestus* s.l. (61%) than *An. gambiae* s.l. (43%). In non-IRS districts, high *An. funestus* s.l. parity rates were observed in Karonga (89%), Salima (61%), and Kasungu (51%) districts. The lowest *An. funestus* s.l. parity rate was recorded in Balaka (6.1%). High *An. gambiae* s.l. parity rates were observed in Salima (76%), Chikwawa (70%), and Kasungu (61%) districts. The lowest *An. gambiae* s.l. parity rate was recorded in Karonga, where all 118 *An. gambiae* s.l. dissected were nulliparous.

In IRS districts overall, high *An. funestus* s.l. parity rates were observed in Nkhotakota (64%) and Nkhata Bay (60%) and Mangochi (16%). The lowest parity rate for this species was recorded in Balaka (6.1%). A high parity rate of *An. gambiae* s.l. was recorded in Nkhata Bay (60%), while the lowest parity rates were recorded in Balaka (36%) and Mangochi (35%).

In the IRS districts of Nkhata Bay and Nkhotakota, *An. funestus* s.l. parity rates were similar before and after spraying, at 60.7% vs 59.2% and 63.3% vs 64.7%, respectively. Among *An. gambiae* s.l., higher parity rates were observed before than after spraying in Nkhata Bay (67.6% vs 50%) and Nkhotakota (83.3% vs 44.5%). No clear trends were observed in Mangochi and Balaka because entomological monitoring in these districts commenced in November, after spraying.

Residual Life of Sprayed Insecticides: Overall, spray quality was satisfactory in the three IRS districts of Nkhotakota (PMI supported) and Nkhata Bay and Mangochi (Global Fund supported). However, one brick house at Kela site in Mangochi recorded less than 100% mosquito mortality 24 hours after spraying. Cone bioassays commenced late in Balaka District; hence, a spray quality assessment was not conducted. Fludora Fusion (FF) is still highly effective (100% mosquito mortality) in Nkhotakota District, 10 months after spraying and in Balaka and Mangochi districts, 9 months after spraying. Similarly, SumiShield (SS) is still effective (>80% World Health Organization threshold) after 10 months in Nkhotakota and 9 months in both Balaka and Mangochi. Both insecticides exhibited a fumigant effect for over a period of six months after spraying in all four districts.

Blood Meal Source: Human blood was the predominant source for both *An. funestus* s.l. and *An. gambiae* s.l. *Anopheles funestus* s.l. mainly fed on human blood (93.2%, n=246), followed by cow (4.2%, n=11), goat (1.5%, n=4), pig (0.7%, n=2), and dog (0.4%, n=1). Similarly, higher proportions of *An. gambiae* s.l. (58.6%, n=78) fed on human blood followed by cow (30%, n=40), goat (6%, n=8), dog (3.8%, n=5), and pig (1.5%, n=2).

Insecticide Resistance: Both *An. funestus* s.l. and *An. gambiae* s.l. are fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Both species are highly resistant to the pyrethroids deltamethrin, permethrin, and alpha-cypermethrin. Pre-exposure of *An. funestus* s.l. and *An. gambiae* s.l. to 4% PBO restored their susceptibility to pyrethroids. *Ace-1* resistant allele was absent in *An. arabiensis* and *An. gambiae* s.s.

Conclusion

Overall, *An. funestus* s.l. was the most abundant vector of all the *Anopheles* mosquitoes collected in the eight entomological monitoring districts. Among the *An. gambiae* s.l., *An. arabiensis* was the predominant member identified to the species-specific level. Among the *An. funestus* s.l., 99.8% were *An. funestus* s.s. with only one *An. leesoni* reported from Salima. Both *An. funestus* s.l. and *An. gambiae* s.l. exhibited endophagic and exophagic behavior. *An. funestus* s.l. predominantly fed indoors while *An. gambiae* s.l. predominantly fed outdoors. The biting activity of *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk in all six districts. Morning/daytime biting after 5:00 am was also observed when people were awake in Nkhata Bay, Kasungu, and Nkhotakota districts. *An. funestus* s.l. is the predominant malaria vector in five districts; in Karonga, Salima, and Chikwawa, *An. gambiae* s.l. was responsible for all observed transmission. The overall SR was also higher for *An. funestus* s.l. (1.0%) than for *An. gambiae* s.l. (0.3%). The overall EIR (18.1 ib/p/yr.) due to *An. funestus* s.l. was also higher than that of *An. gambiae* s.l. (4.1 ib/p/yr.).

Spray quality was satisfactory in Nkhotakota, Nkhata Bay, and Mangochi districts for both FF and SS 50WG. Both insecticides have long residual efficacy as evidenced by the continued effectiveness of the chemicals nine months after spraying in Balaka and Mangochi districts and 10 months after spraying in Nkhotakota and Nkhata Bay districts. *An. funestus* s.l., and *An. gambiae* s.l. were susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin and highly resistant to the three pyrethroids tested. Pre-exposure to 4% PBO followed by pyrethroids greatly improved the efficacy of pyrethroids.

The absence of the Acetylcholinesterase-1 resistant allele is consistent with the absence of phenotypic resistance to pirimiphos-methyl (organophosphate) in *An. arabiensis* and *An. gambiae* s.s. in Malawi.

RECOMMENDATIONS

Based on susceptibility testing results, IRS with clothianidin and insecticide-treated nets with the active ingredient chlorfenapyr (e.g., IG2) are appropriate malaria vector control interventions in Malawi, along with the other current tools. Furthermore, insecticide-treated nets treated with PBO also are recommended for distribution in Malawi.

I. INTRODUCTION

The U.S. President's Malaria Initiative (PMI) VectorLink Project in Malawi, in collaboration with the Malaria Alert Centre (MAC) of the Kamuzu University of Health Sciences, conducted a spray quality assessment and monitoring of the residual lifespan of the insecticides in Nkhotakota (Central Region), Nkhata Bay (Northern Region), Mangochi, and Balaka (Southern Region) districts. In addition, comprehensive longitudinal entomological monitoring was done in 17 sentinel sites in eight districts (the aforementioned four, plus Chikwawa, Kasungu, Salima, Nkhata Bay, and Karonga) across the country to assess vector bionomics (vector density, composition, distribution, and behavior), species identification, infection rates, and insecticide resistance. Resistance mechanism tests were also conducted on major malaria vectors in Malawi.

In 2021, indoor residual spraying (IRS) was carried out in four districts (Nkhotakota, Nkhata Bay, Mangochi, and Balaka). In October 2021, VectorLink Malawi carried out IRS using SS 50WG and FF to spray structures in Nkhotakota District. The Malawian government's National Malaria Control Program, supported by World Vision International (WVI) with funding from the Global Fund, conducted IRS with FF in Mangochi and Balaka districts and with SS 50WG in Nkhata Bay. VectorLink Malawi, in collaboration with MAC, conducted a spray quality assessment and monitoring of the residual lifespan of the insecticides in the four districts.

This report summarizes the key findings of the longitudinal entomological monitoring (density, biting rates, and patterns of biting by malaria vectors, bloodmeal analysis, and infectivity rates), residual efficacy tests of SS 50WG and FF, and the susceptibility status of malaria vectors to different insecticides, in the eight districts.

2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Adult mosquitoes were sampled from July 2021 to June 2022 in 17 sentinel sites located in eight districts across Malawi (Figure 1). Four sites are in the northern region, seven in the central region, and six in the southern region (Table 1). Sampling in four sentinel sites in Balaka and Mangochi (Global Fund districts) began in November 2021, while in all the other sites it started in July 2021.

FIGURE 1: MAP OF MALAWI SHOWING THE ENTOMOLOGICAL MONITORING SITES

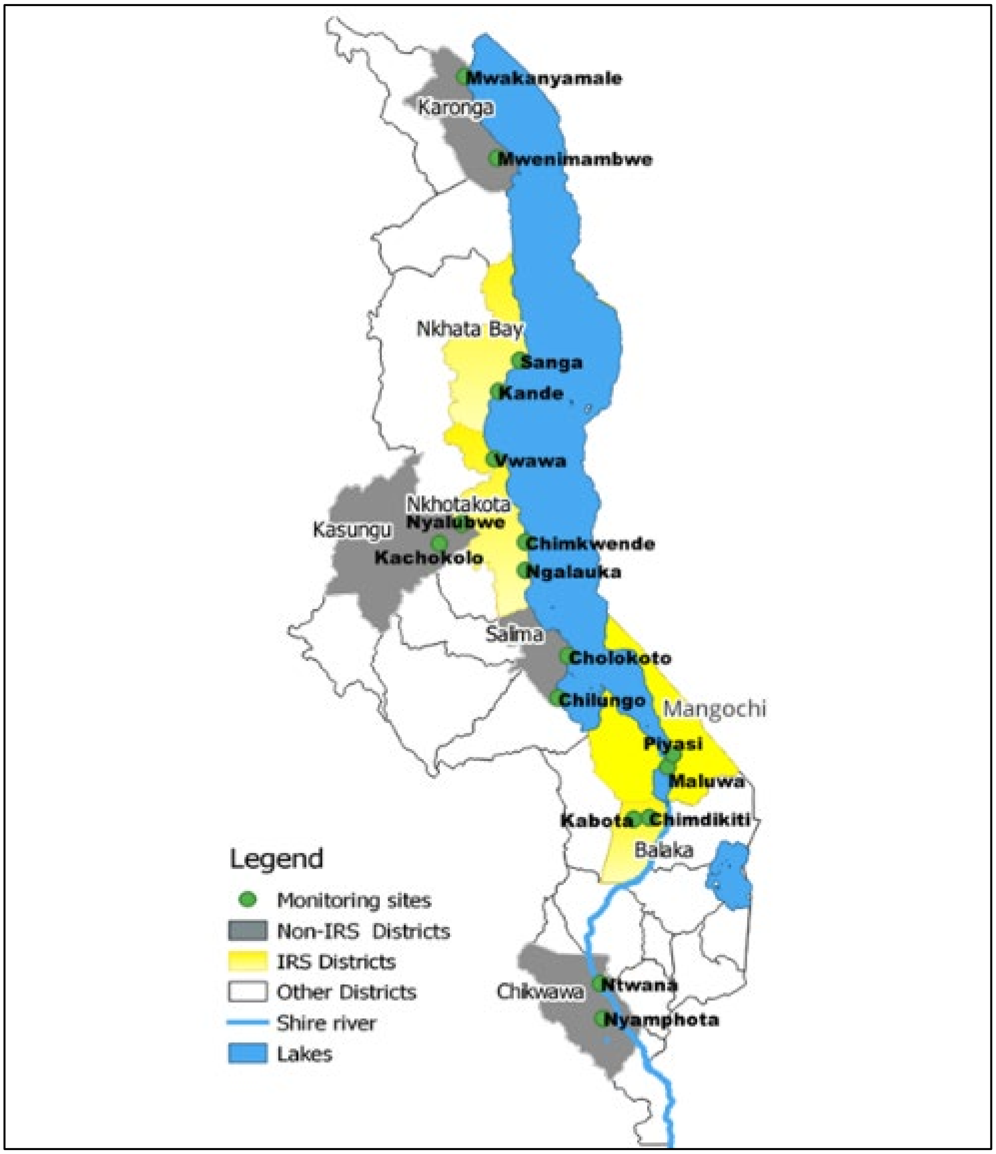


TABLE 1: SENTINEL SITES FOR ENTOMOLOGICAL MONITORING IN MALAWI

		Sentinel Sites	Latitude and Longitude	Collection Method	Malaria Vector Control Interventions
Northern	Karonga	Mwakanyamale	S 9° 47' 1.7"; E 33° 53' 34.36"	PSC, CDC-LT	PBO nets, IG2 nets and RG nets
		Mwenimambwe	S 10° 20' 24.14"; E 34° 6' 41.62"	PSC, CDC-LT	
	Nkhata Bay	Sanga	S 11° 44' 18.58"; E 34° 16' 5.04"	HLC, PSC, CDC-LT	IRS (Sprayed with SS)
		Kande	S 11° 57' 3.3"; E 34° 7' 1.2"	PSC, CDC-LT	
Central	Salima	Chilungo	S 14° 3' 44.41"; E 34° 31' 42.08"	HLC, PSC, CDC-LT	RG nets
		Cholokoto	S 13° 46' 20.77"; E 34° 35' 57.51"	PSC, CDC-LT	
	Nkhotakota	Vwawa	S 12° 24' 54.4"; E 34° 5' 16.44"	HLC, PSC, CDC-LT	IRS (sprayed with FF)
		Chimkwende	S 12° 59' 3.49"; E 34° 18' 13.15"	PSC, CDC-LT	
		Ngalauka	S 13° 10' 38.52"; E 34° 18' 12.84"	HLC, PSC, CDC-LT	
	Kasungu	Kachokolo	S 12° 59' 35.09"; E 33° 43' 4.19"	HLC, PSC, CDC-LT	PBO nets
		Nyalubwe	S 12° 51' 26.44"; E 33° 51' 57.79"	PSC, CDC-LT	
Southern	Mangochi	Piyasi	S 14° 44' 90.21"; E 35° 31' 79.70"	HLC, PSC, CDC-LT	IRS (Sprayed with FF)
		Maluwa	S 14° 53' 13.56"; E 35° 28' 56.78"	PSC, CDC-LT	
	Balaka	Kabota	S 14° 89' 07.52"; E 35° 05' 35.12"	HLC, PSC, CDC-LT	IRS (sprayed with FF)
		Chimdikiti	S 14° 88' 32.32"; E 35° 15' 32.05"	PSC, CDC-LT	
	Chikwawa	Nyamphota	S 16° 15' 31.71"; E 34° 50' 17"	PSC, CDC-LT	IG2 nets
		Ntwana	S 16° 1' 18.05"; E 34° 49' 7.16"	PSC, CDC-LT	

Note: PBO = Piperonyl butoxide, IG2 = Interceptor G2, RG = Royal Guard, IRS = Indoor Residual Spraying

The team used pyrethrum spray catches (PSCs) Standard Operating Procedure (SOP)03/01)[1] and U.S. Centers for Disease Control and Prevention (CDC) miniature light traps (LTs) (SOP01/01) monthly to collect adult mosquitoes in the 17 sentinel sites (Table 2). Collections from the PSC were used to estimate the daily indoor resting density, determination of bloodmeal digestion (abdominal) stages and analysis of bloodmeal source. The CDC-LT collections were used for monthly mosquito population trend analysis and estimating the human biting rate. Mean numbers collected per trap per night were considered as equivalent to mean bites per person per night. Adult mosquitoes were also sampled from July 2021 to June 2022 using human landing catches (HLCs) (SOP02/01) on a quarterly basis from seven sentinel sites in six districts: Sanga (Nkhata Bay), Vwawa and Ngalauka (Nkhotakota), Kachokolo (Kasungu), Chilungo (Salima), Kabota (Balaka), and Piyasi (Mangochi) (Tables 1 and 2). Six households from each village were selected for a single night of collection per quarter. Collection was done from 5:00 pm to 11:00 am. Six volunteers, in pairs, one inside and the other outside a house, sat for six-hour shifts per night. Mosquitoes collected every hour were held in labeled resting cups, provided with 10% sugar solution, and stored in a cooler box. The collection from HLC was analyzed to estimate preferred biting location and peak biting time of the main vectors.

The next morning, ovary dissections were done on a subsample of female *Anopheles* collected from HLC and CDC-LT (proportion depended on the numbers collected) to check their parity status. Samples were then morphologically identified using the method described by (Coetzee 2020) and, finally, sent to the MAC laboratory for further analyses.

TABLE 2: LONGITUDINAL MONITORING OF ADULT MOSQUITOES IN SENTINEL SITES

Collection Method	Time	Frequency	Sample
PSCs	6:00 am to 8:00 am	1 day per site per month	15 houses per site/same houses every month
CDC-LT	6:00 pm to 6:00 am	1 night per site per month	10 houses per site/same houses every month
HLC	5:00 pm to 11:00 am	1 night per house every 3 months	6 houses per site/same houses every quarter

[1] See all PMI VectorLink SOPs at https://pmivectorlink.org/resources/tools-and-innovations/

2.2 Human Behavior Surveys

Human behavior patterns in the six districts were estimated from household members where HLCs were conducted. A questionnaire was administered to the head of the household during each visit to record the time household members went inside their houses, the time they went to sleep in the evening, the time they woke up in the morning, and the time they exited the house in the morning. Weighted estimates of mosquito biting rates according to human behavior were generated using the methods of Monroe et. al. (2020).

2.3 Insecticide Resistance Monitoring

2.3.1 Insecticide Susceptibility Tests

To determine the frequency of insecticide resistance, larval and adult malaria vectors were collected from one sentinel site in each of the eight districts. Additional sites were visited to collect adult malaria vectors when low numbers were encountered at the planned sentinel site. *An. funestus* s.l. is the predominant vector species in many areas and, due to the difficulty of finding larval stages of this species, mosquitoes were collected as adults, allowed to lay eggs, and reared to the adult stage for subsequent testing. Adult and/or larvae of *An. gambiae* s.l. were collected from larval habitats and reared to adult stage for testing.

2.3.2 Mosquito Collections for Susceptibility Test

Adult *Anopheles* mosquitoes were sampled from a single sentinel site in each of four districts (Chikwawa, Salima, Kasungu, and Karonga). Adult mosquitoes were allowed to lay eggs and reared to F_1 generation. In addition, some samples were collected as larvae from their natural habitats in Ntwana and Nkhwazi sites (Chikwawa), Chilungo (Salima), and Chimkwende (Nkhotakota). The collected larvae were reared to adults and subsequently tested for susceptibility to different insecticides. All mosquito-rearing activities were carried out in the insectary at MAC.

2.3.3 Insecticides Tested

Table 3 summarizes the sentinel sites and insecticides tested. World Health Organization (WHO) tube bioassays were used to test both *An. funestus* s.l. and *An. gambiae* s.l. against pyrethroids. CDC bottle assays were used to test resistance in the main malaria vectors by exposing adult mosquitoes to clothianidin 4μg/bottle and chlorfenapyr 100μg/bottle using the newly developed bottle bioassay procedures.

Table 3: Live *Anopheles* Collections (Adults or Larvae), Species Tested, Number of Households Sampled, and Insecticides Tested in Five Sentinel Sites

District	Sentinel Site	Source	Species	# of households where adult mosquitoes were sampled	Test
Chikwawa	Ntwana	F1	*An. gambiae* s.l.	42	Deltamethrin 0.05%
					Permethrin 0.75%
					Alpha-cypermethrin 0.05%
					Pirimiphos methyl 0.25%
		F1	*An. funestus* s.l.		Pirimiphos-methyl 0.25%
		Larvae	*An. gambiae* s.l.	–	Chlorfenapyr 100μg/bottle
	Nkhwazi				Clothianidin 4μg/bottle

District	Sentinel Site	Source	Species	# of households where adult mosquitoes were sampled	Test
Salima	Chilungo	Larvae	*An. gambiae* s.l.	–	Alpha-cypermethrin 0.05%
					Deltamethrin 0.05%
					Permethrin 0.75%
					Clothianidin 4µg/bottle
	Kabumba	F1	*An. gambiae* s.l.	34	Pirimiphos-methyl 0.25%
Kasungu	Kachokolo	F1	*An. funestus* s.l.	55	Permethrin
					PBO + Permethrin 0.75%
					Pirimiphos-methyl 0.25%
Nkhotakota	Chimkwende	Larvae	*An. funestus* s.l.	–	Alpha-cypermethrin 0.05%
					Clothianidin 4µg/bottle
Karonga	Mwenimambwe	F1	*An. gambiae* s.l.	62	Pirimiphos-methyl 0.25%
					Clothianidin 4µg/bottle
					Chlorfenapyr 100µg/bottle

2.3.4 WHO Tube Assays

Tests were performed according to standard WHO procedures (WHO 2016) and SOP06/01[2]. Both F_1 and those collected as larvae from their aquatic habitats were raised to adults and females aged 2–5 days were used for susceptibility tests by exposing them to WHO-recommended diagnostic doses. At the end of each test mosquitoes were placed in an individual tube, which was placed in a Ziploc bag containing desiccants and clearly labeled with the assay date, mosquito species, dead or alive after exposure, insecticide used, and location.

2.3.5 CDC Bottle Assays

The CDC bottle bioassay method (Brogdon and Chan 2010) with modifications and SOP04/01 were also used to test for the susceptibility of malaria vectors (*An. funestus* s.l. and *An. gambiae* s.l.). Knockdown effect was observed at 60 minutes and mortality at 24, 48, and 72 hours after exposure to chlorfenapyr. A parallel test with susceptible strain of *An. gambiae* (Kisumu) was also performed as a control for comparability of testing conditions.

A susceptible strain of *An. gambiae* (Kisumu) was also tested as a control to confirm the quality of insecticide-treated papers and bottles.

2.3.6 Interpretation of Results

Susceptibility of *An. funestus* s.l. and *An. gambiae* s.l. was evaluated based on the WHO criteria of test mortality (WHO 2016): 98–100% mortality indicates susceptibility. Mortality of equal to or more than 90% but less than 98% suggests the existence of resistance and the need for confirmation. If mortality is less than 90%, then the population is resistant. When control mortality was greater than or equal to 5% but less than 20%, the observed mortality was corrected using Abbott's formula (Abbott 1925). If the control mortality was above 20%, the test results were discarded.

[2] See all PMI VectorLink SOPs at https://pmivectorlink.org/resources/tools-and-innovations/

2.3.7 MOLECULAR DETECTION OF *ACETYLCHOLINESTERASE-1*

The protocol used in the detection of *Acetylcholinesterase (Ace*-1*)* mutation in dead mosquitoes from PSCs and CDC-LT collections was adopted from the Malaria Research and Reference Reagent Resource (MR4) Center Manual (2016). DNA was extracted using Livak grinding buffer and the extracted DNA was analyzed using an Intentional Mismatched Primer-Polymerase Chain Reaction (PCR) assay to detect *Ace*-1 gene mutation in *An. gambiae* s.l.

2.3.8 WALL CONE BIOASSAYS

The WHO wall cone bioassay protocol (SOP09/01) was followed during the tests. Three test cones and one control cone were used. The test cones were placed at three different heights (1.5 m; 1.0 m, and 0.5 m) on sprayed wall surfaces and fixed onto uncontaminated cardboard outside of sleeping structures. At least 10 nonblood fed female *An. gambiae* s.s. (Kisumu strain) mosquitoes aged 2–5 days were introduced into each cone. The mosquitoes were exposed to the walls for 30 minutes. After 30 minutes, the mosquitoes were transferred to insecticide-free holding paper cups and provided with 10% sugar solution. The holding cups were then placed in a cool box with a wet towel for appropriate humidity. Knockdown was observed and recorded at 30 and 60 minutes, respectively. For both SS 50WG and FF, mortality was observed every 24 hours throughout the five-day holding period. Test mortality was corrected using Abbott's formula when control mortality was between 5% and 20% (Abbott 1925).

2.3.9 SPRAY QUALITY ASSESSMENT

The 2021 IRS campaign was carried out from October to November 2021 in Nkhotakota, Nkhata Bay, Mangochi, and Balaka districts. VectorLink Malawi, in collaboration with MAC, conducted wall bioassay tests to check spray quality in six villages selected to represent the three entomological sites in Nkhotakota and 2 sites in Nkhata Bay. The tests were carried out on October 5–13 in Nkhotakota District and on October 27–November 2 in Nkhata Bay. In Nkhotakota, Dwambadzi and Ngala operations sites were sprayed with SS 50WG, whereas Boma, Chididi, Mkaika, and Mwansambo sites were sprayed with FF. Nkhata Bay was sprayed with SS 50WG in all operations sites: Kande, Chintheche, Boma, Mpamba, Tukombo, and Mzenga. Spraying in Mangochi was done with FF in all operations sites; spray quality was assessed by World Vision in collaboration with MAC on November 26–December 3, 2021.

A spray quality assessment was conducted in one village selected from each of the six operations sites in each district except for Balaka, where a spray quality assessment was not conducted (Table 4). Cone bioassay tests were performed within 24 hours to one week of spraying in all the villages. Six structures made of different wall surfaces—brick, cement plastered, and mud—were randomly selected at each site to conduct wall bioassay tests for spray quality assessment and for monthly monitoring. In Nkhotakota, five operations sites had six structures made of all three types of wall surfaces: brick, cement plastered, and mud. In Mangochi and Balaka, all structures selected per village had brick, cement plastered, and mud walls. In the Boma operations site, only brick and mud houses were tested due to absence of cement plastered structures. In Nkhata Bay, almost all structures tested were of brick and plastered cement due to absence of mud surfaces. In Nkhotakota (Mkaika), Nkhata Bay (Kande), and Mangochi (Mwambwajira and M'doka villages), 12 structures were randomly selected in one operations site; six were sprayed under close supervision to serve as a positive control and the remaining six structures were sprayed without close supervision (normal spraying). The Kisumu strain of *An. gambiae* s.s. (2–5 days old) reared at the MAC insectary was used for the wall cone bioassays.

TABLE 4: SPRAY QUALITY ASSESSMENT VILLAGES IN NKHOTAKOTA, NKHATA BAY, MANGOCHI, AND BALAKA DISTRICTS

District	Operations site	Group Village Head	Village	Longitude and Latitude	Insecticide
Nkhotakota	Dwambadzi	Thung'unda	Thung'unda	S 12° 16' 7"; E 33°58' 19"	SumiShield 50WG
	Ngala	Vwawa	Vwawa†	S 12° 24' 47"; E 34° 2' 5"	SumiShield 50WG
	Boma	Chimkwende	Chimkwende†	S 12° 59' 3"; E 34° 18' 12"	Fludora Fusion
	Chididi	Zamangwe	Zamangwe 1	S 13° 5' 59"; E 34° 16 ' 33"	Fludora Fusion
	Mkaika	Ngalauka	Ngalauka*†	S 13° 10' 29"; E 34° 18' 3"	Fludora Fusion
	Mwansambo	Kapongola	Kapongola	S 13° 12' 53"; E 34° 11' 0"	Fludora Fusion
Nkhata Bay	Kande	Kande	Kande*†	S 11 ° 57' 4"; E 34° 7'1"	SumiShield 50WG
	Chintheche	Sanga	Sanga†	S 11°44'16"; E 34° 16' 13"	SumiShield 50WG
	Boma	Chadagha	Khoza	S 11° 39' 21"; E 34° 18 ' 17"	SumiShield 50WG
	Mpamba	Chigwere	Mbama	S 11° 21' 36"; E 34° 5' 35"	SumiShield 50WG
	Tukombo	Chivuma	Mtiti	S 12° 0' 12"; E 34° 2' 53"	SumiShield 50WG
	Mzenga	Chakupompha	Chioda	S 11° 45' 34"; E 33° 56' 7"	SumiShield 50WG
Mangochi	-	Kela	Kela	S 14° 18' 3"; E 35° 6' 55"	Fludora Fusion
	-	Mwambwajira	Mwambwajira*	S 14° 25' 21"; E 35° 36' 11"	Fludora Fusion
	-	Mwambwajira	M'doka	S 14° 24' 47"; E 35° 35' 50"	Fludora Fusion
	-	Chapola	Kawaya	S 14° 44' 22"; E 35° 11' 17"	Fludora Fusion
	-	Chapola	Kalichelo	S 14° 44' 1"; E 35° 11' 27"	Fludora Fusion
	-	-	Nombo	S 14° 26' 10"; E 35° 35' 7"	Fludora Fusion
	-	Matuwi	Makokola	S 14° 18' 24"; E 35° 7' 44"	Fludora Fusion
Balaka	Kankao	Siliya	Siliya	S 15° 1' 42"; E 34° 53' 34"	Fludora Fusion
	Mpulura	Matola	Domoka	S 14° 55' 48"; E 34° 57' 20"	Fludora Fusion
	Chiendausiku	Maliwata	Mwanyali	S 14° 55' 37"; E 35° 1' 16"	Fludora Fusion
	Mdala	Chagunda	Silili (Chagunda)	S 14° 56' 12"; E 35° 11' 53"	Fludora Fusion

*Positive control site, †Entomological monitoring sites

2.3.10 MONITORING RESIDUAL EFFICACY

Monthly monitoring of insecticide residual efficacy was performed in four villages in Nkhotakota: Vwawa, sprayed with SS, and Chimkwende, Ngalauka, and Zamangwe 1, sprayed with FF. Monthly monitoring also was performed in four villages in Nkhata Bay: Kande, Sanga, Mbama, and Mtiti, sprayed with SS. In Mangochi, monitoring was done in five villages sprayed with FF: Mwambwajira, M'doka, Kuwaya, Kalichero, and Makokola. And in Balaka, it was done in four villages sprayed with FF: Siliya, Domoka, Mwanyali, and Silili.

The fumigation effect of SS 50WG and FF was also assessed in the above villages. A total of 14–20 non-blood fed female *An. gambiae* Kisumu were placed in a wire cage (covered with untreated net) 10 cm away from the sprayed walls to assess the fumigation effect of FF and SS 50WG. Knockdown and mortality were recorded in the same way as described for wall bioassays (Section 2.5).

2.3.11 LABORATORY ANALYSIS

PCR was used to identify members of *An. gambiae* s.l. and *An. funestus* s.l. to the species level as described by Benedict (2007). A total of 2,750 mosquitoes samples [CDC-LTs (600), HLC (650), PSCs (650), live collections (300), dissected (250) and Bbochemical assays (300)] were targeted for species identification by PCR. The heads and thoraxes of a sample of the *An. gambiae* s.l., *An. funestus* s.l., *An. pharoensis* and *An, coustani* were sorted and tested for the presence of circumsporozoite antigens of *Plasmodium falciparum* (*Pf*) using enzyme-linked immunosorbent assays (ELISA) described by Wirtz et al. (1987) to determine the sporozoite rate (SR). HBRs and, subsequently entomological inoculation rates (EIRs), were estimated from CDC-LT collections and not HLC collections. A total of 15,600 mosquitoes samples were targeted for ELISA analysis. Conventional PCR as described in the MR4 2016 manual was used to determine the source of bloodmeal in freshly fed *Anopheles* mosquitoes targeting a total of 300 mosquito samples.

2.4 DATA ANALYSIS

The following parameters were calculated:

- Indoor resting density (IRD) = number of adult *Anopheles*/house/day
- Human biting rate (HBR) from HLC = total number of vectors collected divided by number of collectors per hour or night.
- Human biting rate (HBR) from CDC-LT = total number collected divided by number of traps per night. Mean number per trap per night was considered as equivalent to mean bites per person per night.
- The proportion of vector bites occurring indoors for an unprotected individual (πI, u)
- The proportion of vector bites occurring indoors during sleeping hours, for an unprotected individual (πI, s)
- SR = *Anopheles* found positive for the presence of circumsporozoite proteins (CSP) / total number tested*100)
- EIRs = number of infectious bites/per person /per unit time
- Nightly EIR = Daily HBRs * SRs
- Monthly EIR = Nightly EIR * No. of days per month
- Annual EIR = ∑Monthly EIRs

3. RESULTS AND DISCUSSION

3.1 SPECIES COMPOSITION

A total of 34,869 female *Anopheles* mosquitoes were collected from 17 sentinel sites in all the eight monitoring districts from July 2021 to June 2022. Out of these, 20,168 (57.9%) were collected using PSCs, 11,972 (34.3%) using CDC-LTs, and 2,729 (7.8%) using HLCs (Figures 2–4).

Overall, 49.8% (n=17,364) of the *Anopheles* collected were identified morphologically as *An. funestus* s.l.*,* 46.8% (n=16,316) were *An. gambiae* s.l., and 3.4% (n=1,169) were *An. coustani.* However, species composition varied by sentinel site: *An. funestus* s.l. was predominant in Sanga and Kande sites (Nkhata Bay District), Ngalauka, Chimkwende, and Vwawa sites (Nkhotakota) Nyalubwe and Kachokolo sites (Kasungu), Chilungo site (Salima), and Kabota site (Balaka). *An. gambiae* s.l. was predominant in Mwenimambwe and Mwakanyamale sites (Karonga), Ntwana and Nyamphota sites (Chikwawa), Chimdikiti site (Balaka), and Maluwa and Piyasi sites (Mangochi). Species composition also varied based on indoor or outdoor location. Overall, more *An. funestus* s.l. were collected indoors (69.1%, n=1087), whereas more *An. gambiae* s.l. and *An. coustani* were collected outdoors (68.1%, n=759) and (78.1%, n=883), respectively (Figure 4). It is also interesting to note that the proportion of *An. coustani* collected from HLC was higher than from PSC and CDC-LTs collections, probably indicating that this mosquito is significantly involved in human biting but mostly feeding and/or resting outdoors.

Another 346 other female *Anopheles* and 6,321 other mosquito genera were collected during the monitoring period using the three methods. Among *Anopheles* mosquitoes collected were *An. pharoensis* (n=202), *An. tenebrosus* (n=67), *An. pretoriensis* (n=60), *An. maculipalpis* (n=9), and *An. squamosus* (n=8). Other mosquito genera collected were: 3,773 *Culex*, 2,548 *Mansonia* species, and 40 *Aedes.*

FIGURE 2: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL EIGHT MONITORING DISTRICTS, FROM PSC COLLECTION

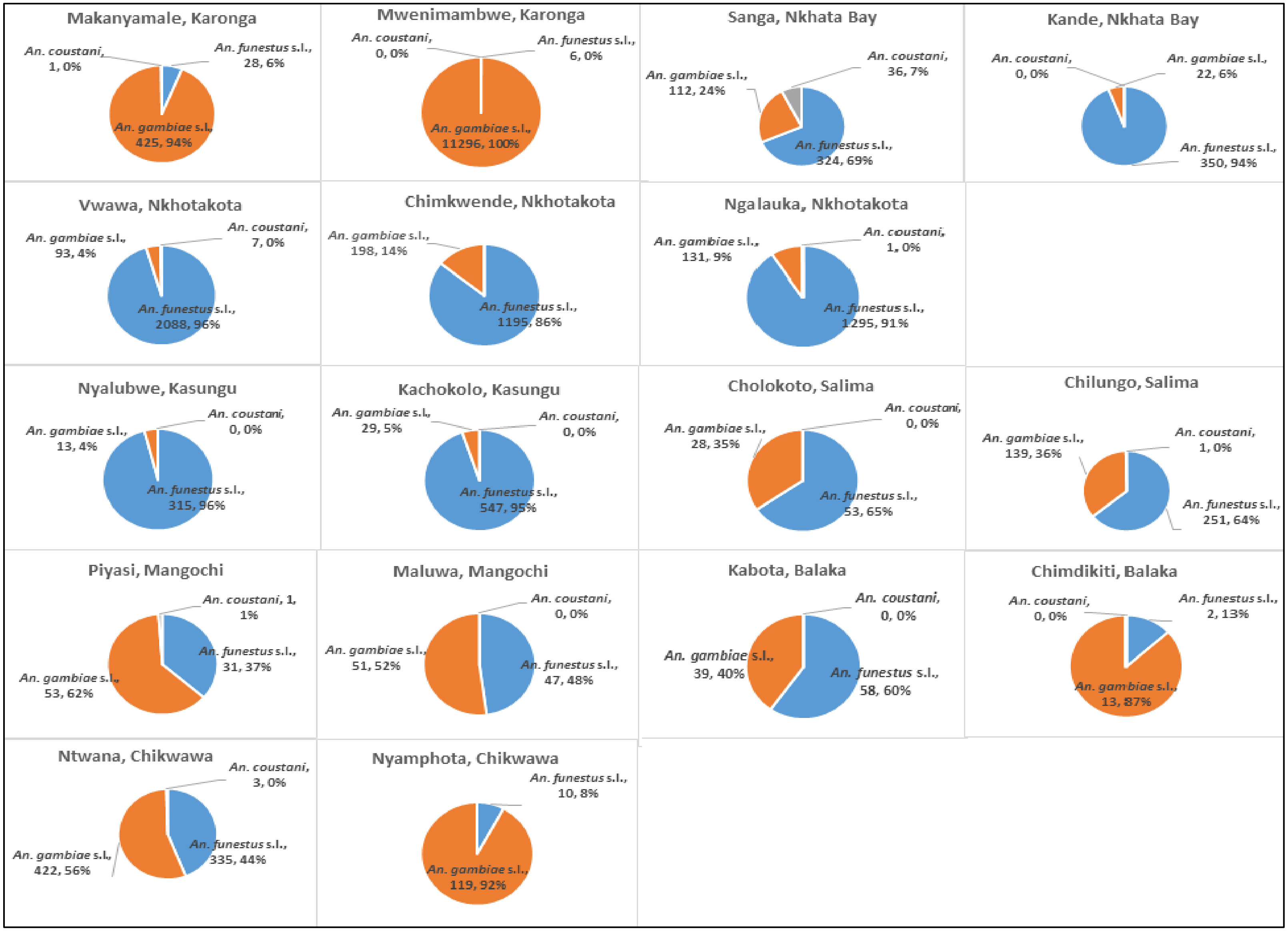
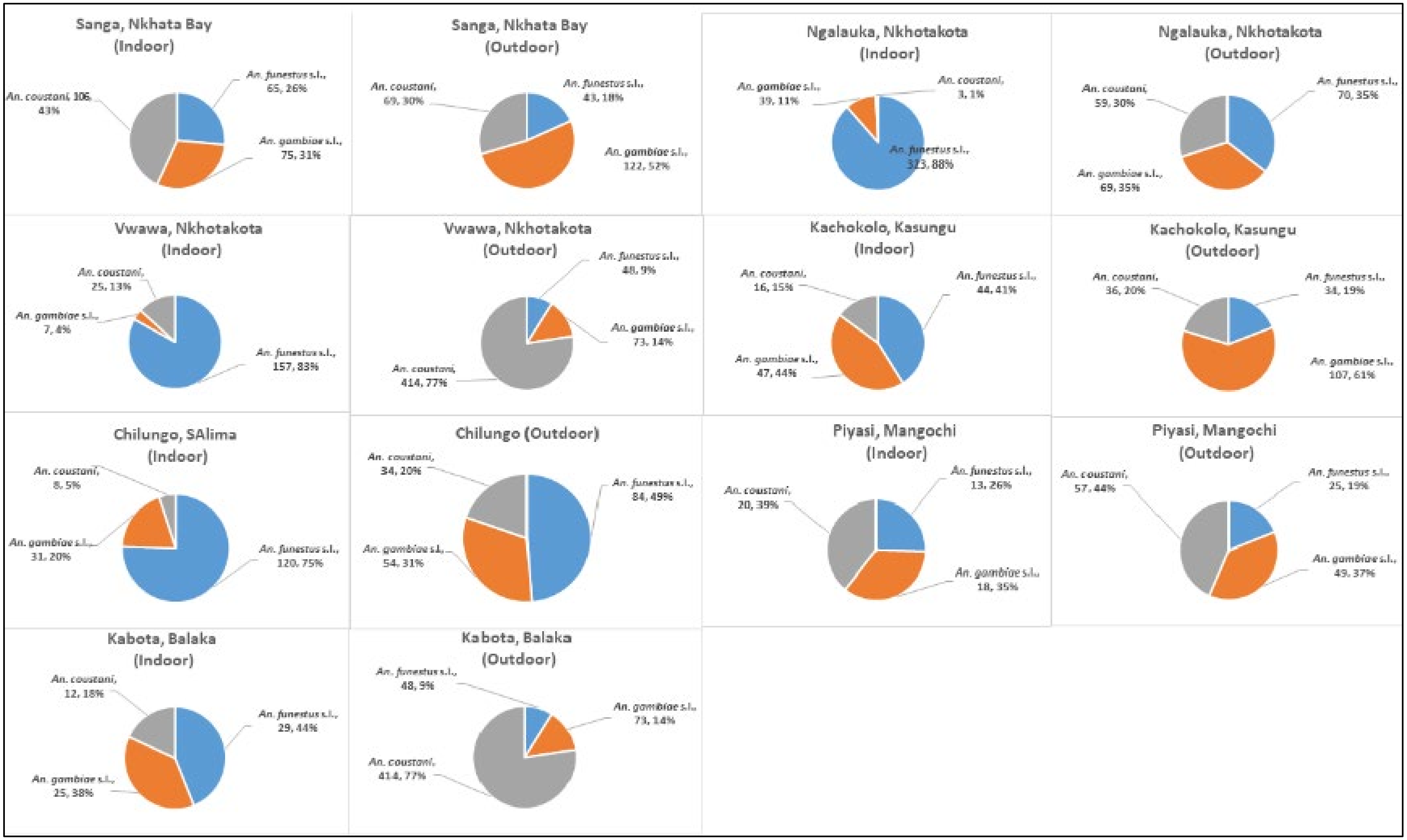


FIGURE 3: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL EIGHT MONITORING DISTRICTS, FROM CDC-LT COLLECTION

FIGURE 4: *ANOPHELES* COMPOSITION, BY SENTINEL SITE ACROSS ALL FOUR MONITORING DISTRICTS, FROM HLC COLLECTION

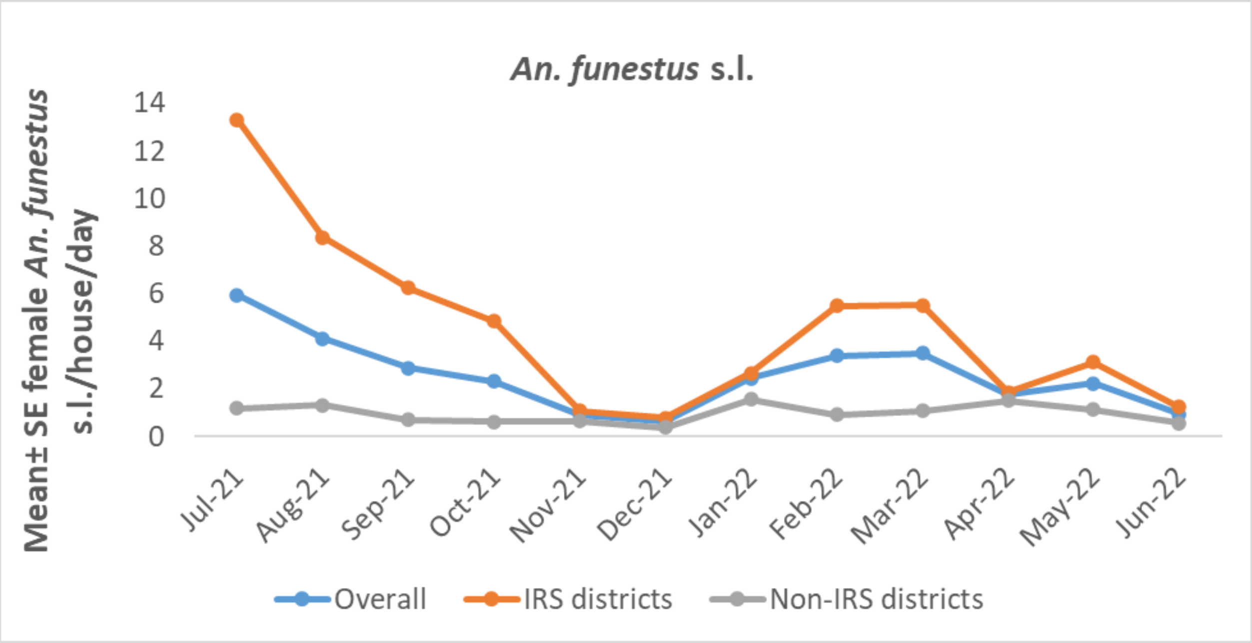


3.2 INDOOR RESTING DENSITY OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. COLLECTED BY PSCS

3.2.1 INDOOR RESTING DENSITY OF *AN. FUNESTUS* S.L.

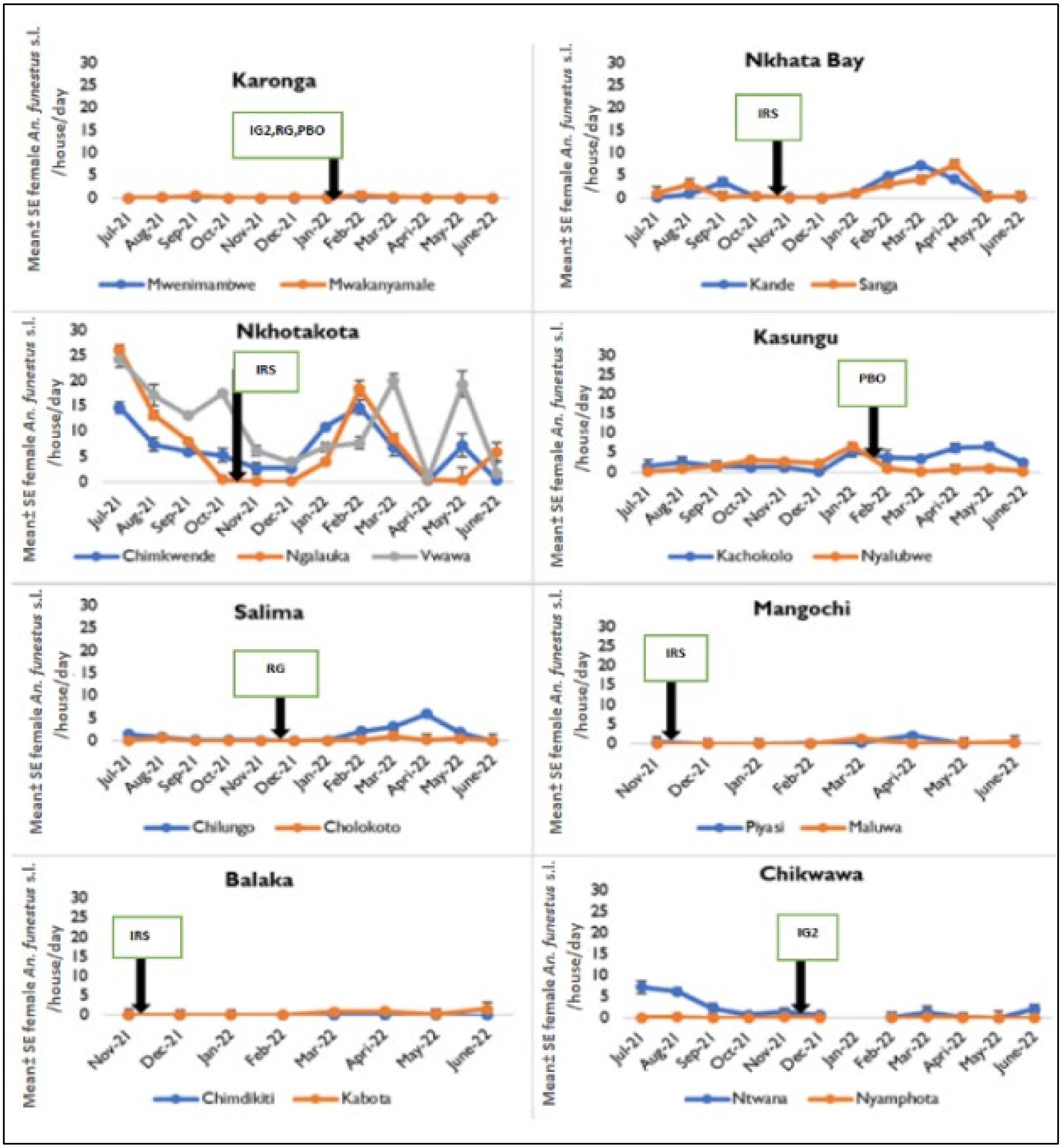
The overall IRD across all 17 sentinel sites from July 2021 to June 2022 was 2.59 *An. funestus* s.l. per house per day. The highest mean IRD of *An. funestus* s.l. from PSC collections was observed in Julywith a mean catch of 5.9 mosquitoes per house per day (m/h/d). The lowest was observed in December with a mean catch of 0.6 m/h/d. Similarly, in the IRS district, the peak mean *An. funestus* s.l. IRD was observed in July (13.3 m/h/d) while in the non-IRS district, the mean *An. funestus* s.l. IRD was very low throughout the collection period (<2 m/h/d) (Figure 5).

FIGURE 5: MEAN IRD OF *AN. FUNESTUS* S.L. COLLECTED BY PSCS ACROSS FOUR IRS AND FOUR NON-IRS DISTRICTS (17 SENTINEL SITES), JULY 2021–JUNE 2022



The highest mean IRD of *An. funestus* s.l. from PSCs collection was observed in Nkhotakota District (at Ngalauka site), with 26.3 m/h/d in July 2021. In this district, *An. funestus* s.l. mean IRD was high before spraying (July to October); it gradually decreased soon after spraying (November to December) before rising again during the rainy season (January). The density decreased at the two sites (Chimkwende and Ngalauka) between February and June. The density at Vwawa fluctuated between February and May before declining in June. The mean IRD of *An. funestus* s.l. was very low throughout the collection period in the remaining seven districts (15 sentinel sites), with a mean density of <6.0 m/h/d (Figure 6).

FIGURE 6: MEAN IRD± SE OF *AN. FUNESTUS* S.L. COLLECTED BY PSCS ACROSS EIGHT DISTRICTS, JULY 2021–JUNE 2022

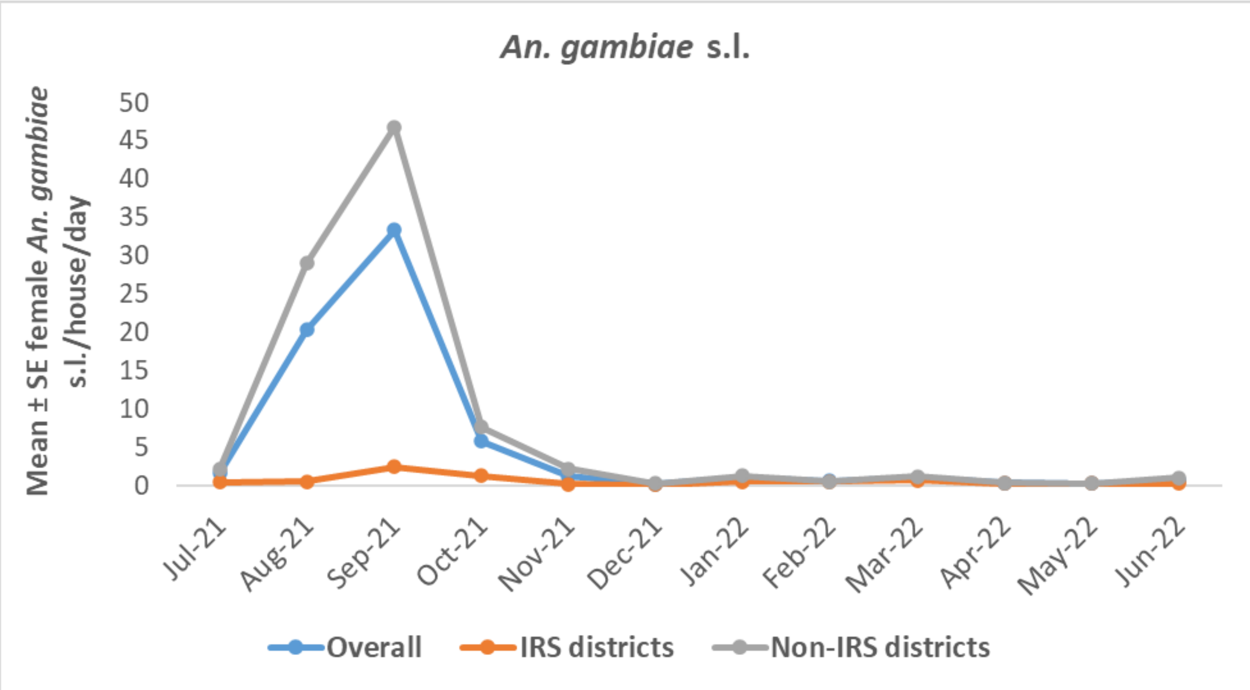


3.2.2 INDOOR RESTING DENSITY OF *AN. GAMBIAE*

The overall mean IRD of *An. gambiae* s.l. collected using PSC was 5.5 m/h/d across all 17 sentinel sites. The highest mean IRD of *An. gambiae* s.l. (33.4 m/h/d) was observed in September

Similarly, in non–IRS districts, peak mean IRD was observed in September (46.8 m/h/d). In IRS districts, mean *An. gambiae* s.l. IRD was very low throughout the collection period (<3 m/h/d) (Figure 7).

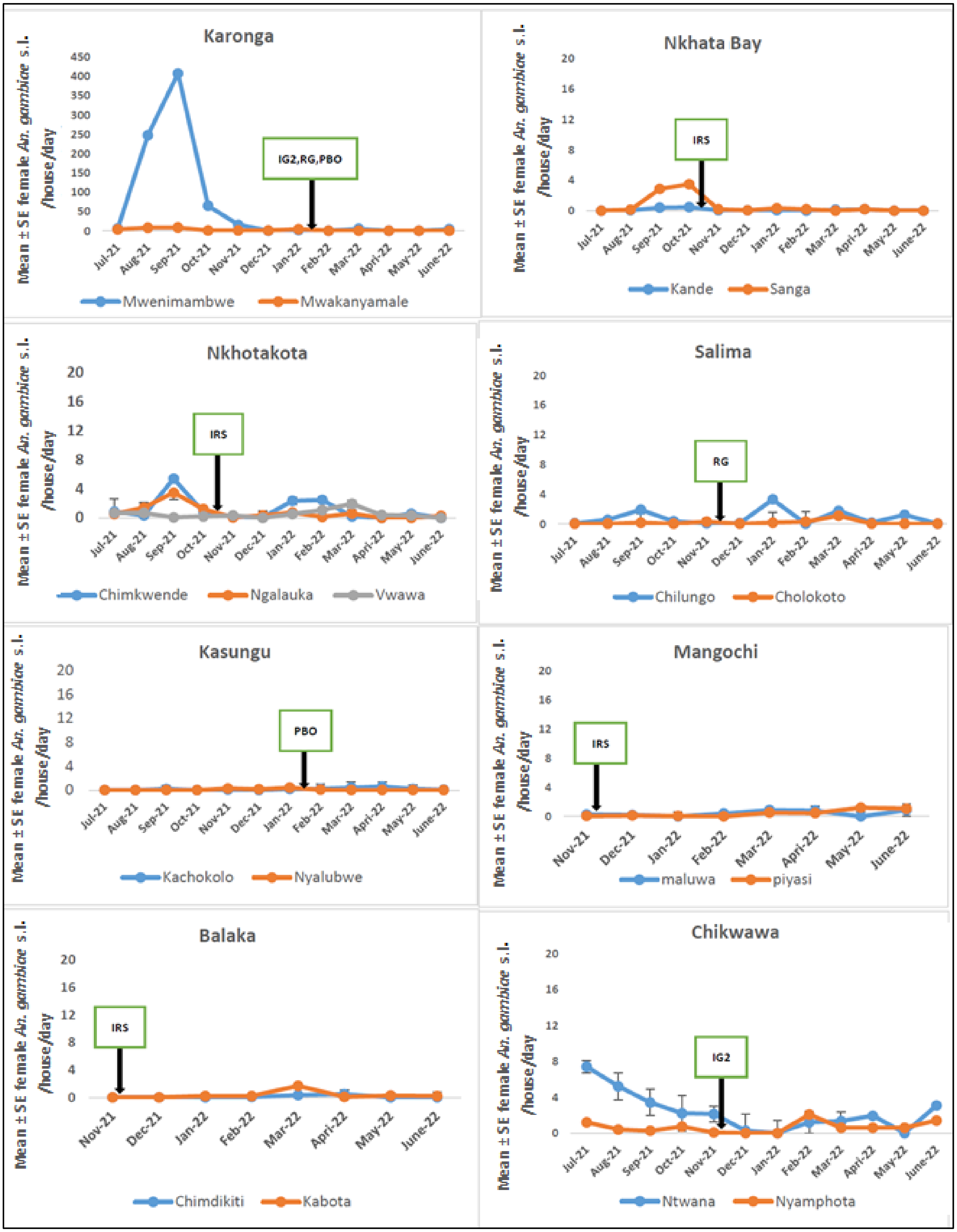
FIGURE 7: MEAN IRD± SE OF *AN. GAMBIAE* S.L. COLLECTED BY PSCS ACROSS FOUR IRS AND FOUR NON-IRS DISTRICTS (17 SENTINEL SITES), JULY 2021–JUNE 2022



The highest mean IRD of *An. gambiae* s.l. was recorded in September 2021 at Mwenimambwe[3] site (Karonga) with a mean catch of 407.3 m/h/d. The IRD of *An. gambiae* s.l. remained low throughout the monitoring period in the other 16 sentinel sites (<8.0 mosquitoes/house/day (Figure 8).

[3] At Mwenimambwe site, there is a rice scheme (Wovwe), mosquito density drastically increases when this scheme opens e.g., in September.

FIGURE 8: MEAN IRD± SE OF *AN. GAMBIAE* S.L. COLLECTED BY PSCS ACROSS EIGHT DISTRICTS, JULY 2021–JUNE 2022



3.3 Gonotrophic Status of *An. funestus* s.l. and *An. gambiae* s.l. Collected by PSC

A total of 20,168 female *Anopheles* mosquitoes were collected using PSCs. Of these, 6,935 were *An. funestus* s.l., 13,183 were *An. gambiae* s.l. and 50 were *An. coustani*. The gonotrophic status of *An. coustani* was not determined because it is not confirmed as a vector in Malawi. Out of 20,118 *An. funestus* s.l., and *An. gambiae* s.l. collected, 5,500 (27.3%) were unfed, the highest proportion were blood fed (n=11,572, 57.5%), 1,243 (6.2%) were half gravid, and 1,727 (8.6%) were gravid. The gonotrophic status for 76 (0.4%) samples could not be determined because their abdomens were damaged. The proportion of gravid mosquitoes was higher among *An. funestus* s.l. (16.5%) than among *An. gambiae* s.l. (4.4%), indicating the more endophilic behavior of *An. funestus* s.l. (Table 5).

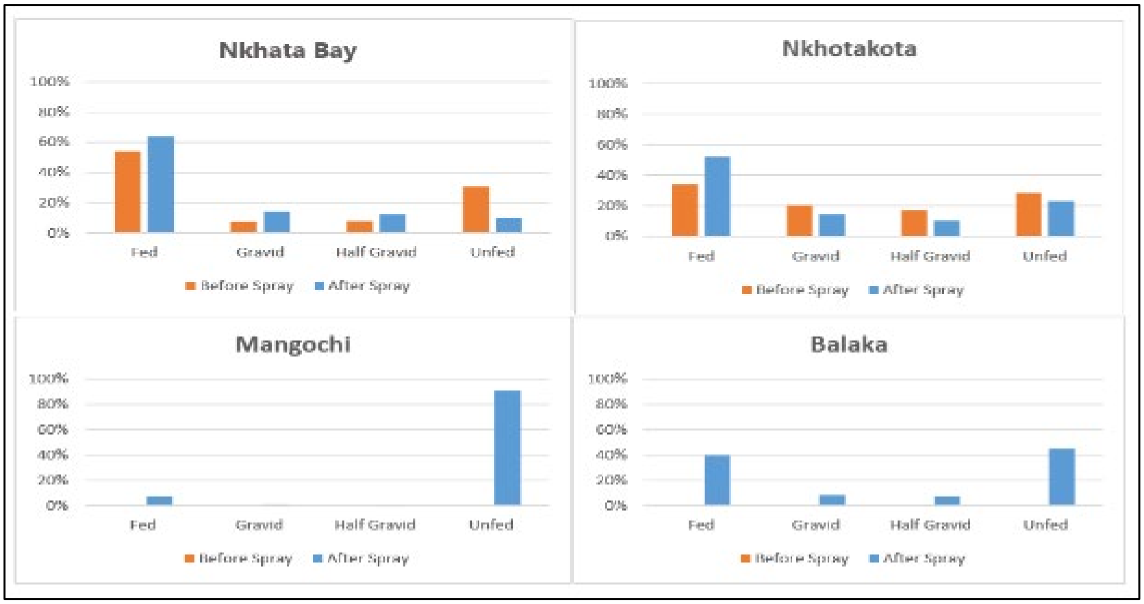
Table 5: Gonotrophic Status of *An. funestus* s.l. and *An. gambiae* s.l. Sampled by PSCs in All 17 Sentinel Sites

Species	Gonotrophic Status					
	Unfed	Fed	Half-gravid	Gravid	Undetermined	Total
An. funestus s.l.	1,484 (21.4%)	3,345 (48.2%)	919 (13.3%)	1,148 (16.5%)	39 (0.6 %)	6,935
An. gambiae s.l.	4,016 (30.5%)	8,227 (62.4%)	324 (2.4%)	579 (4.4%)	37 (0.3%)	13,183
Grand total	5,500 (27.3%)	11,572 (57.5%)	1,243 (6.2%)	1,727 (8.6%)	76 (0.4%)	20,118

In Nkhotakota, an IRS district, the proportion of blood-fed mosquitoes was higher after spraying (November to June) than before spraying (June to September) (Figure 9). The proportion of gravid mosquitoes was lower after spraying than before spraying. Similarly, in Nkhata Bay IRS district, the proportion of blood-fed mosquitoes was higher after spraying (November to June) than before spraying (July to September). For Mangochi and Balaka IRS districts, sample collection began in November, after spraying. Both districts recorded high proportions of unfed mosquitoes compared to the other abdominal stages.

Overall, the decreasing proportions of half gravid and gravid mosquitoes may indicate a tendency to rest outdoors after spray but also the killing effect of IRS on resting mosquitoes before they reach the gravid stage.

FIGURE 9: GONOTROPHIC STATUS OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED BY PSCS IN THE FOUR IRS DISTRICTS



In the non-IRS districts of Chikwawa, Kasungu, and Salima, the proportion of blood-fed *Anopheles* mosquitoes was higher during the rainy season (November to May) than during dry season (June to October) (Figure 10). In Karonga District, the proportion of blood-fed mosquitoes collected in the dry similar season was similar to the proportions collected in the rainy season.

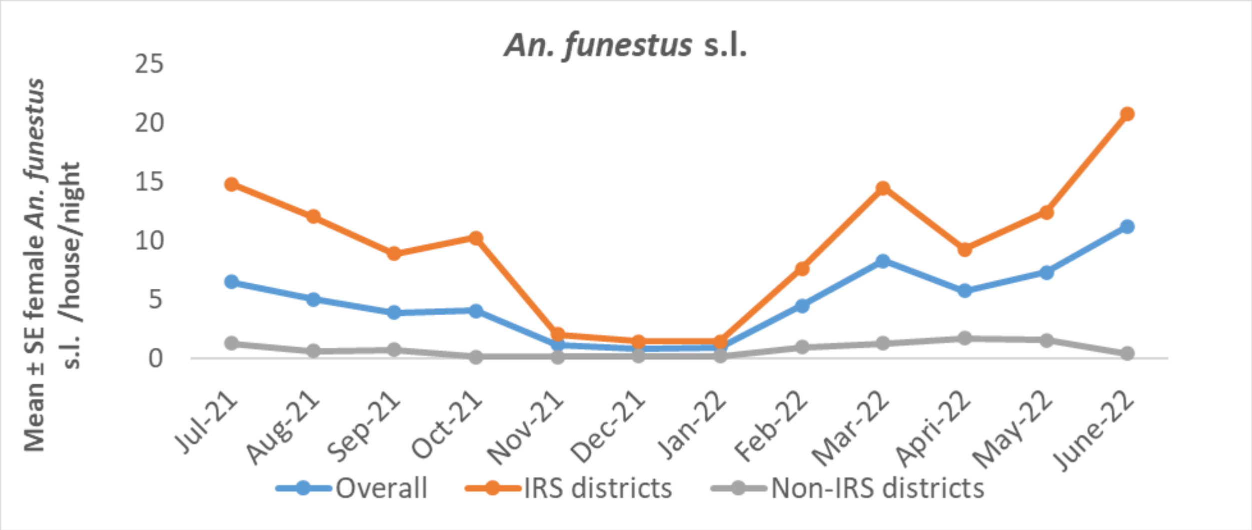
FIGURE 10: GONOTROPHIC STATUS OF FEMALE *ANOPHELES* MOSQUITOES COLLECTED BY PSCS IN THE FOUR NON-IRS MONITORING DISTRICTS

3.1 NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC-LT INDOORS

3.1.3 *AN. FUNESTUS* S.L. NUMBERS COLLECTED BY CDC-LT

The overall mean density of *An. funestus* s.l. was 5.0 mosquitoes per trap per night (m/t/n). The highest mean number of *An. funestus* s.l. was collected in June (11.2 m/t/n) and the lowest was recorded in December and January (<1 m/t/n). The trend was the same in IRS districts where the peak *An. funestus* s.l. mean density was recorded in June (20.9 m/t/n) and the lowest in December and January (<2 m/t/n). In non–IRS districts, the *An. funestus* s.l. mean density was very low throughout the collection period (<2 m/t/n) (Figure 11).

FIGURE 11: MEAN DENSITY OF *AN. FUNESTUS* S.L. COLLECTED. COLLECTED BY CDC-LT ACROSS FOUR IRS AND FOUR NON-IRS DISTRICTS (17 SENTINEL SITES), JULY 2021–JUNE 2022

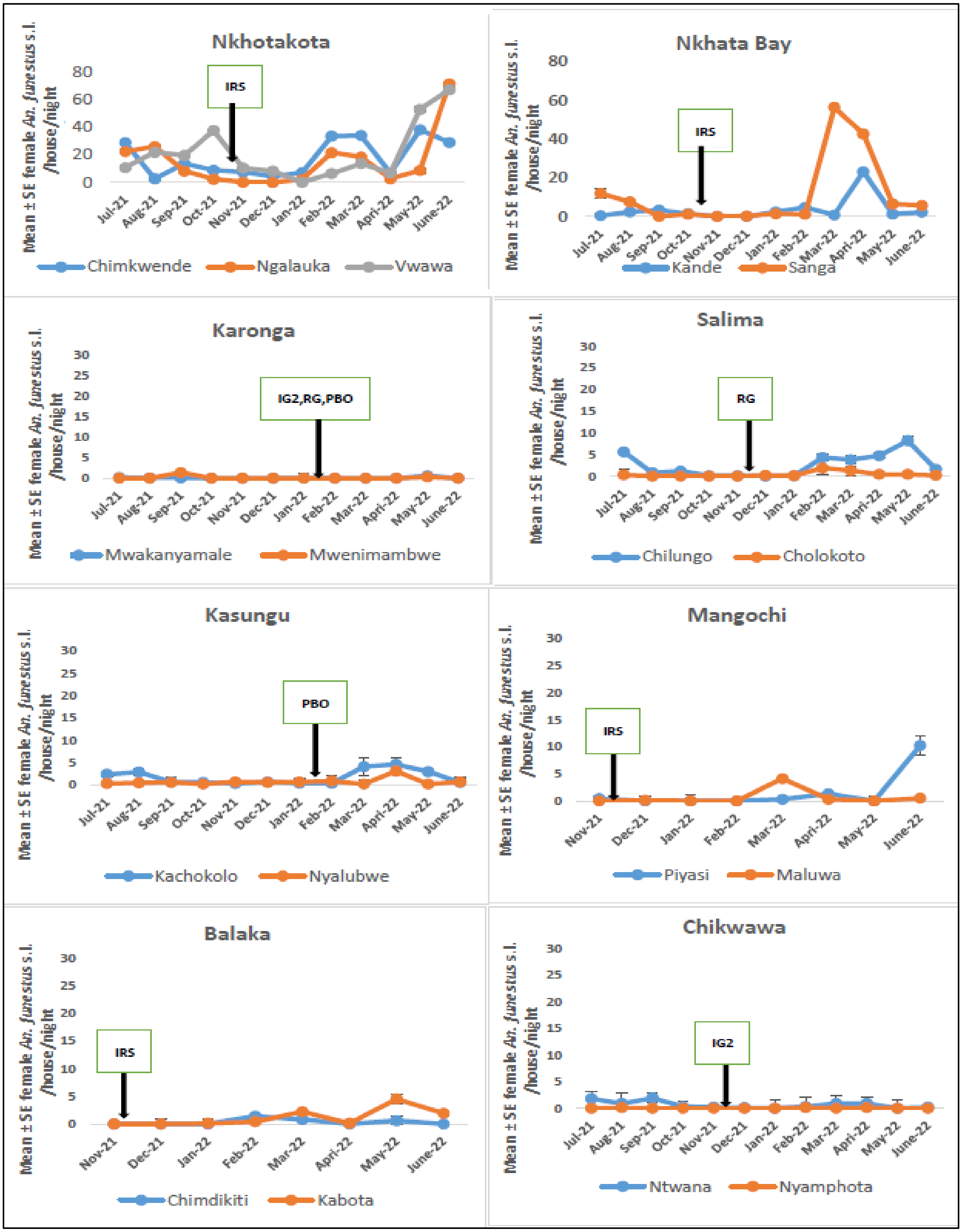


The highest mean density of *An. funestus* s.l. from CDC-LTs was observed in June 2021 from Ngalauka and Vwawa sites (Nkhotakota IRS district), with a mean density of 71.3 m/t/n and 67.0 m/t/n, respectively. The density of this species was also high in Nkhata Bay (IRS district) in March 2022 at Sanga site, with 56.4 m/t/n.

In Nkhotakota IRS district, the density of *An. funestus* s.l. was relatively high before spraying (July to September). However, after spraying in October, the mean density steadily decreased from November to January. In February, the mean density began to rise and remained relatively high through March. It then dropped in April (<7 m/t/n) before it rose again in May and remained high until June. This trend was observed in the three sites of Vwawa, Chimkwende, and Ngalauka. In Nkhata Bay IRS district, the mean density at Sanga site was high in July (before spraying), with *An. funestus* s.l. density of 11.9 m/t/n. Density declined from September to February (<1.0 m/t/n) before rising in March and then decreasing from April to June

The mean number of *An. funestus* s.l. remained low (<9.0 m/t/n) throughout the sampling period in the IRS districts of Mangochi (Piyasi and Nyalubwe sites) and Balaka (Chimdikiti and Kabota sites) and in non-IRS districts of Karonga (Mwenimambwe and Mwakanyamale sites), Chikwawa (Ntwana and Nyamphota), Kasungu (Kachokolo and Nyalubwe), and Salima (Chilungo and Cholokoto) (Figure 12).

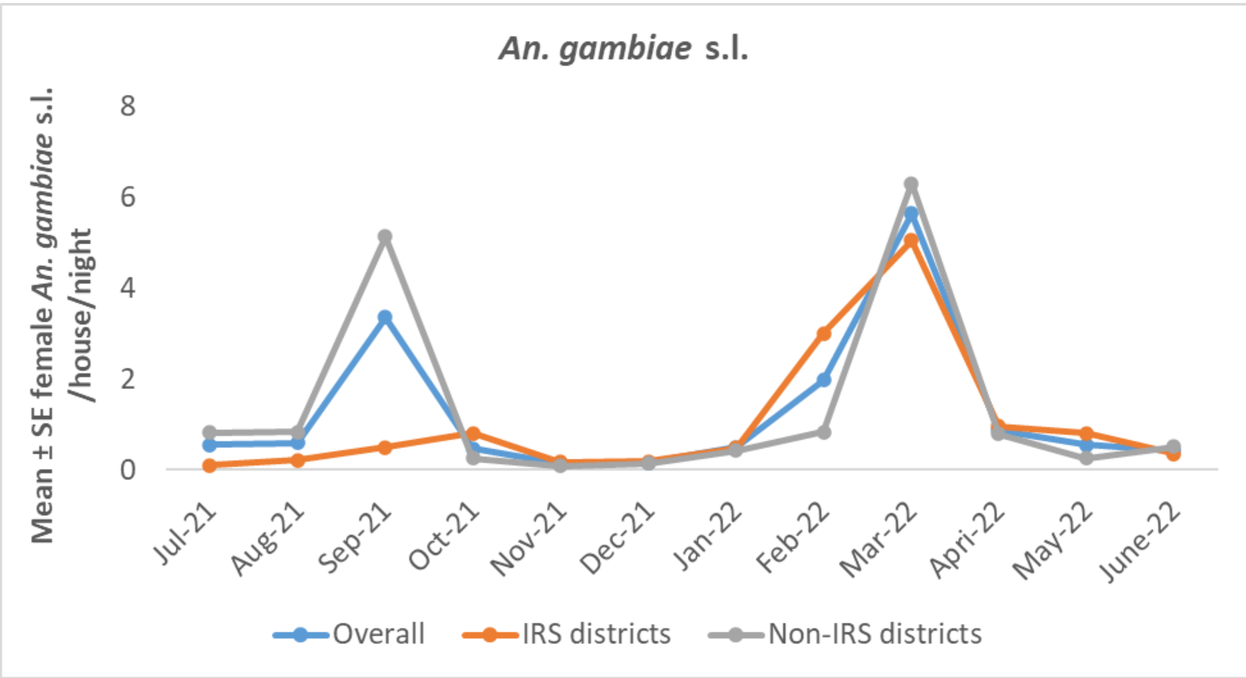
FIGURE 12: MEAN NUMBER OF *AN. FUNESTUS* S.L. COLLECTED BY CDC-LTS ACROSS EIGHT DISTRICTS, JULY 2021–JUNE 2022



3.1.2 *AN. GAMBIAE* S.L. NUMBERS COLLECTED BY CDC-LT

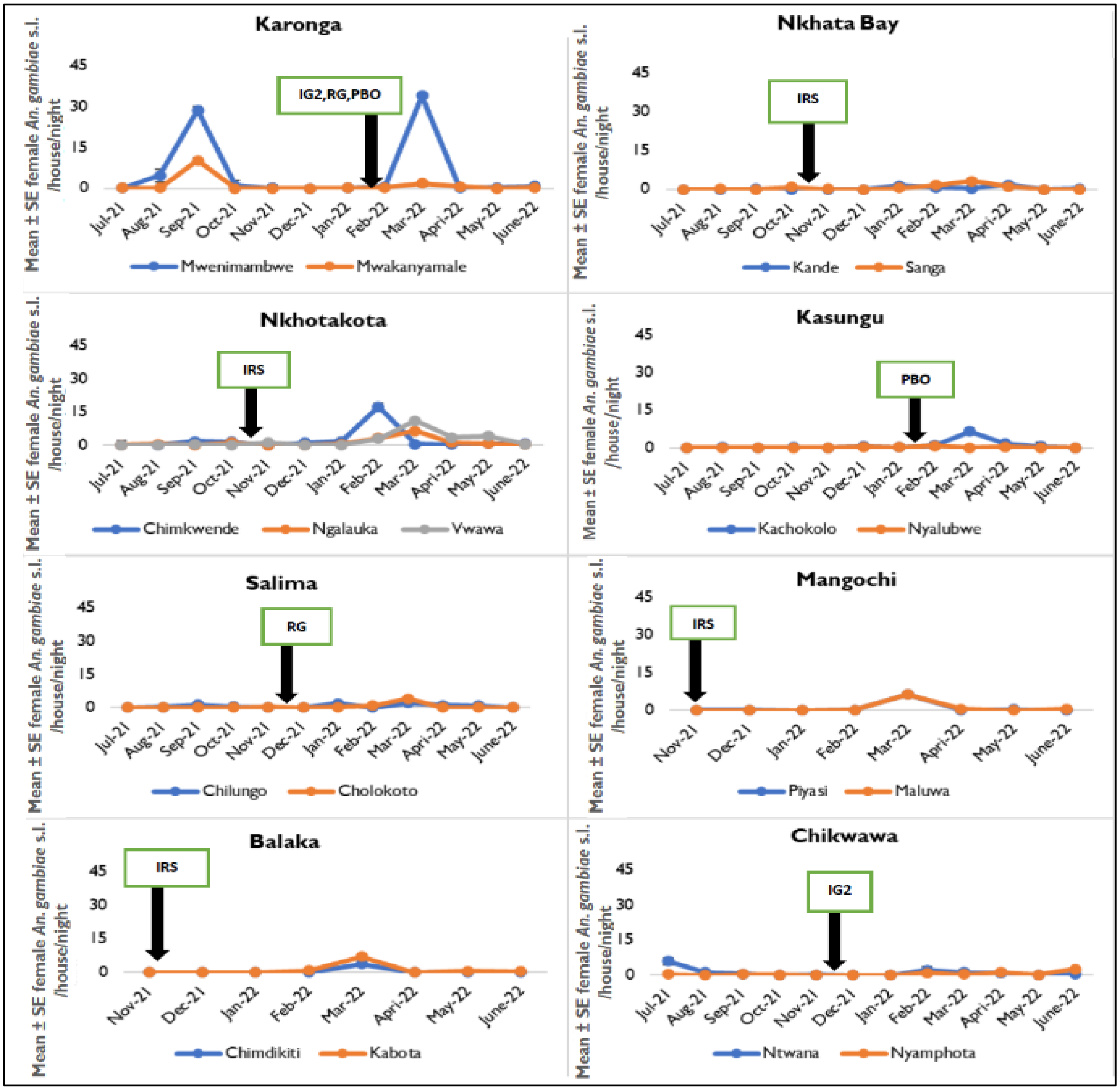
The number of *An. gambiae* s.l. collected indoors using CDC-LTs in the 17 sentinel sites is presented in Figure 13. The overall mean density of *An. gambiae* s.l. was 1.27 m/t/n. The peak *An. gambiae* s.l. density was in September (3.4 m/t/n) and in March (5.6 m/t/n). Similarly, in non-IRS districts, the peak *An. gambiae* s.l. density was observed in September (5.1 m/t/n) and in March (6.3 m/t/n). However, IRS districts, the peak *An. gambiae* s.l. density was only observed in March (5 m/t/n).

FIGURE 13: MEAN DENSITY OF *AN. GAMBIAE* S.L. COLLECTED BY CDC-LT ACROSS FOUR IRS AND FOUR NON-IRS DISTRICTS (17 SENTINEL SITES), JULY 2021–JUNE 2022



Mwenimambwe site in Karonga District recorded the highest density of *An. gambiae* s.l. in March 2022 with a mean density of 34.1 m/t/n, followed by Chimkwende site in Nkhotakota District, which recorded *An. gambiae* s.l. density of 17.5 m/t/n. In the remaining 15 sentinel sites, in both IRS and non-IRS districts, *An. gambiae* s.l. density was very low throughout the collection period, with a density of <5 m/t/n. (Figure 14).

FIGURE 14: MEAN NUMBER OF *AN. GAMBIAE* S.L. COLLECTED BY CDC-LTS ACROSS EIGHT DISTRICTS ACROSS EIGHT DISTRICTS (17 SENTINEL SITES) SENTINEL SITES), JULY 2021—JUNE 2022.



3.1.3 GONOTROPHIC STATUS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC-LTS

A total of 11,972 female *Anopheles* mosquitoes were collected using CDC-LTs. Of these, 9,354 were *An. funestus* s.l., 2,381 *An. gambiae* s.l., and 237 *An. coustani*. The gonotrophic status of *An. coustani* was not determined because it is not confirmed as vector in Malawi. Out of the 11,735 *An. funestus* s.l., and *An. gambiae* s.l. collected, the highest proportion were unfed (n=9,986, 85.1%), 743 (6.3%) were blood fed, 465 (4 %) were gravid, and 137 (1.2%) were half gravid. The gonotrophic status for 404 (3.4%) samples could not be determined because their abdomens were damaged. CDC-LTs captured more host-seeking (unfed) females than fed and gravid females (Table 6).

TABLE 6: GONOTROPHIC STATUS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. SAMPLED BY CDC-LTS IN ALL 17 SENTINEL SITES

	Gonotrophic Status					
Species	Unfed	Fed	Half-gravid	Gravid	Undetermined	Total
An. funestus s.l.	8,047 (86%)	529 (5.6%)	107 (1.1%)	359 (3.8%)	312 (3.3%)	9,354
An. gambiae s.l.	1,939 (81.4%)	214 (9%)	30 (1.3%)	106 (4.4%)	92 (3.9%)	2,381
Grand total	9,986 (85.1%)	743(6.3%)	137 (1.2%)	465 (4%)	404 (3.4%)	11,735

3.2 LABORATORY ANALYSIS

3.2.1 SPECIES IDENTIFICATION

A) AN. GAMBIAE S.L.

A total of 2,585 female *An. gambiae* s.l. were randomly sampled and identified to the species level by PCR. Mosquito samples were collected using four methods: PSC (n=1,290, 49.9%), CDC-LT (n=354,13.7%), HLC (n=122, 4.7%), and Prokopack aspirator (n=819, 31.7%). Out of the 2,585 *An. gambiae* s.l., 2,579 (99.8%) were identified as *An. arabiensis* and 6 (0.2%) as *An. gambiae* s.s.. *An. arabiensis* was found in all the eight districts and was predominant member of the *An. gambiae* complex, while *An. gambiae* s.s. was found in three districts: Chikwawa (n=1, 0.9%), Karonga (n=1, 0.8%), and Salima (n=3, 2.1%).

B) AN. FUNESTUS S.L.

A total of 566 female *An. funestus* s.l. were identified to the species-specific level using PCR (Table 2). The *An. funestus* s.l. were collected from PSCs (n=36, 6.4%), CDC-LTs (n=299, 52.8 %), HLCs (n=75, 13.3 %), and Prokopack (156, 27.6%). Virtually all of the *An. funestus* s.l. (565, 99.8%) were identified as *An. funestus* s.s., the predominant member of *An. funestus* s.l. and present in all eight districts. The one *An. funestus* s.l. from Salima (n=1, 0.2%) was identified as *An. leesoni*.

A total of 2750 moquitoes (both *An. gambiae* s.l. and *An. funestus* s.l.) were targeted for species identification by PCR.

3.2.2 BLOOD MEAL ANALYSIS

A total of 800 blood-fed mosquitoes from eight districts were analyzed for blood meal using PCR to identify the source of their blood meal. A total of 397 samples (49.6%) amplified while 403 (50.4%) did not amplify. The target for bloodmeal analysis was 300 samples. Even though blood meal protocol was optimized, there is still a problem with this analysis. Technical assistance is needed to fully resolve this issue.

Out of the amplified samples, 66.5% (n=264) were *An. funestus* s.l. while 33.5% (n=133) were *An. gambiae* s.l. Human blood was the predominant source for both *An. funestus* s.l. and *An. gambiae* s.l. The highest number of *An. funestus* s.l. (n=246, 93.2%) fed on human blood, followed by cow (11, 4.2%), goat (4, 1,5%), pig (2, 0.7%), and dog (1, 0.4%). Similarly, the highest number of *An. gambiae* s.l. (78, 58.6%) fed on human blood followed by cow (40, 30%), goat (8, 6%), dog (5, 3.8%), and pig (2, 1.5%) (Table 7). The human blood index of *An. funestus* s.l. (93.2%) was significantly higher ($p<0.0001$) than the human blood index of *An. gambiae* s.l., indicating that *An. funestus* s.l. is more anthropophilic. Even though it is not ideal to estimate the degree of anthropophagy for each species without similar data from outdoor collected mosquitoes, given the same collection methods and places were used for both, the data suggests that *An. funestus* s.l. is more anthropophilic than *An. gambiae* s.l.

TABLE 7: NUMBER AND PROPORTION OF FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. TESTED TO DETERMINE BLOOD MEAL SOURCE

		An. funestus s.l. Blood meal source						*An. gambiae* s.l. Blood meal source					
District	Site	N (%) Cow	N (%) Dog	N (%) Goat	N (%) Human	N (%) Pig	Total Tested	N (%) Cow	N (%) Dog	N (%) Goat	N (%) Human	N (%) Pig	Total Tested
Balaka	Chimdikiti	0	0	0	0	0	0	0	0	1	0	0	**1**
	Kabota	0	0	0	0	0	0	0	0	0	2	0	2
Chikwawa	Ntwana	1	0	0	10	1	12	2	0	0	2	0	4
	Nyamphota	0	1	0	1	0	2	1	0	0	3	0	4
Karonga	Mwakanyamale	0	0	1	3	0	4	14	3	5	6	1	29
	Mwenimambwe	0	0	0	2	0	2	18	2	2	35	0	57
Kasungu	Kachokolo	3	0	0	32	1	36	0	0	0	1	0	1
	Nyalubwe	2	0	0	21	0	23	0	0	0	1	0	1
Mangochi	Maluwa	0	0	0	1	0	1	0	0	0	2	0	2
	Piyasi	1	0	0	6	0	7	0	0	0	2	0	2
Nkhata Bay	Kande	3	0	1	68	0	72	1	0	0	1	0	2
	Sanga	0	0	1	25	0	26	0	0	0	8	1	9
Nkhotakota	Chimkwende	0	0	0	8	0	8	0	0	0	1	0	1
	Ngalauka	0	0	0	17	0	17	2	0	0	1	0	3
	Vwawa	0	0	0	15	0	15	0	0	0	2	0	2
Salima	Chilungo	1	0	1	33	0	35	2	0	0	11	0	13
	Cholokoto	0	0	0	4	0	4	0	0	0	0	0	0
		11 (4.2)	1 (0.4)	4 (1.5)	246 (93.2)	2 (0.7)	264	40 (30.1)	5 (3.8)	8 (6)	78 (58.6)	2 (1.5)	133

3.2.3 DETECTION OF *ACE-1*

Of 62 (out of the targeted 300) female *An. gambiae* s.l., 61 (98.4%, 61) *An. arabiensis* and 1 (1.6%) *An. gambiae* s.s. were analyzed for *Ace-1* mutation. The processed samples were collected from six districts (Chikwawa, Karonga, Kasungu, Nkhata Bay, Nkhotakota, and Salima) using PSCs and CDC-LTs. Out of the 62 processed samples, 20 (32.2%) were collected from Chikwawa, 24 (38.7%) from Karonga, 1 (1.6%) from Kasungu, 1 (1.6%) from Nkhata Bay, 12 (19.4%) from Nkhotakota, and 4 (6.5%) from Salima. All the samples analyzed were homozygous susceptible and no G119S mutation was detected (Table 8).

TABLE 8: *ACE-1* RESULTS

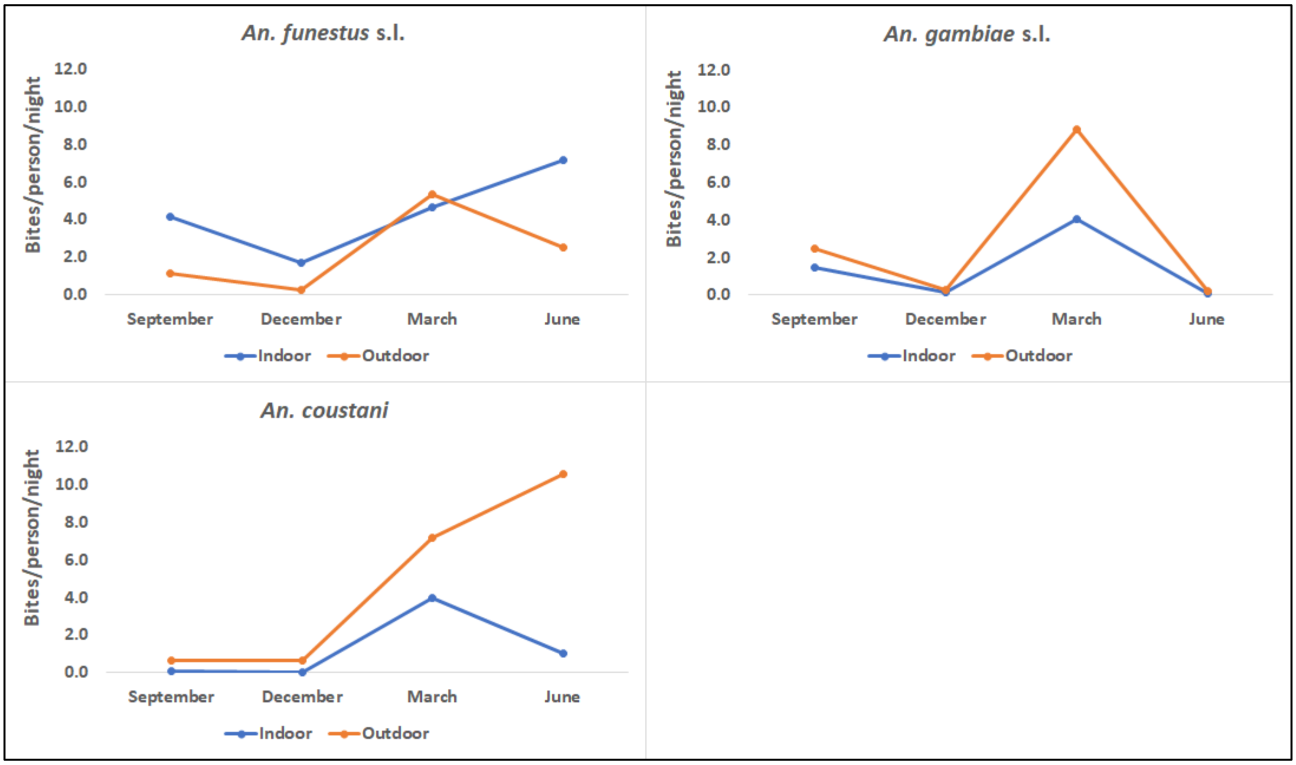
		Ace - 1		
District	Species	RR	RS	SS
Chikwawa	*An. arabiensis*	0	0	20
Karonga	*An. gambiae* s.s.	0	0	1
	An. arabiensis	0	0	23
Kasungu	*An. arabiensis*	0	0	1
Nkhata Bay	*An. arabiensis*	0	0	1
Nkhotakota	*An. arabiensis*	0	0	12
Salima	*An. arabiensis*	0	0	4
Number of genotypes		0	0	62
Number of alleles		0	0	124
Total number of alleles		124		
Frequency of the resistance R allele [2RR+RS)/2[RR+RS+SS)]		**0%**		

3.3 Biting Rates of Malaria Vectors

3.3.1 Biting Rates of Malaria Vectors by District from HLC Collections

HBR was measured quarterly in six districts: Nkhata Bay, Nkhotakota, Salima, Kasungu, Mangochi, and Balaka. Overall, the HBRs for *An. funestus* s.l. were 4.6 b/p/n) indoors and 2.5 b/p/n outdoors. The HBRs for *An. gambiae* s.l. were 1.5 b/p/n indoors and 3.1 b/p/n outdoors. The HBRs for *An. coustani* were 1.4 b/p/n indoors and 5.2 outdoors (Annex A, Table A7). Overall, the highest indoor biting activity of *An. funestus* s.l., 7.2 b/p/n, was recorded in June. The highest outdoor biting activity of *An. funestus* s.l., 5.3 b/p/n, was observed in March. Both the highest indoor and outdoor biting activity of *An. gambiae* s.l. were observed in March, 4.1 b/p/n and 8.8 b/p/n respectively. The highest indoor and outdoor human biting activity of *An. coustani* were observed in March (4.0 b/p/n) and (10.6 b/p/n), respectively (Figure 15).

Figure 15: Average Bites of *Anopheles* Mosquitoes per Person per Night in Seven Sentinel Sites



Overall, the highest indoor biting activity of *An. funestus* s.l. was recorded in June, in Nkhotakota District (30.2 b/p/n and 16.8 b/p/n in September) and in Salima District (10.3 b/p/n) in March. The highest biting activity of *An. gambiae* s.l. was observed in Nkhata Bay District in March (6.3 b/p/n), indoors, and in Kasungu District (11.9 b/p/n), outdoors. The highest indoor and outdoor human biting activity of *An. coustani* was observed in March in Nkhata Bay (14.2 b/p/n) and in June in Nkhotakota (61.7 b/p/n) District (Annex A, Table A7).

3.3.2 BITING RATES OF MALARIA VECTORS BY DISTRICT FROM CDC-LT COLLECTIONS

Seven of the monitoring districts had two sentinel sites while Nkhotakota District had three sites. Ten CDC-LTs were set at each sentinel site. Hence, 20 CDC-LTs were set in each of the seven districts and Nkhotakota had 30. Annex A, Table 8 and Figure 16 summarize the estimated HBRs of *Anopheles* mosquitoes from the CDC-LT collections. Overall, the highest biting activity of *An. funestus* s.l. was observed in June, 8.5 bites per person per night (b/p/n) and the lowest in December-January, 0.7 b/p/n. The highest biting activity of *An. gambiae* s.l. was observed in March, 5.6 b/p/n and the lowest in November-December, 0.1 b/p/n.(Annex A, Table A8). High *An. funestus* s.l. biting rates were recorded in June, in Nkhotakota District (55.8 b/p/n) and in March and April in Nkhata Bay District (28.6 b/p/n and 32.9 b/p/n respectively). In the remaining six districts, *An. funestus* s.l. biting activity was very low (<7 b/p/n). The highest *An. gambiae* s.l. biting activity was recorded in September (19.5 b/p/n) and in March (18.0 b/p/n) in Karonga District. In the remaining seven districts, *An. gambiae* s.l. biting activity was very low (<8 b/p/n) (Figure 16).

FIGURE 16: ESTIMATE OF HBRS OF *ANOPHELES* MOSQUITOES FROM CDC-LT COLLECTION IN EIGHT DISTRICTS, JULY 2021 – JUNE 2022

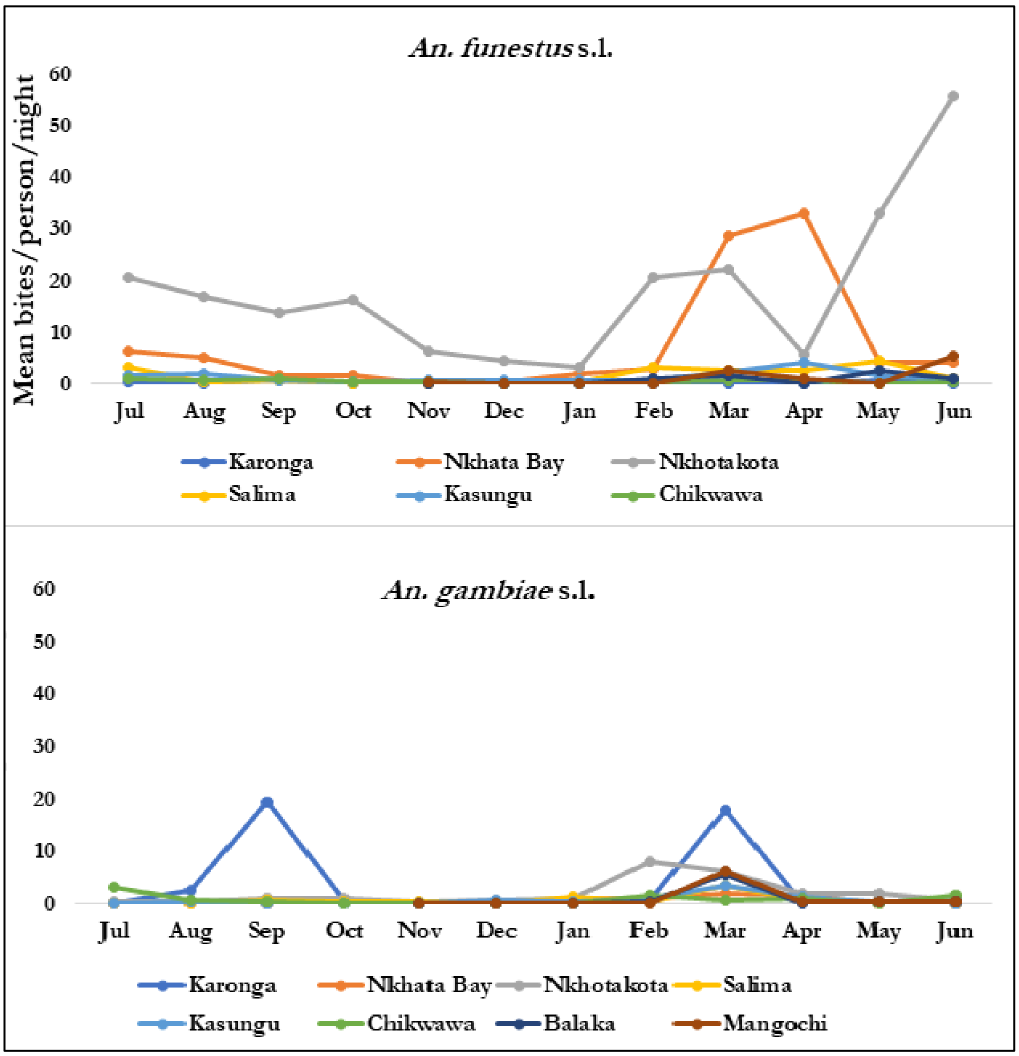


TABLE 9: DIFFERENCE IN HBR WHEN CALCULATED FROM CDC-LT AND HLC COLLECTIONS IN SIX SENTINEL DISTRICTS, 2022

District	*Month*	*An. funestus* s.l.			*An. gambiae* s.l.			*Total*		
		HLC	*CDC*	*Diff*	*HLC*	*CDC*	*Diff*	*HLC*	*CDC*	*Diff*
Nkhata Bay	September	0.83	0.05	0.78	6	1.65	4.35	6.83	1.7	5.13
	December	0.17	0	0.17	0	0.1	-0.1	0.17	0.1	0.07
	March	6.5	1.7	4.8	6.33	28.6	-22.27	12.83	30.3	-17.47
	June	3.33	0.1	3.23	0.17	4	-3.83	3.5	4.1	-0.6
Kasungu	September	2.5	0	2.5	0	0.65	-0.65	2.5	0.65	1.85
	December	0.67	0.5	0.17	0	0.65	-0.65	0.67	1.15	-0.48
	March	2.11	3.3	-1.19	5.22	2.15	3.07	7.33	5.45	1.88
	June	0.5	0.05	0.45	0	0.65	-0.65	0.5	0.7	-0.2
Nkhotakota	September	16.83	0.8	16.03	0.42	13.8	-13.38	17.25	14.6	2.65
	December	9.5	0.53	8.97	0.67	4.27	-3.6	10.17	4.8	5.37
	March	3	6.13	-3.13	2.42	22.07	-19.65	5.42	28.2	-22.78
	June	30.17	0.6	29.57	0.33	55.77	-55.44	30.5	56.37	-25.87
Salima	September	0.5	0.65	-0.15	0.83	0.6	0.23	1.33	1.25	0.08
	December	0	0.05	-0.05	0.17	0.1	0.07	0.17	0.15	0.02
	March	10.33	3.25	7.08	4	2.55	1.45	14.33	5.8	8.53
	June	9.17	0	9.17	0.17	0.9	-0.73	9.34	0.9	8.44
Mangochi	December	0	0.05	-0.05	0	0.05	-0.05	0	0.1	-0.1
	March	1.17	6.25	-5.08	2.67	2.3	0.37	3.84	8.55	-4.71
	June	0	0.3	-0.3	0	5.35	-5.35	0	5.65	-5.65
Balaka	December	0	0	0	0	0	0	0	0	0
	March	4.83	5.55	-0.72	3.67	1.5	2.17	8.5	7.05	1.45
	June	0	0.3	-0.3	0	0.95	-0.95	0	1.25	-1.25
Overall, 22 months		**102.11**	**30.16**	**71.95**	**33.07**	**148.66**	**-115.59**	**135.18**	**178.82**	**-43.64**
Bites/person/night		**4.64**	**1.37**	**3.27**	**1.50**	**6.76**	**-5.25**	**6.14**	**8.13**	**-1.98**

HBRs estimated from HLC, and CDC-LT methods were compared for the four months in the year both methods were used to collect host seeking mosquitoes indoors. The difference in HBR by the two methods were not consistent and across the collection months and different sites. The difference in HBR for both vectors shows some variation by site and time. However, the two vectors show a notable difference in overall HBR when estimated from the two collection methods. While the HBR from HLC is much higher (4.64 b/p/n) than the HBR from CDC-LT (1.37 b/p/n) for *An. funestus* s.l. the HBR for *An. gambiae* s.l. is higher when estimated from CDC-LT (6.76 b/p/n) than from HLC (1.50 b/p/n). The overall HBR was higher for CDC-LT than HLC; 8.1 vs 6.1 b/p/n. The estimates assume all CDC-LT collected females were seeking hosts. However, only 85% of the CDC-LT mosquitoes were unfed. When this is adjusted for the unfed mosquitoes only, the HBR estimated by HLC (6.1 b/p/n) is similar to the HBR estimated from CDC-LT collection (6.9 b/p/n). Though CDC-LT seems to slightly overestimate HBR, in this Malawi case, it appears that estimating HBR from CDC-LT is almost as good as HLC. Infection Detection

Overall, the total number of mosquitoes targeted for sporozoite infection detection was 15, 600. However, 87% (13,592) mosquitoes were screened for sporozoite infection.

A total of 8,557 *An. funestus* s.l. collected from the eight monitoring districts using PSC, CDC-LTs, and HLCs were screened for sporozoite infection. The overall SR was 1.0%. A total of 4,098 *An. gambiae* s.l. from all three collection methods were tested for *Pf,* and the overall SR was 0.3%.

A) SPOROZOITE RATES FROM HLC COLLECTIONS (JULY 2021–JUNE 2022)

Tables in Annex A summarize the sporozoite infections of *An. gambiae* s.l. (Annex A, Table A1) and *An. funestus* s.l. (Annex A, Table A2) captured by HLC. SRs were recorded from the four districts during the sampling period. No ELISA tests were conducted on samples from Balaka and Mangochi districts.

No sporozoite-positive *An. gambiae* s.l. were found either indoors or outdoors in the four districts.

The overall *An. funestus* s.l. SR from the four districts was 0.5% (n=807). SR was 0.7% (n=567) indoors; no sporozoite-positive *An. funestus* s.l. were found outdoors (n=237). In non-IRS districts, high *An. funestus* s.l. SR was recorded in Kasungu (PBO) district in March, 14.3% (n=14) indoors. In IRS districts, *An. funestus* s.l. SRs were recorded only indoors in Nkhotakota, in September before spraying (2.4%, n=41) and in December after spraying (3.0%, n=33). No sporozoite-positive *An. funestus* s.l. were found outdoors in either month.

A total of 750 *An. coustani* and 187 *An. pharoensis* (collected both indoors and outdoors) were tested for infection and no sporozoite-positive were found in either species from the four IRS districts.

B) Sporozoite Rates from PSC and CDC-LT Collections (July 2021–June 2022)

i) From PSC collections (July 2021–June 2022)

***An. gambiae* s.l.:** Annex A, Table A3 summarizes the SRs of *An. gambiae* s.l. collected from PSCs. A total of 2,155 *An. gambiae* s.l. were tested for *Pf* infection. The overall *An. gambiae* s.l. SR from the eight districts was 0.1%. High *An. gambiae* s.l. SRs were recorded in Nkhotakota (1.6%) and Karonga (0.1%). No sporozoite-positive *An. gambiae* s.l. were detected in Nkhata Bay, Kasungu, Salima, Balaka, Mangochi, and Chikwawa districts.

***An. funestus* s.l.:** Annex A, Table A4 summarizes the SRs of *An. funestus* s.l. collected from PSCs. A total of 2,752 *An. funestus* s.l. were tested for *Pf* infection. The overall *An. funestus* s.l. SR from the eight districts was 0.4%. *An. funestus* s.l. SRs were generally low in all the eight districts (<1%).

A total of 38 *An. coustani* from PSCs collections were tested for infection and no sporozoite-positive *An. coustani* were found.

ii) From CDC-LT collections (July 2021–June 2022)

***An. gambiae* s.l.:** Annex A, Table A5 summarizes the SRs of *An. gambiae* s.l. collected from CDC-LTs. A total of 1398 *An. gambiae* s.l. were tested for *Pf* infection. The overall *An. gambiae* s.l. SR from all eight districts was 0.3%. Generally, *An. gambiae* s.l. SRs were low in all the districts; the highest was recorded in Chikwawa (1.6%) followed by Karonga (1%).

***An. funestus* s.l.:** Annex A, Table A6 summarizes the SRs of *An. funestus* s.l. collected from CDC-LTs. A total of 4891 *An. funestus* s.l. were tested for *Pf* infection. The overall *An. funestus* s.l. SR from the eight districts was 1.4%. *An. funestus* s.l. SRs were low in all the eight districts; the highest was recorded in Balaka (2.2%) followed by Nkhata Bay (1.7%), Nkhotakota (1.5%), and Kasungu (0.8%). No sporozoite-positive *An. funestus* s.l. were recorded in Karonga, Salima, Chikwawa, and Mangochi districts. A total of 52 *An. coustani* from PSCs collections were tested for infection and no sporozoite-positive *An. coustani* were found.

3.4 EIRs of *An. gambiae* s.l. and *An. funestus* s.l. Estimated from CDC-LT Collections, July 2021–June 2022

Monthly trends in HBR were estimated from CDC-LT collections that were conducted monthly.[4] The number of vectors collected per trap per night was considered equivalent to the number of bites per person per night. The HBRs from CDC-LT collections were then used to estimate monthly and annual EIRs. Figure 17 and Annex A, Table A9 summarizes the SRs and monthly EIRs of *An. gambiae* s.l. and *An. funestus* s.l. estimated from CDC-LTs collections over a 12-month period (July 2021–June 2022) in the six districts of Karonga, Nkhotakota, Nkhata Bay, Kasungu, Salima, and Chikwawa. Entomological monitoring for Balaka and Mangochi districts was conducted for eight months (November 2021–June 2022).

The estimated risk of malaria transmission for the 12 months (annual EIR) was highest in Nkhotakota District, at 94.5 infective bites/person/year (ib/p/yr.)), all from *An. funestus* s.l. The second highest EIR was recorded in Nkhata Bay District (27.4 ib/p/yr.; all from *An. funestus* s.l.) followed by Kasungu (22.0 ib/p/yr.; all from *An. funestus* s.l.), Karonga (14.3 ib/p/yr.; all from *An. gambiae* s.l.), and Chikwawa (4.7 ib/p/yr.; all from *An. gambiae* s.l.).

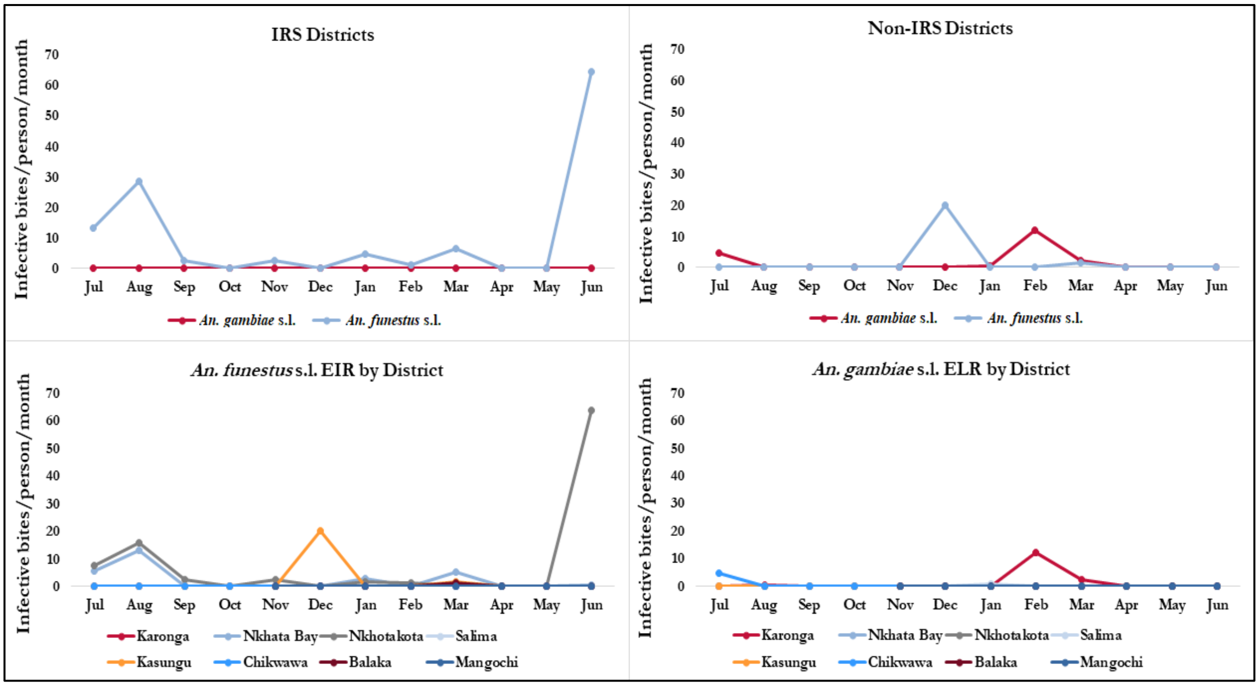
In the two districts supported by Global Fund (Balaka and Mangochi), entomological monitoring started in November and data was collected for eight months. The estimated risk of malaria transmission for the eight months (infective bites/person /8 months EIR) was low for both Balaka (*An. funestus* s.l. = 1.2 ib/p/8 months) and Mangochi (no infective bites from either *An. gambiae* s.l. and *An. funestus* s.l. over the eight months). NB: Low numbers of samples processed from these two districts might have affected the results.

In the IRS districts of Nkhotakota and Nkhata Bay, the estimated risk of malaria transmission over a 12-month period was 94.5 ib/p/yr. and 27.4 ib/p/yr., respectively. However, in Nkhotakota, a relatively high (25.5 ib/p/3 months) EIR was observed before spraying (July–September); it greatly declined soon after spraying (October–May) to 5.3 ib/p/8 months, before rapidly rising in June (63.8 ib/p/month), nine months after spraying. In Nkhata Bay, a higher EIR was recorded before spraying (July–September), 18.8 ib/p/3 months, than after spraying (November–June), 10 ib/p/8 months.

In non-IRS districts, there was variation in the monthly EIRs of *Anopheles* mosquitoes. The highest estimated risk of malaria transmission over a 12-month period was observed in Kasungu, a PBO net distribution district, (22.0 ib/p/yr.), followed by Karonga (14.3 ib/p/yr.), where all three types of nets were distributed (PBO, IG2, and Royal Guard); Chikwawa (4.7 ib/p/yr.), where IG2 nets were distributed; and Salima (0.5 ib/p/yr.), where Royal Guard nets were distributed.

[4] HLC collections were performed quarterly and were not used to estimate trends in monthly HBR and EIR. They were used to show biting location and biting time preference.

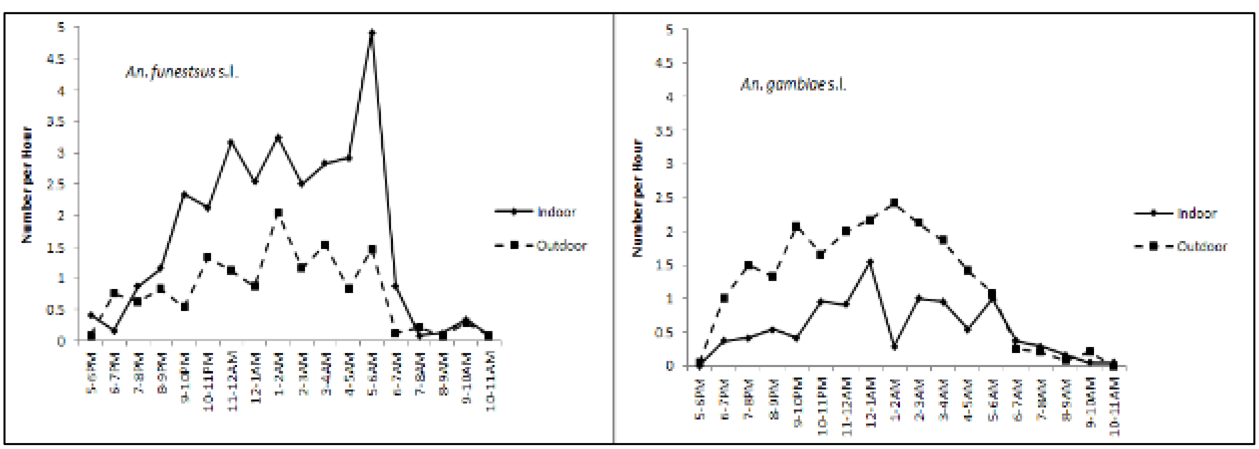
FIGURE 17: EIRS OF *AN. GAMBIAE* S.L AND *AN. FUNESTUS* S.L. FROM CDC-LT COLLECTIONS BY DISTRICT, JULY 2021 -JUNE 2022



3.4.1 TIME AND LOCATION OF BITING OF MALARIA VECTORS

The overall biting activity of *An. funestus* s.l. and *An. gambiae* s.l. from the six districts of Nkhata Bay, Nkhotakota (two sites), Kasungu, Salima, Mangochi, and Balaka are presented in Figure 18. *An. funestus* s.l. predominantly fed indoors while *An. gambiae* s.l. predominantly fed outdoors. The biting activity of *An. funestus* s.l. occurred throughout the night, from 5:00 pm to 5:00 am both indoors and outdoors. There was a rise in indoor biting of this species from 9:00 pm, reaching a peak between 5:00 am and 6:00 am before rapidly declining after 6:00 am until 11:00 am. The biting activity of *An. gambiae* s.l. showed a similar pattern, albeit without a distinct peak. Morning/daytime indoor and outdoor biting activity of this species was observed from 7:00 am until 11:00 am.

FIGURE 18: AVERAGE HOURLY INDOOR AND OUTDOOR BITING RATES BY TIME OF NIGHT FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. FROM THE SIX DISTRICTS, JULY 2021–JUNE 2022



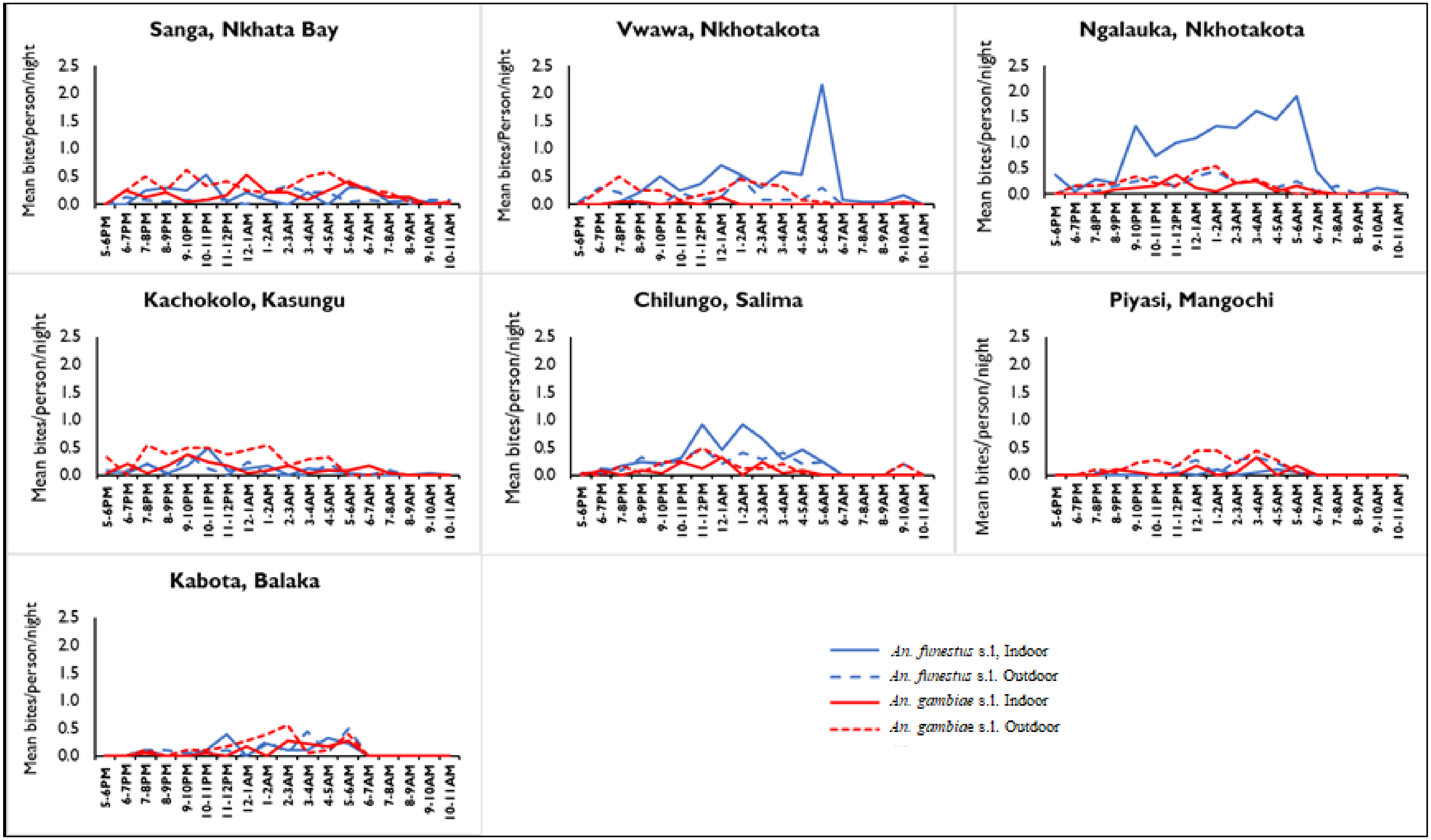
The biting pattern of *An. funestus* s.l. and *An. gambiae* s.l. in the same six districts collected quarterly from July 2021 to June 2022 is summarized in Figure 19.

In Nkhotakota District, *An. funestus* s.l. indoor biting at Vwawa site was low throughout the night and peaked at 5:00 am. At Ngalauka site, *An. funestus* s.l. biting occurred throughout the night until 6:00 am before rapidly declining from 7:00 am to 11:00 am. The outdoor biting activity of both *An. funestus* s.l. and *An. gambiae* s.l. remained relatively low throughout the night.

In Salima District, the biting activity of *An. funestus* s.l. occurred both indoors and outdoors from 5:00 pm to 5:00 am. The biting activity rose between 11:00 pm and 5:00 am before declining after 6 am. The outdoor biting activity of this species in this district was very low throughout the night till morning. Similarly, the indoor and outdoor biting activity of *An. gambiae* s.l. in the district was very low throughout the night till the morning.

In Nkhata Bay, Kasungu, Mangochi, and Balaka districts, the human biting activity (indoor and outdoor) of *An. gambiae* s.l. and *An. funestus* s.l. was very low throughout the night, showing no pattern.

FIGURE 19: AVERAGE HOURLY INDOOR AND OUTDOOR BITING RATES BY TIME OF NIGHT FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. FROM EACH OF THE SIX DISTRICTS (JULY 2021–JUNE 2022).



3.4.2 INTERSECTION BETWEEN MOSQUITO AND HUMAN BEHAVIOR

Human behavior surveys were conducted in six districts: Nkhata Bay, Nkhotakota, Kasungu, Salima, Mangochi, and Balaka. Across these districts, the percentage of *An. funestus* s.l. and *An. gambiae* s.l. bites occurring indoors were 89% and 65%, respectively (Table 12). The percentage of exposure to bites while asleep was 86% for *An. funestus* s.l. and 62% for *An. gambiae* s.l. We estimated the exposure of persons according to their behavior and the time and location of mosquito biting using models described by Monroe et al. (2020).

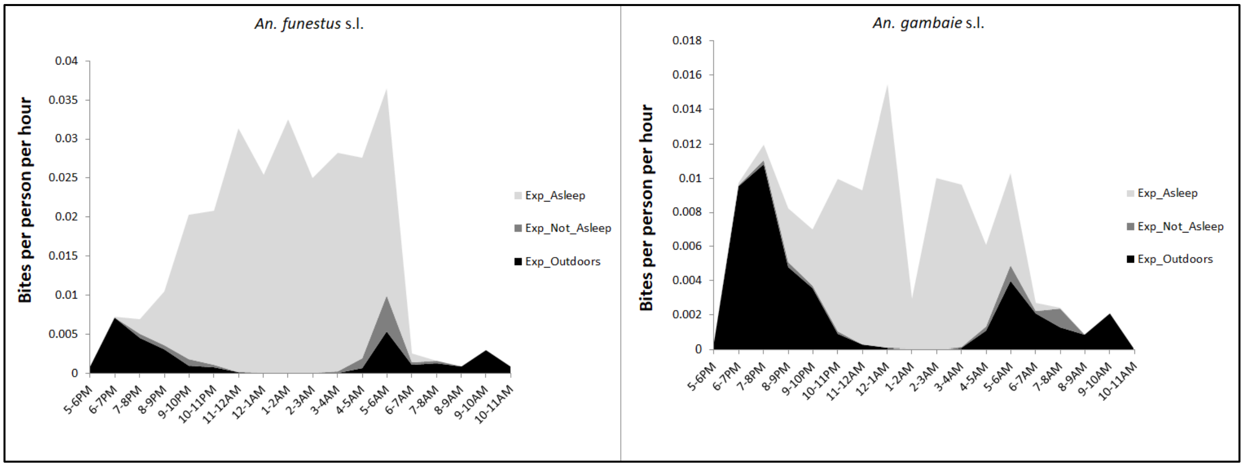
Overall*, An. funestus* s.l. biting indoors was twice that of outdoors. However, a smaller proportion of indoor *An. funestus* s.l. bites were observed after 6:00 am (4.9%) and 7:00 am (2.0%) while people were waking up. Similarly, a lower percentage of *An. funestus* s.l. bites occurred outdoors after 6:00 am (5.7%) and 7:00 am (4.8%). When mapped with human behavior, 89% of *An. funestus* s.l. bites occurred when people were indoors (πi, i) and 86% occurred when people were sleeping (πi, s) (Table 12 and Figure 18).

High *An. gambiae* s.l. biting activity occurred outdoors. Morning bite of *An. gambiae* s.l. was also observed after 5:00 am; 19.4% and 8.5% indoors and outdoors, respectively (Table 8). When mapped with human behavior, 65% of *An. gambiae* s.l. bites occurred when people were indoors (πi, i) and 62% occurred when people were sleeping (πi, s) (Table 10 and Figure 20).

TABLE 10. SUMMARY OF INTERACTION BETWEEN MOSQUITO AND HUMAN BEHAVIOR BY SPECIES

Parameter	*An. funestus* s.l.	*An. gambiae* s.l.
Proportion of human exposure indoors (πi, i)	0.89	0.65
Proportion of human exposure while asleep (πi, s):	0.86	0.62
% Biting indoors	68.7	31.5
Indoor/Outdoor ratio	2.2	0.5
% Biting before 9 pm (Indoor)	8.5	13.5
% Biting before 10 pm (Indoor)	16.1	17.7
% Biting before 9 pm (outdoor)	16.4	17.9
% Biting before 10 pm (outdoor)	20.2	27.6
% Biting after 5 am (indoor)	20.9	19.4
% Biting after 6 am (indoor)	4.9	9.3
% Biting after 7 am (indoor)	2.0	5.5
% Biting after 5 am (outdoor)	16.1	8.5
% Biting after 6 am (outdoor)	5.7	3.5
% Biting after 7 am (outdoor)	4.8	2.3

FIGURE 20: PROFILES OF BITING BY *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. EXPERIENCED BY THE HUMAN POPULATION IN THE SIX DISTRICTS



3.5 PARITY RATES

Mosquito ovary dissections of *An. funestus* s.l. and *An. gambiae* s.l. from the CDC-LT and HLC are presented in Figures 21, 22 and Annex B, Tables B1 and B2. The total number of mosquitoes dissected depended on the numbers collected. All mosquitoes that were fresh/not brittle were dissected at each sentinel site. Moist mutton cloth was placed in CDC-LT collection cups to maintain mosquito freshness. A total of 1,506 (14%) *An. funestus* s.l. and 613 (20%) *An. gambiae* s.l. were dissected. Very few mosquitoes were dissected from CDC-LT collections and the parity results need to be cautiously interpreted.

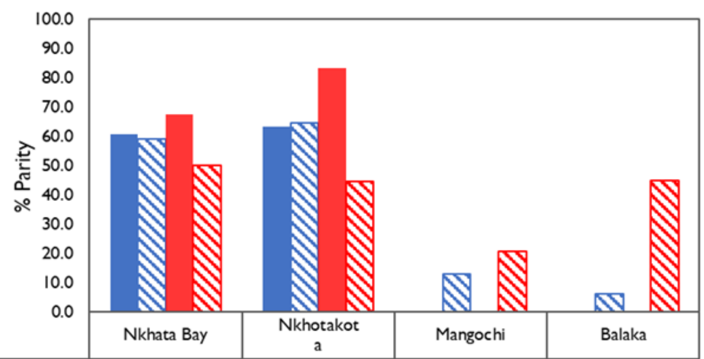
Overall, higher proportions of parous females were recorded among *An. funestus* s.l. (60.6%) than *An. gambiae* s.l. (43.1%). In non-IRS districts, the highest *An. funestus* s.l. parity rate was observed in Karonga (88.9%) followed by Salima (61.7%), Kasungu (51.5%), and Chikwawa (42.9%); the lowest parity rate was recorded in Balaka (6.3%). The highest parity rate for *An. gambiae* s.l. in these districts was observed in Salima (76.0%) followed by Chikwawa (70.4%) and Kasungu (61.1%). In Karonga, all 118 *An. gambiae* s.l. dissected were nulli parous.

In IRS districts, the overall highest *An. funestus* s.l. parity rates were observed in Nkhotakota (64.4%) and Nkhata Bay (59.6%). The lowest parity rate for this species was recorded in Balaka (6.1%). The highest parity rate of *An. gambiae* s.l. was recorded in Nkhata Bay (60.0%), followed by Nkhotakota (46.3%), Balaka (35.9%), and Mangochi (34.6%) districts.

Figure 21 summarizes the proportion of parous female *An. funestus* s.l. and *An. gambiae* s.l. before spray vs after spraying in IRS districts. In Nkhata Bay, the proportion of parous *An. funestus* s.l. was similar before (60.7%) and after spraying (59.2%). This was also the case in Nkhotakota, where the parity rates were 63/3% before spraying and 64.7% after.

In Nkhata Bay, the *An. gambiae* s.l. parity rate was higher before spraying (67.6%) than after spraying (50%). Similarly, in Nkhotakota, the *An. gambiae* s.l. parity rate was higher before spraying (83.3%) than after spraying (44.5%). No clear trends could be observed in Mangochi and Balaka because entomological monitoring in these districts began after spraying in November 2021.

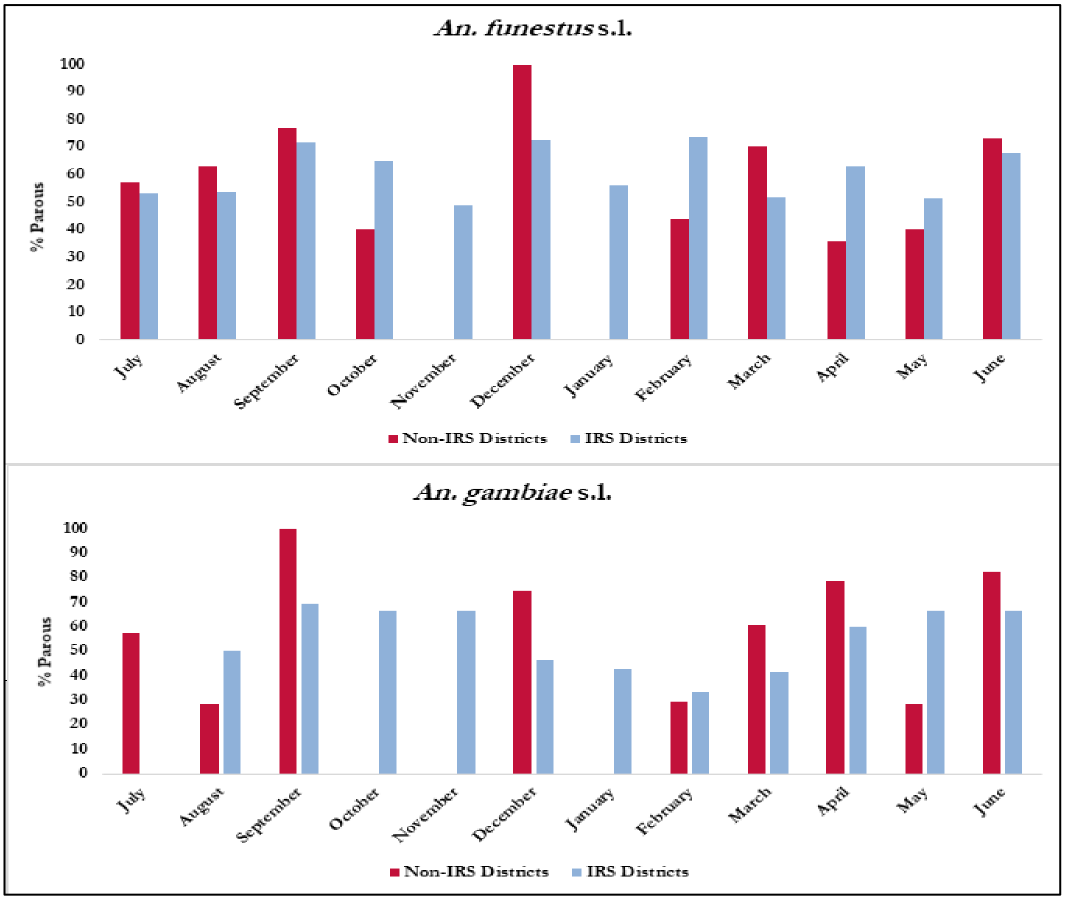
FIGURE 21: PROPORTION OF PAROUS FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN IRS DISTRICTS BEFORE AND AFTER IRS



	Nkhata Bay	Nkhotakota	Mangochi	Balaka
An. funestus s.l. before spray	60.7	63.3	0	0
An. funestus s.l. after spray	59.2	64.7	12.9	6.3
An. gambiae s.l. before spray	67.6	83.3	0	0
An. gambiae s.l. after spray	50.0	44.5	20.7	45.0

Note: Before spray = July 2021–September 2022; "After spray" = November 2021–June 2022

FIGURE 22: PROPORTION OF PAROUS FEMALE *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN IRS AND NON-IRS DISTRICTS



3.6 Cone Bioassays

3.6.1 Spray Quality Assessment

Spray quality at all sites sprayed with SS and FF in Nkhotakota, Nkhata Bay, and Mangochi districts was satisfactory (mosquito mortality >80% WHO threshold) (Annex Table C1). A mosquito mortality of 100% was recorded after 24 hours of observation from all sites except one brick structure at Thung'unda, a SS site in Nkhotakota, which recorded 100% mosquito mortality after 48 hours of observation, and one brick structure at Kela in Mangochi, which recorded 86.3% mosquito mortality after 24 hours. Quality assessment was not conducted in Balaka because entomological monitoring commenced two weeks after the IRS campaign began. It is recommended that spray quality assessment be conducted within two weeks of spraying.

3.6.2 Residual Life of Fludora Fusion and SumiShield 50WG

Results of monthly follow-up of the residual life of FF in the four districts of Nkhotakota, Nkhata Bay, Balaka, and Mangochi are presented in Annex Figures C1 to C10. FF is still effective, with 100% mosquito mortality recorded within the recommended five-day observation period 10 months (M10) after spraying in Nkhotakota (Annex Figures C1 and C2). FF is still effective in Mangochi and Balaka at nine months (M9) after spraying, with 100% mortality recorded within the five-day observation period in both districts (Annex Figures C3–C7). No bioassays were conducted in Mangochi in May (M6) due to a shortage of mosquitoes at the MAC insectary.

In Nkhata Bay, SS is still effective at M10, with 100% mosquito mortality within the five-day observation period in 97% of the 30 structures sprayed (Annex Figures C8–C10). No bioassays were conducted in May (M7) due to a shortage of mosquitoes at the MAC insectary. In Vwawa village (Nkhotakota), SS is still effective with 100% mosquito mortality recorded two days after mosquito exposure (Annex Figure C10). Residual life assessment is continuing in all the four IRS districts.

3.6.3 Fumigation Effect of Fludora Fusion and SumiShield 50WG

Fludora Fusion exhibited a fumigant effect 10 months after spraying in Nkhotakota District, with 100% mosquito mortality recorded within the five-day observation period (Annex Table C2). In Mangochi, FF showed a fumigant effect for five months, with 100% mortality recorded within the five-day observation period. At M7, the fumigant effect was very low for most of the surfaces, with half of the structures recording mortalities below 80%. However, the fumigant effect increased to 100% mortalty in months 8 and 9. In Balaka District, FF exhibited a fumigant effect at M9 with 83% of the structures recording 100% mosquito mortality within the five-day observation period (Annex Table C3).

The fumigation effect of SS in Nkhata Bay was observed for six months after spraying; 100% moquito mortality was observed within the five-day observation period. At M8, mosquito mortality in half of the structures fell below the 80% WHO threshold. The fumigation effect increased in M10, when 100% mosquito mortality was recorded. However, at Vwawa site (Nkhotakota), SS showed a fumigant effect 10 months after spraying, recording 100% mosquito mortality within the five-day observation period (Annex Table C2).

3.7 INSECTICIDE RESISTANCE MONITORING

This section discusses *An. funestus* s.l. and *An. gambiae* s.l. susceptibility to various insecticides in the five districts of Chikwawa, Salima, Kasungu, Nkhotakota, and Karonga. Susceptibility test results are summarized in Annex Table D1. Several attempts were made to collect live adult female mosquitoes from Nkhata Bay District, but they died before reaching the MAC laboratory; hence no tests were conducted for this district.

3.7.1 *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN CHIKWAWA DISTRICT

In Chikwawa, *An. gambiae* s.l. was resistant to deltamethrin 0.05%, permethrin 0.75%, and alpha-cypermethrin 0.05%. However, *An. gambiae* s.l. remains fully susceptible (100% mortality) to pirimiphos-methyl 0.25% (Figure 23a). *An. funestus* s.l. likewise remains fully susceptible (100% mortality) to pirimiphos-methyl (Figure 23b). *An. gambiae* s.l. also remains susceptible to chlorfenapyr (100% mortality) and clothianidin (98% mortality) (Figure 23a).

FIGURE 23A: *AN. GAMBIAE* S.L. EXPOSED TO DIFFERENT INSECTICIDES IN CHIKWAWA DISTRICT

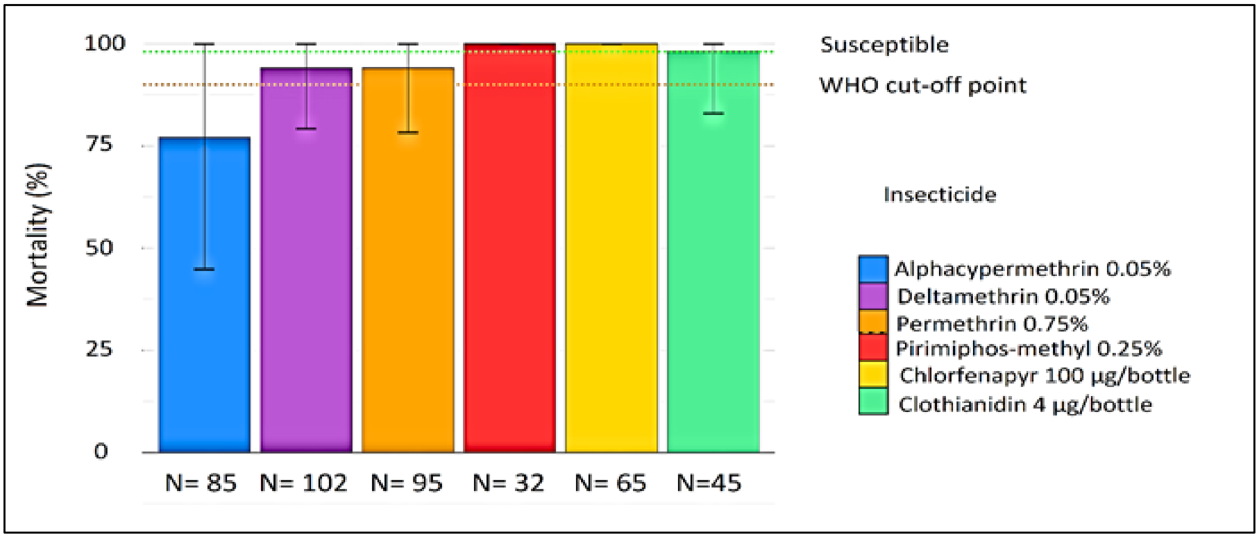
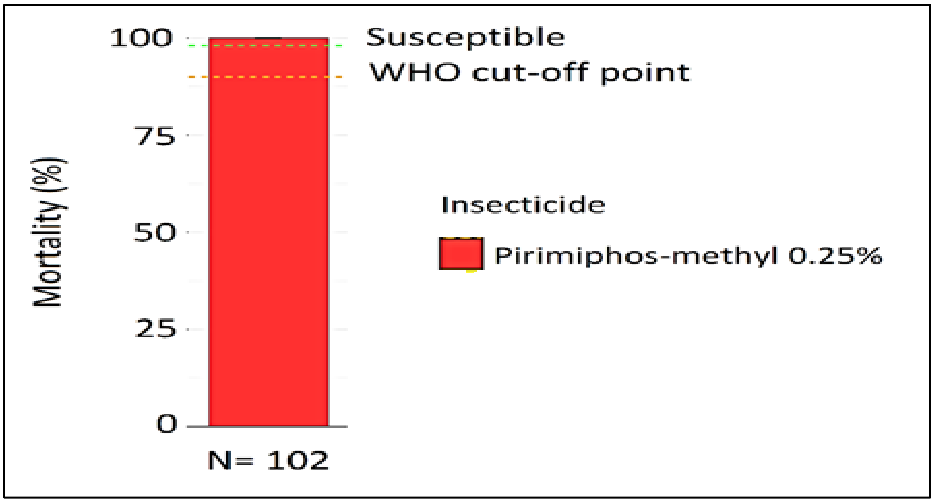


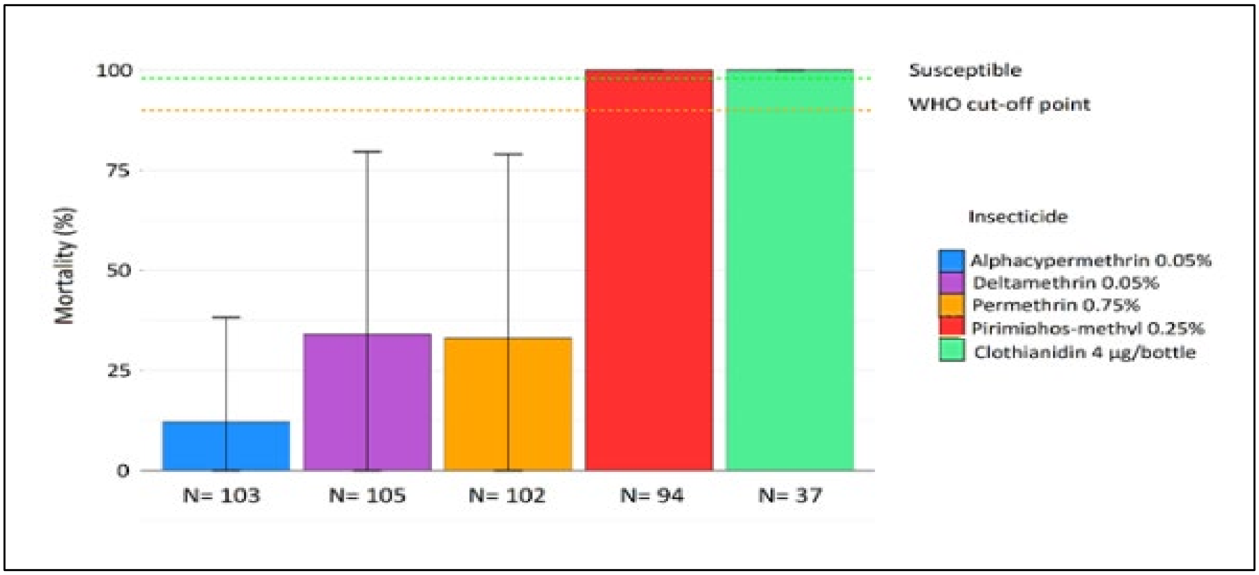
FIGURE 23B: *AN. FUNESTUS* S.L. RESPONSE TO PIRIMIPHOS-METHYL IN CHIKWAWA DISTRICT



3.7.2 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN SALIMA DISTRICT

In Salima, *An. gambiae* s.l. was fully susceptible to clothianidin (100% mortality) and pirimiphos-methyl 0.25% (100% mortality), but resistant to alpha-cypermethrin 0.05% (11.7% mortality), deltamethrin 0.05% (34.6% mortality), and permethrin 0.75% (33.3% mortality) (Figure 24).

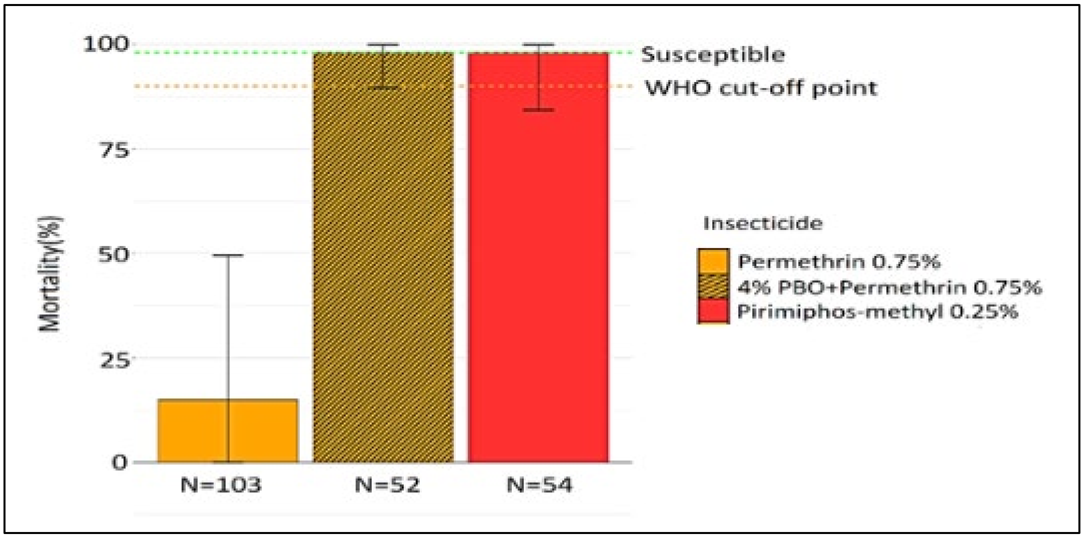
FIGURE 24: *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN SALIMA DISTRICT



3.7.3 *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN KASUNGU DISTRICT

In Kasungu, *An. funestus* s.l. was resistant to permethrin 0.75% (15.5% mortality) but susceptible to 4% PBO + permethrin 0.75% (98.1% mortality) and pirimiphos-methyl 0.25% (98% mortality) (Figure 25).

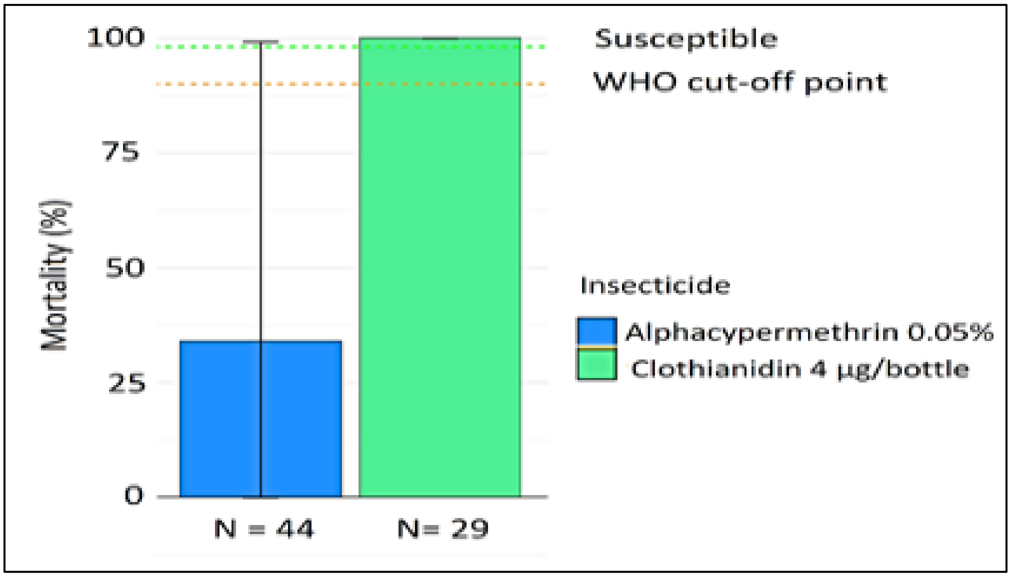
FIGURE 25: *AN. FUNESTUS* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN KASUNGU DISTRICT



AN. GAMBIAE S.L. AND *AN. FUNESTUS* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHOTAKOTA DISTRICT

In Nkhotakota, *An. funestus* s.l. was resistant to alpha-cypermethrin 0.05% (34% mortality) but susceptible to clothianidin (100% mortality) (Figure 26).

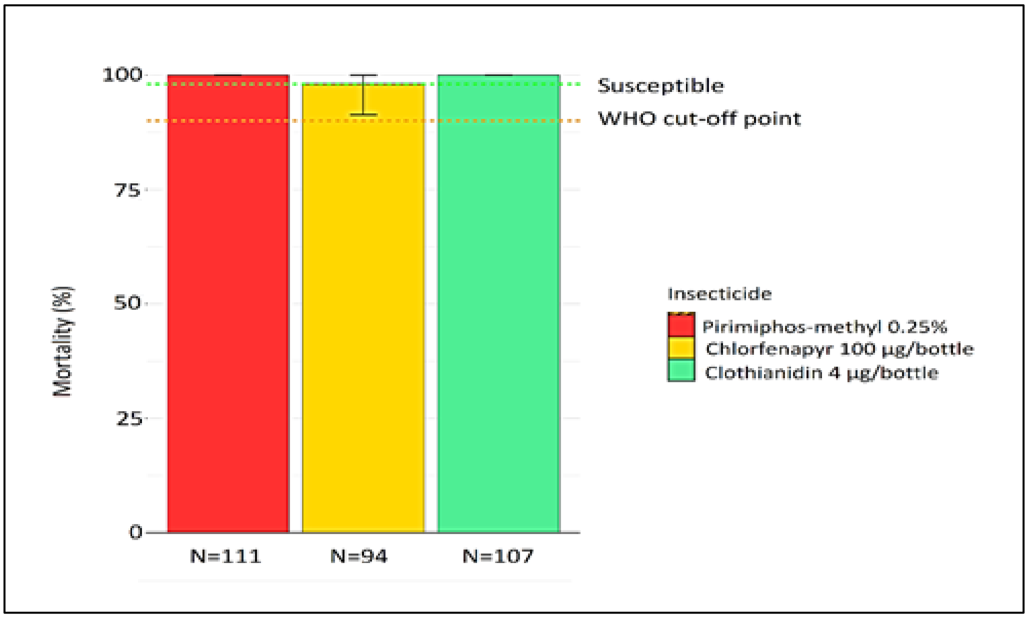
FIGURE 26: *AN. FUNESTUS* S.L. RESPONSE TO ALPHA-CYPERMETHRIN AND CLOTHIANIDIN IN NKHOTAKOTA DISTRICT



3.7.4 *AN. GAMBIAE* S.L. SUSCEPTIBILITY TO DIFFERENT INSECTICIDES IN NKHATA BAY DISTRICT

In Nkhata Bay, *An. gambiae* s.l. was susceptible to pirimiphos-methyl 0.25% (100% mortality), clothianidin 4µg/bottle (100% mortality), and chlorfenapyr 100µg/bottle (98% mortality) (Figure 27).

FIGURE 27: *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES IN NKHATA BAY DISTRICT



4. Conclusion and Recommendations

4.1 Summary of Entomological Findings

Overall, in the 2021/22 collections, *An. funestus* s.l. was the most abundant vector of all the *Anopheles* mosquitoes collected. Unlike the previous years, more *An. funestus* s.l. was collected in the 2021/22 entomological monitoring reporting period compared to the past four years where more *An. gambiae* s.l. than *An. funestus* s.l. were collected. Even in Nkhotakota, the district that has received IRS since 2018, there were more *An. funestus* s.l. collected than *An. gambiae* s.l. in 2021/2022 than in 2020/2021 and prior years. It is not clear, if this is indication of shift or just a yearly fluctuation in the species compostion. However, this dominance in *An. funestus* s.l. in Nkhotakota is not expected given that this vector is considerd more endophilic and thus impacted more by IRS than the *An. gambiae* s.l. which is predominantly *An. arabiensis*. The reason for this remains unclear. The preference for feeding location remains the same, with more *An. funestus* s.l. biting indoors (endphagic) than outdoors and more *An. gamniae* s.l. bting outdoors (exophagic) than indoors. s.

- *Anopheles funestus* s.l. was dominant in the following districts: Nkhata Bay, Nkhotakota, Kasungu and Salima. Two species were identified to species-specific level from the *An. funestus* group, *An. funestus* s.s. and *An. leesoni* (Salima District) was detected for the first time.*Anopheles gambiae* s.l. was dominant in the following districts: Karonga Chikwawa, Mangochi, and Balaka (Chimdikiti site). The proportions are similar in the other districts. Two species from the *An. gambiae* complex were identified: *An. arabiensis* and *An. gambiae* s.s. *An. arabiensis* was the predominant, accounting for 99.8% of all mosquitoes that were identified to species-specific level by PCR.
- *Anopheles gambiae* s.l. seems to be highly dominant in the most northern district (Karonga), with 90 to 100% in the PSC or CDC-LT collections and the southern district, Chikwawa, with 56 to 95% in either of the two methods. Proximity to the lake and latitude do not seem to determine the difference in the distibution of these two malaria vectors in Malawi. Other differences in the micro habitat are probably involved in driving the spatial variation in distribution of these two vectors. High biting rates as measured by CDC-LTs were observed in *An. gambiae* s.l. (6.76 (b/p/n) compared to *An. funestus* s.l. (1.37 b/p/n). From HLC collections, *An. funestus* s.l. were 4.6 b/p/n) indoors and 2.5 b/p/n outdoors. The HBRs for *An. gambiae* s.l. were 1.5 b/p/n indoors and 3.1 b/p/n outdoors. The HBRs for *An. coustani* were 1.4 b/p/n indoors and 5.2 outdoors .
- Both *An. funestus* s.l. and *An. gambiae* s.l. exhibited endophagic and exophagic behavior. *An. funestus* s.l. predominantly fed indoors while *An. gambiae* s.l. predominantly fed outdoors. This feeding behavior has been consistent since 2018 to present, covering four IRS spray seasons.
- The biting activity of *An. funestus* s.l. and *An. gambiae* s.l. occurred from dawn to dusk in all the six districts. Morning/ daytime biting after 5am was also observed when people were

awake in Nkhata Bay, Kasungu, and Nkhotakota. The biting behavior of both species has been consistent over the past four IRS seasons. Morning biting is still being observed in all districts where HLCs are being conducted.

- Overall, higher proportions of parous females were recorded among *An. funestus* s.l. (61%) than *An. gambiae* s.l. (43%). There was no consistent pattern in the difference of parity rate neitherby IRS vs non-IRS districtsnor pre and post IRS. Unfed CDC-LT collected mosquitoes rather than HLC are used for parity dissection and in many cases most of the mosquitoes become dry and brittle and unsuitable for dissection by morning. Given these challenges, few are dissected, and it is difficult to do any meaningful analysis by month from such the small sample size. The human blood index of *An. funestus* s.l. was higher than that of the *An. gambiae* s.l. In some cases both mosquitoes were collected from the same houses (indoors) and had the same access for human and animal bloodmeal but the proportion of *An. funestus* s.l. with human bloodmeal is higher than that of *An. gambiae* s.l.. Overall, 93.2% of the *An. funestus* s.l. and 58.6% of the *An gambiae* s.l. had human bloodmeal indicating that *An. funestus* s.l. is more anthropophilic than *An. gambiae* s.l. In 2020/2021 the proprtion of human bloodmeal was 89.3% for *An. funestus* s.l. and only 28.6% for *An. gambiae* s.l., .
- The absence of the *Ace-1* resistant allele is consistent with the absence of phenotypic resistance to pirimiphos-methyl (organophosphate) in *An. gambiae* s.l. in Malawi.
- *Anopheles. funestus* s.l. is the most important malaria vector in the five districts, except in Karonga, Salima, and Chikwawa, where *An. gambiae* s.l. was responsible for all observed transmission. The overall SR was also higher for *An, funestus* s.l. (1.0%) than for *An. gambiae* s.l. (0.3%). The overall EIR rate (18.1 ib/p/yr) due to *An. funestus* s.l. was also higher than that of *An. gambiae* s.l. (4.1 ib/p/yr). Over the past years, the overall numbers collected were higher for *An. gambiae* s.l. than *Anopheles funestus* s.l.. However, the contribution of *An. funestus* s.l. was higher despite their lower numbers. This year, *An. funestus* s.l. numbers were higher and they contributed more to the EIR compared to *An. gambiae* s.l.
- EIR was calculated from the monthly CDC-LT collections rather than HLCs which were conducted quarterly. When HBRs estimated from CDC-LT were compared with HBRs from HLCs, for the four months in the year that both methods were performed, CDC-LT seems to underestimate HBRs for *An. gambiae* s.l. (1.4 b/p/n for CDC-LT vs 4.6 b/p/n for HLC) and overestimate the HBRs for *An. funestus* s.l (6.8 b/p/n for CDC-LT vs 1.5 b/p/n for HLC). Overall, HBRs from CDC-LT were higher than HLC. Howver, the calculation of HBR from CDC-LT used all female mosquitoes trapped and assumed the numbers per trap per night are equivalent to the bites per person per night. These included about 15% of fed and gravid mosquitoes which may not have been in a state of host seeking but considered as biting. If only the trapped unfed mosquitoes were to be used, the difference between the CDC-LT and HLC estimates is narrower (6.1 vs 6.9 b/p/n) and if consistently used, CDC-LT could be considered as good as HLC in estimating and comparing EIR across space and time in Malawi.

4.1.1 IRS QUALITY AND EFFICACY

- Spray quality was satisfactory in Nkhotakota, Nkhata Bay, and Mangochi for both FF and SS 50WG.

- Both FF and SS 50WG have long residual efficacy as evidenced by the continued effectiveness of the chemicals nine months after spraying in Balaka and Mangochi districts and 10 months after spraying in Nkhotakota and Nkhata Bay districts.
- In terms of residual efficacy, both insecticides have long life that seems to cover almost the entire year. Therefore, both are equally recommended for IRS in Malawi. Though FF is a combination of deltamethrin and clothianidin, and SS is formulated as only clothianidin, the residual efficacy data shows no evidence to suggest one is better than the other. This observation is consistent over the years.

4.1.2 INSECTICIDE RESISTANCE

- *Anopheles funestus* s.l. and *An. gambiae* s.l. were highly resistant to the three pyrethroids tested: deltamethrin, permethrin, and alpha-cypermethrin.
- Pre-exposure of *An. funestus* s.l. and *An. gambiae* s.l. to 4% PBO followed by pyrethroids greatly improved the efficacy of pyrethroids. Pyrethroid resitance from both species has been observed for the past 5 years and beyond. This has led to policy change in Malawi where only LLINs with PBO are recommended for mass distribution not standard nets that contain pyrethroids only.
- *Anopheles funestus* s.l. and *An. gambiae* s.l. were susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. Thus, clothianidin for IRS and insecticide-treated nets with the active ingredient of chlorfenapyr can be used as part the malaria vector control interventions in Malawi. Since 2018, no resistance to clothianidin, pirimiphos-methyl or chlorfenapyr has been observed from either species.

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ANNEX A: SPOROZOITE RATE IN *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. FROM PSC AND CDC-LT COLLECTIONS

TABLE A1: SRS IN *AN. GAMBIAE* S.L. BY LOCATION FROM HLC COLLECTIONS, JULY 2021–JUNE 2022

District	Month	*An. gambiae* s.l.								
		Indoor			Outdoor			Total		
		Total tested	Total +ve	Sporozoite rate (%)	Total tested	Total +ve	Sporozoite rate (%)	Total tested	Total +ve	Sporozoite rate (%)
Nkhata Bay	September	35	0	0	55	0	0	90	0	0
	December	0	0	0	0	0	0	0	0	0
	March	42	0	0	58	0	0	100	0	0
	June	2	0	0	2	0	0	4	0	0
Nkhotakota	September	6	0	0	3	0	0	9	0	0
	December	8	0	0	9	0	0	17	0	0.0
	March	15	0	0	76	0	0	91	0	0
	June	1	0	0	0	0	0	1	0	0
Salima	September	0	0	0	0	0	0	0	0	0
	December	0	0	0	4	0	0	4	0	0
	March	23	0	0	37	0	0	60	0	0
	June	0	0	0	3	0	0	3	0	0
Kasungu	September	0	0	0	0	0	0	0	0	0
	December	0	0	0	0	0	0	0	0	0
	March	36	0	0.0	111	0	0	147	0	0
	June	2	0	0	1	0	0	3	0	0
Total		170	0	**0.0**	359	0	**0**	529	0	**0.0**

TABLE A2: SRS IN *AN. FUNESTUS* S.L. BY LOCATION FROM HLC COLLECTIONS BY DISTRICT, JULY 2021–JUNE 2022

District	Month	*An. funestus* s.l.								
		Indoor			Outdoor			Total		
		Total tested	Total +ve	Sporozoite rate (%)	Total tested	Total +ve	Sporozoite rate (%)	Total tested	Total +ve	Sporozoite rate (%)
Nkhata Bay	September	4	0	0	3	0	0	7	0	0
	December	0	0	0	0	0	0	0	0	0
	March	44	0	0	34	0	0	78	0	0
	June	35	0	0	41	0	0	76	0	0
Nkhotakota	September	41	1	**2.4**	11	0	0	52	1	1.9
	December	33	1	3.0	6	0	0	39	1	2.6
	March	79	0	0	34	0	0	113	0	0.0
	June	183	0	0	37	0	0	220	0	0.0
Salima	September	0	0	0	0	0	0	0	0	0.0
	December	0	0	0	0	0	0	0	0	0.0
	March	63	0	0	28	0	0	91	0	0.0
	June	30	0	0	8	0	0	38	0	0.0
Kasungu	September	14	0	0	8	0	0	22	0	0.0
	December	0	0	0	0	0	0	0	0	0.0
	March	14	2	**14.3**	19	0	0	33	2	6.1
	June	27	0	0	8	0	0	35	0	0.0
Total		567	4	**0.7**	237	0	**0**	804	4	**0.5**

Table A3: SRS of *An. gambiae* s.l. from PSC Collections by District

District	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Karonga	Total No. tested	131	801	110	112	92	1	53	13	49	0	110	61	1533
	No. sporozoites (+ve)	1	1	0	0	0	0	0	0	0	0	0	0	2
	Sporozoite rate (%)	0.8	0.1	0	0	0	0	0	0	0	0	0	0	0.1
Nkhata Bay	Total No. tested		2	49	50	6	1	1	5	2	0	7	1	124
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Nkhotakota	Total No. tested	3	16	7	1	7	0	7	5	0	5	3	8	62
	No. sporozoites (+ve)	0	0	0	0	0	0	0	1	0	0	0	0	1
	Sporozoite rate (%)	0	0.0	0	0	0	0	0	20	0	0	0	0	1.6
Salima	Total No. tested	0	6	1	4	6	1	50	4	0	1	5	0	78
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Kasungu	Total No. tested	1	1	2	0	3	0	11	3	1	1	0	0	23
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Chikwawa	Total No. tested	19	26	26	0	34	2	0	47	18	23	0	66	261
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Balaka	Total No. tested	0	0	0	0	0	0	5	5	33	0	0	0	43
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Mangochi	Total No. tested	0	0	0	0	4	2	1	5	19	0	0	0	31
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Total	Total No. tested	154	852	195	167	152	7	128	87	122	30	125	136	2155
	No. sporozoites (+ve)	1	1	0	0	0	0	0	1	0	0	0	0	3
	Sporozoite rate (%)	0.6	0.1	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.1

TABLE A4: SRS OF *AN. FUNESTUS* S.L. FROM PSC COLLECTIONS BY DISTRICT

District	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Karonga	Total No. tested	0	3	0	0	0	0	0	12	0	0	0	0	15
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0.0	0.0	0	0	0	0	0	0	0	0	0	0	0.0
Nkhata Bay	Total No. tested	22	53	35	53	109	1	130	69	13	12	21	12	530
	No. sporozoites (+ve)	0	0	0	0	0	1	0	0	0	0	0	0	1
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.2
Nkhotakota	Total No. tested	231	200	67	120	106	6	67	133	77	25	51	242	1325
	No. sporozoites (+ve)	2	1	2	0	0	1	0	2	1	0	0	0	9
	Sporozoite rate (%)	0.9	0.5	3.0	0	0	0	0	1.5	1.3	0	0	0	0.7
Salima	Total No. tested	3	25		4	2		5	37		24	6	44	150
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Kasungu	Total No. tested	22	53	35	53	109	1	130	69	13	12	21	12	530
	No. sporozoites (+ve)	0	0	0	0	0	1	0	0	0	0	0	0	1
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.2
Chikwawa	Total No. tested	9	51	22		24	3			22	2		30	163
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Balaka	Total No. tested	0	0	0	0	0	0	0	0	12	0	0	0	12
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Mangochi	Total No. tested	0	0	0	0	3	0	0	4	20	0	0	0	27
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	0
	Sporozoite rate (%)	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Total	Total No. tested	287	385	159	230	353	11	332	324	157	75	99	340	2752
	No. sporozoites (+ve)	2	1	2	0	0	3	0	2	1	0	0	0	11
	Sporozoite rate (%)	0.7	0.3	1.3	0.0	0.0	27.3	0.0	0.6	0.6	0.0	0.0	0.0	0.4

TABLE A5: SRS OF *AN. GAMBIAE* S.L. FROM CDC-LT COLLECTIONS BY DISTRICT

District	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Karonga	Total No. tested	3	47	44	8	1		3	8	209	0	0	10	**333**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	1	0	0	0	**1**
	Sporozoite rate (%)	**0.0**	**0.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.5**	**0**	**0**	**0**	**0.3**
Nkhata Bay	Total No. tested	0	0	0	4	0	0	18	22	28	5	2	2	**81**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Nkhotakota	Total No. tested	3	9	0	10	13	7	14	184	23	31	37	5	**336**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Salima	Total No. tested	0	2	15	0	0	2	19	8	55	12	0	0	**113**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Kasungu	Total No. tested	1	0	0	3	0	2	3	15	66	19	7	1	**117**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Chikwawa	Total No. tested	43	2	9	0	2	0	0	31	12	7	51	30	**187**
	No. sporozoites (+ve)	3	0	0	0	0	0	0	0	0	0	0	0	**3**
	Sporozoite rate (%)	**7.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1.6**
Balaka	Total No. tested	0	0	0	0	0	0	1	8	100	0	0	0	**109**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0.0**
Mangochi	Total No. tested	0	0	0	0	1	1	0	1	119	0	0	0	**122**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Total	Total No. tested	50	60	68	25	17	12	58	277	612	74	97	48	**1398**
	No. sporozoites (+ve)	3	0	0	0	0	0	0	0	1	0	0	0	**4**
	Sporozoite rate (%)	**6.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.2**	**0.0**	**0.0**	**0.0**	**0.3**

TABLE A6: SRS OF *AN. FUNESTUS* S.L. FROM CDC-LT COLLECTIONS BY DISTRICT

District	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Total
Karonga	Total No. tested	2	0	1	0	0	0	1	0	0	0	0	0	**4**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0.0**	**0.0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Nkhata Bay	Total No. tested	93	81	33	21	0	0	36	55	461	94	79	73	**1026**
	No. sporozoites (+ve)	3	8	0	0	0	0	2	0	4	0	0	0	**17**
	Sporozoite rate (%)	**0**	**9.9**	**0**	**0**	**0**	**0**	**5.6**	**0**	**0.9**	**0**	**0**	**0**	**1.7**
Nkhotakota	Total No. tested	503	401	115	395	175	100	65	490	284	102	382	86	**3098**
	No. sporozoites (+ve)	26	6	4	3	0	0	0	8	0	0	0	0	**47**
	Sporozoite rate (%)	**5.2**	**1.5**	**3.5**	**0.8**	**0**	**0**	**0**	**1.6**	**0**	**0**	**0**	**0**	**1.5**
Salima	Total No. tested	58	8	6	2	0	3	2	60	31	42	51	71	**334**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Kasungu	Total No. tested	28	33	12	7	4	1	7	8	41	63	38	10	**252**
	No. sporozoites (+ve)	0	0	1	0	0	1	0	0	0	0	0	0	**2**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.8**
Chikwawa	Total No. tested	27	16	14	0	2	1	0	3	9	7	3	5	**87**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Balaka	Total No. tested	0	0	0	0	0	0	1	18	26	0	0	0	**45**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	1	0	0	0	**1**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3.8**	**0**	**0**	**0**	**2.2**
Mangochi	Total No. tested	0	0	0	0	2	1	0	0	42	0	0	0	**45**
	No. sporozoites (+ve)	0	0	0	0	0	0	0	0	0	0	0	0	**0**
	Sporozoite rate (%)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0.0**
Total	Total No. tested	711	539	181	425	183	106	112	634	894	308	553	245	**4891**
	No. sporozoites (+ve)	29	14	5	3	0	1	2	8	5	0	0	0	**67**
	Sporozoite rate (%)	**4.1**	**2.6**	**2.8**	**0.7**	**0.0**	**0.9**	**1.8**	**1.3**	**0.6**	**0.0**	**0.0**	**0.0**	**1.4**

TABLE A7: HBRS OF *ANOPHELES* MOSQUITOES FROM SIX DISTRICTS COLLECTED BY HLC, JULY 2021–JUNE 2022

District	Month	*An. funestus* s.l		*An. gambiae* s.l.		*An. coustani*	
		Indoor	*Outdoor*	*Indoor*	*Outdoor*	*Indoor*	*Outdoor*
Nkhata Bay	September	0.83	0.5	6	9.83	0.17	1
	December	0.17	0	0	0	0.33	0.33
	March	6.5	5.33	6.33	10.17	14.17	8.33
	June	3.33	1.33	0.17	0.33	2.83	1.83
Kasungu	September	2.5	1.33	0	0	0	0.33
	December	0.67	0.5	0	0	0	0
	March	2.11	2.33	5.22	11.89	1.78	3.78
	June	0.5	0.33	0	0	0	0
Nkhotakota	September	16.83	3.17	0.42	0.5	0.17	2.0
	December	9.5	1.17	0.67	0.83	0.0	3.5
	March	3.0	8.83	2.42	10.0	1.17	11.67
	June	30.17	6.5	0.33	0.5	3.33	61.67
Salima	September	0.5	0.83	0.83	2	0	0
	December	0	0	0.17	0.83	0	0
	March	10.33	6	4	5.83	1.33	5.67
	June	9.17	7.17	0.17	0.33	0	0
Mangochi	December	0	0	0	0	0	0
	March	1.17	4.17	2.67	8.17	3.33	9.5
	June	0	0	0	0	0	0
Balaka	December	0	0	0	0	0	0
	March	4.83	5.33	3.67	6.83	2	4
	June	0	0	0	0	0	0
Overall		**4.64**	**2.49**	**1.5**	**3.09**	**1.39**	**5.16**

TABLE A8: ESTIMATE OF HBRS OF *ANOPHELES* MOSQUITOES FROM CDC-LT COLLECTION IN EIGHT SENTINEL DISTRICTS, JULY 2021–JUNE 2022

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Karonga													
An. gambiae s.l.	Total collected by CDC-LTs	3	50	389	10	1	0	3	10	359	8	4	11
	HBR/night	0.15	2.50	19.45	0.50	0.05	0	0.15	0.50	17.95	0.40	0.20	0.55
An. funestus s.l.	Total collected by CDC-LTs	2	1	15	0	0	0	1	0	0	0	9	0
	HBR/night	0.1	0.05	0.75	0	0	0	0.05	0	0	0	0.45	0
Nkhata Bay													
An. gambiae s.l.	Total collected by CDC-LTs	0	1	1	8	1	0	18	21	34	28	0	2
	HBR/night	0.00	0.05	0.05	0.40	0.05	0.00	0.90	1.05	1.70	1.40	0.00	0.10
	Total collected by CDC-LTs	123	100	33	27	0	2	38	58	572	658	78	80
An. funestus s.l.	HBR/night	6.15	5.00	1.65	1.35	0.00	0.10	1.90	2.90	28.60	32.90	3.90	4.00
Nkhotakota													
An. gambiae s.l.	Total collected by CDC-LTs	5	10	24	32	14	16	25	238	184	52	59	18
	HBR/night	0.17	0.33	0.80	1.07	0.47	0.53	0.83	7.93	6.13	1.73	1.97	0.60
An. funestus s.l.	Total collected by CDC-LTs	619	505	414	488	182	128	94	614	662	165	994	1673
	HBR/night	20.63	16.83	13.80	16.27	6.07	4.27	3.13	20.47	22.07	5.50	33.13	55.77
Salima													
An. gambiae s.l.	Total collected by CDC-LTs	0	2	13	5	4	1	22	9	65	12	9	0
	HBR/night	0.00	0.10	0.65	0.25	0.20	0.05	1.10	0.45	3.25	0.60	0.45	0.00
An. funestus s.l.	Total collected by CDC-LTs	59	8	12	1	1	2	2	62	51	51	86	18
	HBR/night	2.95	0.40	0.60	0.05	0.05	0.10	0.10	3.10	2.55	2.55	4.30	0.90
Kasungu													
An. gambiae s.l.	Total collected by CDC-LTs	1	4	0	3	0	10	5	17	66	23	7	1
	HBR/night	0.05	0.20	0.00	0.15	0.00	0.50	0.25	0.85	3.30	1.15	0.35	0.05
An. funestus s.l.	Total No. CDC-LT	27	34	13	8	10	13	11	13	43	77	32	13
	HBR/night	1.35	1.70	0.65	0.40	0.50	0.65	0.55	0.65	2.15	3.85	1.60	0.65
Chikwawa													
An. gambiae s.l.	Total collected by CDC-LTs	62	11	9	2	2	0	0	31	14	20	1	29
	HBR/night	3.10	0.55	0.45	0.10	0.10	0.00	0.00	1.55	0.70	1.00	0.05	1.45
An. funestus s.l.	Total collected by CDC-LTs	18	10	19	3	2	1	0	4	9	10	0	2
	HBR/night	0.90	0.50	0.95	0.15	0.10	0.05	0.00	0.20	0.45	0.50	0.00	0.10

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Balaka													
An. gambiae s.l.	Total collected by CDC-LTs	*	*	*	*	0	0	1	8	111	0	9	6
	HBR/night	*	*	*	*	0.00	0.00	0.05	0.40	5.55	0.00	0.45	0.30
An. funestus s.l.	Total collected by CDC-LTs	*	*	*	*	0	0	1	18	30	1	51	19
	HBR/night	*	*	*	*	0	0	0.05	0.9	1.5	0.05	2.55	0.95
Mangochi													
An. gambiae s.l.	Total collected by CDC-LTs	*	*	*	*	1	1	0	3	125	7	4	6
	HBR/night	*	*	*	*	0.05	0.05	0.00	0.15	6.25	0.35	0.20	0.30
An. funestus s.l.	Total collected by CDC-LTs	*	*	*	*	4	1	0	0	46	16	0	107
	HBR/night	*	*	*	*	0.20	0.05	0.00	0.00	2.30	0.80	0.00	5.35
***An. gambiae* s.l.**	**Overall HBR/night**	**0.58**	**0.62**	**3.57**	**0.41**	**0.12**	**0.14**	**0.41**	**1.61**	**5.60**	**0.83**	**0.46**	**0.42**
***An. funestus* s.l.**	**Overall HBR/night**	**5.35**	**4.08**	**3.07**	**3.04**	**0.87**	**0.65**	**0.72**	**3.53**	**7.45**	**5.77**	**5.74**	**8.47**

* No entomological activities were conducted.

TABLE A9: SRs AND EIRs OF *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. FROM CDC-LT COLLECTIONS BY DISTRICT, JULY 2022–JUNE 2022

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Eight Months EIR	Total Annual EIR
Karonga															
An. gambiae s.l.	Total tested	134	848	154	120	93	1	56	21	258	0	110	71		
	No. sporozoite positive	1	1	0	0	0	0	0	0	1	0	0	0		
	Sporozoite rate	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Total CDC-LTs	3	50	389	10	1	0	3	10	359	8	4	11		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.15	2.50	19.45	0.50	0.05	0	0.15	0.50	17.95	0.40	0.20	0.55		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00		
	Monthly EIR	0.03	0.09	0.00	0.00	0.00	0.00	0.00	12.00	2.16	0.00	0.00	0.00		14.28
An. funestus s.l.	Total tested	2	3	1	0	0	0	1	12	0	0	0	0		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Total CDC-LTs	2	1	15	0	0	0	1	0	0	0	9	0		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.1	0.05	0.75	0	0	0	0.05	0	0	0	0.45	0		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0	0	0	0	0	0	0	0	0.00	0	0	0		0.00
	Total monthly EIR	0.03	0.09	0.00	0.00	0.00	0.00	0.00	12.00	2.16	0.00	0.00	0.00		14.28
Nkhata Bay															
An. gambiae s.l.	Total tested	0	2	139	54	6	1	19	27	132	5	9	7		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0	0	0	0	0	0	0	0	0	0	0	0		
	Total CDC-LTs	0	1	1	8	1	0	18	21	34	28	0	2		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.00	0.05	0.05	0.40	0.05	0.00	0.90	1.05	1.70	1.40	0.00	0.10		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0	0	0	0	0	0	0	0	0.00	0	0	0		
An. funestus s.l.	Total tested	100	95	92	33	4	0	39	156	698	94	186	233		
	No. sporozoite positive	3	8	0	0	0	0	2	0	4	0	0	1		
	Sporozoite rate	0.03	0.08	0.00	0.00	0.00	0.00	0.05	0.00	0.01	0.00	0.00	0.00		
	Total CDC-LTs	123	100	33	27	0	2	38	58	572	658	78	80		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	6.15	5.00	1.65	1.35	0.00	0.10	1.90	2.90	28.60	32.90	3.90	4.00		
	Nightly EIR	0.18	0.42	0.00	0.00	0.00	0.00	0.10	0.00	0.16	0.00	0.00	0.02		
	Monthly EIR	5.72	13.05	0.00	0.00	0.00	0.00	3.02	0.00	5.08	0.00	0.00	0.52		27.39
	Total monthly EIR	5.72	13.05	0.00	0.00	0.00	0.00	3.02	0.00	5.08	0.00	0.00	0.52		27.39
Nkhotakota															
An. gambiae s.l.	Total tested	25	17	11	20	24	21	189	118	36	40	14	6		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0.00	0.00	0	0.0	0	0.00	0	0.00	0.000	0	0	0		
	Total CDC-LTs	5	10	24	32	14	16	25	238	184	52	59	18		
	Total traps	30	30	30	30	30	30	30	30	30	30	30	30		
	HBR/night	0.17	0.33	0.80	1.07	0.47	0.53	0.83	7.93	6.13	1.73	1.97	0.60		

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Eight Months EIR	Total Annual EIR
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
An. funestus s.l.	Total tested	601	234	515	281	145	132	623	486	127	433	550	734		
	No. sporozoite positive	7	7	3	0	2	0	10	1	0	0	0	28		
	Sporozoite rate	0.01	0.03	0.01	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.04		
	Total CDC-LTs	619	505	414	488	182	128	94	614	662	165	994	1673		
	Total traps	30	30	30	30	30	30	30	30	30	30	30	30		
	HBR/night	20.63	16.83	13.80	16.27	6.07	4.27	3.13	20.47	22.07	5.50	33.13	55.77		
	Nightly EIR	0.24	0.50	0.08	0.00	0.08	0.00	0.05	0.04	0.00	0.00	0.00	2.13		
	Monthly EIR	7.45	15.61	2.41	0.00	2.51	0.00	1.56	1.18	0.00	0.00	0.00	63.82		94.54
	Total monthly EIR	7.45	15.61	2.41	0.00	2.51	0.00	1.56	1.18	0.00	0.00	0.00	63.82		94.54
Salima															
An. gambiae s.l.	Total tested	0	8	16	4	6	8	68	12	115	13	5	3		
	No. sporozoite positive	0	0	0	0	0	0	1	0	0	0	0	0		
	Sporozoite rate	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00		
	Total CDC-LTs	0	2	13	5	4	1	22	9	65	12	9	0		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.00	0.10	0.65	0.25	0.20	0.05	1.10	0.45	3.25	0.60	0.45	0.00		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00		0.50
An. funestus s.l.	Total tested	61	33	6	6	2	3	7	97	123	66	57	153		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0	0	0	0	0	0	0	0	0	0	0	0		
	Total CDC-LTs	59	8	12	1	1	2	2	62	51	51	86	18		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	2.95	0.40	0.60	0.05	0.05	0.10	0.10	3.10	2.55	2.55	4.30	0.90		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
	Total monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00		0.50
Kasungu															
An. gambiae s.l.	Total tested	2	1	2	3	3	0	14	18	215	20	7	4		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0	0	0	0	0	0	0	0	0	0	0	0		
	Total CDC-LTs	1	4	0	3	0	10	5	17	66	23	7	1		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.05	0.20	0.00	0.15	0.00	0.50	0.25	0.85	3.30	1.15	0.35	0.05		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
An. funestus s.l.	Total tested	50	86	69	60	113	2	137	77	87	75	59	57		
	No. sporozoite positive	0	0	1	0	0	2	0	0	2	0	0	0		
	Sporozoite rate	0.00	0.00	0.01	0.00	0.00	1.00	0.00	0.00	0.02	0.00	0.00	0.00		
	Total CDC-LTs	27	34	13	8	10	13	11	13	43	77	32	13		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	1.35	1.70	0.65	0.40	0.50	0.65	0.55	0.65	2.15	3.85	1.60	0.65		
	Nightly EIR	0.00	0.00	0.01	0.00	0.00	0.65	0.00	0.00	0.05	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.28	0.00	0.00	20.15	0.00	0.00	1.53	0.00	0.00	0.00		21.96
	Total monthly EIR	0.00	0.00	0.28	0.00	0.00	20.15	0.00	0.00	1.53	0.00	0.00	0.00		21.96

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Eight Months EIR	Total Annual EIR
Chikwawa															
An. gambiae s.l.	Total tested	62	28	35	0	36	2	0	78	30	30	51	96		
	No. sporozoite positive	3	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Total CDC-LTs	62	11	9	2	2	0	0	31	14	20	1	29		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	3.10	0.55	0.45	0.10	0.10	0.00	0.00	1.55	0.70	1.00	0.05	1.45		
	Nightly EIR	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	4.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		4.65
An. funestus s.l.	Total tested	36	67	36	0	26	4	0	3	31	9	3	35		
	No. sporozoite positive	0	0	0	0	0	0	0	0	0	0	0	0		
	Sporozoite rate	0	0	0	0	0	0	0	0	0	0	0	0		
	Total CDC-LTs	18	10	19	3	2	1	0	4	9	10	0	2		
	Total traps	20	20	20	20	20	20	20	20	20	20	20	20		
	HBR/night	0.90	0.50	0.95	0.15	0.10	0.05	0.00	0.20	0.45	0.50	0.00	0.10		
	Nightly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
	Total monthly EIR	4.65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		4.65
Balaka															
An. gambiae s.l.	Total tested	*	*	*	*	0	0	6	13	133	0	0	0		
	No. sporozoite positive	*	*	*	*	0	0	0	0	0	0	0	0		
	Sporozoite rate	*	*	*	*	0	0	0	0	0	0	0	0		
	Total CDC-LTs	*	*	*	*	0	0	1	8	111	0	9	6		
	Total traps	*	*	*	*	20	20	20	20	20	20	20	20		
	HBR/night	*	*	*	*	0.00	0.00	0.05	0.40	5.55	0.00	0.45	0.30		
	Nightly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
An. funestus s.l.	Total tested	*	*	*	*	0	0	1	18	38	0	0	0		
	No. sporozoite positive	*	*	*	*	0	0	0	0	1	0	0	0		
	Sporozoite rate	*	*	*	*	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00		
	Total CDC-LTs	*	*	*	*	0	0	1	18	30	1	51	19		
	Total traps	*	*	*	*	20	20	20	20	20	20	20	20		
	HBR/night	*	*	*	*	0	0	0.05	0.9	1.5	0.05	2.55	0.95		
	Nightly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00		
	Monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	1.22	0.00	0.00	0.00	1.22	
	Total monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	1.22	0.00	0.00	0.00	1.22	
Mangochi															
An. gambiae s.l.	Total tested	*	*	*	*	5	3	1	6	138	0	0	0		
	No. sporozoite positive	*	*	*	*	0	0	0	0	0	0	0	0		
	Sporozoite rate	*	*	*	*	0	0	0	0	0	0	0	0		
	Total CDC-LTs	*	*	*	*	1	1	0	3	125	7	4	6		
	Total traps	*	*	*	*	20	20	20	20	20	20	20	20		
	HBR/night	*	*	*	*	0.05	0.05	0.00	0.15	6.25	0.35	0.20	0.30		
	Nightly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

Species	Indicator	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Eight Months EIR	Total Annual EIR
An. funestus s.l.	Total tested	*	*	*	*	5	1		4	62	0	0	0		
	No. sporozoite positive	*	*	*	*	0	0	0	0	0	0	0	0		
	Sporozoite rate	*	*	*	*	0	0	0	0	0	0	0	0		
	Total CDC-LTs	*	*	*	*	4	1	0	0	46	16	0	107		
	Total traps	*	*	*	*	20	20	20	20	20	20	20	20		
	HBR/night	*	*	*	*	0.20	0.05	0.00	0.00	2.30	0.80	0.00	5.35		
	Nightly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	Monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	Total monthly EIR	*	*	*	*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

*No entomological monitoring was conducted

ANNEX B: PARITY OF *ANOPHELES* MOSQUITOES

TABLE B1: TOTAL NUMBER AND PROPORTION PAROUS FEMALE *AN. FUNESTUS* S.L. COLLECTED BY CDC-LT AND HLC ACROSS ALL SEVEN MONITORING DISTRICTS

District	Status	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Total
Karonga	No. collected	2	1	15	0	0	0	1	0	0	0	9	0	28
	No. dissected	1	0	8	0	0	0	0	0	0	0	0	0	9
	No. parous	0	0	8	0	0	0	0	0	0	0	0	0	8
	Parity rate (%)	**0.0**	**0.0**	**100.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**88.9**
Nkhata Bay	No. collected	123	100	41	27	0	3	38	58	643	658	78	108	**1877**
	No. dissected	47	17	14	11	0	0	15	3	99	37	19	45	307
	No. parous	25	12	11	6	0	0	11	0	61	25	6	26	183
	Parity rate (%)	**53.2**	**70.6**	**78.6**	**54.5**	**0.0**	**0.0**	**73.3**	**0.0**	**61.6**	**67.6**	**31.6**	**57.8**	**59.6**
Nkhotakota	No. collected	619	505	534	488	182	192	94	614	853	165	994	1893	7133
	No. dissected	0	46	78	23	51	55	10	123	96	14	139	262	897
	No. parous	0	22	55	16	26	40	3	93	57	7	75	184	**578**
	Parity rate (%)	**0.0**	**47.8**	**70.5**	**69.6**	**51.0**	**72.7**	**30.0**	**75.6**	**59.4**	**50.0**	**54.0**	**70.2**	**64.4**
Kasungu	No. collected	27	34	36	8	10	20	11	13	83	77	32	18	369
	No. dissected	13	21	4	5	1	1	0	5	35	33	6	8	132
	No. parous	8	14	2	2	0	1	0	4	22	12	1	2	68
	Parity rate (%)	**61.5**	**66.7**	**50.0**	**40.0**	**0.0**	**100.0**	**0.0**	**80.0**	**62.9**	**36.4**	**16.7**	**25.0**	**51.5**
Salima	No. collected	59	8	20	1	1	2	2	62	149	51	86	116	557
	No. dissected	0	3	1	0	0	0	0	29	15	17	9	33	107
	No. parous	0	3	0	0	0	0	0	11	13	6	5	28	66
	Parity rate (%)	**0.0**	**100.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**37.9**	**86.7**	**35.3**	**55.6**	**84.8**	**61.7**
Mangochi	No. collected	0	0	0	0	4	1	0	0	76	10	0	107	204
	No. dissected	0	0	0	0	2	0	0	0	27	0	0	2	31
	No. parous	0	0	0	0	0	0	0	0	4	0	0	0	5
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**14.8**	**0.0**	**0.0**	**0.0**	**16.1**
Balaka	No. collected	0	0	0	0	0	0	1	18	93	1	51	12	183
	No. dissected	0	0	0	0	0	0	0	0	16	0	0	0	16
	No. parous	0	0	0	0	0	0	0	0	1	0	0	0	1
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**6.3**	**0.0**	**0.0**	**0.0**	**6.3**

District	Status	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Total
Chikwawa	No. collected	18	10	19	3	2	1	0	4	9	10	0	2	78
	No. dissected	0	3	0	0	0	0	0	0	4	0	0	0	7
	No. parous	0	0	0	0	0	0	0	0	3	0	0	0	3
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**75.0**	**0.0**	**0.0**	**0.0**	**42.9**
Overall	No. collected	848	658	665	527	199	219	147	769	1906	972	1250	2256	10429
	No. dissected	61	90	105	39	54	56	25	160	292	101	173	350	1506
	No. parous	33	51	76	24	26	41	14	108	161	50	87	240	912
	Parity rate (%)	**54.1**	**56.7**	**72.4**	**61.5**	**48.1**	**73.2**	**56.0**	**67.5**	**55.1**	**49.5**	**50.3**	**68.6**	**60.6**

Table B2: Total Number and Proportion Parous Female *An. gambiae* s.l. Collected by CDC-LT and HLC Across All Six IRS Districts

District	Status	Jul-21	Aug-21	Sep-21	Oct-21	Nov-21	Dec-21	Jan-22	Feb-22	Mar-22	Apr-22	May-22	Jun-22	Total
Karonga	No. collected	3	50	389	10	1	0	3	10	359	8	4	11	848
	No. dissected	1	3	25	4	0	0	0	2	74	0	4	5	118
	No. parous	1	2	25	0	0	0	0	2	35	0	0	4	0
	Parity rate (%)	**100.0**	**66.7**	**100.0**	**0.0**	**0.0**	**0.0**	**0.0**	**100.0**	**47.3**	**0.0**	**0.0**	**80.0**	**0.0**
Nkhata Bay	No. collected	0	1	96	8	1	0	18	21	99	28	0	5	311
	No. dissected	0	0	65	3	0	0	6	6	25	0	0	3	110
	No. parous	0	0	44	2	0	0	3	2	13	0	0	2	66
	Parity rate (%)	**0.0**	**0.0**	**67.7**	**66.7**	**0.0**	**0.0**	**50.0**	**33.3**	**52.0**	**0.0**	**0.0**	**66.7**	**60.0**
Nkhotakota	No. collected	5	10	35	32	14	34	25	238	333	52	59	28	865
	No. dissected	0	2	4	0	6	15	1	39	56	5	3	3	134
	No. parous	0	1	4	0	4	7	0	13	26	3	2	2	62
	Parity rate (%)	**0.0**	**50.0**	**100.0**	**0.0**	**66.7**	**46.7**	**0.0**	**33.3**	**46.4**	**60.0**	**66.7**	**66.7**	**46.3**
Kasungu	No. collected	1	4	0	3	0	10	5	17	220	23	7	1	291
	No. dissected	0	2	0	2	0	0	0	7	87	9	1	0	108
	No. parous	0	0	0	0	0	0	0	3	55	7	1	0	66
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**42.9**	**63.2**	**77.8**	**100.0**	**0.0**	**61.1**
Salima	No. collected	0	2	30	5	4	7	22	9	124	12	9	3	227
	No. dissected	0	1	2	0	0	4	0	2	9	5	2	0	25
	No. parous	0	**0**	2	0	0	3	0	0	9	4	1	0	19
	Parity rate (%)	**0.0**	**0.0**	**100.0**	**0.0**	**0.0**	**75.0**	**0.0**	**0.0**	**100.0**	**80.0**	**50.0**	**0.0**	**76.0**
Mangochi	No. collected	0	0	0	0	1	1	0	3	69	7	4	6	212
	No. dissected	0	0	0	0	0	0	0	0	29	0	0	0	52
	No. parous	0	0	0	0	0	0	0	0	6	0	0	0	18
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**20.7**	**0.0**	**0.0**	**0.0**	**34.6**
Balaka	No. collected	0	0	0	0	0	0	1	8	72	0	9	6	198
	No. dissected	0	0	0	0	0	0	0	0	20	0	0	0	39
	No. parous	0	0	0	0	0	0	0	0	9	0	0	0	14
	Parity rate (%)	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**45.0**	**0.0**	**0.0**	**0.0**	**35.9**
Chikwawa	No. collected	62	11	9	2	3	0		31	14	20	1	29	181
	No. dissected	6	1	0	0	3	0	0	6	0	0	0	12	27
	No. parous	3	0	0	0	3	0	0	0	4	0	0	10	19
	Parity rate (%)	**50.0**	**0.0**	**0.0**	**0.0**	**100.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**83.3**	**70.4**
Overall	No. collected	71	78	559	60	24	52	74	337	1290	150	93	89	3133
	No. dissected	7	9	96	9	9	19	7	62	300	19	10	23	613
	No. parous	4	3	75	2	7	10	3	20	157	14	4	18	264
	Parity rate (%)	**57.1**	**33.3**	**78.1**	**22.2**	**77.8**	**52.6**	**42.9**	**32.3**	**52.3**	**73.7**	**40.0**	**78.3**	**43.1**

ANNEX C: SPRAY QUALITY AND RESIDUAL LIFE ASSESSMENT OF FLUDORA FUSION AND SUMISHIELD 50WG IN THE FOUR IRS DISTRICTS

TABLE C1: SPRAY QUALITY ASSESSMENT IN NKHOTAKOTA, NKHATA BAY AND MANGOCHI

District	Insecticide	Site	Surface	Mortality (%)		Total # Dead	Total # Exposed	Mortality (%)	
				Day 1	Day 2			Test	Control
Nkhotakota	SumiShield	Thung'unda	Cement	100	-	34	34	100	0
			Brick	99	100	103	103	100	0
			Mud	100	-	69	69	100	0
		Vwawa	Cement	100	-	66	66	100	0
			Brick	100	-	62	62	100	0
			Mud	100	-	64	64	100	0
	Fludora Fusion	Chimkwende	Brick	100	-	99	99	100	0
			Mud	100	-	92	92	100	0
		Zamangwe 1	Brick	100	-	90	90	100	0
			Mud	100	-	91	91	100	0
		Ngalauka 1 - positive control	Cement	100	-	62	62	100	0
			Brick	100	-	33	33	100	0
			Mud	100	-	93	93	100	0
		Ngalauka 1 - normal spraying	Cement	100	-	57	57	100	0
			Brick	100	-	62	62	100	0
			Mud	100	-	62	62	100	0

District	Insecticide	Site	Surface	Mortality (%)		Total # Dead	Total # Exposed	Mortality (%)	
				Day 1	Day 2			Test	Control
Nkhata Bay	Fludora Fusion	Kapolonga	Cement	100	-	32	32	100	0
			Brick	100	-	100	100	100	0
			Mud	100	-	65	65	100	0
		Sanga	Cement	100	-	104	104	100	0
			Brick	100	-	105	105	100	0
		Kande - positive control	Cement	100	-	88	88	100	0
			Brick	100	-	90	90	100	0
		Kande - normal spraying	Cement	100	-	65	65	100	0
			Brick	100	-	99	99	100	0
			Mud	100	-	28	28	100	0
		Khoza	Cement	100	-	87	87	100	0
			Brick	100	-	103	103	100	0
		Chioda	Cement	100	-	96	96	100	0
			Brick	100	-	100	100	100	0
		M'bama	Cement	100	-	102	102	100	0
			Brick	100	-	93	93	100	0
		Mtiti	Cement	100	-	108	108	100	0
			Brick	100	-	65	65	100	0
			Mud	100	-	34	34	100	0
Mangochi	Fludora Fusion	Mwambwajira - positive control	Cement	100	-	68	68	100	1.6
			Brick	100	-	60	60	100	8.7
			Mud	100	-	69	69	100	0
		M'doka	Cement	100	-	67	67	100	11.3
			Brick	100	-	61	61	100	7.6
			Mud	100	-	62	62	100	0
		Kuwaya	Cement	100	-	65	65	100	12.6
			Brick	100	-	51	51	100	8.4
			Mud	100	-	67	67	100	8.2
		Kalichelo	Cement	100	-	51	51	100	2.3
			Brick	100	-	56	56	100	11.9
			Mud	100	-	64	64	100	8.5
		Makokola	Cement	100	-	74	74	100	17.2
			Brick	100	-	73	73	100	9
			Mud	100	-	82	82	100	19.3
		Kela	Cement	100	-	67	67	100	2.2
			Brick	86.3	-	59	70	86.3	5.4
			Mud	100	-	70	70	100	1.8
		Nombo	Cement	100	-	65	65	100	15.4
			Brick	100	-	51	51	100	11.9
			Mud	100	-	67	67	100	15.3

Table C2: Summary of Fumigation Effect in Nkhotakota and Nkhata Bay

District	Insecticide	Site/Village	Wall/Surface Type	Mortality (%) M0 (Oct)	M1 (Nov)	M2 (Dec)	M3 (Jan)	M4 (Feb)	M5 (Mar)	M6 (Apr)	M7 (May)	M8 (Jun)
Nkhotakota	SumiShield	Vwawa	Cement	100	100	100	100	100	100	100	100	100
			Brick	100	100	100	100	100	100	100	100	100
			Mud	100	100	100	100	100	100	100	100	100
	Fludora Fusion	Chimkwende	Brick	100	100	100	100	100	100	100	100	100
			Mud	100	100	100	100	100	100	100	100	100
		Ngalauka - Positive control	Cement	100	100	100	100	100	100	100	100	100
			Brick	100	100	100	100	100	100	100	100	100
			Mud	100	100	100	100	100	100	100	100	94.4
		Ngalauka - Normal spraying	Cement	100	100	100	100	100	100	100	100	100
			Brick	100	100	100	100	100	100	100	100	100
			Mud	100	100	100	100	100	100	100	100	100
		Zamangwe 1	Brick	100	100	100	100	100	100	100	100	86
			Mud	100	100	100	100	100	100	100	100	97.7
Nkhata Bay	SumiShield	Kande - Positive control	Cement	100	100	100	100	100	100	100	-	59.1
			Brick	100	100	100	100	100	100	100	-	40.4
		Kande - Normal spraying	Cement	100	100	100	100	100	100	100	-	90
			Brick	100	100	100	100	100	100	100	-	87.7
			Mud	100	100	100	100	100	94.5	100	-	26.7
		Sanga	Cement	100	100	100	100	100	71.7	70	-	78.6
			Brick	100	100	100	100	100	100	100	-	73.5
		M'bama	Cement	100	100	100	100	100	100	100	-	76.3
			Brick	100	100	100	100	100	100	100	-	84
		Mtiti	Cement	100	100	100	100	100	100	100	-	80.7
			Brick	100	100	100	100	100	100	100	-	100
			Mud	100	100	100	100	100	100	100	-	82.4

TABLE C3: SUMMARY OF FUMIGATION EFFECT IN MANGOCHI AND BALAKA

District	Insecticide	Site/Village	Wall/Surface Type	Mortality (%)							
				M0 (Nov)	M1 (Dec)	M2 (Jan)	M3 (Feb)	M4 (Mar)	M5 (Apr)	M6 (May)	M7 (Jun)
Mangochi	Fludora Fusion	Mwambwajira	Cement	100	100	100	100	100	100	-	80.5
			Brick	81	100	100	100	97.4	100	-	84.1
			Mud	100	100	100	100	100	100	-	86.6
		M'doka	Cement	100	100	100	100	97.3	100	-	100
			Brick	100	74.4	100	100	100	100	-	57.7
			Mud	100	66.7	100	100	100	100	-	92.7
		Kuwaya	Cement	69.3	100	100	60.4	100	100	-	72.2
			Brick	100	100	100	100	100	100	-	68
			Mud	100	100	100	100	100	100	-	100
		Kalichero	Cement	100	100	100	100	100	100	-	100
			Brick	100	100	100	100	100	100	-	65.1
			Mud	100	86.4	100	100	100	100	-	73.3
		Makokola	Cement	100	100	100	100	100	100	-	64
			Brick	97.5	100	100	100	100	100	-	87
			Mud	100	100	96.7	100	100	100	-	64
Balaka	Fludora Fusion	Siliya	Cement	-	100	86.9	100	100	28	100	97.8
			Brick	-	100	100	100	100	70.6	100	89.2
			Mud	-	100	100	100	100	5.6	100	82
		Domoka	Cement	-	100	100	100	100	97.2	100	100
			Brick	-	100	100	100	68.8	100	100	100
			Mud	-	100	100	100	97.7	97.4	100	100
		Mwanyali	Cement	-	100	100	100	65	100	100	100
			Brick	-	100	100	100	90.5	70.2	100	100
			Mud	-	100	100	100	94.5	83.5	100	100
		Silili	Cement	-	100	100	100	57.1	100	100	100
			Brick	-	100	100	100	100	100	100	100
			Mud	-	100	100	100	100	52.7	100	100

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FIGURE C1: RESIDUAL EFFICACY OF FLUDORA FUSION AT NGALAUKA VILLAGE IN NKHOTAKOTA DISTRICT

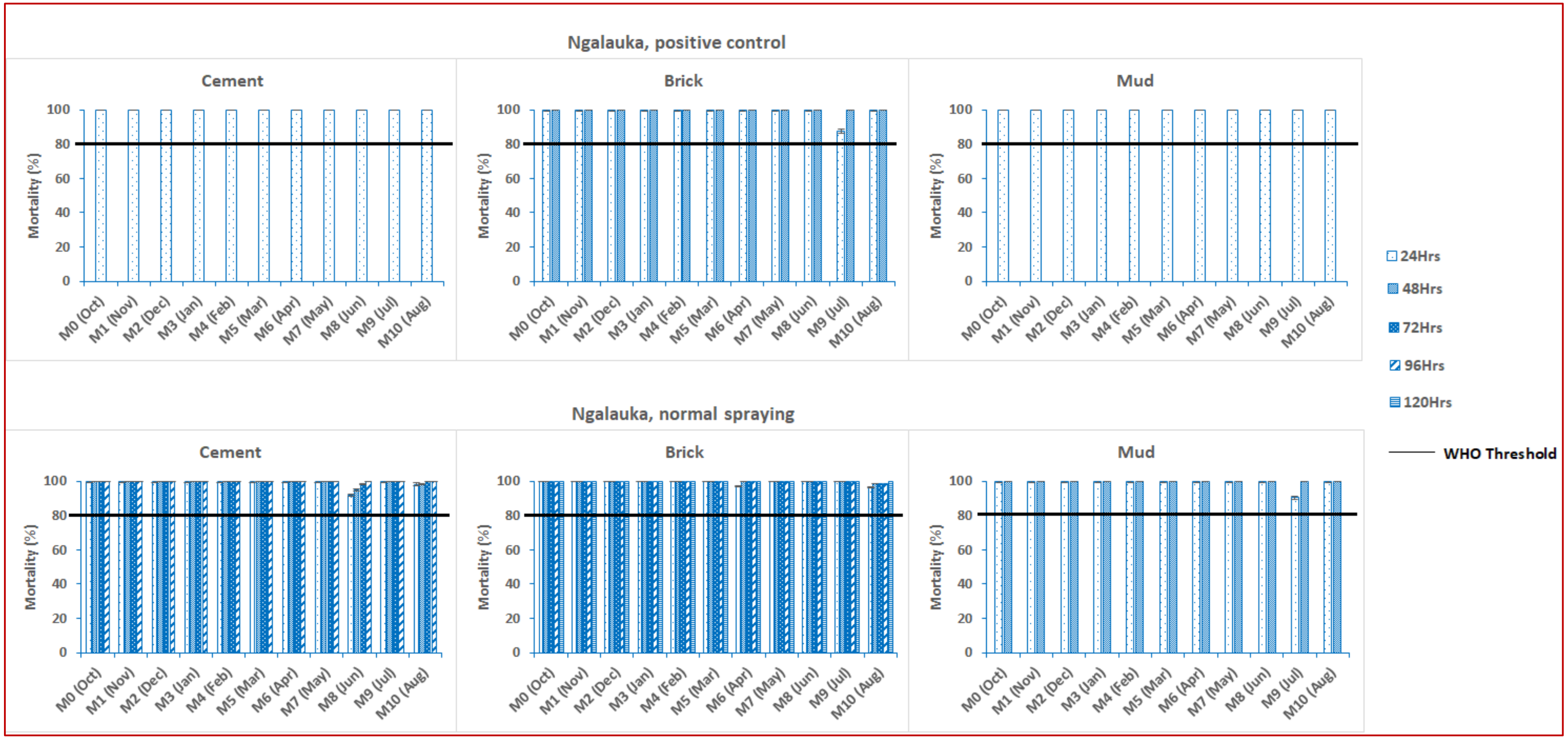


FIGURE C2: RESIDUAL EFFICACY OF FLUDORA FUSION AT CHIMKWENDE AND ZAMANGWE I VILLAGES IN NKHOTAKOTA DISTRICT

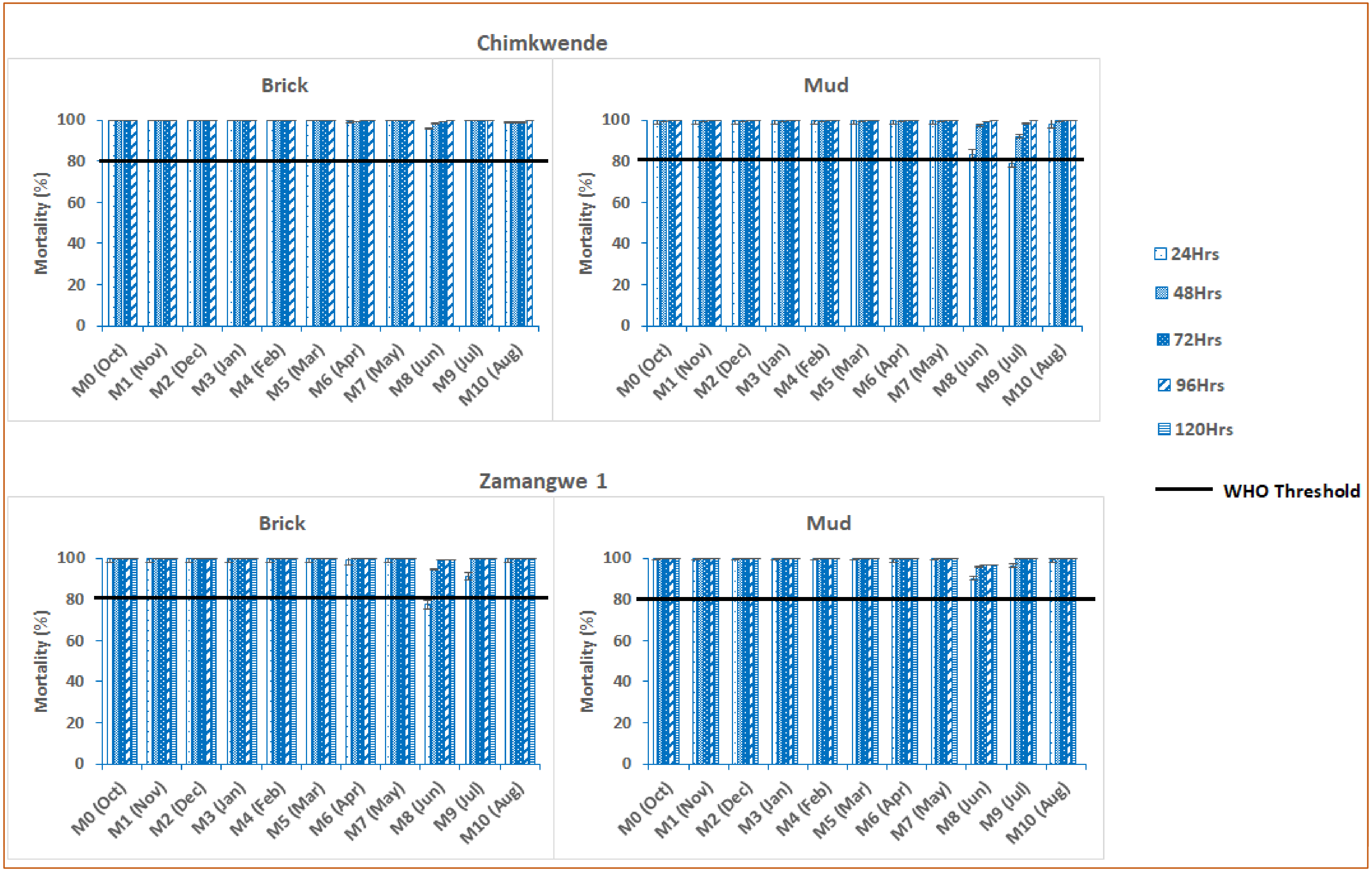


FIGURE C3: RESIDUAL EFFICACY OF FLUDORA FUSION AT MWAMBWAJIRA AND M'DOKA VILLAGES IN MANGOCHI DISTRICT

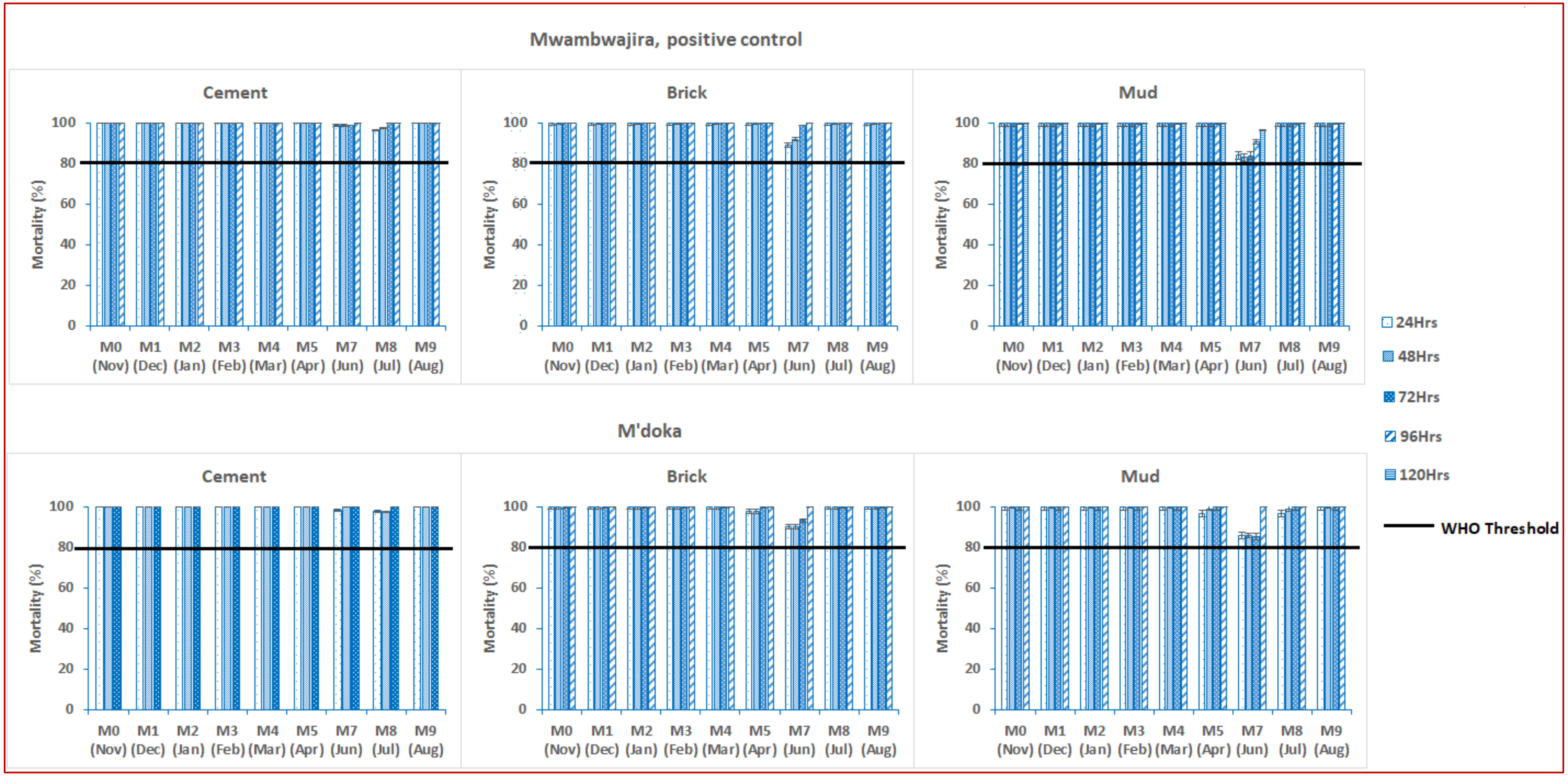


Figure C4: Residual Efficacy of Fludora Fusion at Kuwaya and Kalichero Villages in Mangochi District

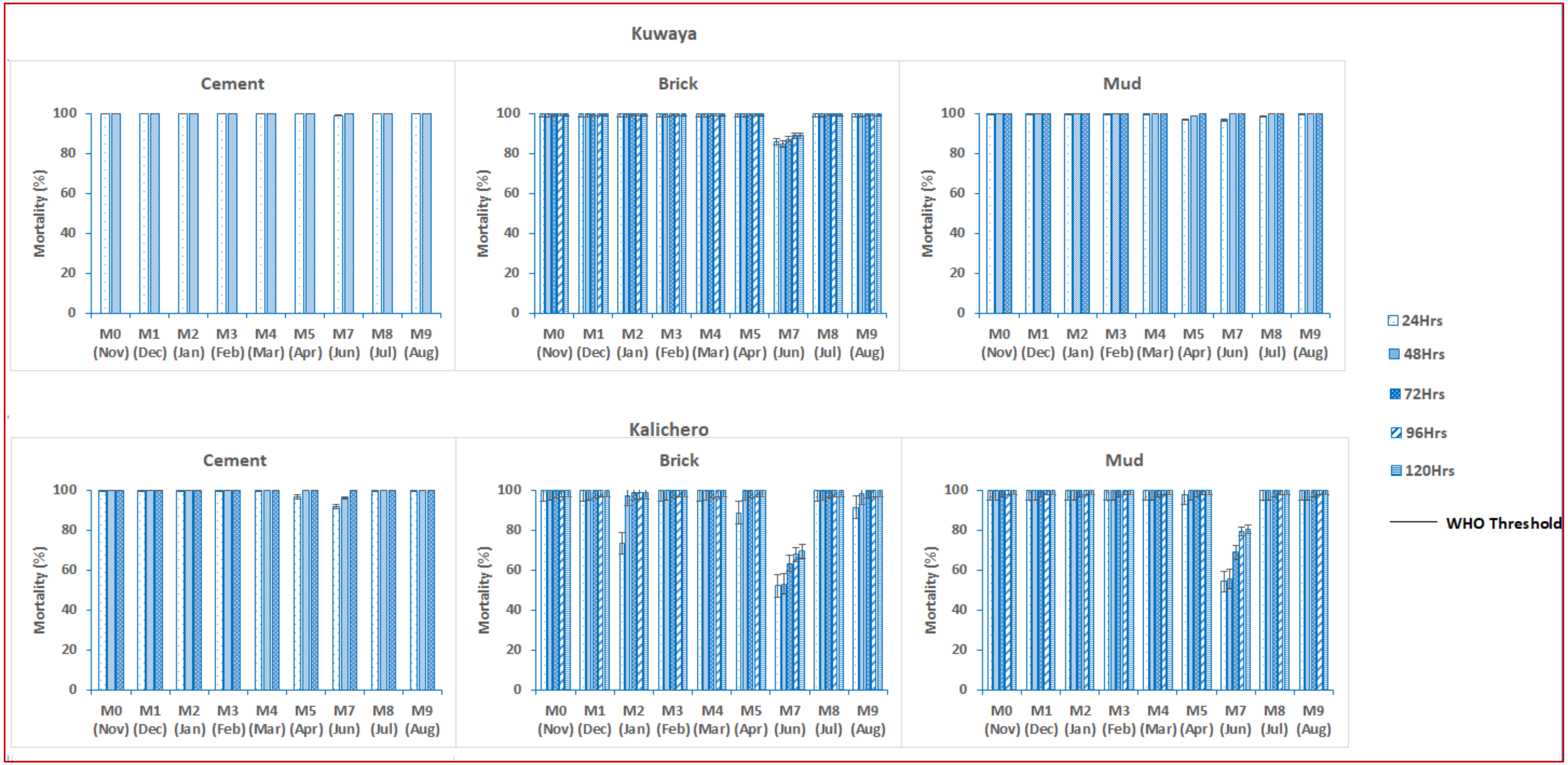


FIGURE C5: RESIDUAL EFFICACY OF FLUDORA FUSION AT MAKOKOLA VILLAGE IN MANGOCHI DISTRICT

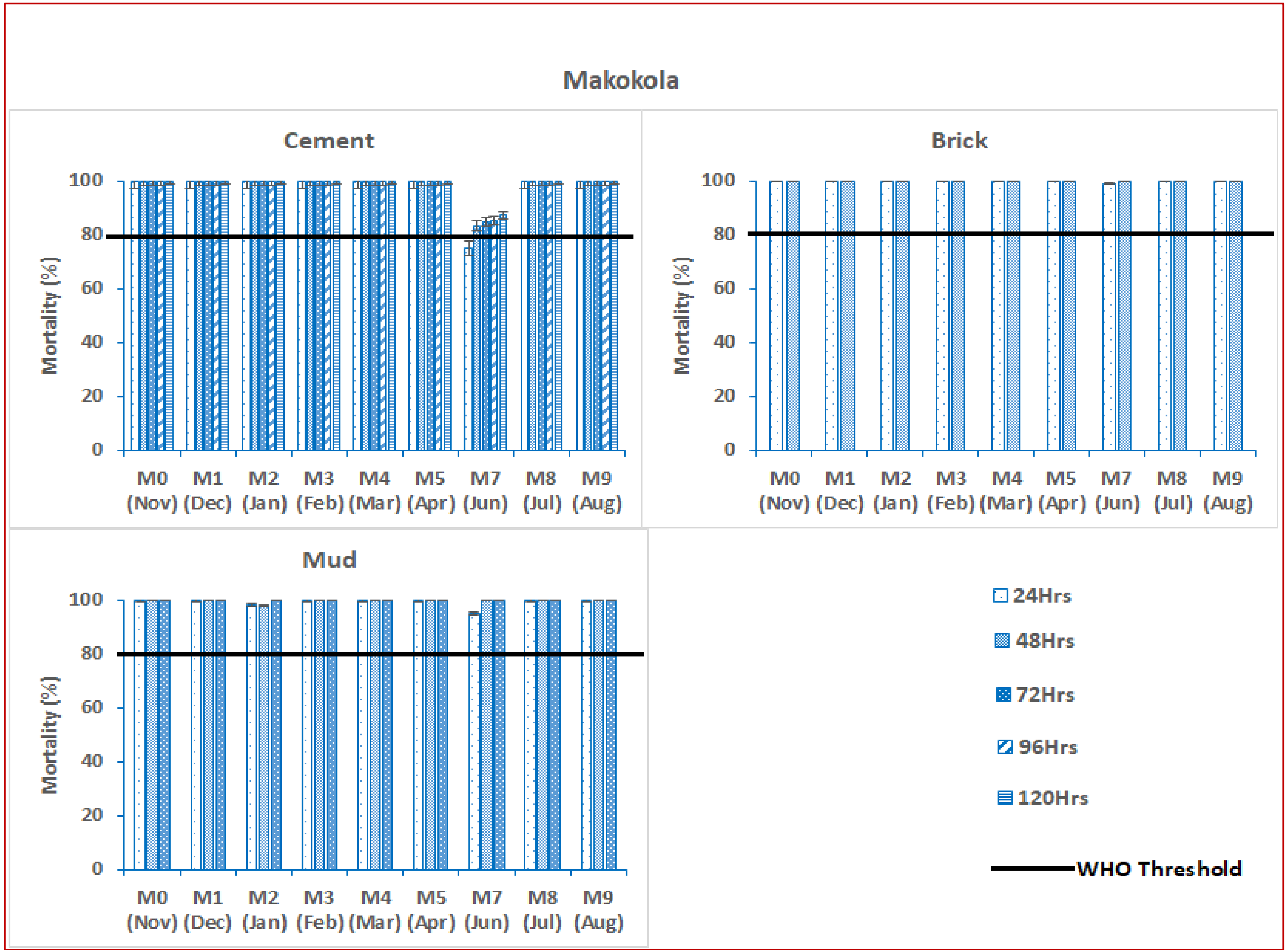


FIGURE C6: RESIDUAL EFFICACY OF FLUDORA FUSION AT SILIYA AND DOMOKA VILLAGES IN BALAKA DISTRICT

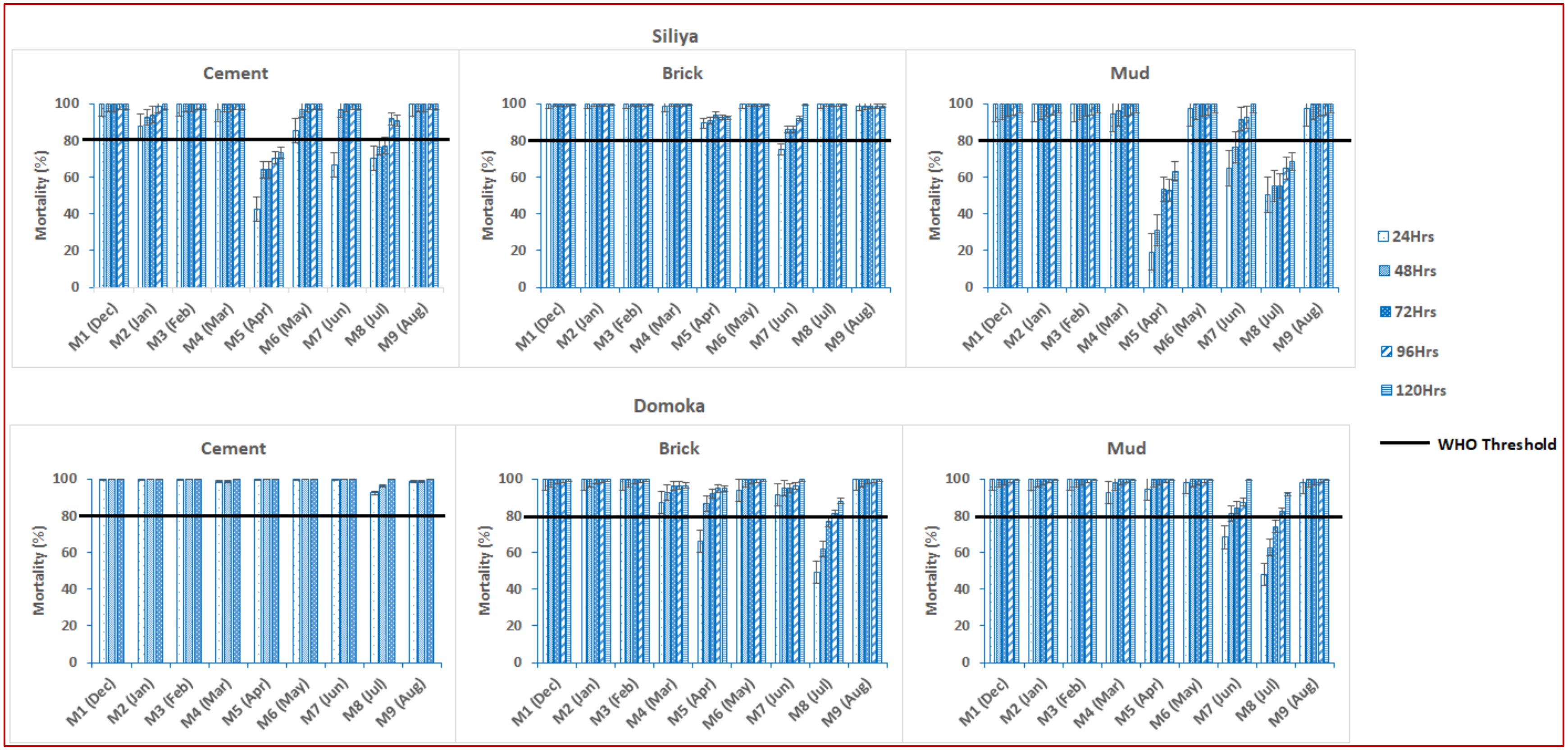


FIGURE C7: RESIDUAL EFFICACY OF FLUDORA FUSION AT MWANYALI AND SILILI VILLAGES IN BALAKA DISTRICT

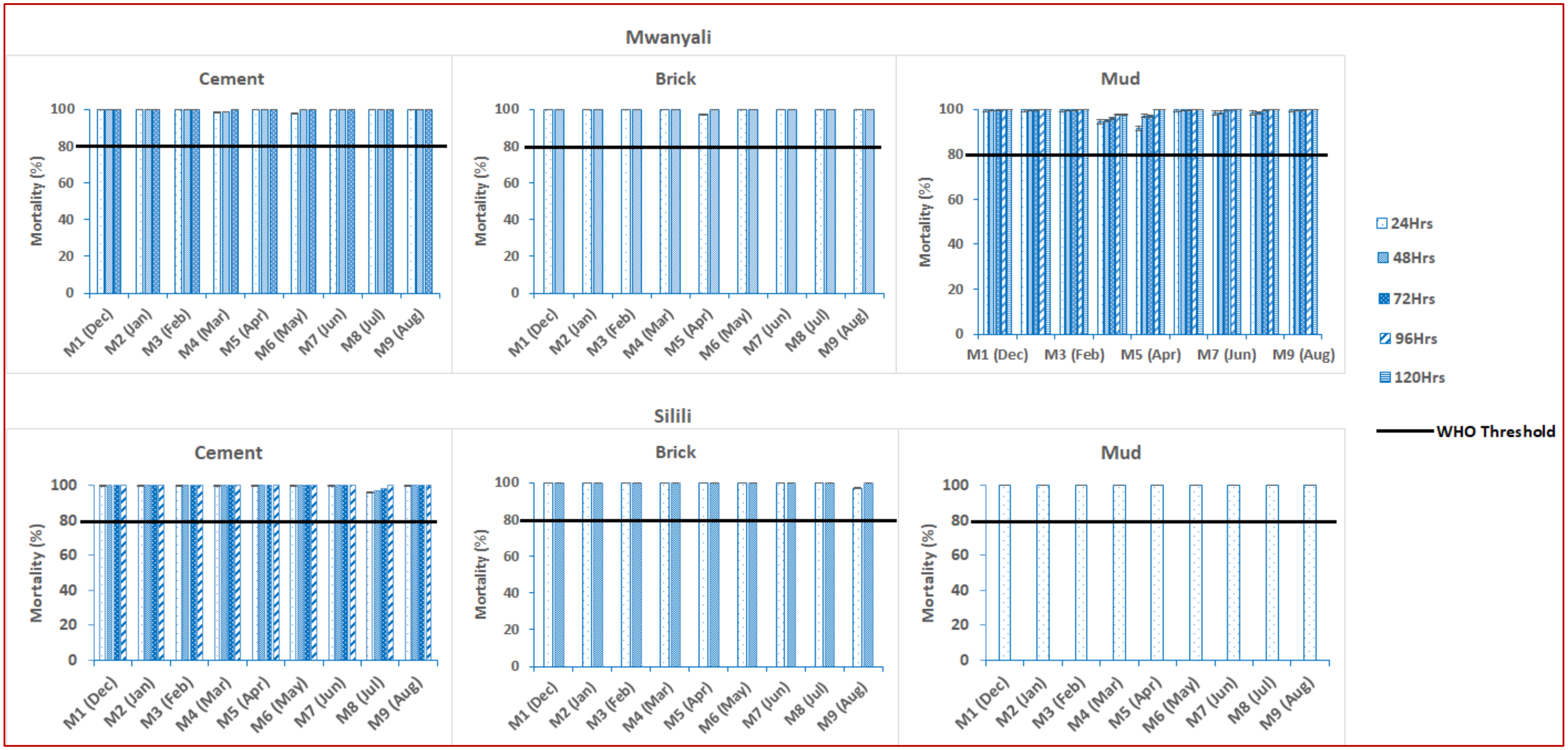


FIGURE C8: RESIDUAL EFFICACY OF SUMISHIELD 50WG AT KANDE VILLAGE IN NKHATA BAY DISTRICT

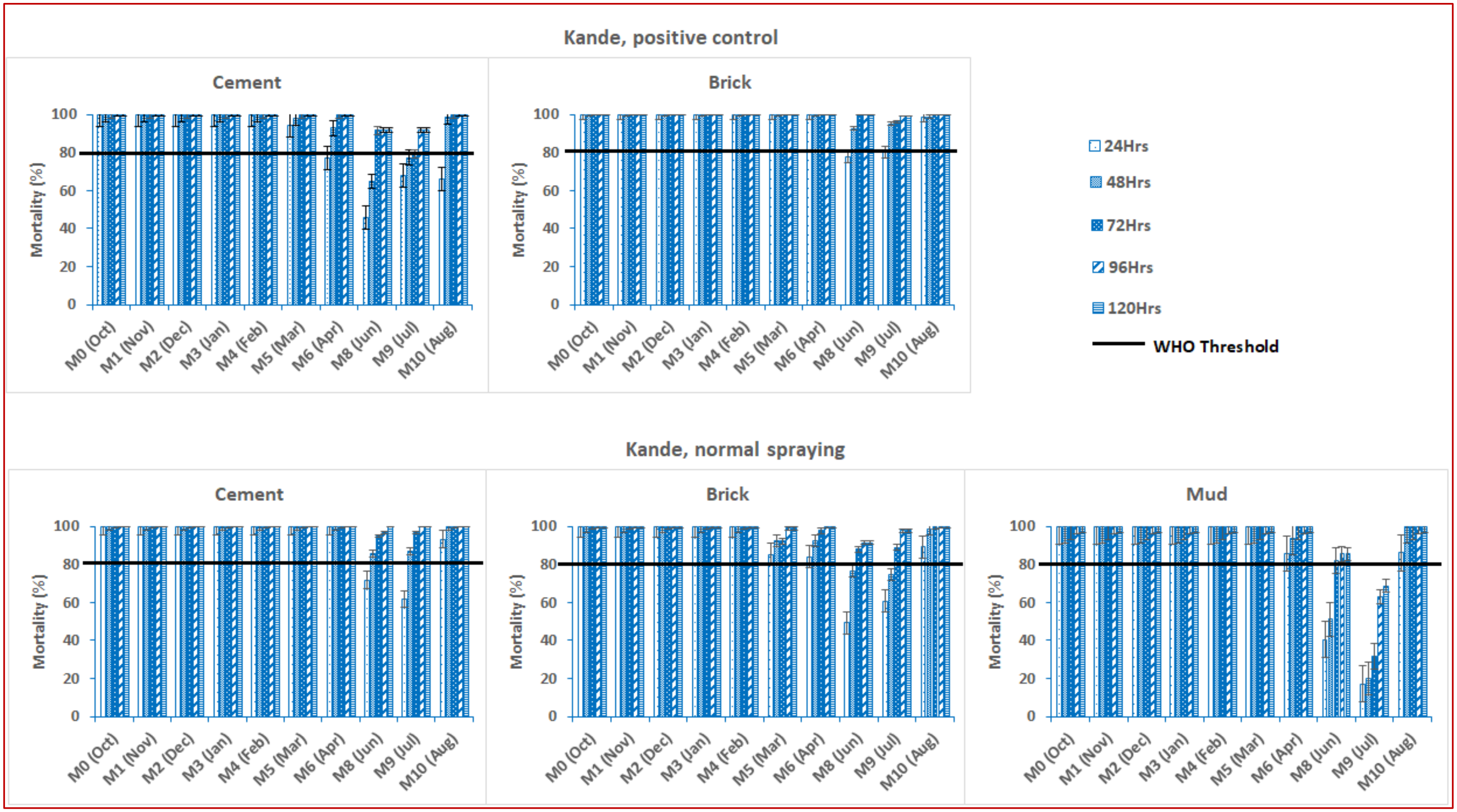


FIGURE C9: RESIDUAL EFFICACY OF SUMISHIELD AT SANGA AND MBAMA VILLAGES IN NKHATA BAY DISTRICT

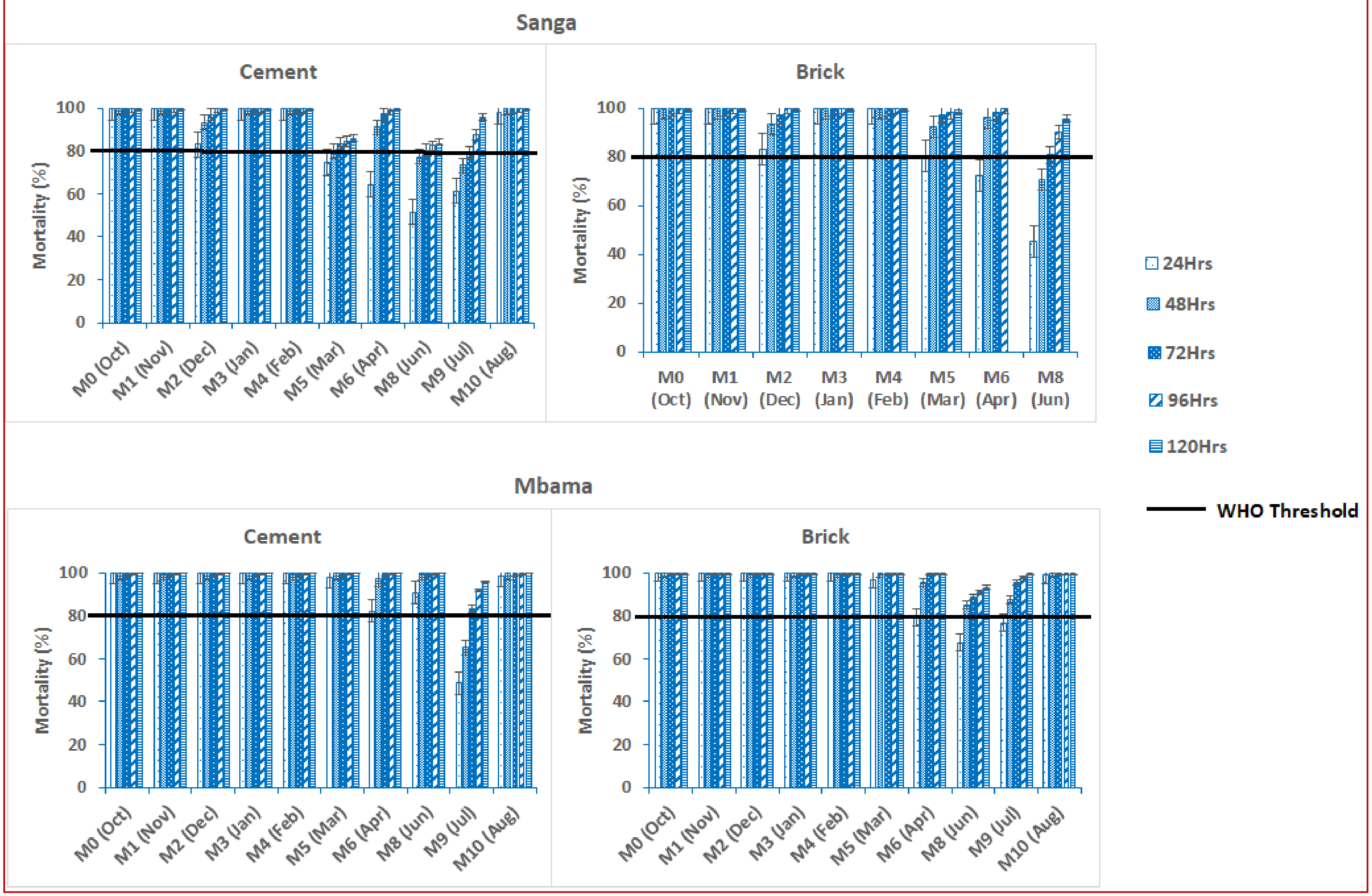
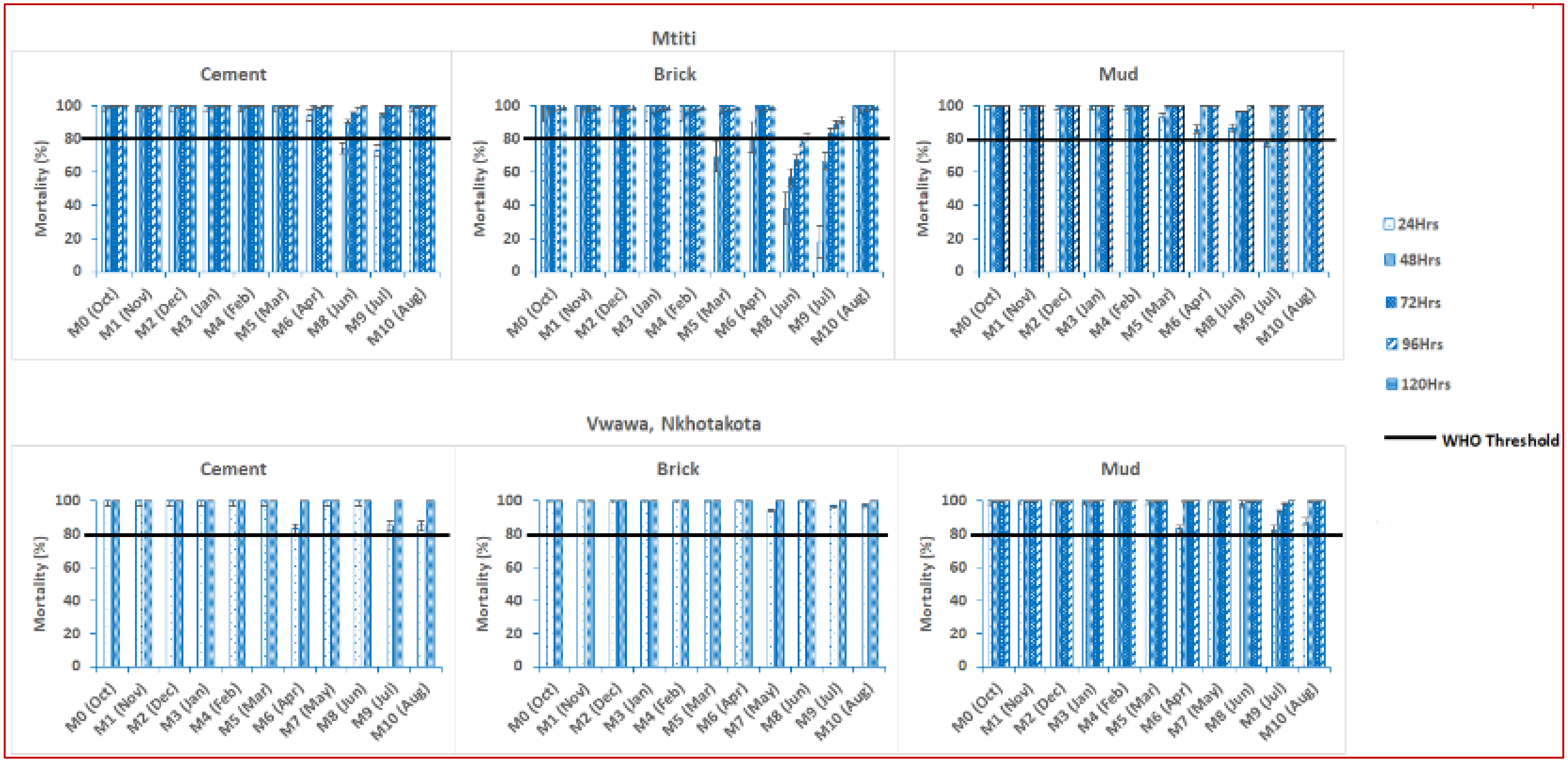


FIGURE C10: RESIDUAL EFFICACY OF SUMISHIELD AT MTITI AND VWAWA VILLAGES IN NKHATA BAY AND NKHOTAKOTA DISTRICTS



ANNEX D: *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. RESPONSE TO DIFFERENT INSECTICIDES

TABLE D1: *AN. FUNESTUS* S.S., *AN. ARABIENSIS* AND *AN. GAMBIAE S.S.* RESPONSE TO DIFFERENT INSECTICIDES IN CHIKWAWA, SALIMA, KASUNGU, NKHOTAKOTA AND KARONGA DISTRICTS

District	Site	Insecticide	Species	Source	Total Tested	Total Dead	% Mortality	Time at Final Mortality	Vector Control Intervention
Chikwawa	Ntwana	Deltamethrin 0.05%	*An. gambiae* s.l.	F1	102	97	94.3	24Hrs	IG2
		Permethrin 0.75%	*An. gambiae* s.l.	F1	95	91	95.3	24Hrs	
		Alpha-cypermethrin 0.05%	*An. gambiae* s.l.	F1	85	64	72.5	24Hrs	
		Pirimiphos methyl 0.25%	*An. gambiae* s.l.	F1	32	32	100	24Hrs	
		Pirimiphos-methyl 0.25%	*An. funestus* s.l.	F1	102	102	100	24Hrs	
		Chlorfenapyr 100µg/bottle	*An. gambiae* s.l.	Larvae	65	65	100	24Hrs	
	Nkhwazi	Clothianidin 4µg/bottle	*An. gambiae* s.l.	Larvae	45	44	98	24Hrs	
Salima	Chilungo	Alpha-cypermethrin 0.05%	*An. gambiae* s.l.	Larvae	103	12	11.7	24Hrs	Royal Guard
		Deltamethrin 0.05%	*An. gambiae* s.l.	Larvae	104	36	34.6	24Hrs	
		Permethrin 0.75%	*An. gambiae* s.l.	Larvae	102	34	33.3	24Hrs	
		Clothianidin 4µg/bottle	*An. gambiae* s.l.	Larvae	37	37	100	24Hrs	
	Kabumba	Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	F1	94	94	100	24Hrs	
Kasungu	Kachokolo	Permethrin	*An. funestus* s.l.	F1	103	16	15.5	24Hrs	Olyset Plus
		PBO + Permethrin 0.75%	*An. funestus* s.l.	F1	52	51	98.1	24Hrs	
		Pirimiphos-methyl 0.25%	*An. funestus* s.l.	F1	54	53	98	24Hrs	
Nkhotakota	Chimkwende	Alpha-cypermethrin 0.05%	*An. funestus* s.l.	Larvae	44	15	34	24Hrs	IRS
		Clothianidin 4µg/bottle	*An. funestus* s.l.	Larvae	29	29	100	24Hrs	
Karonga	Mwenimambwe	Pirimiphos-methyl 0.25%	*An. gambiae* s.l.	F1	111	111	100	24Hrs	Royal Guard, IG2 & Olyset Plus
		Clothianidin 4µg/bottle	*An. gambiae* s.l.	F1	107	107	100	24Hrs	
		Chlorfenapyr 100µg/bottle	*An. gambiae* s.l.	F1	94	92	98	3 Days	