



U.S. President's Malaria Initiative

USAID *Okoa Maisha Dhibiti Malaria* (OMDM)/Save Lives, End Malaria

2019 Entomological Monitoring Annual Report

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ACRONYMS AND ABBREVIATIONS

ai/m ²	active ingredient per meter squared
AOR	agreement officer's representative
°C	degree Celsius
CBR	CDC light trap with bottle rotator
CDC	US Centers for Disease Control and Prevention
CI	confidence interval
cm	centimeters
CS	circumsporozoite antigens
DC	district council
DDT	dichlorodiphenyltrichloroethane
ELISA	enzyme-linked immunosorbent assay
HBR	human biting rate
HLC	human landing catch
IBR	indoor biting rate
IRS	indoor residual spraying
KD	knock down
kdr	knockdown resistance
LLIN	long-lasting insecticidal net
LTC	light trap collection
m	meter
MBR	man biting rate
mg	milligram
ml	milliliter
NIMR	National Institute for Medical Research
NMCP	National Malaria Control Programme
OMDM	Okoa Maisha Dhibiti Malaria/Save Lives, End Malaria
PBO	piperonyl butoxide
PCR	polymerase chain reaction
PMI	US President's Malaria Initiative
PSC	pyrethrum spray catches
PTC	pit trap catches
s.l.	sensu lato
S/N	serial number

s.s.	sensu stricto
USAID	United States Agency for International Development
WHO	World Health Organization
ZAMEP	Zanzibar Malaria Elimination Programme

Executive Summary

Background. Malaria vector control in sub-Saharan Africa is highly dependent on the use of insecticides deployed through indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs). However, the effectiveness of these malaria vector control interventions relies on our knowledge of local vector species and their susceptibility to the insecticides being used. Periodic collection of such data is essential to inform vector control strategies and track their impacts on malaria transmission. The United States Agency for International Development (USAID)–funded *Okoa Maisha Dhibiti Malaria* (OMDM)/Save Lives, End Malaria Activity conducted routine entomological surveillance and insecticide residual efficacy testing in Mainland Tanzania’s Lake Zone (11 sites) and Zanzibar (10 sites) to assess the efficacy of IRS from September 2018 through October 2019. Similarly, routine monitoring of the susceptibility status of major malaria vectors to insecticides were conducted in 22 sentinel districts of Mainland Tanzania and 8 in Zanzibar. These entomological monitoring activities were implemented in partnership with the National Institute for Medical Research (NIMR) Mwanza Centre, NIMR Amani, and the Zanzibar Malaria Elimination Programme (ZAMEP).

Insecticide residual efficacy (Mainland Tanzania). In the Lake Zone, 10 months of post spraying with clothianidin, mud surfaces effectively retained the insecticide, averaging 85.6% mortality. Other surfaces recorded an average of 84.6% for cement, 84.3% for burnt brick, 83.6% for painted surfaces, and 81.8% for whitewashed surfaces.

Entomological surveillance (Mainland Tanzania). A total of 14,035 female *Anopheles* were collected in the Lake Zone. The female *Anopheles* were morphologically identified as *Anopheles gambiae* s.l.; 9,183 [65.4%], *An. funestus* s.l. (2,597 [18.5%]), *An. coustani* (1,896 [13.5%]), *An. pharoensis* (217 [1.5%]), and *An. rufipes*. (142 [1.0%]). *An. gambiae* s.l. was the most abundant vector species sampled by all collection methods. Identification of species (n=4,373) by polymerase chain reaction (PCR)–based methods showed the local vector population across sites to be predominantly *An. arabiensis* (2,318 [53%]), *An. funestus* s.s.; (1,186 [27.1%]), *An. gambiae* s.s. (606 [13.9%]), and *An. parensis* (100 [2.3%]). *An. arabiensis* was the most abundant species in four sites (i.e., Misenyi, Bukoba Rural, Chato, and Kakonko), and *An. funestus* s.s. was the dominant species in three sites (i.e., Ngara, Nyang’hwale, and Buchosa). Sporozoite rates were found to vary across the sites, ranging from 0% to 2.7% in sprayed sites and 1.2% to 3.7% in unsprayed sites. There was a general decrease in indoor biting rates (IBRs) in all sprayed sentinel sites following IRS operations between October 2018 to September 2019.

The IRS application shifted biting patterns of *An. gambiae* s.l. to the outdoors; there were unclear trends for *An. funestus* s.l. There was a general reduction of IBRs from 0.29 bites per person per hour (before IRS) to less than 0.05 bites per person per hour after IRS. Before IRS, the highest outdoor biting rates were observed at dusk (1900–2000 hours) and dawn (0500–0600 hours), suggesting high exophagic behavior in *An. arabiensis*. At non-IRS sites, *An. gambiae* s.l. biting rates were higher indoors because of the absence of insecticides. Also, higher biting rates of *An. gambiae* s.l. were found outdoors, likely due to people staying outside for longer hours or, potentially, the mosquitoes exhibiting zoophilic behavior. Both indoor and outdoor biting rates were relatively constant throughout the night for *An. funestus* s.l.

In sprayed districts, two months post-IRS operations, indoor resting density decreased in all districts except for Kakonko (increased trends from January through May 2019) and Chato

(peaking in December 2018 and April 2019). The OMDM team also observed higher trends of indoor resting density in non-IRS districts throughout the monitoring period.

Anopheles that fed on humans (including mixed-blood meals on both humans and other animals) was 52.2% in IRS districts and 58.1% in non-IRS districts. IRS also showed a significant effect in reducing the longevity of *Anopheles* vector species.

Insecticide resistance monitoring (Mainland Tanzania). *An. gambiae* s.l. were resistant to permethrin (mortality rate less than 90%) in 14 out of the 22 sentinel sites. The 14 sites are Biharamulo, Geita, Igunga, Kakonko, Kasulu, Kibaha, Kibondo, Kilwa, Misenyi, Newala, Rorya, Tandahimba, Tunduru, and Ukerewe. Malaria vectors were resistant to deltamethrin in all districts that showed resistance to permethrin. Resistance to dichlorodiphenyltrichloroethane (DDT) was recorded in Biharamulo, Kasulu, Kibondo, Misenyi, Tandahimba, and Ukerewe. Resistance to bendiocarb was recorded in Biharamulo, and resistance to pirimiphos-methyl was recorded in Kilosa. Vectors were fully susceptible to clothianidin in all seven sentinel sites where it was tested (i.e., Geita, Ukerewe, Rorya, Kibondo, Kakonko, Kasulu, Biharamulo). The *Anopheles* mosquitoes showed 100% mortality on Day 6, 24 hours before the recommended post-exposure observation period of 7 days.

The resistance intensity to permethrin was high in Kibondo and Ukerewe. There was also a high resistance intensity to deltamethrin in Misenyi and Rorya. Full restoration of susceptibility after pre-exposure to piperonyl butoxide (PBO) was observed against permethrin and deltamethrin exposure at all sites; the only exceptions were for permethrin in Ukerewe and Misenyi and for deltamethrin in Biharamulo and Misenyi. These responses suggest that insecticide resistance in the *An. gambiae* population is primarily mediated by an oxidase-based metabolic resistance (cytochrome P450s), with minor contributions from other mechanisms, including knockdown resistance (*kdr*) gene mutations.

Molecular and biochemical tests revealed the occurrence of both target site mutations (*kdr*-east [L1014S]) and metabolic (cytochrome P450) resistance mechanisms in the resistant sentinel districts, respectively. Also, *kdr*-east (L1014S) were detected in mosquitoes in more than half (14 out of 22) of the sentinel districts, with allelic frequencies ranging from 2% in Nachingwea and Geita to almost 90% in Kibondo. Likewise, the mean levels of oxidase enzymes were significantly higher in mosquitoes from almost all resistant sentinel districts, demonstrating the existence of oxidase types of metabolic resistance.

Insecticide residual efficacy (Zanzibar). In Zanzibar, 6 months post-IRS, pirimiphos-methyl was still effective with a mortality of greater than or equal to 80% on all wall types.

Entomological surveillance (Zanzibar). A total of 2,418 *Anopheles* mosquitoes were collected: 1,328 from Pemba and 1,090 from Unguja. The highest number of mosquitoes were collected from human landing catches (HLC), contributing to 71% of the total *Anopheles* catches in Pemba and to 60% in Unguja. Out of the 2,418 *Anopheles* mosquitoes collected, 98.8% (n=2,388) were morphologically identified as *An. gambiae* s.l. and 1.1% (n=26) as *An. coustani*. Other *Anopheles* mosquitoes collected constituting a total of 0.2% (n=4) were *An. ziemanni*, *An. nili*, *An. funestus* s.l., and *An. Maculipalpis*, with 1 each. Out of the total mosquitoes collected, 2,256 samples (i.e., Pemba = 1,246 and Unguja = 1,010) were analyzed for species identification. The results showed that *An. arabiensis* was the predominant vector in both Pemba (86%) and Unguja (78%), followed by *An. lesoni*. Other *Anopheles* species found in small proportions were *An. merus*, *An. gambiae* s.s., *An. parensis*, *An. rivulorum*, *An. funestus* s.s., and *An. quadriannulatus*. The results show that the outdoor biting density of *An. arabiensis* is higher earlier in the day when people are

active outdoors. The human biting rate (HBR; also known as the man biting rate [MBR]) is slightly higher in unsprayed sites than sprayed sites.

Insecticide resistance monitoring (Zanzibar). *An. gambiae* s.l. was resistant to deltamethrin, permethrin, and alphacypermethrin (mortality less than 90%) but remained susceptible to bendiocarb and pirimiphos-methyl (mortality greater than 98%) across all sites. The resistance intensity varied between the two islands, being higher in Pemba than Unguja. Pre-exposing *An. gambiae* s.l. to PBO restored complete susceptibility to the three pyrethroids tested, indicating the involvement of cytochrome P450 in observed pyrethroid resistance.

1. Introduction

Globally, vector control has significantly contributed to reducing malaria morbidity and mortality (Bhatt, 2015) and accounts for most of the estimated costs required to implement the World Health Organization's (WHO's) *Global Technical Strategy for Malaria 2016–2030* (WHO, 2016).

Insecticide-based interventions, namely indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs), are the core vector control interventions for malaria prevention. Since 2006, the U.S. President's Malaria Initiative has supported IRS in selected districts of Mainland Tanzania and Zanzibar. The recent success in malaria control in Mainland Tanzania—where malaria has been reduced by 50% over the past decade—as well as in other sub-Saharan African countries, has been largely attributed to the scale-up of such vector control interventions (Lim et al., 2011; WHO, 2015). The mosquito populations targeted by these interventions to reduce malaria transmission often rely on the use of four classes of insecticides—pyrethroids, carbamates, organochlorines, and organophosphate insecticides—all of which are also used for agricultural purposes. The effectiveness of current malaria vector control interventions depends entirely on the continued susceptibility of *Anopheles* mosquitoes to these four classes of insecticides.

The dramatic increase of insecticide resistance in mosquitoes throughout sub-Saharan Africa, including Tanzania (Kabula et al., 2013; Kisinza et al., 2018), poses a threat to the sustainability of insecticidal vector control methods (Ranson et al., 2011). Insecticide resistance creates significant challenges to malaria vector control interventions in several areas of the world, especially in sub-Saharan Africa, where widespread resistance to the four classes of insecticides has been (Antonio-Nkodjio et al., 2015; Matowo et al., 2014; Nkya et al., 2014). Additionally, variations in the impact of interventions on individual mosquito vectors due to differences in species susceptibility to insecticides can result in the more efficient killing of specific species while altering the composition of others. As a result, the overall effectiveness of interventions against the remaining vectors may change over time, necessitating alternative or supplementary interventions. Therefore, it is essential to systematically track vector species and their characteristics and to monitor interventions to identify modifications that might be required in vector control strategies. Understanding the local malaria vector species, their behavior, and their disease incrimination is vital for planning effective malaria control interventions.

The United States Agency for International Development (USAID)–funded *Okoa Maisha Dhibiti Malaria* (OMDM)/Save Lives, End Malaria Activity implements malaria entomological monitoring activities in Mainland Tanzania and Zanzibar. The activities are implemented in partnership with the National Institute for Medical Research (NIMR) Mwanza Centre, NIMR Amani, and the Zanzibar Malaria Elimination Programme (ZAMEP). This report covers the entomological monitoring activities that OMDM conducted during its first year (Year 1), from October 2018 through September 2019. Primary entomological monitoring objectives included the following:

- To determine malaria vector bionomics and IRS quality monitoring in Mainland Tanzania and Zanzibar. This effort involved the following:
 - Assessing malaria vector density and species composition in intervention and selected control areas;
 - Understanding vector preference for feeding times and locations and estimating human biting rates (HBRs);

- Assessing the impacts of IRS on the lifespan of malaria vectors through ovary dissection for parity;
- Monitoring the quality of insecticide application and insecticide decay rates; and
- Determining sporozoite rates and blood-meal sources.
- To detect and monitor insecticide resistance in malaria vectors, which involved learning the
 - Susceptibility status of malaria vectors to insecticides commonly used in public health;
 - Susceptibility status of malaria vectors to the new IRS insecticide clothianidin (SumiShield™);
 - Pyrethroid resistance intensity in malaria vectors; and
 - Mechanisms of insecticide resistance.

2. Entomological Monitoring in Mainland Tanzania

2.1 Malaria Vector Bionomics and IRS Quality Monitoring

The NIMR Mwanza Centre led entomological monitoring activities, for Mainland Tanzania. The four objectives of the entomological monitoring were to:

1. Identify malaria vector species in IRS intervention and control districts;
2. Assess the vector ecology (density, distribution, and seasonality) in IRS intervention and control sentinel sites;
3. Monitor vector feeding and resting behavior; and
4. Assess insecticide residual efficacy post-IRS through cone wall bioassays.

2.1.1 Methods

Study sites

Entomological data were collected from October 2018 through September 2019 from 11 districts (one sentinel site in each district) in Mainland Tanzania's Lake Zone. Eight sites were IRS sites with three control non-IRS sites. Entomological sentinel sites were selected based on the following criteria:

- Disease incidence and /or prevalence;
- The topography of the area;
- Agricultural practices (e.g., rain-fed rice, irrigation); and
- Urban or rural setting.

Figure 1 shows the geographical distribution of the study districts in the Lake Zone of Mainland Tanzania. **Table 1** highlights the summary of entomological activities conducted per sentinel district.

Figure 1. Eleven districts selected for entomological surveillance and insecticide residual efficacy monitoring of Mainland Tanzania's Lake Zone

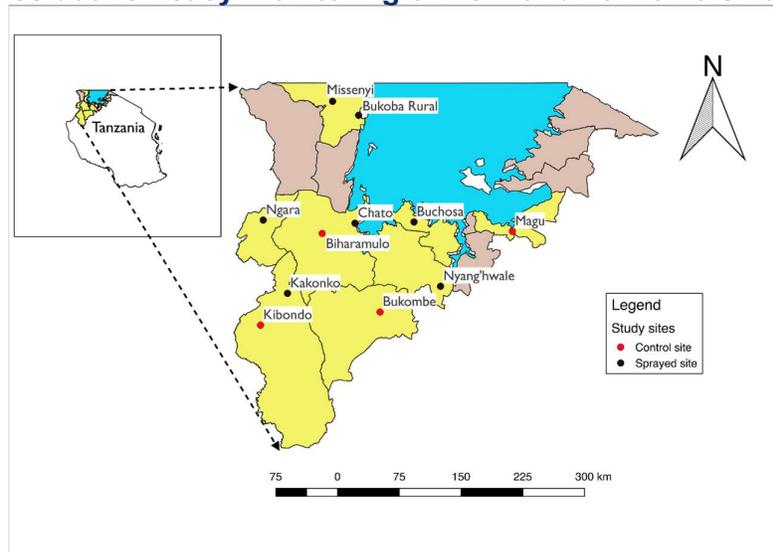


Table 1. Entomological surveillance and insecticide residual efficacy monitoring sites and activities in Mainland Tanzania's Lake Zone

Region	District	Sentinel Site Location	Sentinel Site Status	Intervention Status	Indicator Data Collected
Kagera	Ngara	Nterungwe	Existing	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Bukoba Rural	Kangabusharo	Existing	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Misenyi	Gabulanga	Existing	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Biharamulo	Kalebezo	Existing	Unsprayed control	Species composition, vector abundance, distribution and seasonality, and feeding time and location
Geita	Nyang'hwale	Izunya	Existing	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Chato	Nyamirembe	Existing	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location

Region	District	Sentinel Site Location	Sentinel Site Status	Intervention Status	Indicator Data Collected
	Bukombe	Lyambamgongo	Existing	Unsprayed control	Species composition, vector abundance, distribution and seasonality, and feeding time and location
Kigoma	Kakonko	Itumbiko	Proposed	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Kibondo	Rugunga	Proposed	Unsprayed control	Species composition, vector abundance, distribution and seasonality, and feeding time and location
Mwanza	Buchosa	Kalebezo	Proposed	SumiShield 50WG sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Magu	Kipeja	Proposed	Unsprayed control	Species composition, vector abundance, distribution and seasonality, and feeding time and location

Rearing of susceptible *An. gambiae* s.s. (Kisumu strain)

The NIMR Mwanza Centre insectary reared adult *An. gambiae* s.s. (susceptible Kisumu strain) according to standard protocols in sufficient numbers to meet the demands of monthly cone wall bioassay field activities. The adult mosquito rooms were maintained at 27±1 degree Celsius (°C) and 60%–80% relative humidity; the larval rooms were maintained at 30±1°C and 60%–80% relative humidity.

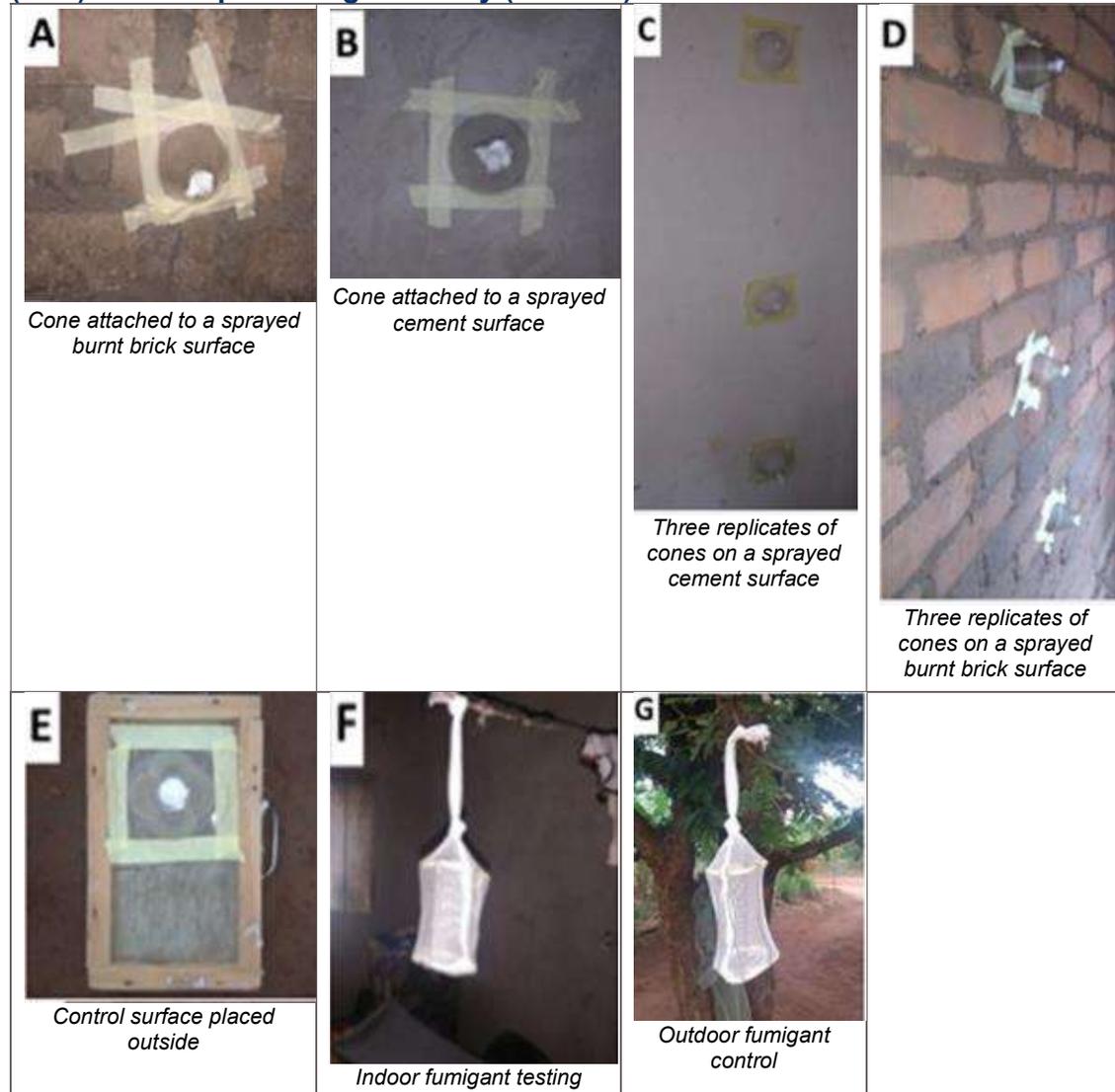
Insecticide residual efficacy monitoring

Cone bioassays were conducted as per standard WHO protocols (1981). The tests were carried out by using 2- to 5-day-old, sucrose-fed, laboratory-reared, known susceptible *An. gambiae* s.s. mosquitoes. Batches of 10 mosquitoes were exposed for 30 minutes inside a WHO plastic cone on sprayed wall surfaces in each of the rooms and houses sampled (**Figure 2**). Generally, clothianidin is regarded as a slow-acting insecticide. As a result, the usual WHO protocol for cone bioassays was modified so that mortality was recorded every 24 hours for 6 consecutive days after insecticide exposure lasting 30 minutes. For the initial IRS quality assessment, three locations on walls were sampled: low (0.5 meters [m] above the floor), middle (1.0 m above the floor), and upper levels (1.5 m above the floor). After the initial IRS quality assessment, only one room per household was sampled to measure insecticide residual efficacy, with only two locations on walls sampled: low (0.5 m above the floor) and upper level (1.5 m above the floor).

A control cone bioassay was conducted for every house tested by exposing mosquitoes to an unsprayed surface of a similar wall material. Some insecticide formulations used for IRS, such as clothianidin, have a fumigant airborne effect that can last for several months after spraying. A strong fumigant effect in sprayed rooms can result in cone bioassays on unsprayed walls showing 100% mosquito mortality. As a result, to avoid the possibility of control mortality increasing because of clothianidin's fumigant effect, bioassays on the unsprayed portable surface were conducted in the shade outside sprayed houses (**Figure**

E). To test the airborne efficacy of clothianidin, fumigant assays using nets suspended in a wire cylinder were conducted in all villages inside and outside of sampled houses and used 10 female mosquitoes per assay (**Figure F** and **3G**).

Figure 2. Cone bioassays conducted on different wall surfaces during testing (A–E) and setup of fumigant assay (F and G)



At the end of each bioassay, mosquitoes were transferred via aspirator to paper cups containing a 10% glucose solution. Cups were placed in a cool box and covered with a wet towel. The team assessed knockdown 60 minutes post-exposure. A mosquito was considered to be alive if it could fly. Final mortality was scored 6 days after initial exposure. When control mortality was between 5% and 20%, experimental mortality was corrected using Abbott's formula (1925).

Vector ecology

The monitoring team conducted entomological surveillance to determine vector ecology in IRS and non-IRS sites. Sampling methods used across all Mainland Tanzania sites included

Centers for Disease Control and Prevention (CDC) light traps, clay pots, Prokopack aspirators, and CDC light traps with bottle rotator (CBR). Sentinel houses were selected randomly at the beginning of the surveillance period and used throughout the monitoring program. **Figure** shows each collection method in more detail. Adult mosquitoes collected via these methods were used to determine basic entomological indicators, including species distribution and abundance, resting behavior, feeding, and biting behavior, and seasonality (

Table).

The team identified collected specimens in the field and species-sorted mosquitoes according to standard morphological keys (Gillies et al., 1987). Sub-samples of host-seeking unfed female *An. gambiae* and *An. funestus* were dissected for determination of the parity rate. The sub-sample included 10% of all unfed *An. gambiae* and *An. funestus* collected by using all collection methods. Blood-fed female mosquitoes were independently preserved in filter paper wraps for determination of the blood-meal source, with the remainder preserved for further laboratory analysis, including species identification and detection of malaria infection.

Figure 3. Entomological sampling collection methods used in Mainland Tanzania



Clockwise from top left: CDC light traps, clay pots, CBR, and Prokopack aspirators

Table 2. Mosquito collection methods used for entomological surveillance in Mainland Tanzania

Collection Method	Purpose	Districts	Sites	Number of Households	Days per Month	Time (Hours)	Sample Processing (Identification of)			
							Species	Parous Rate	Sporozoite Rate	Blood Meal Source
CDC light trap	Indoor abundance	11	1 per District	2 Houses per site	28	1800–0600	✓	✓	✓	
Clay pot	Outdoor abundance	11	1 per District	2 Houses per site	28	1800–0600	✓	✓	✓	✓
Prokopack aspirator	Resting behavior	11	1 per District	5 Houses per site	10	0600–0800	✓	✓	✓	✓
CBR	Indoor and outdoor biting behavior	11	1 per District	2 Houses per site	10	1800–0600	✓	✓	✓	✓

CDC light traps

This collection method is used for indoor biting (endophagic) mosquitoes. The team conducted the study in two houses per night in each village (for mosquito collection) in a district; CDC light traps were placed for 28 consecutive nights each month. Traps were installed approximately 1.5 m above the floor, next to the head of a sleeping person(s) in the room. The person or people were requested to sleep overnight under an untreated mosquito net or nets provided by the project. CDC light traps were set-up to operate from 1800 to 0600 to trap mosquitoes. Captured mosquitoes were transferred into mosquito-holding cups.

Clay pot method

This collection method is used for outdoor resting mosquitoes. The team used locally made clay pots with a diameter of approximately 0.5 m, an opening of 20 centimeters (cm), and a 2-cm hole at the bottom, allowing rainwater to drain. Four clay pots were positioned outdoors overnight from 1800 to 0600; the same houses selected for CDC light trap collections were used for the clay pot studies. The pots were positioned at an inclined angle to let mosquitoes enter and rest inside the dark inner wall surface of the pot. At 0600, community mosquito collectors covered the opening with a piece of netting with a small hole into which they inserted an aspirator to suck out mosquitoes and individually transfer them into a mosquito-holding cup.

Prokopack aspirator

This collection method is used to sample indoor resting (endophilic) mosquitoes. The Prokopack aspirator model 1419 was used. Mosquitoes were collected from five selected houses (two of the houses selected for the CDC light trap collection and three additional randomly selected houses) for 10 days per month. Aspiration was conducted between 0600 and 0800. Aspiration of resting adults produced collections of both sexes and all physiological stages directly from their resting sites, allowing for better estimations of species diversity, abundance, sex ratio, and physiological status. The team used data sheets to record the number of people who slept in the house the previous night.

The monitoring team put the mosquitoes in well-labeled, moist petri dishes and took them to the field office where they were sorted morphologically by species. The team noted the abdominal status of all female anophelines that were collected and sorted the mosquitoes into the following categories: gravid, semi-gravid, unfed, and blood-fed females.

CDC light traps with bottle rotator (CBR)

This collection method is used to monitor indoor and outdoor mosquito biting times. One CBR each was placed both inside and outside of randomly selected houses.

CBR sampling was conducted 10 nights each month. The sampling was scheduled for nights near a new moon to minimize the moonlight's effect on the outdoor collection and to reduce bias when comparing species distribution across seasons. The team estimated the presence of moonlight and phase of the moon using a lunar calendar.¹ The team assumed that the mosquitoes that entered a trap during any hour during collection were those actively seeking hosts and, in most cases, would bite human hosts in the same hour and in the same room or house if the bed nets were absent. Indoor and outdoor human biting fractions and times of biting of the *Anopheles* mosquitoes were determined and recorded throughout the entire

¹ The moon phase lunar calendar (2018/2019) is an online lunar calendar similar to the one here: <http://www.timeanddate.com/calendar/moonphases.html>.

sampling period. CBRs were set indoors—with a person sleeping under an untreated net—and outdoors from 1800 to 0600; the collection of mosquitoes occurred at 1-hour intervals.

Morphological identification of mosquitoes

The team conducted morphological identification of the mosquitoes collected following the taxonomic keys of Gillies and Coetzee (1987) in the field and reconfirmed the identification at the laboratory. Based on morphological characteristics, *Anopheles* mosquitoes were sorted to generally known species (e.g., *An. gambiae* s.l., *An. funestus*, *An. coustani*, *An. pharoensis*). Identified *Anopheles* mosquitoes were preserved individually with silica gel in Eppendorf tubes, labeled, and then kept in the laboratory for further enzyme-linked immunosorbent assay (ELISA) and molecular analyses.

Laboratory analyses

An ELISA circumsporozoite assay was used to determine the sporozoite index in a sub-sample of collected *An. gambiae* s.l. (Burkot et al., 1984; Wirtz et al., 1987). A polymerase chain reaction (PCR)-based assay was used to identify sibling species existing within *An. gambiae* s.l. (Scott et al., 1993) and *An. funestus* s.l. mosquitoes (MR4). The PCR assay selected for species identification was based on the morphological identification of the specimen.

Additionally, the team performed blood-meal analysis by using direct ELISA to determine host preferences among the collected mosquitoes (Beier et al., 1988). The team obtained mosquitoes for blood-meal analysis from clay pots, Prokopack aspirators, and CBR.

Data analysis

Vector density was calculated as the number of adult female vectors collected per sampling method and unit time. HBR was determined as the proportion of adult female vectors that attempted to feed or were freshly blood-fed per person, per unit time. Biting time was estimated as the number of adult female vectors that attempted to feed or were freshly blood-fed per person, per unit time expressed as 1-hour increments. Indoor resting density was calculated as the proportion of adult female vectors collected resting either indoors or outdoors.

2.1.2 Results

Insecticide residual efficacy monitoring

Clothianidin is a slow-acting insecticide whose maximum killing effect on mosquitoes is measured 6 days after initial exposure of the susceptible mosquito on the sprayed surface. Cone bioassays using the susceptible Kisumu strain of *An. gambiae* showed complete mortality 6 days post-exposure for up to 7 months on all wall surfaces in all sites. The wall surfaces assessed were cement, burnt brick, painted, mud, and whitewash. Ten months post-spraying with clothianidin, all surface types continued to retain an effective insecticide above the WHO recommended threshold. At -10 months post-IRS, mud surfaces effectively retained the insecticide with an average mortality of 86% on Day 6 of the bioassay. Other surfaces recorded the following mortality rates: cement (85%), burnt brick (84%), painted (84%), and whitewash (82%). The fumigant effect of clothianidin remained high up to 5 months post-spraying. The fumigant effect showed significant decline in the mortality at 10 months after IRS when the mortality was less than 80% on all surfaces. The detailed results per site and per wall surface are shown in **Annexes I and II**.

Vector ecology

Abundance, distribution, and species composition in Mainland Tanzania

A total of 14,035 female *Anopheles* mosquitoes were collected by using all collection methods from the period of October 2018 through September 2019. Of these mosquitoes, 9,183 (65.4%) were morphologically identified as *An. gambiae* s.l.; 2,597 (18.5%) as *An. funestus* s.l.; 1,896 (13.5%) as *An. coustani*; 217 (1.5%) as *An. pharoensis*, and 142 (1%) as *An. rufipes*.

A total of 5,584 (39.8%) female *Anopheles* mosquitoes were collected by using CDC light traps; 4,694 (33.4%) by CBR; 1,922 (13.7%) by Prokopack aspirators; and 1,835 (13.1%) by clay pots. **Table 3** shows the number of *An. gambiae* s.l. and *An. funestus* s.l. by collection method and location, and **Table 4** displays the number of *An. coustani*, *An. pharoensis*, and *An. rufipes* collected from each site.

An. gambiae s.l. was the most abundant vector species sampled by all collection methods in each IRS district, except for Nyang'hwale and Kibondo where *An. funestus* s.l. was the most abundant.

Table 3. Number of *An. gambiae* s.l. and *An. funestus* s.l. by collection method and location

Location			CDC Light Trap		CBR		Prokopack		Outdoor Clay Pot		Total	
Region	District	Study Site	<i>An. gambiae</i> s.l. (n)	<i>An. funestus</i> s.l. (n)	<i>An. gambiae</i> s.l. (n)	<i>An. funestus</i> s.l. (n)	<i>An. gambiae</i> s.l. (n)	<i>An. funestus</i> s.l. (n)	<i>An. gambiae</i> s.l. (n)	<i>An. funestus</i> s.l. (n)	<i>An. gambiae</i> s.l. (n)	<i>An. funestus</i> s.l. (n)
Sprayed Sites												
Geita	Chato	Nyamirembe	631 (1.88)	76 (0.23)	1,753 (14.61)	267 (2.23)	380 (3.17)	56 (0.47)	481 (1.43)	70 (0.21)	3,245 (3.56)	469 (0.51)
	Nyang'hwale	Izunya	23 (0.07)	78 (0.23)	13 (0.11)	22 (0.18)	16 (0.13)	38 (0.32)	0 (0)	11 (0.03)	52 (0.06)	149 (0.16)
Kagera	Misenyi	Gabulanga	471 (1.4)	154 (0.46)	122 (1.02)	22 (0.18)	24 (0.2)	8 (0.07)	10 (0.03)	0 (0)	627 (0.69)	184 (0.2)
	Ngara	Nterungwe	16 (0.05)	6 (0.02)	22 (0.18)	17 (0.14)	2 (0.02)	0 (0)	11 (0.03)	1 (0)	51 (0.06)	24 (0.03)
	Bukoba Rural	Kangabusharo	42 (0.13)	2 (0.01)	134 (1.12)	11 (0.09)	29 (0.24)	0 (0)	36 (0.11)	0 (0)	241 (0.26)	13 (0.01)
Mwanza	Buchosa	Kalebezo	106 (0.33)	8 (0.03)	56 (0.49)	8 (0.07)	31 (0.27)	1 (0.01)	32 (0.1)	1 (0)	225 (0.26)	18 (0.02)
Kigoma	Kakonko	Itumbiko	1,088 (3.43)	352 (1.11)	263 (2.19)	138 (1.15)	299 (2.49)	135 (1.13)	356 (1.12)	168 (0.53)	2,006 (2.30)	793 (0.91)
Total Sprayed Sites			2,377 (7.19)	676 (2.04)	2,363 (19.81)	485 (4.07)	781 (6.55)	238 (2)	926 (2.8)	251 (0.76)	6,447 (7.17)	1,650 (1.83)
Unsprayed Sites												
Geita	Bukombe	Lyambamgongo	514 (1.53)	123 (0.37)	299 (2.49)	67 (0.56)	175 (1.46)	16 (0.13)	122 (0.36)	22 (0.07)	1,110 (1.22)	228 (0.25)
Kagera	Biharamulo	Kalebezo	358 (1.07)	98 (0.29)	473 (3.94)	192 (1.6)	282 (2.35)	96 (0.8)	135 (0.4)	35 (0.1)	1,248 (1.37)	421 (0.46)
Mwanza	Magu	Kipeja	247 (0.78)	12 (0.04)	53 (0.46)	21 (0.18)	30 (0.26)	1 (0.01)	0 (0)	0 (0)	330 (0.38)	34 (0.04)
Kigoma	Kibondo	Kitahana	46 (0.16)	243 (0.82)	1 (0.01)	19 (0.17)	1 (0.01)	2 (0.02)	0 (0)	0 (0)	48 (0.06)	264 (0.33)
Total Unsprayed Sites			1,165 (3.63)	476 (1.48)	826 (7.14)	299 (2.58)	488 (4.22)	115 (0.99)	257 (0.8)	57 (0.18)	2,736 (3.13)	947 (1.08)

Note: (n)=mean number of anopheles mosquitoes per trap night.

Table 4. Number of *An. coustani*, *An. pharoensis*, and *An. rufipes* collected by location

Region	District	Study Site	<i>An. coustani</i> (n)	<i>An. pharoensis</i> (n)	<i>An. rufipes</i> (n)
Sprayed Sites					
Geita	Chato	Nyamirembe	499	14	0
	Nyang'hwale	Izunya	14	0	0
Kagera	Misenyi	Gabulanga	709	3	0
	Ngara	Nterungwe	0	0	0
	Bukoba Rural	Kangabusharo	201	37	48
Mwanza	Buchosa	Kalebezo	10	4	0
Kigoma	Kakonko	Itumbiko	291	109	76
Total Sprayed Sites			1,724	167	124
Unsprayed Sites					
Geita	Bukombe	Lyambamgongo	11	2	0
Kagera	Biharamulo	Kalebezo	201	46	14
Mwanza	Magu	Kipeja	2	2	0
Kigoma	Kibondo	Kitahana	5	0	4
Total Unsprayed Sites			219	50	18

Molecular analysis for mosquito species composition and sporozoite rate

Mosquito species composition. The team analyzed a sub-sample of 4,373 female *Anopheles* mosquitoes by using PCR for species identification and ELISA for detection of sporozoites during the monitoring period (**Table**). PCR showed that the local vector population across sites was predominantly *An. arabiensis* (53%), *An. funestus* s.s. (27.1%), *An. gambiae* s.s. (13.9%), and *An. parensis* (2.3%). Out of the processed samples, 3.7% were not amplified by PCR (**Table**). Note that of the samples that were morphologically identified as *An. coustani*, *An. pharoensis*, and *An. rufipes* were not included in species identification by PCR because of a lack of their specific primers.

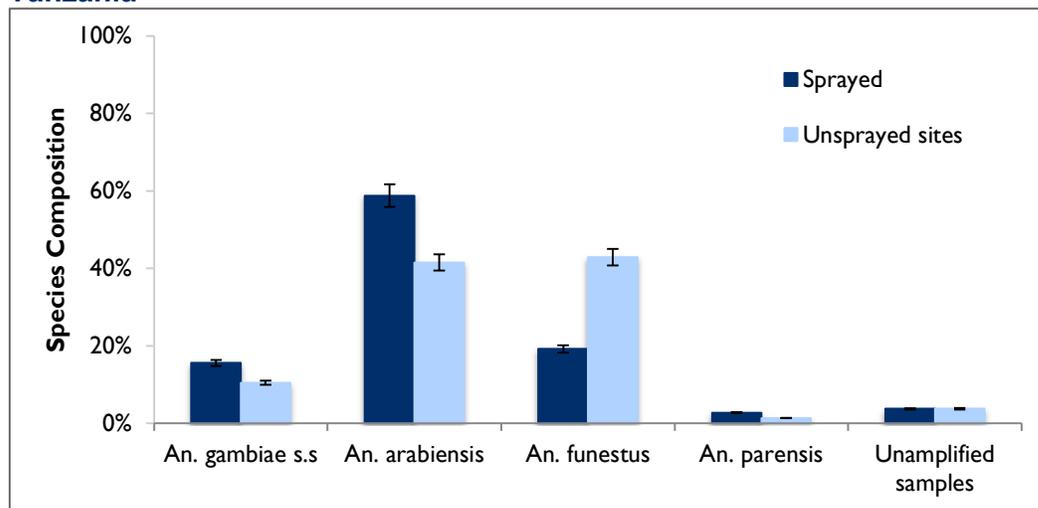
Sprayed sites. *An. arabiensis* was the most abundant species in four sites (i.e., Bukoba Rural, Chato, Kakonko, and Misenyi,) and *An. funestus* s.s. was the dominant species at three sites (Buchosa, Ngara, and Nyang'hwale). *An. parensis* was also identified at three sites (Bukoba Rural, Chato, and Kakonko).

Unsprayed sites. Although *An. parensis* was found at all sites except Magu, *An. arabiensis* was the predominant species in Bukombe and Magu, and *An. funestus* s.s. was the predominant species in Biharamulo and Kibondo. The proportions of species diversity in all sprayed and unsprayed site are presented in **Figure**.

Table 5. Species identification by PCR results and ELISA sporozoite results in sprayed and unsprayed districts in Mainland Tanzania

District	Species Identification PCR						ELISA Results		
	N Tested (n)	<i>An. gambiae</i> s.s. (n [%])	<i>An. arabiensis</i> (n [%])	<i>An. funestus</i> s.s. (n [%])	<i>An. parensis</i> (n [%])	Negative PCR (N [%])	Total Tested (n)	Number Positive (n)	Sporozoite Rate (%) (95% Confidence Interval [CI])
Sprayed Sites									
Ngara	30	3 (10)	6 (20)	19 (63.3)	0	2 (6.7)	30	0	0
Misenyi	594	247 (41.6)	257 (43.3)	85 (14.3)	0	5 (0.8)	594	16	2.7 (1.5–4.3)
Bukoba Rural	42	2 (4.8)	12 (28.6)	5 (11.9)	1 (2.4)	22 (52.4)	42	1	2.4 (0–12.5)
Chato	496	1 (0.2)	410 (82.7)	15 (3)	50 (10.1)	20 (4)	496	0	0
Nyang'hwale	111	5 (4.5)	14 (12.6)	78 (70.3)	0	14 (12.6)	111	1	0.9 (0–4.9)
Buchosa	187	40 (21.4)	66 (35.3)	69 (36.9)	0	12 (6.4)	187	1	0.5 (0–2.9)
Kakonko	1,451	155 (10.7)	946 (65.2)	288 (19.8)	29 (2)	33 (2.3)	1,482	22	1.5 (0.9–2.2)
Total—Sprayed Sites	2,911	453 (15.6)	1,711 (58.8)	559 (19.2)	80 (2.7)	108 (3.7)	2,942	41	1.4 (1.0–1.9)
Unsprayed Sites									
Biharamulo	510	117 (22.9)	96 (18.8)	250 (49)	14 (2.7)	33 (6.5)	510	19	3.7 (2.2–5.7)
Bukombe	485	4 (0.8)	298 (61.4)	179 (36.9)	2 (0.4)	2 (0.4)	485	6	1.2 (0.4–2.7)
Magu	239	7 (2.9)	200 (83.7)	24 (10)	0	8 (3.3)	239	6	2.5 (0.9–5.4)
Kibondo	228	25 (11)	13 (5.7)	174 (76.3)	4 (1.8)	12 (5.3)	288	3	1.3 (0.2–3.0)
Total—Non-sprayed Sites	1,462	153 (10.5)	607 (41.5)	402 (42.9)	20 (1.4)	55 (3.8)	1522	34	2.3 (1.6–3.2)
Total—All Sites	4,373	606 (13.9)	2,318 (53)	961(27.1)	100 (2.3)	163 (3.7)	4,404	75	1.7 (1.3–2.1)

Figure 2. Species composition in sprayed and unsprayed sites in Mainland Tanzania



Mosquito sporozoite rate. Overall, the sporozoite rates varied across sites, ranging from 0% to 2.7% at sprayed sites and 1.2% to 3.7% at unsprayed sites, with a mean sporozoite rate of 1.7% in the study area (**Table 5**). The mean sporozoite rate was considerably lower at 1.4% (95% Confidence Interval [CI]: 1–1.9) at the sprayed sites compared with 2.3% (95% CI: 1.6–3.2) at unsprayed sites. The team’s analysis indicated that *An. gambiae* s.s. had a higher sporozoite rate overall (2.8%), followed by *An. funestus* s.s. (2.1%; **Table**).

Table 6. Sporozoite results by mosquito species identified by PCR in Mainland Tanzania

Mosquito Species	Number of Samples Analyzed	Number of Positive Sporozoite	Sporozoite Rate (%)
<i>An. gambiae</i> s.s.	606	17	2.8
<i>An. arabiensis</i>	2,318	33	1.4
<i>An. funestus</i> s.s.	1,186	25	2.1
<i>An. parensis</i>	100	0	0
Unidentified by PCR	163	0	0

As shown in **Table** , the sporozoite rate was greater in unsprayed sites compared with sprayed sites for all species combined.

Table 7. Species sporozoite results in sprayed and non-sprayed sites in Mainland Tanzania

Mosquito Species	Spray Status	Number of Samples Analyzed	Number of Positive Sporozoite	Sporozoite Rate % (95% CI)	P Value
<i>An. gambiae</i> s.s.	Sprayed	453	12	2.6 (1.4–4.6)	0.6487
	Non-sprayed	153	5	3.3 (1.1–7.5)	
<i>An. arabiensis</i>	Sprayed	1,711	20	1.2 (0.7–1.8)	0.1093
	Non-sprayed	607	13	2.1 (1.1–3.6)	
<i>An. funestus</i> s.s.	Sprayed	559	9	1.6 (0.7–3.0)	0.2336
	Non-sprayed	627	16	2.6 (1.5–4.1)	
<i>An. parensis</i>	Sprayed	80	0	0	Not applicable
	Non-sprayed	20	0	0	
Unidentified by PCR	Sprayed	108	0	0	Not applicable
	Non-sprayed	55	0	0	

Biting and resting behavior in Mainland Tanzania

Indoor biting rates of An. gambiae s.l. and An. funestus s.l.

Sprayed districts. There was a general indoor biting rate (IBR) decrease in all sprayed sentinel sites following IRS operations from October 2018 through September 2019. However, IBRs increased in Kakonko—the highest increase to date—reaching up to 15 bites per person per night between March and May 2019. Misenyi had the highest IBR peak in June 2019 with 5.3 bites per person per night (**Figure** and **Table**).

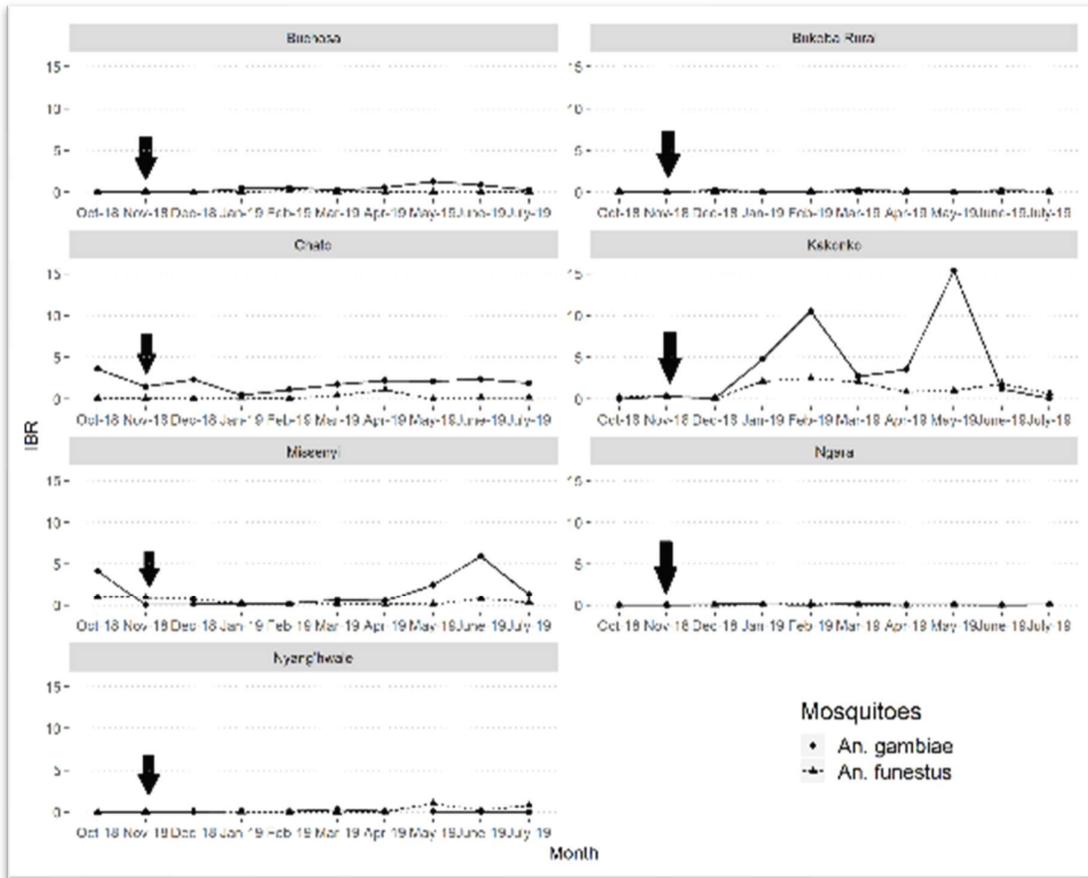
Table 8. Biting rates for mosquitoes collected by CBR

District	Indoor CBR		Outdoor CBR	
	Number of <i>An. gambiae</i> s.l. N (n) ^a	Number of <i>An. funestus</i> s.l. N (n) ^a	Number of <i>An. gambiae</i> s.l. N (n) ^a	Number of <i>An. funestus</i> s.l. N (n) ^a
Ngara	11 (0.09)	8 (0.07)	11 (0.09)	9 (0.08)
Misenyi	108 (0.85)	22 (0.18)	14 (0.12)	0
Bukoba Rural	18 (0.15)	0	116 (0.97)	11 (0.09)
Chato	612 (5.1)	102 (0.85)	1141 (9.51)	165 (1.38)
Nyang'hwale	12 (0.1)	18 (0.15)	1 (0.01)	4 (0.03)
Buchosa	14 (0.12)	8 (0.07)	42 (0.36)	0
Kakonko	166 (1.38)	85 (0.71)	97 (0.81)	53 (0.44)
Biharamulo ^b	349 (2.91)	172 (1.43)	124 (1.03)	20 (0.17)
Bukombe ^b	221 (1.84)	43 (0.36)	78 (0.65)	24 (0.2)
Magu ^b	43 (0.38)	21 (0.18)	10 (0.09)	0
Kibondo ^b	0	7 (0.06)	1 (0.01)	12 (0.11)
	1,554 (13.17)	486 (4.12)	1,635 (13.86)	298 (2.53)

^a N (n) = The number of mosquitoes collected (mean number per trap night).

^b Control sites.

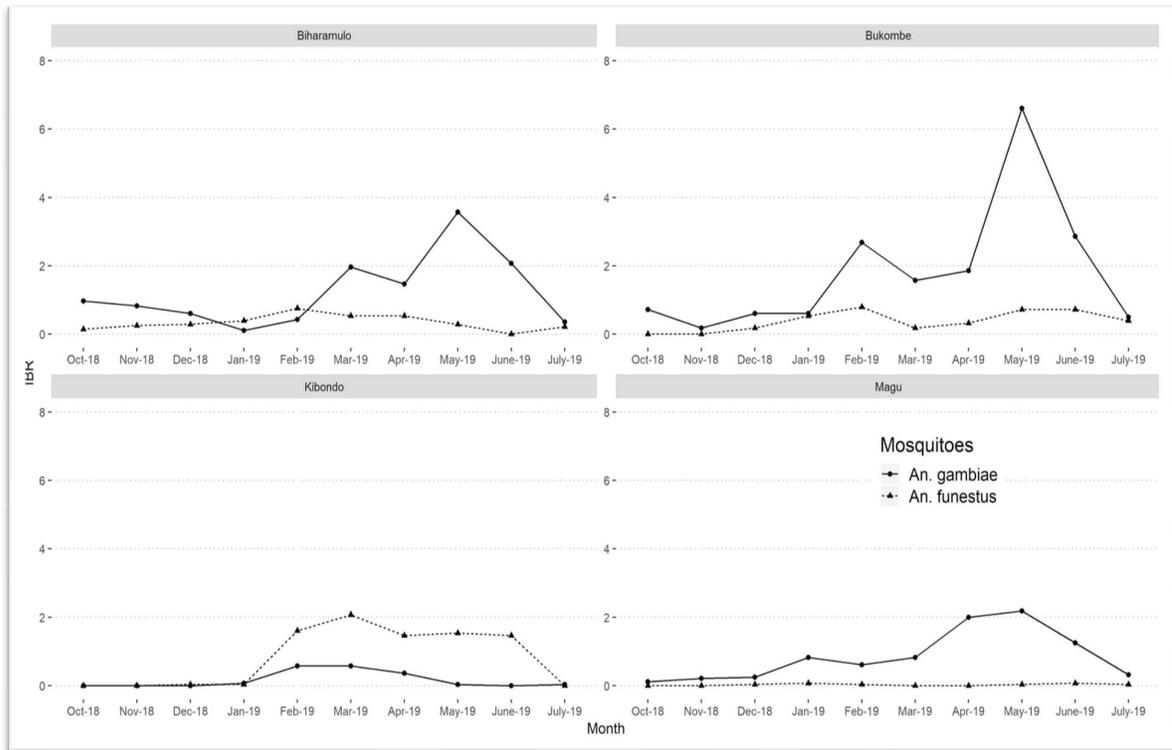
Figure 3. Monthly IBRs by using CDC light traps of *Anopheles* mosquitoes in sprayed districts (n=7) in Mainland Tanzania



Note: Arrows indicate the month when the IRS operations were carried out.

Unsprayed districts. In unsprayed districts, the IBR for both *An. gambiae* s.l. and *An. funestus* s.l. increased at all sites, especially starting in January 2019, because of prolonged rainfall between November and December 2018 (Figure).

Figure 6. Monthly IBRs by using CDC light traps of *Anopheles* mosquitoes in unsprayed districts in Mainland Tanzania

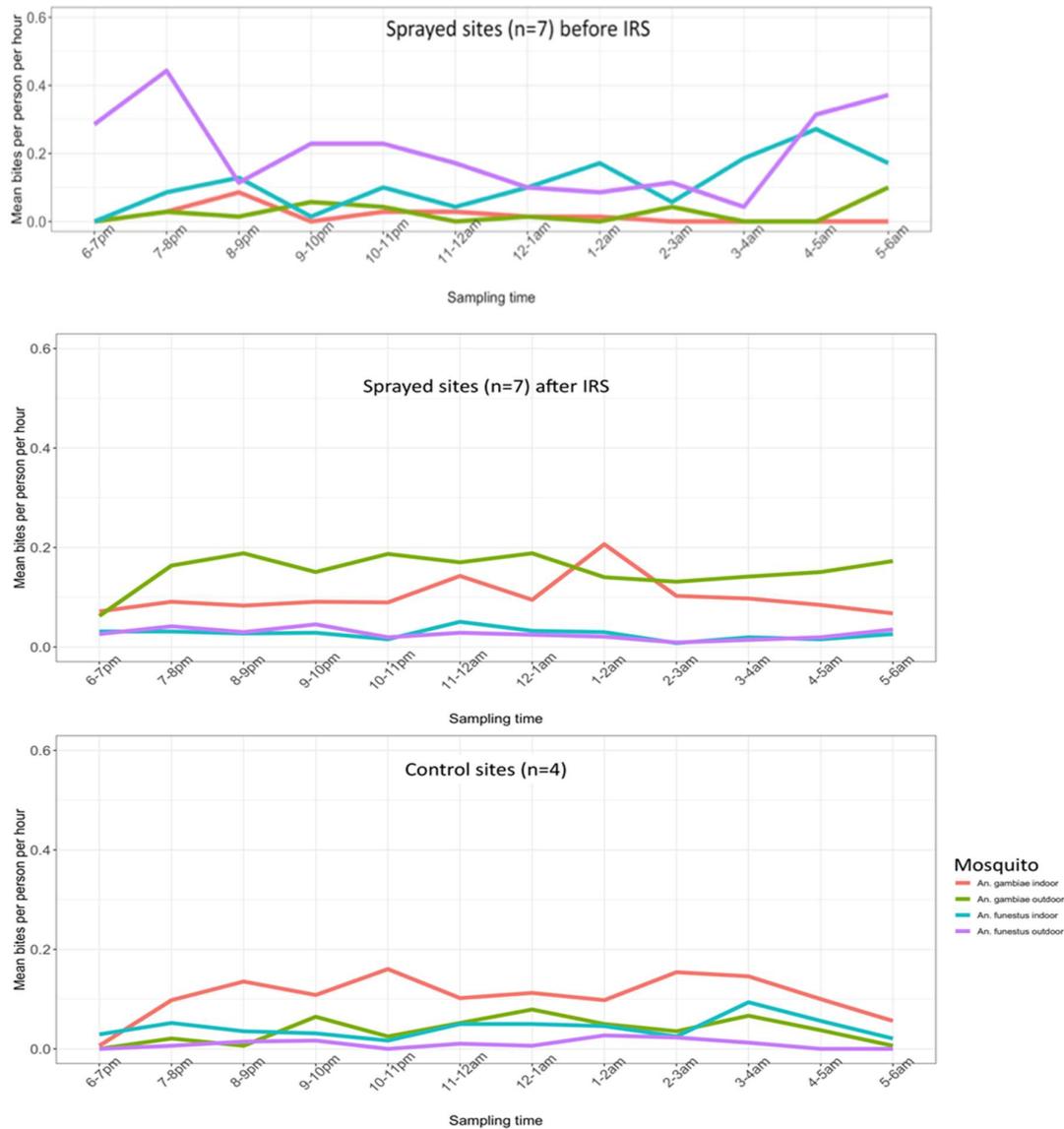


Biting times of An. gambiae s.l. and An. funestus s.l.

Sprayed sites before and after IRS. There were higher numbers of *An. funestus* s.l. before IRS, peaking between 0400 and 0500 indoors and 0700 and 0800 outdoors. This trend dropped after IRS (Figure). However, the team still recorded a considerable number of indoor biting *An. gambiae* s.l., peaking between 0100 and 0200 after IRS.

Unsprayed sites. *An. gambiae* s.l. biting rates were higher indoors because of the absence of insecticides. Higher biting rates of *An. gambiae* s.l. were also exhibited outdoors likely due to people staying outside for longer hours or the mosquitoes exhibiting zoophilic behavior. Both indoor and outdoor biting rates remained relatively constant throughout the night for *An. funestus* s.l.

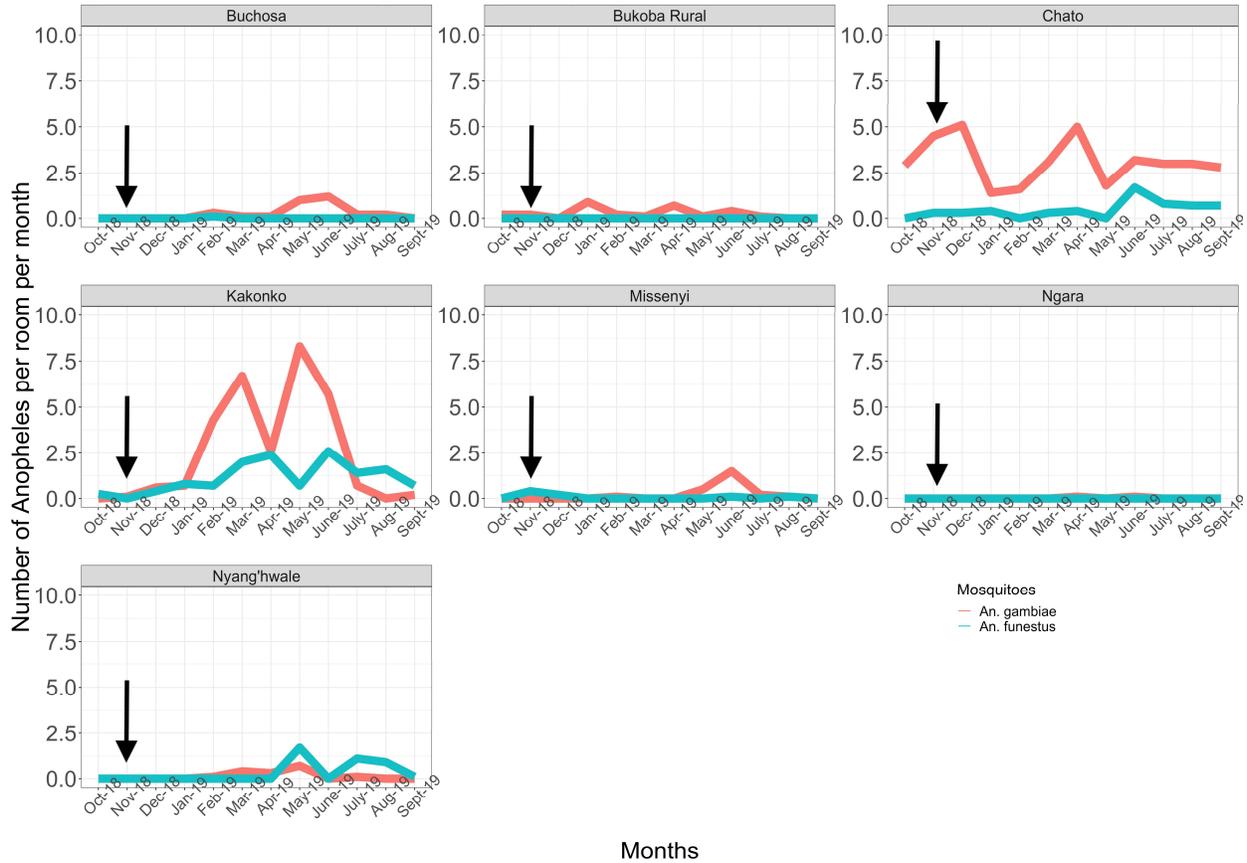
Figure 7. Mean indoor and outdoor biting rates of *Anopheles* collected by CBR in sprayed and unsprayed districts



Indoor resting density of *An. gambiae* s.l. and *An. funestus* s.l.

In sprayed districts, indoor resting density remained low following IRS with the exception of the Chato and Kakonko districts. In Kakonko, there was an increase in indoor resting density 2 months post-IRS; in Chato, increases were observed both pre- and post-IRS. Moreover, there was consistent low indoor resting density from October 2018 through May 2019 at all sites except Kakonko District (Figure).

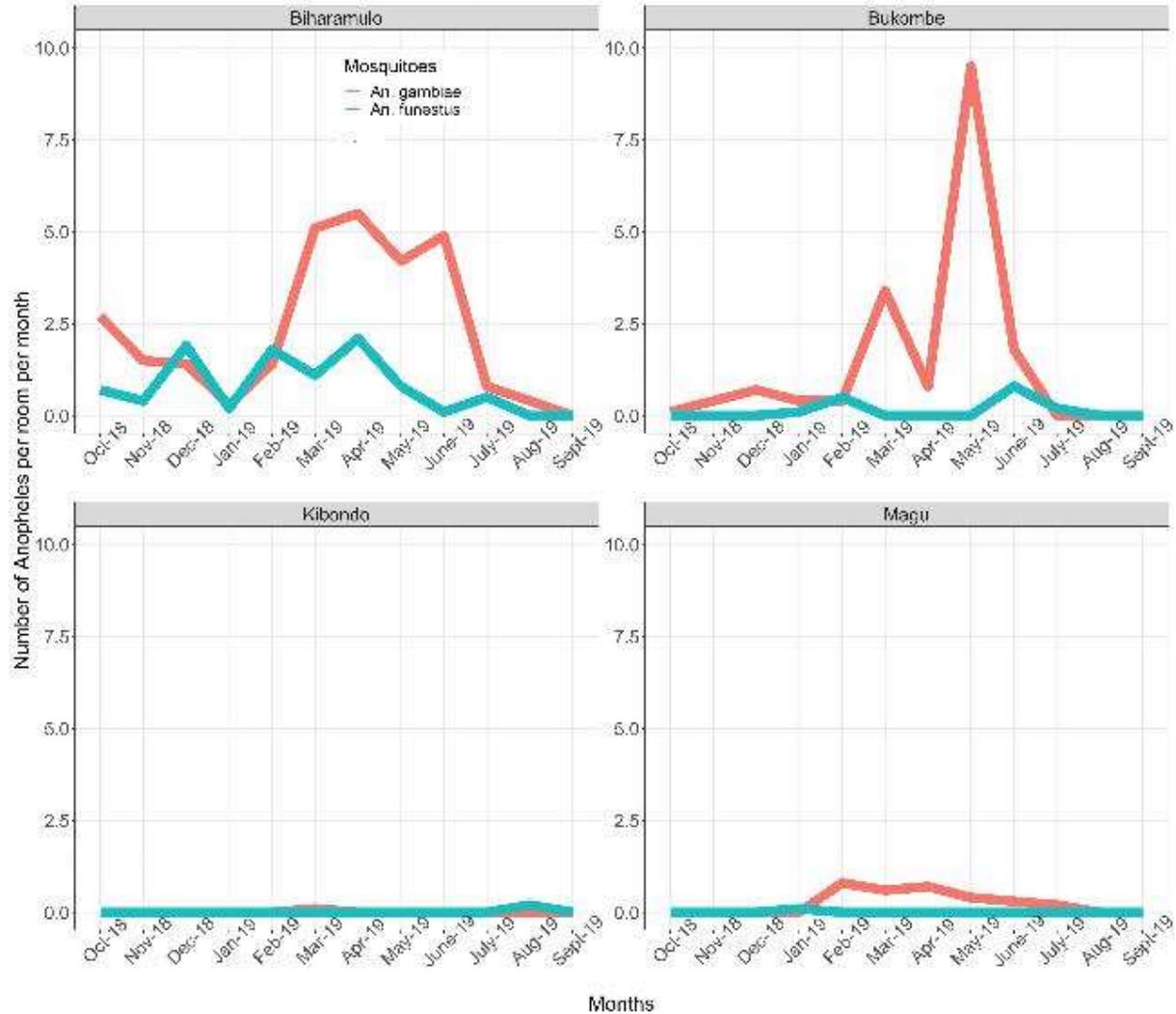
Figure 4. Indoor resting density in sprayed districts



Note: Arrows indicated the period before IRS operations.

Throughout the monitoring period, the indoor resting density was higher in non-IRS districts compared with IRS districts, except for Magu and Kibondo. Bukombe District recorded the highest indoor resting density when compared with other sites (**Figure 5**).

Figure 5. Indoor resting density in four unsprayed districts



Host preference

The team tested a total of 1,061 *Anopheles* from IRS and non-IRS districts to identify vertebrate host blood source (i.e., human, cow, goat, or dog). Overall, the proportion of *Anopheles* that fed on humans, including mixed-blood meals on humans and other animals, was 52.2% in IRS districts and 58.1% in non-IRS districts.

Parous rates

A total of 6,055 *Anopheles* (43.1% of mosquitoes collected during the surveillance period) were dissected to estimate their parity. The average parity in *Anopheles* mosquitoes before IRS was 61.4% and reduced to 22.69% after IRS. However, before IRS, there were no mosquitoes for parity dissections in five (Table).

Table 9. Parity rates in IRS and non-IRS sites

Region	District	Study Site	Parity Rate % (Number of Mosquitoes Dissected and Examined)	
			Before IRS	After IRS
Sprayed Sites				
Geita	Chato	Nyamirembe	62.5 (104)	28.21 (1,260)
	Nyang'hwale	Izunya	Not applicable	9.95 (63)
Kagera	Misenyi	Gabulanga	Not applicable	0.5 (402)
	Ngara	Nterungwe	Not applicable	69.71 (48)
	Bukoba Rural	Kangabusharo	55.6 (9)	16.78 (123)
Mwanza	Buchosa	Kalebezo	Not applicable	22.36 (178)
Kigoma	Kakonko	Itumbiko	Not applicable	11.3 (1,830)
Total			61.4 (114)	22.69 (3,904)
Unsprayed Sites				
Geita	Bukombe	Lyambamgongo	8.48 (832)	
Kagera	Biharamulo	Kalebezo	39.42 (895)	
Mwanza	Magu	Kipeja	10.23 (275)	
Kigoma	Kibondo	Kitahana	8.33 (35)	
Total			16.61 (2,037)	

Note: "Not applicable" means that no parity dissections were conducted because there were no mosquitoes in the collections in these sites before IRS.

2.2. Detection and monitoring of insecticide resistance in malaria vectors

This activity was conducted by NIMR Amani for Mainland Tanzania. The study aimed to monitor insecticide resistance among malaria vectors to selected insecticides in 22 sentinel districts in Mainland Tanzania. The specific objectives of these monitoring activities were to determine the following:

1. Susceptibility status of *An. gambiae* s.l. to deltamethrin (0.05%), permethrin (0.75%), bendiocarb (0.1%), dichlorodiphenyltrichloroethane (DDT; 4%) and pirimiphos-methyl (0.25%) in selected sentinel sites;
2. Susceptibility status of *An. gambiae* s.l. to the new IRS insecticide clothianidin (SumiShield) used for IRS in seven districts of Mainland Tanzania;
3. Pyrethroid resistance intensity in *An. gambiae* s.l. in the districts recorded to have high permethrin and deltamethrin resistance;
4. Involvement of mixed function oxidases in the observed phenotypic resistance determined by conducting PBO synergist bioassays in selected sentinel sites with pyrethroid resistance;
5. Distribution of malaria vector species by using molecular techniques; and
6. Resistance mechanisms by using PCR.

2.2.1 Methods

Study design

This study was a cross-sectional countrywide survey conducted between April and June 2019 using 22 established sentinel districts in Mainland Tanzania. The survey aimed to detect and monitor the susceptibility status of malaria vectors to insecticides, establish species composition of local malaria vectors, and determine the underlying mechanisms of resistance. Before conducting surveillance activities, a one-week training workshop to equip the data collectors (field workers and district malaria vector surveillance officers) with basic knowledge on malaria entomology; mosquito collection techniques; field susceptibility tests of malaria vectors; and rearing, preserving, and transporting samples for laboratory analyses and molecular characterizations.

Context and sites selection

Sentinel districts for insecticide resistance monitoring in Mainland Tanzania were selected following WHO-recommended selection criteria. OMDM also prioritized the following criteria:

- Evidence of presence or absence of insecticide resistance detected by previous surveys;
- The use of insecticides for IRS (including all IRS sites);
- Districts bordering other countries with known insecticide resistance; and
- Malaria endemicity in the area (priority was given to zones with the highest malaria burden).

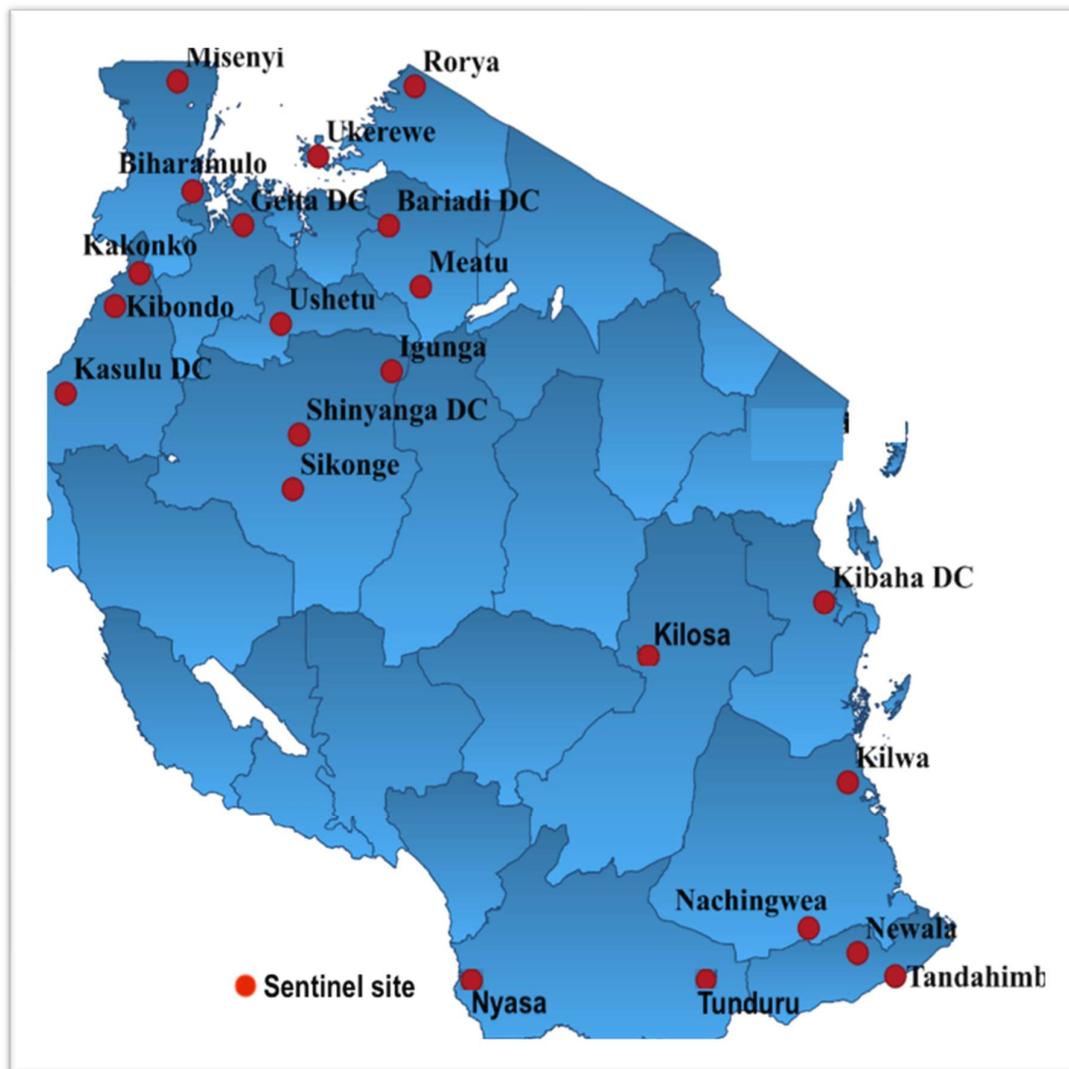
Table and

Figure 6 highlight the selected districts for Mainland Tanzania.

Table 10. 2019 sentinel districts for insecticide resistance monitoring in Mainland Tanzania

S/N	Region	District	S/N	Region	District
1	Tabora	Igunga	12	Kigoma	Kakonko
2	Tabora	Sikonge	13	Kigoma	Kibondo
3	Pwani	Kibaha District Council	14	Kigoma	Kasulu
4	Simiyu	Bariadi District Council	15	Mtwara	Newala
5	Simiyu	Meatu	16	Mtwara	Tandahimba
6	Lindi	Kilwa	17	Ruvuma	Nyasa
7	Lindi	Nachingwea	18	Ruvuma	Tunduru
8	Mara	Rorya	19	Shinyanga	Shinyanga District Council
9	Geita	Geita District Council	20	Shinyanga	Ushetu
10	Mwanza	Ukerewe	21	Morogoro	Kilosa
11	Kagera	Misenyi	22	Kagera	Biharamulo

Figure 6. Distribution of sentinel districts for insecticide resistance monitoring in Mainland Tanzania



Training of field workers and malaria focal persons

Training objectives

Surveillance is typically preceded by a hands-on training workshop to harmonize surveillance techniques, tools, and standard operating procedures. NIMR Amani, in collaboration with the National Malaria Control Programme (NMCP), conducted a 3-day hands-on training workshop April 15–17, 2019, for field implementers on the standardized protocols for malaria entomological vector surveillance at the Amani Medical Research Centre in Muheza, Tanga.

The training’s main objective was to provide basic entomological skills to conduct insecticide resistance monitoring of malaria vectors, including mapping and characterizing breeding sites; larval collecting and rearing; adult mosquito collecting and morphological identification; vector density; susceptibility testing; insecticide resistance intensity testing, including PBO-

pyrethroid synergy testing; malaria vector control; and, resistance management. The training also focused on providing a basic overview and skills in specimen preservation and transportation of mosquito samples for laboratory analyses and molecular characterization. Targeted participants included research scientists, field workers, technical staff, and district mosquito vector control practitioners.

Training manual and materials

NIMR and the NMCP's medical entomologists and vector control specialists updated the training manual *Malaria Entomological Surveillance and Insecticide Monitoring* before the April 2019 training. The training manual was prepared according to WHO's standards and adapted from standard WHO training materials, as well as from the Global Plan for Insecticide Resistance Management, including the new version of testing procedures for insecticide resistance monitoring in malaria vector mosquitoes. Topics covered in the manual include an introduction to malaria entomology, identification of malaria vectors, sampling and preservation techniques of malaria vectors, and detection and monitoring susceptibility of malaria vectors to insecticides used for malaria control. Additionally, trainees received instruction on insecticide resistance intensity testing, as well as malaria vector control and resistance management.

Course participants

Facilitators. Facilitators for the training were researchers and lecturers from local institutions in Tanzania, including the NMCP, NIMR, and the University of Dar es Salaam.

Trainees. In 2019, 30 participants from district and municipal councils and research institutions in Tanzania attended the training, including NIMR Amani (16), NIMR Tabora (1), NIMR Tukuyu (2), district councils from sentinel sites (6), University of Dar es Salaam (2), Muheza Vector Control Training School (1), and the Tropical Pesticides Research Institute (2). Course participants included research scientists, laboratory scientists, district vector control officers, and field entomologists.

Course content and training approaches

The training began with introductory lectures on mosquito vectors, ecology, population biology, and control techniques. This was immediately followed by theory and practical demonstrations on larval and adult mosquito sampling and identification techniques, molecular identification techniques, and insecticide resistance monitoring and management. Facilitators also covered vector mapping and data management and via lecture format that was supplemented with exercises where participants could practice testing insecticide resistance and susceptibility using CDC bottle bioassays and synergists.

Mosquito collections in the field

The field team conducted mosquito larvae searches at the selected sites. They carefully collected *Anopheles* larvae using a 350-milliliter (ml) dipper and transferred the larvae into plastic containers, which were then loosely capped to allow aeration. These containers were transported in cool boxes to the field laboratory where the larvae were reared at 27°C–30°C. Larvae collected from several breeding sites in the same village were pooled together for rearing and testing and fed with TetraMin® fish food. The team regularly monitored larvae development and transferred the pupae into shallow plastic cups and small beakers using Pasteur pipettes. The samples were then placed in appropriately labeled cages for adult emergence. Female adult mosquitoes aged 2 to 5 days were used for WHO susceptibility tests and PBO synergy testing. Geographic coordinates of each sampling site were recorded using calibrated smart phones.

WHO insecticide susceptibility testing in the field

Susceptibility tests were carried out using the WHO's test kits for adult mosquitoes (WHO, 2016), comprising insecticide impregnated test papers and non-impregnated papers for control and plastic tubes marked red for exposure and green for holding. Two- to 5-day old female mosquitoes were tested using standard WHO insecticide susceptibility procedures with four replicates of 15 to 25 wild adult female mosquitoes per test tube. Mosquitoes were exposed to papers impregnated with the WHO-recommended concentrations of deltamethrin (0.05%), bendiocarb (0.1%), permethrin (0.75%), DDT (4%), and pirimiphos-methyl (0.25%) prepared at Universiti Sains Malaysia (WHO, 2016). During the exposure period, knockdown rates were recorded after exposure times of 10, 15, 20, 30, 40, 50, and 60 minutes for pyrethroids and DDT. The time for mosquito knockdowns was recorded at pre-determined intervals during exposure to the pyrethroid insecticides only. A mosquito was considered knocked down if it laid on its side at the bottom of the exposure tube and was unable to fly.

At the end of the exposure period, mosquitoes were transferred into holding tubes lined with untreated papers by gently blowing them through the open space between the exposure and holding tubes. A cotton ball soaked in 10% sugar was placed on top of the holding tube to avoid mosquito death by starvation. The mortality was scored 24 hours after exposure. The susceptibility status was evaluated based on the following WHO criteria: 98%–100% mortality indicates susceptibility; 90%–97% mortality requires additional confirmation; and < 90% mortality indicates resistance (WHO, 2016). When control mortality between 5% and 20% was recorded, the mean observed mortality was corrected using Abbott's formula (Abbott, 1925).

Susceptibility testing of malaria vectors to clothianidin

Whatman® No. 1 filter papers measuring 12 cm by 15 cm were treated with the diagnostic dose of clothianidin (2% weight/volume) of 13.2 milligrams (mg) of active ingredient per paper, equivalent to 734 mg active ingredient per meter squared (ai/m²). For one paper, 26.4 mg of SumiShield 50WG (containing 50% clothianidin) was suspended in 2 ml of distilled water; the resulting suspension was shaken well before pipetting it onto the filter paper. After evenly treating each filter paper, the papers were left to dry at room temperature and later wrapped in aluminum foil and stored in the refrigerator at 4°C. Filter papers were freshly prepared for each test. An untreated filter paper pipetted with 2 ml of distilled water was used as the negative control.

Treated filter papers were inserted into WHO plastic tubes and tested according to standard WHO susceptibility test protocols. Exposure time was 60 minutes, after which mosquitoes were transferred into the untreated holding tubes and provided with lightly moistened cotton wool containing 10% sugar water. A fresh moistened cotton wool containing 10% sugar was changed daily. Knockdown rates were recorded halfway through the test (i.e., 30 minutes) and again at 60 minutes. Mortality was recorded on Days 1–6 after exposure. The negative control mortality was similarly recorded on Days 1–6 to calculate mortality. The testing and post-exposure holding temperature was 27°C ± 2°C and relative humidity of 75% ± 10%.

Insecticide resistance intensity testing

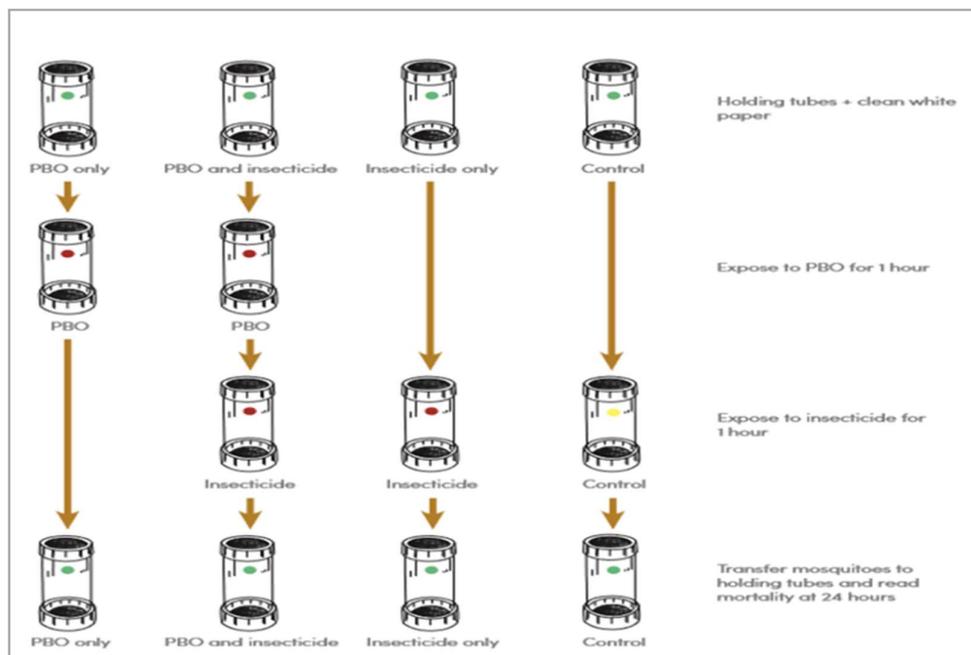
Any resistance phenotypes detected using the discriminating concentrations was further assessed for their potential operational significance. This was done by exposing subsequent mosquito samples from the same target vector population to substantially higher concentrations of the insecticide. The WHO susceptibility test papers using 5× and 10× the discriminating concentrations in a stepwise manner was used to assess the intensity of resistance to provide information on the range, if any, of resistance phenotypes present in a

target vector population and their potential operational significance. This was conducted in 10 sentinel districts, which had recorded potentially high resistance levels to permethrin and deltamethrin. Similar procedures as to those described in section 3.1.5 were used to perform insecticide resistance intensity testing.

PBO–synergist bioassays

PBO synergist tests were conducted at sentinel sites where mosquitoes were found to be resistant to permethrin or deltamethrin to ascertain the involvement of mixed function oxidases in the observed phenotypic resistance. In this test, F1 adult mosquitoes aged 2–5 days were pre-exposed to 4% PBO paper for 1 hour, after which they were immediately exposed to either 0.75% permethrin or 0.05% deltamethrin. Two controls were used during this experiment: control one consisted of mosquitoes exposed to clean papers without insecticides or PBO and control two consisted of mosquitoes exposed to papers treated with PBO only (**Figure**). The number of mosquitoes tested for each insecticide varied between 40 to 80. Mortalities were assessed after exposure. The PBO synergized group was compared to the un-synergized group 24 hours post-exposure. This comparison was used to evaluate the potential role of cytochrome P450 genes in the observed resistance.

Figure 7. Synergist-insecticide bioassay test using WHO testing procedures



Source: WHO, 2016

Mosquito preservation for molecular and biochemical analyses

All tested mosquitoes were preserved with silica gel in 1.5-ml Eppendorf tubes and transported to NIMR Amani for further laboratory analyses, including molecular species identification and detection of molecular mechanisms of insecticide resistance. Adult mosquitoes used for biochemical assays were not exposed to insecticides but were freshly frozen when they were 4 days old. They were kept under -80°C until ready for the assays. Cryo Express dry shippers were used to transport the frozen mosquito samples from the field to the laboratory. Storage temperature inside the shipping cavity remains at approximately -190°C until the liquid nitrogen evaporated from the absorbent material. A

minimum of 100 freshly frozen adult female mosquitoes were preserved for subsequent biochemical assays from each sentinel district.

Laboratory analyses

Identification of *An. gambiae* s.l. and detection of target site resistance mechanisms

For each sentinel site, researchers extracted genomic DNA from 40 to 130 female adult mosquitoes using the method described by Collins et al. (1987). Sibling species of *An. gambiae* s.l. were identified using the standard PCR method described by Scott et al. (1993). From each site, 30–40 female *An. gambiae* s.l. were screened for target site mutations using TaqMan assay (Bass et al., 2007). The aim was to screen all resistant mosquitoes and 30% of the susceptible samples. In places where there were more resistant mosquitoes, at least 50% of resistant mosquitoes were analyzed.

Detection of metabolic resistance mechanisms

Adult female mosquitoes aged 3–4 days that were not previously exposed to insecticide and who were reared in an insecticide-free environment were used in the assay following the CDC plate bioassay method (CDC, 2000). The mosquito samples, which were freshly frozen and kept at –80°C, were transported in liquid nitrogen at approximately –190°C to respective laboratories for analysis of biochemical resistance mechanisms. Biochemical assays were carried out to quantify levels of cytochrome P450 monooxygenases/mixed function oxidase, non-specific esterase, acetylcholinesterase, and glutathione-S-transferase in individual mosquitoes.

2.2.2 Results

Susceptibility status of *An. gambiae* s.l. to insecticides in Mainland Tanzania

An. gambiae s.l. were resistant to permethrin (mortality rate <90%) in Biharamulo, Geita, Igunga, Ukerewe, Rorya, Kibondo, Kakonko, Kasulu, Misenyi, Kibaha, Kilwa, Newala, Tandahimba, and Tunduru. Malaria vectors were resistant to deltamethrin in all districts that also showed resistance to permethrin. Resistance to DDT (mortality rate <90%) was recorded in Biharamulo, Kibondo, Kasulu, Misenyi, Ukerewe, and Tandahimba. Resistance to bendiocarb was recorded in Biharamulo, while resistance to pirimiphos-methyl was recorded in Kilosa (mortality rate <90%). The detailed susceptibility status of all tested insecticides in the 22 sentinel sites are shown in

Table . The local malaria vectors were also fully susceptible to clothianidin in the seven sentinel sites where the tests were conducted. The *Anopheles* mosquitoes showed 100% mortality on Day 6, which was 24 hours before the recommended post-exposure observation period of 7 days. **Figure 12** provides detailed results of *An. gambiae* s.l. susceptibility tests to clothianidin.

Figure 12. Mortality rates (%) trend of *An. gambiae* s.l. to the WHO diagnostic dose of clothianidin 24–144 hours after exposure

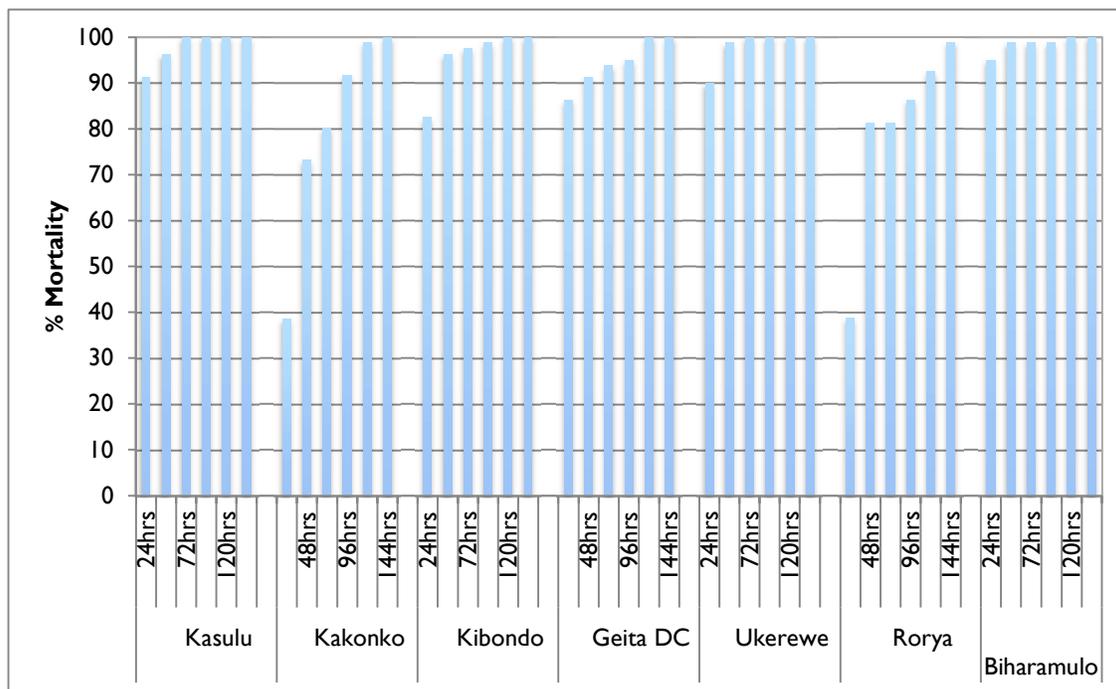
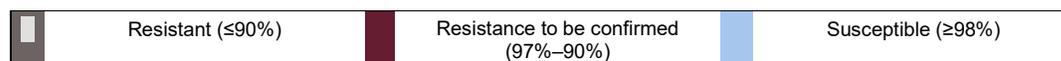


Table 11. Susceptibility status (mortality rates²) of *An. gambiae s.l.* to the WHO discriminating concentrations of four different insecticides

District	Percentage Mortalities (95% CI)				
	Permethrin	Deltamethrin	DDT	Bendiocarb	Actellic
Bariadi	99 (97–99.75)	98 (95–99.1)	100 (100–100)	100 (100–100)	100 (100–100)
Biharamulo	15 (3–27)	12 (4–20)	76 (66–86)	88 (80–96)	75 (66–84)
Geita	53 (47–59)	66 (56–76)	100 (100–100)	100 (100–100)	100 (100–100)
Igunga	84 (76–92)	82 (64–100)	100 (100–100)	100 (100–100)	100 (100–100)
Kibaha	10 (6–14)	5 (1–10)	98 (96–100)	96 (92–100)	100 (100–100)
Kilwa	33 (9–57)	78 (72–84)	100 (100–100)	100 (100–100)	100 (100–100)
Kilosa	98 (95–100)	100 (100–100)	100 (100–100)	93 (87–99)	50 (29–71)
Kibondo	10 (10–10)	25 (17–33)	25 (18–32)	96 (91–99)	100 (100–100)
Kakonko	46 (41–51)	80 (76–84)	96 (91–99)	100 (100–100)	100 (100–100)
Kasulu	15 (9–21)	19 (13–25)	29 (24–34)	100 (100–100)	100 (100–100)
Meatu	96 (94–98)	98 (95–99)	100 (100–100)	100 (100–100)	100 (100–100)
Misenyi	32 (15–49)	25 (15–35)	61 (51–71)	89 (75–99)	100 (100–100)
Newala	54 (46–62)	50 (41–59)	96 (91–99)	100 (100–100)	100 (100–100)
Nyasa	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
Rorya	43 (30–56)	58 (47–69)	96 (91–99)	99 (97–100)	100 (100–100)
Shinyanga District Council	99 (97–100)	98 (96–100)	100 (100–100)	100 (100–100)	100 (100–100)
Tandahimba	39 (34–44)	30 (21–39)	85 (81–89)	100 (100–100)	100 (100–100)
Tunduru	65 (40–90)	70 (51–89)	100 (100–100)	100 (100–100)	100 (100–100)
Ukerewe	71 (57–85)	53 (47–59)	91 (85–97)	100 (100–100)	100 (100–100)
Ushetu	73 (61–85)	64 (57–71)	100 (100–100)	100 (100–100)	100 (100–100)
Nachingwea	90 (87–93)	83 (80–86)	100 (100–100)	100 (100–100)	100 (100–100)
Sikonge	74 (68–80)	98 (96–100)	92 (84–100)	100 (100–100)	100 (100–100)



Intensity of insecticides resistance

Malaria vectors were still resistant to permethrin (mortality rate $<90\%$) at 10 times the diagnostic concentration in Kibondo and Ukerewe. They also showed high resistance intensity to deltamethrin in Misenyi and Rorya (mortality rate $<90\%$) at 10 times the

²Based on WHO criteria for insecticide susceptibility levels, i.e., mortality-rate based criteria was used to determine the levels of mosquito susceptibilities: Susceptible ($\geq 98\%$); resistant to be confirmed (97%–90%), and resistant ($\leq 90\%$).

diagnostic concentrations. The detailed intensity of resistance results is highlighted in **Figure** and **Figure** .

Figure 13. Percentage mortality of wild female *An. gambiae* s.l. to different concentrations of permethrin in eight sentinel districts in Mainland Tanzania

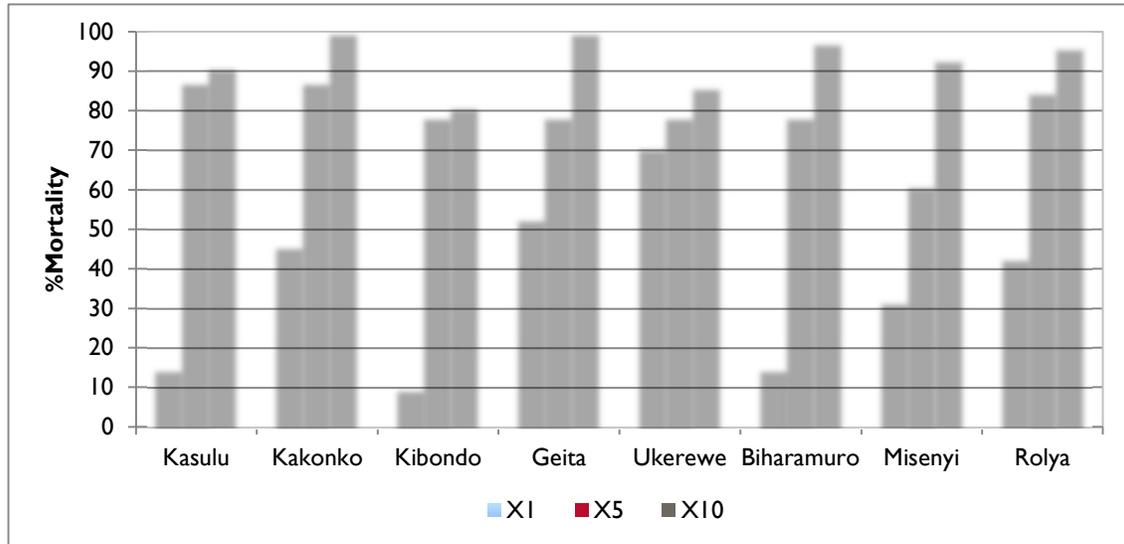
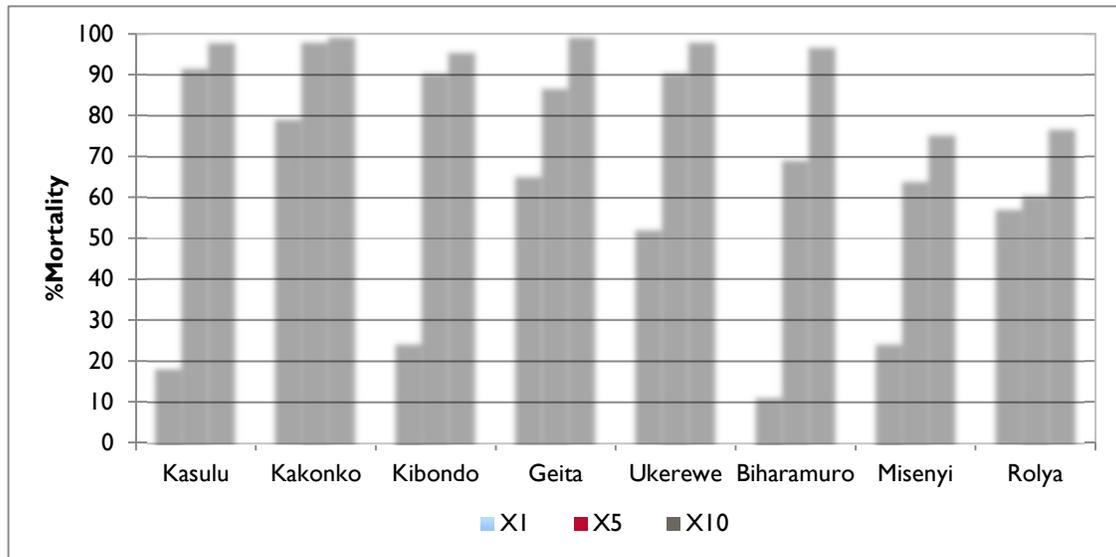


Figure 14. Percentage mortality of wild female *An. gambiae* s.l. to different concentrations of deltamethrin in eight sentinel districts in Mainland Tanzania



Synergist tests with PBO

When mosquitoes from the eight sites were exposed to permethrin and deltamethrin alone, their mortalities were <80%. However, there was a significant increase in mortality to both permethrin and deltamethrin at all eight sites following pre-exposure to PBO. This suggests that cytochrome P450 likely plays a significant role in the observed pyrethroid resistance phenotype. Detailed synergist test results are shown in **Figure** and **Figure** .

Figure 85. Comparison of mortality rates of *An. gambiae* s.l. exposed to permethrin (0.75%) alone and in combination with PBO per site

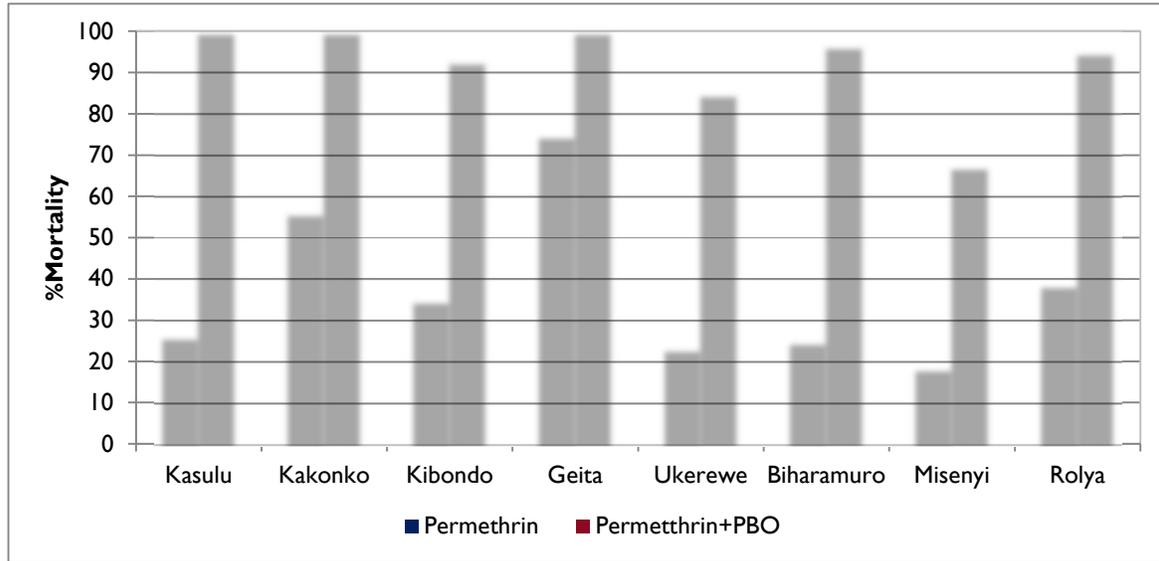
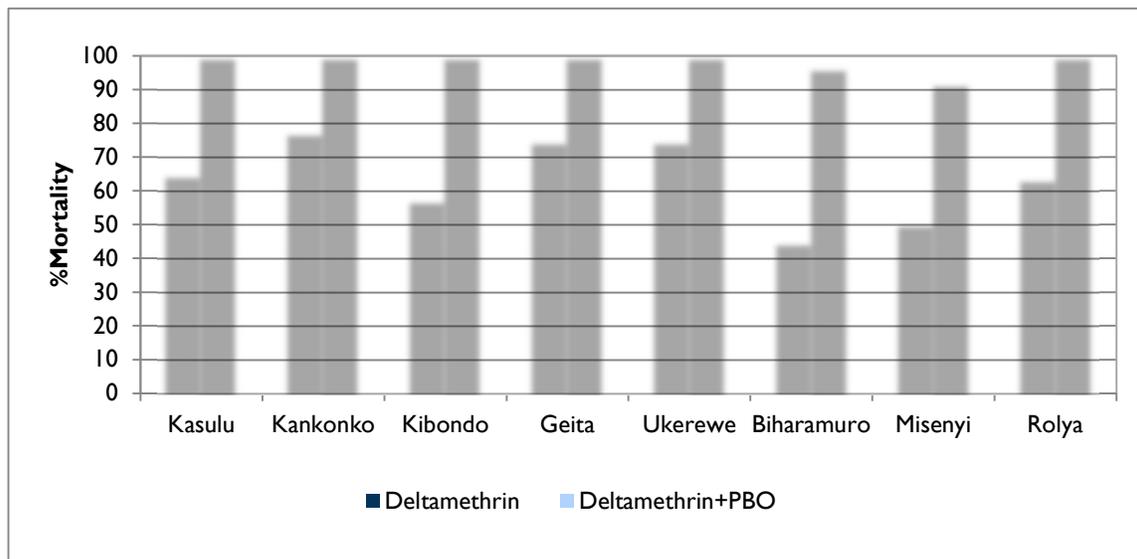


Figure 16. Comparison of mortality rates of *An. gambiae* s.l. exposed to deltamethrin (0.05%) alone and in combination with PBO per site

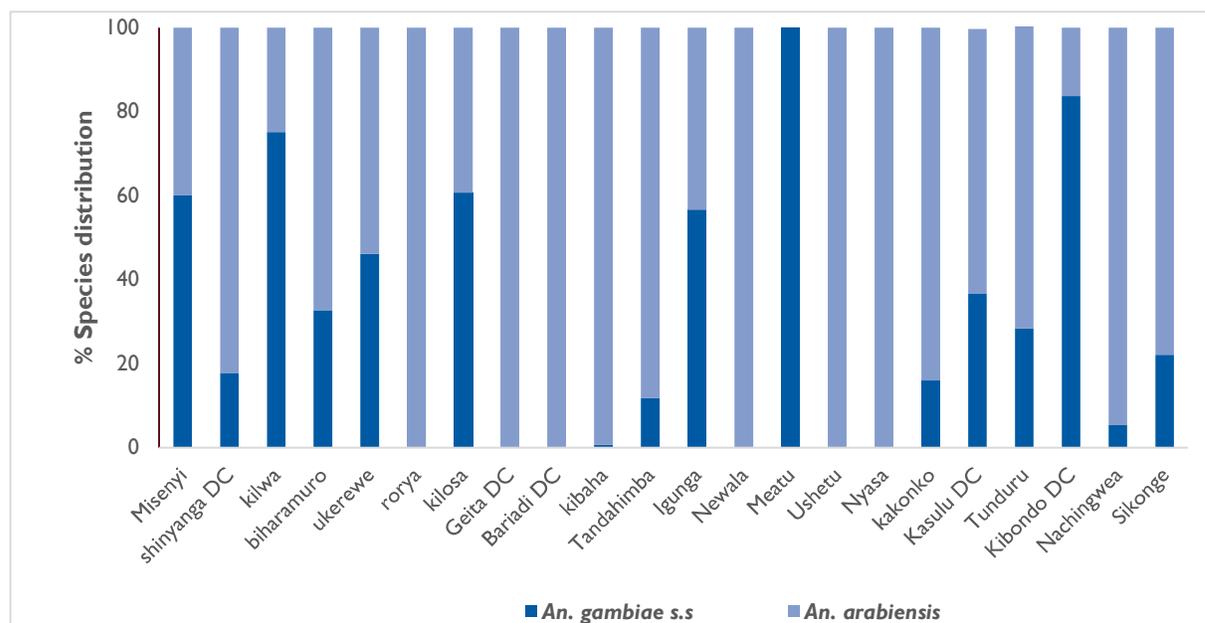


Species composition of malaria vectors in insecticide resistance tests

Overall, 10,340 mosquitoes were collected and morphologically identified as *An. gambiae* s.l.; out of these, 3,000 underwent PCR analysis to identify the *An. gambiae* s.l. sibling species. DNA amplification was possible in 2,972 *An. gambiae* s.l. Of these, 48% and 52% were identified as *An. gambiae* s.s. and *An. arabiensis*, respectively.

The distribution of these two sibling species at each of the sentinel districts is shown in **Figure .**

Figure 17. Percentage distribution of the *An. gambiae* s.s. and *An. arabiensis* in surveyed sentinel districts



Insecticide resistance mechanisms

Target site resistance mutations

Mosquitoes from all sentinel districts were analyzed using TaqMan assay (Bass et al., 2007) for *kdr*-east and *kdr*-west. *Kdr*-east mutation was detected in mosquitoes from more than half of the sentinel districts (14 of 22) with allelic frequencies ranging from 2% in Nachingwea and Geita to about 90% in Kibondo (Table). This shows that the *kdr* gene mutation intensity is increasing and spreading widely geographically. This increasing trend indicates that the resistance selection pressure is still not adequately contained suggesting the need for additional emphasis on resistance management.

Table 2. Distribution and allelic frequencies of *kdr*-east (L1014S) mutation genotypes

Sentinel District	N	<i>An. gambiae</i> s.l.			Allelic frequency
		Genotype count			
		RR	RS	SS	R
Kilosa	74	0	8	66	0.05
Geita	72	1	1	70	0.02
Kilwa	67	0	7	60	0.05
Kakonko	67	3	3	61	0.07
Ukerewe	67	40	8	19	0.66
Newwala	67	2	12	53	0.12
Meatu	67	4	3	60	0.08
Tandahimba	67	26	32	9	0.63
Kasulu	67	0	4	63	0.03
Tunduru	68	0	16	52	0.12
Kibondo	67	53	10	4	0.87

Sentinel District	N	<i>An. gambiae</i> s.l.			Allelic frequency
		Genotype count			
		RR	RS	SS	R
Misenyi	73	45	3	25	0.64
Nachingwea	67	0	3	64	0.02
Biharamulo	67	31	0	36	0.46

RR, homozygous resistant; RS, heterozygous resistant; SS, homozygous susceptible.

Biochemical resistance mechanisms

Biochemical tests to analyze enzyme activity (**Figure** and **Figure**) were done on mosquitoes from 20 sentinel districts. The tests were performed according to techniques described by the CDC (2000). Enzyme expression levels between wild mosquitoes from these sites and the susceptible Kisumu strain were compared using t-tests. The means for the optical density values with a *p*-value of ≤ 0.05 were considered significantly elevated for both oxidase (cytochromes P450) and non-specific esterase enzymes. Mean levels of oxidase activities were significantly higher in mosquitoes from almost all resistant sentinel districts showing that the oxidase type of metabolic resistance has a greater role in phenotypic resistance recorded and indicates that tools with PBO may be the appropriate choice to support malaria vector control in Mainland Tanzania.

Figure 18. Mean optical density values for significantly elevated enzyme activity for oxidase

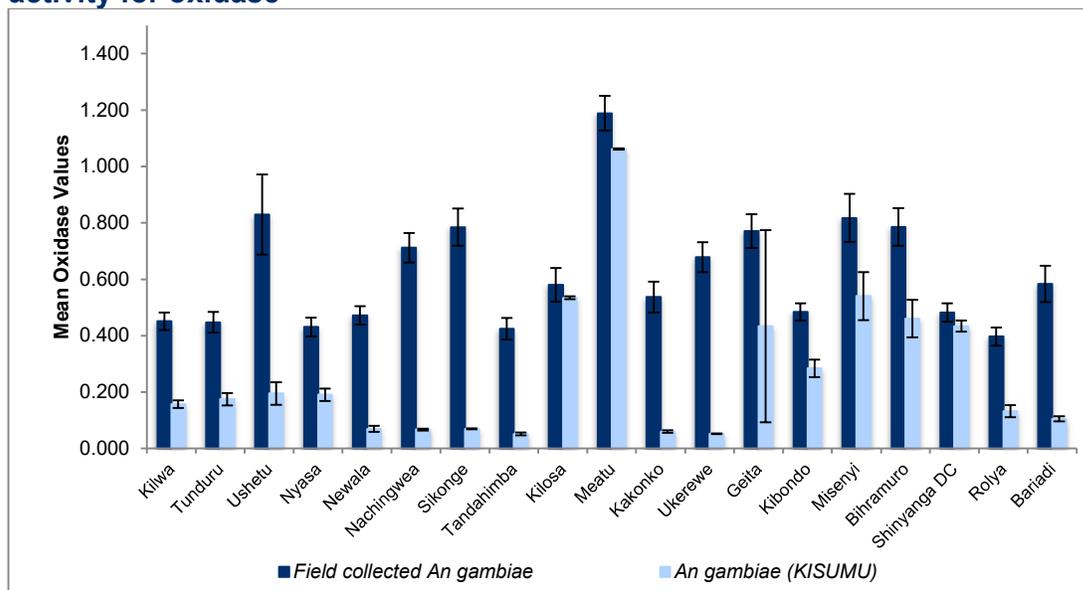
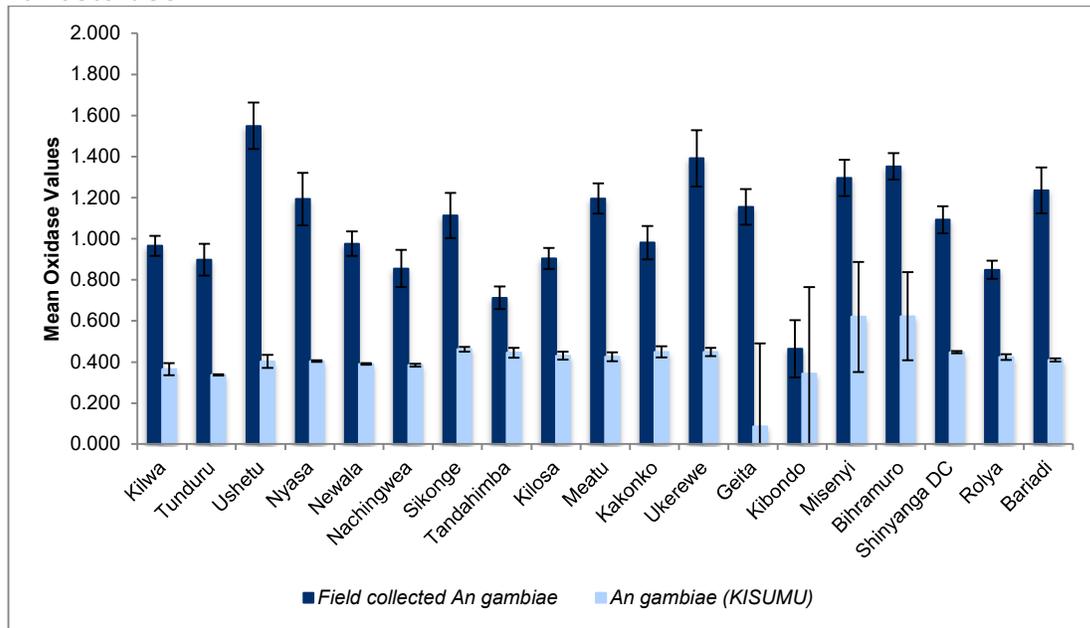


Figure 9. Mean optical density values for significantly elevated enzyme activity for esterase



3. Entomological Monitoring in Zanzibar

3.1. Malaria vector bionomics and IRS quality monitoring

ZAMEP conducted entomological monitoring activities Zanzibar with the following objectives:

1. Identifying malaria vector species in IRS intervention and control districts;
2. Assessing the vector ecology (i.e., density, distribution, and seasonality) in IRS intervention and control sentinel sites;
3. Monitoring vector feeding and resting behavior; and,
4. Assessing the quality of IRS and insecticide residual efficacy post-IRS through cone wall bioassays.

3.1.1 Methods

Study sites

Entomological data collected ranged from October 2018 to September 2019, covering 10 entomological sentinel sites in Zanzibar (6 in Unguja and 4 in Pemba). Selection criteria of entomological sentinel sites included the following:

- Disease incidence/prevalence
- The topography of the area
- Agricultural practices (rain-fed rice, irrigation, etc.)
- Urban or rural setting

Error! Reference source not found. displays the geographical distribution of study districts in Zanzibar. Error! Reference source not found. highlights the summary of entomological activities per sentinel district.

Figure 20. Entomological monitoring sentinel sites, Zanzibar

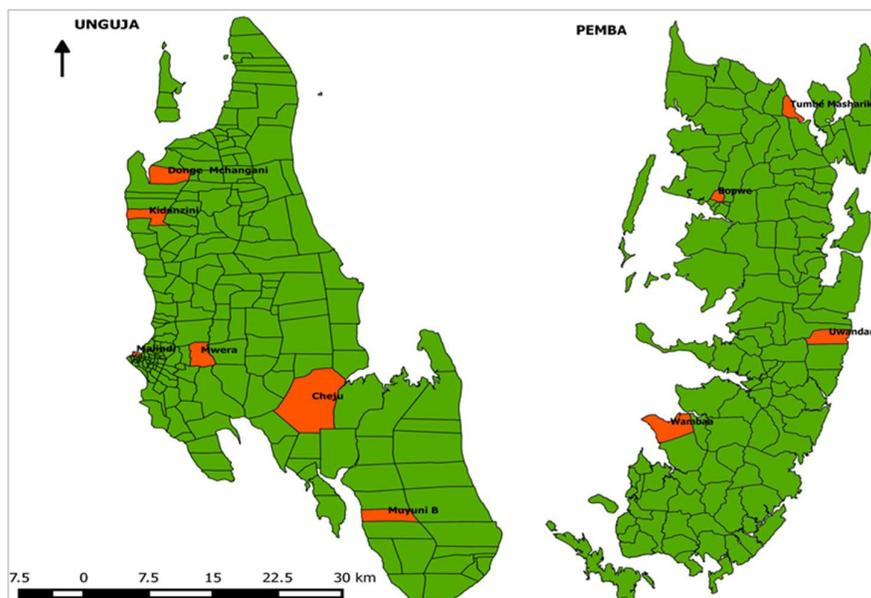


Table 13. Entomological and insecticide residual efficacy monitoring sentinel sites in Zanzibar

Location	District	Sentinel Site	IRS Status in 2019	Entomological Parameters Collected
Unguja	Mjini	Stone town	Not sprayed	Species composition, vector abundance, distribution and seasonality, and feeding time and location
	Kaskazini A	Donge	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Kaskazini B	Bumbwini	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Magharibi A	Mwera	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Kusini	Muyuni	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Kati	Cheju	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
Pemba	Micheweni	Tumbe	Sprayed	IRS quality, insecticide decay rate, species composition, vector abundance, distribution and seasonality, and feeding time and location
	Wete	Bopwe	Not sprayed	Species composition, vector abundance, distribution and seasonality, feeding time and location
	Chake	Uwandani	Not sprayed	Species composition, vector abundance, distribution and seasonality, feeding time and location
	Mkoani	Wambaa	Not sprayed	Species composition, vector abundance, distribution and seasonality, feeding time and location

Rearing of susceptible *An. gambiae* s.s. (R70 strain)

The ZAMEP insectary reared adult *An. gambiae* s.s. (susceptible R70 strain) according to standard protocols in sufficient numbers to meet the demands of monthly cone wall bioassay field activities. The adult mosquito rooms were maintained at $27 \pm 1^\circ\text{C}$ and 60%–80%

relative humidity; the larval rooms were maintained at $30 \pm 1^\circ\text{C}$ and 60%–80% relative humidity.

Insecticide residual efficacy monitoring

Cone bioassays were carried out as per standard WHO protocols (1981). The tests were carried out using 2- to 5-day old, sucrose-fed, laboratory-reared, known susceptible *An. gambiae* s.s. R70 mosquitoes. Batches of 10 mosquitoes were exposed for 30 minutes inside a WHO plastic cone on sprayed wall surfaces in each of the rooms and houses sampled. Three replicates of the WHO cones (one at each position) were adhered to the top, middle, and bottom of different sprayed surfaces. After 30 minutes, mosquitoes were transferred into paper cups and held for 24 hours. During holding, mosquitoes were provided with 10% glucose solution-soaked cotton wool. The team assessed knockdown 60 minutes after the end of exposure and percentage mortality after 24 hours.

Vector ecology

The monitoring team conducted entomological surveillance to determine vector ecology in IRS and non-IRS sites. Mosquitoes were collected via pyrethrum spray catches (PSC), HLC, CDC light traps, and pit traps. **Figure** shows each collection method in further detail. Adult mosquitoes collected via these methods were used to determine basic entomological indicators, including species distribution and abundance, resting behavior, feeding and biting behavior, and seasonality (

Table and Error! Reference source not found.).

The team identified the collected specimens in the field; mosquitoes were sorted by species according to standard morphological keys (Gillies et al., 1987). Blood-fed females were independently preserved in filter paper wraps for determination of the blood-meal source, with the rest preserved for further laboratory analysis, including species-specific identification and detection of malaria infection.

Figure 21. Entomological sampling methods used in Zanzibar

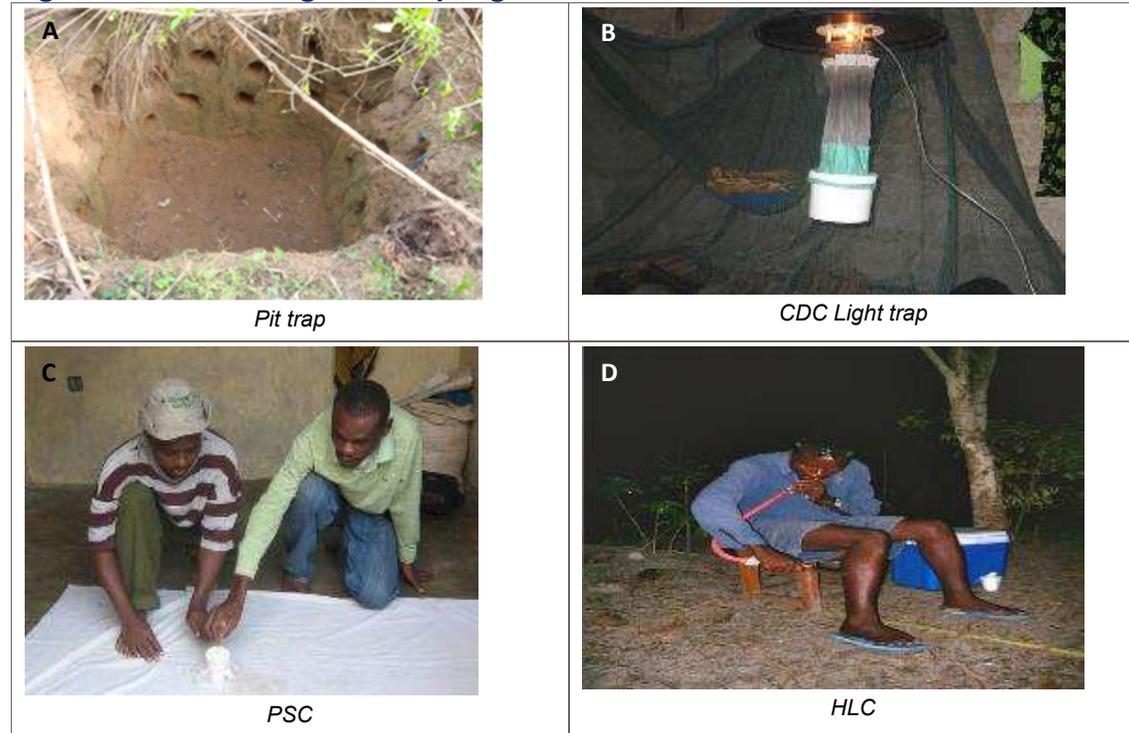


Table 14. Vector sampling methods at sentinel sites in Zanzibar

Method	Purpose	Sentinel Site	Number of Households	Days per Month	Time (Hours)	Sample Processing
HLC	Indoor and outdoor biting behavior	10	2 houses per site	2 days per site	1800–0600	Species, sporozoite rate
PSC	Indoor resting behavior	10	5 houses per site	2 days per site	0600–0800	Species, sporozoite rate
CDC light trap	Indoor abundance	10	2 houses per site	2 days per site	1800–0600	Species, sporozoite rate
Pit trap collection (PTC)	Outdoor resting behavior	10	2 pits per site	2 days per site	0600–0800	Species, sporozoite rate

CDC light traps

This collection method is used for indoor biting (endophagic) mosquitoes. The team randomly selected two houses per night in each village in a study district; CDC light traps were placed for two consecutive nights each month. The traps were installed roughly 1.5 m above the floor, next to the head of a sleeping person(s) in the room. The person(s) was requested to sleep under an untreated mosquito net(s), provided by ZAMEP, overnight. CDC light traps were set to operate from 1800 to 0600 to trap mosquitoes. Captured mosquitoes were transferred into mosquito-holding cups.

HLC

HLCs (WHO, 1975) were conducted between 1800 and 0600 outdoors and indoors twice per month at each site. Two team members collected mosquitoes outdoors; the other two collected sample indoors at two houses per site. Samples from the collections were kept in paper cups, organized by hour collected, and the collector. They were then kept in a cool box until they were sorted, counted, and recorded the following morning.

PSC

PSCs were conducted twice per month in five houses during each mosquito collection morning. White sheets were laid on the entire floor and over the furniture in one room where people had slept the previous night. White sheets facilitate the visibility of the knocked down mosquitoes. The doors and windows of the houses were shut, then the rooms sprayed with pyrethrum (0.3%), synergized with piperonyl butoxide (PBO) as described by Gimnig et al. (2003). A collector outside the house sprayed around the eaves with insecticide to prevent the mosquitoes inside the houses from escaping and another collector sprayed the roofs and the walls inside the house. The houses were then closed for 10 to 15 minutes. Afterwards, the white sheets were removed from the room and the knocked down mosquitoes collected using forceps/tweezers. Knocked down mosquitoes for each household were recorded and then transferred onto moist filter paper inside petri dishes labeled with the date and house number. Collected mosquitoes were put in a cool box and transported to the laboratory for further processing.

PTC

A rectangular pit was dug in the ground (1.5 m in depth, 1.2 m in length, and 1 m in width) within 10 m of each selected residential house. In each of the four sides, roughly

50 cm to 60 cm and 90 cm to 100 cm from the bottom of the pit, between five and eight 30 cm cavities were dug. The main pits were then shaded by an artificial framework thatched with locally available coconut palms. Resting mosquitoes were sampled from 0600 to 0900 inside the cavities using handheld mouth aspirators and an intensive visual search. Collected mosquito samples were kept in paper cups before being processed in the laboratory. The collection was done twice per month at each site.

Morphological identification of mosquitoes

All mosquito samples were transported to the laboratory where the team conducted morphological identification of the mosquitoes collected following the taxonomic keys of Gillies and Coetzee (1987). Based on morphological characteristics, *Anopheles* mosquitoes were sorted to generally known species (i.e., *An. gambiae* s.l., *An. funestus*, *An. coustani*, *An. pharoensis*, etc.). The identified *Anopheles* mosquitoes were preserved with silica gel individually in Eppendorf tubes, labeled, and then kept into the laboratory for further analyses.

Laboratory analyses

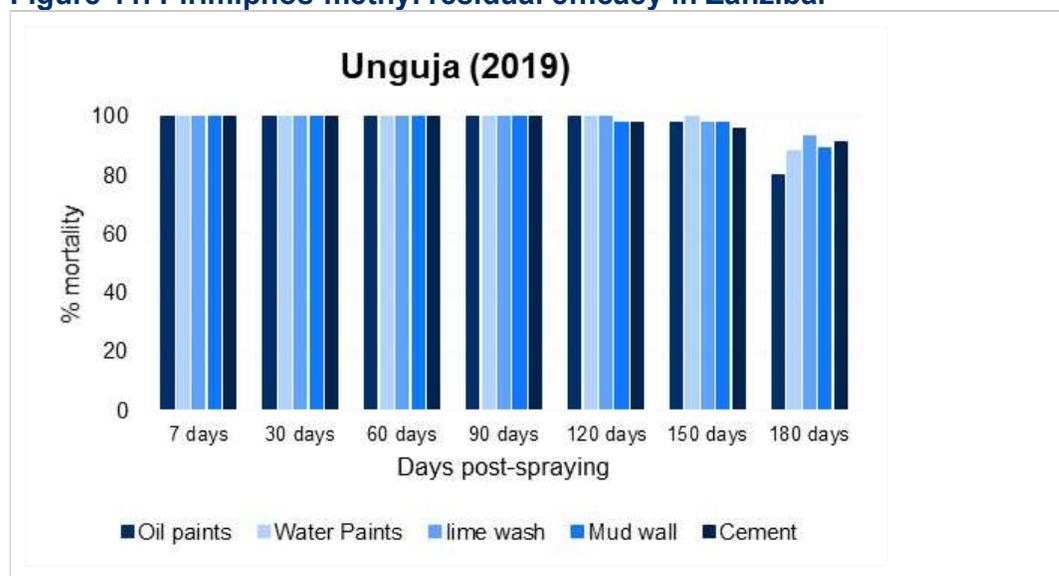
A PCR-based method was used to identify sibling species of *An. gambiae* s.l. (Wilkins et al., 2006) and *An. funestus* s.l. mosquitoes (Koekemoer et al., 2002). The PCR assay was selected based of the morphological identification of the mosquito specimen.

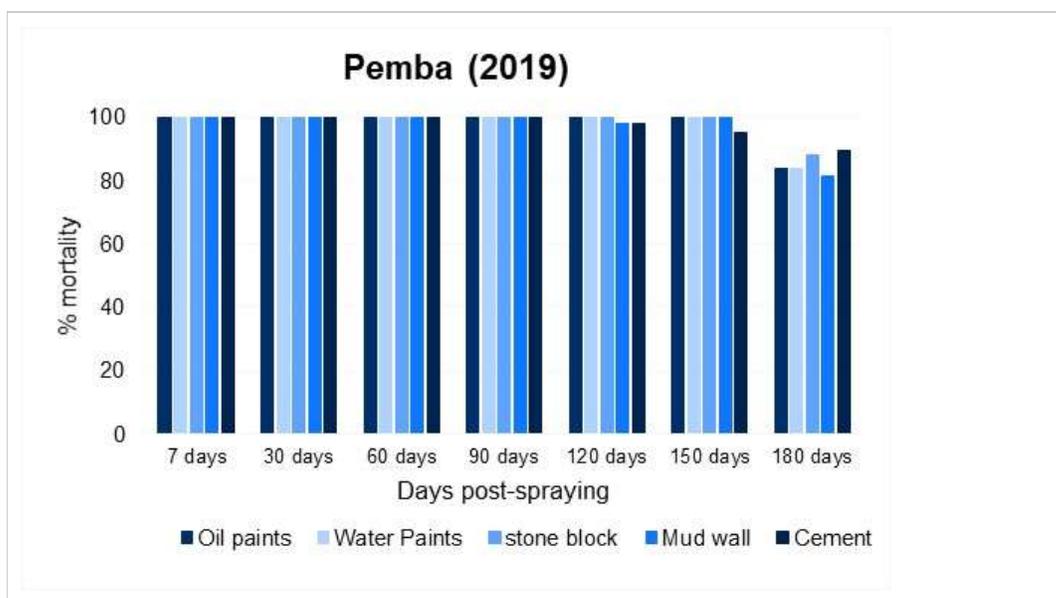
3.1.2 Results

Insecticide residual efficacy monitoring

Bioefficacy tests conducted 7 days post-IRS indicates that there was high quality of the IRS for both Unguja and Pemba. Results of pirimiphos-methyl (Actellic® 300 CS) decaying rates across all wall surfaces in both Unguja and Pemba still maintained the WHO's threshold of greater than 80% mortality for malaria vectors at 180 days (6 months) post-IRS (Figure 10), indicating that the residual efficacy of this insecticide lasts long enough to cover the two main transmission seasons.

Figure 11. Pirimiphos-methyl residual efficacy in Zanzibar





Vector ecology

Abundance, distribution and species composition in Zanzibar

In Zanzibar, a total of 2,418 *Anopheles* mosquitoes were collected, 1,328 from Pemba and 1,090 from Unguja. HLCs proved to be the best sampling technique, as demonstrated through the collection of 71% and 60% of the total *Anopheles* catches in Pemba and Unguja, respectively. The second most successful collection method was through the use of pit traps. The distribution of mosquitoes by collection method is highlighted in Error! Reference source not found. and Error! Reference source not found..

Table 15. Number of *Anopheles* collected by collection method and location in Pemba, Zanzibar

Sentinel Site	Morphological ID	LTC	HLC	PSC	PTC	Total
Bopwe	<i>An. gambiae</i> s. l	1	485	1	81	568
	<i>An. ziemanni</i>	0	1	0	0	1
Bopwe Total		1	486	1	81	569
Tumbe	<i>An. costani</i>	0	12	0	7	19
	<i>An. gambiae</i> s.l	12	288	48	134	482
	<i>An. nili</i>	0	1	0	0	1
	<i>An. funestus</i> s.l	0	0	0	1	1
Tumbe Total		12	301	48	142	503
Uwandani	<i>An. gambiae</i> s.l.	1	12	10	46	69
Uwandani Total		1	12	10	46	69
Wambaa	<i>An. coustani</i>	0	4	3	0	7
	<i>An. gambiae</i> s.l.	0	143	8	28	179
	<i>An. maculipalpis</i>	0	1	0	0	1
Wambaa Total		0	148	11	28	187

Sentinel Site	Morphological ID	LTC	HLC	PSC	PTC	Total
Grand Total		14 (2%)	947 (71%)	70 (5%)	297 (22%)	1,328

Table 16. Number of *Anopheles* collected by collection method and location in Unguja, Zanzibar

Sentinel Site	Morphological ID	LTC	HLC	PTC	PSC	Total
Bumbwini	<i>An. gambiae</i> s.l.	0	13	0	0	13
Cheju	<i>An. gambiae</i> s.l.	1	485	0	0	486
Donge	<i>An. gambiae</i> s.l.	0	140	0	0	140
Muyuni	<i>An. gambiae</i> s.l.	0	1	0	0	1
Mwera	<i>An. gambiae</i> s.l.	0	13	412	22	447
Stone Town	<i>An. gambiae</i> s.l.	0	3	0	0	3
Grand Total		1 (0.01%)	655 (60%)	412 (38%)	22 (2%)	1,090

Mosquito species composition

Of the 2,256 samples analyzed for species identification (Pemba, 1,246; Unguja, 1,010), *An. arabiensis* was identified as the predominant vector in both Pemba (86%) and Unguja (78%). Other malaria vectors found in Zanzibar were *An. leesonii*, *An. merus*, *An. gambiae* s.s., *An. parensis*, *An. rivulorum*, *An. funestus* s.s., and *An. quadriannulatus*. A small proportion of the samples did not amplify to the *An. gambiae* complex and *An. funestus* primers (see Error! Reference source not found. and Error! Reference source not found.).

Table 3. Molecular identification of malaria vectors in Pemba

Sentinel Site	<i>An. arabiensis</i>	<i>An. leesonii</i>	<i>An. merus</i>	<i>An. parensis</i>	<i>An. quadriannulatus</i>	<i>An. rivulorum</i>	Unamplified	Total
Tumbe	359	30	21	1	1	7	30	449
Bopwe	512	36	0	1	0	1	6	556
Uwandani	49	12	1	0	0	0	3	65
Wambaa	148	15	3	0	0	5	5	176
Total	1,068	93	25	2	1	13	44	1,246
%	86	7	2	0.2	0.1	1	4	

Table 4. Molecular identification of malaria vectors in Unguja

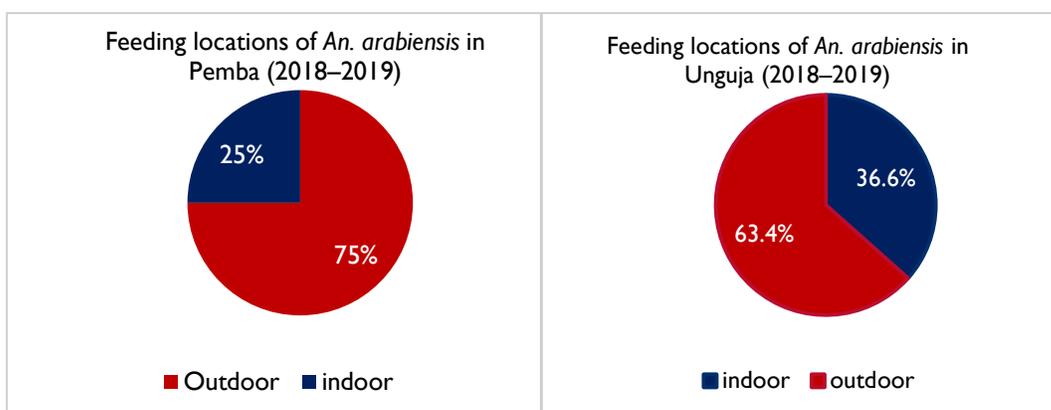
Sentinel Site	<i>An. arabiensis</i>	<i>An. gambiae</i> s.s.	<i>An. merus</i>	Unamplified	Total
Bumbwini	10	0	1	1	12
Cheju	396	2	22	40	460
Donge	101	8	7	12	128
Muyuni	1	0	0	0	1
Mwera	275	2	1	128	406

Sentinel Site	<i>An. arabiensis</i>	<i>An. gambiae s.s.</i>	<i>An. merus</i>	Unamplified	Total
Stone Town	1	1		1	3
Grand Total	784	13	31	182	1010
%	77.6	1.3	3.1	18.0	

Biting and resting behavior in Zanzibar

Generally, outdoor biting behavior of *An. arabiensis* was significantly higher in both Pemba and Unguja, suggesting that the current residual transmission takes place outdoors. Error! Reference source not found. shows the biting behavior of *An. arabiensis* as assessed using HLC.

Figure 12. Biting behavior of *An. arabiensis* in Zanzibar



Hourly biting rate of *An. arabiensis*

The outdoor biting density of *An. arabiensis* is higher earlier in the night when people are active outdoors. IBR is maintained at a lower level throughout the night. In general, the HBR (also known as MBR) is slightly higher in unsprayed sites than sprayed sites. All collected data suggest earlier outdoor transmission of malaria (Error! Reference source not found., Error! Reference source not found., and Error! Reference source not found.). Very few mosquitoes were collected in non-IRS sites in Unguja; therefore, were not included in the analyses.

Figure 13. HBRs of *An. arabiensis* in sprayed sites in Pemba (n = 1)

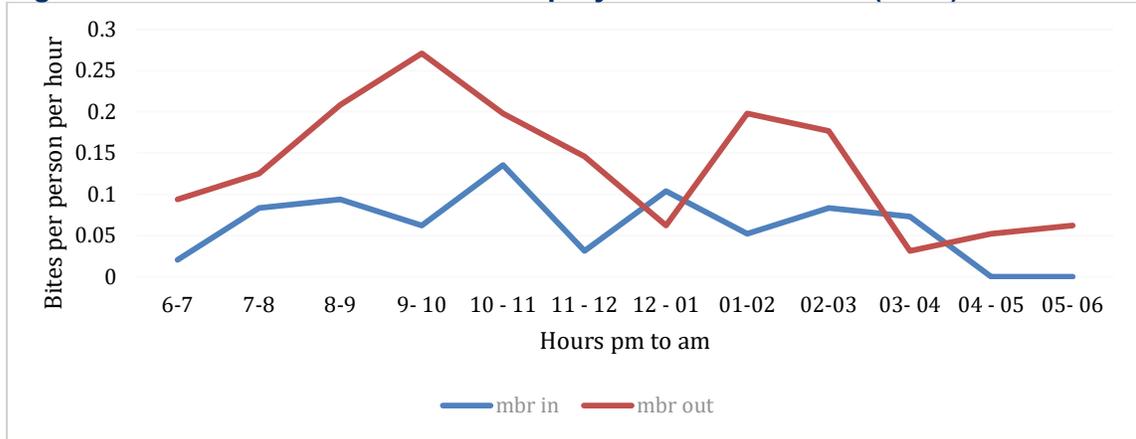


Figure 14. HBRs of *An. arabiensis* at unsprayed sites in Pemba (n=3)

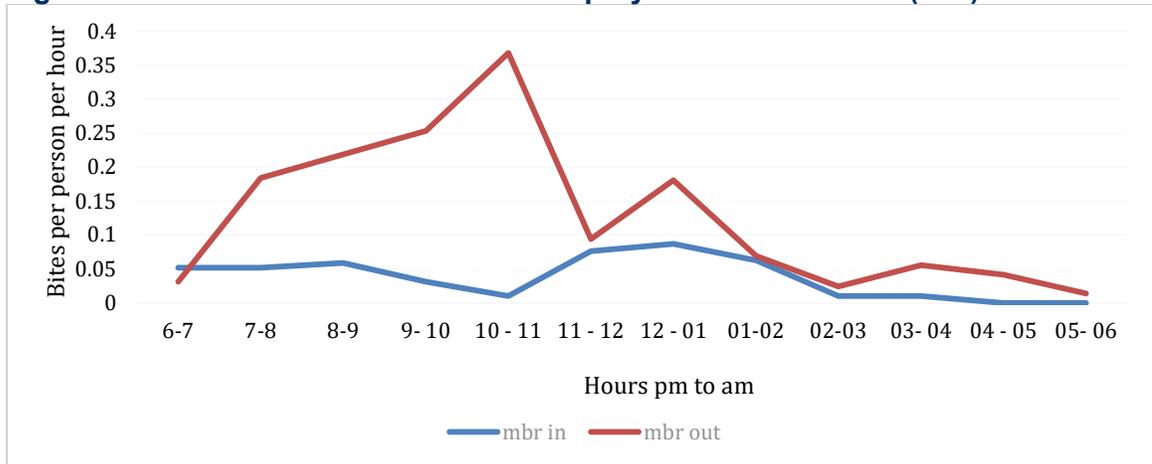
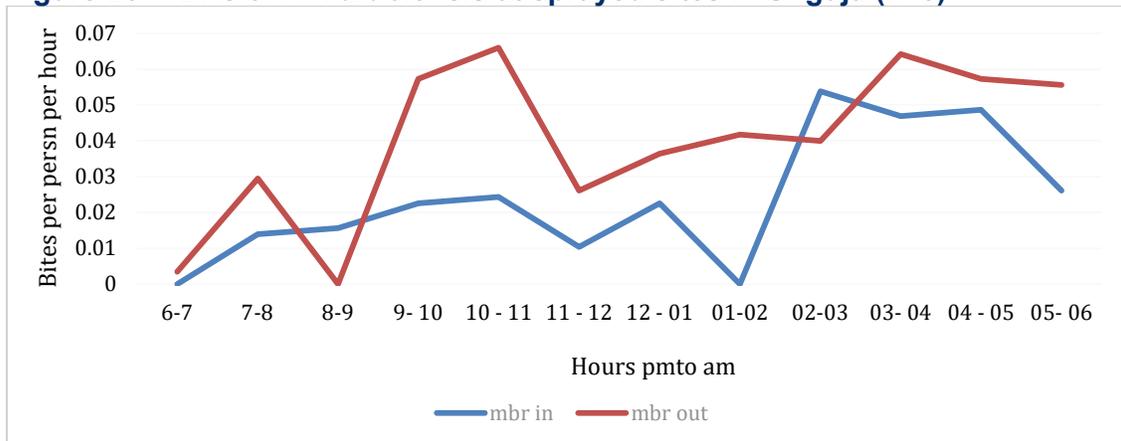


Figure 26. HBRs of *An. arabiensis* at sprayed sites in Unguja (n=5)



Sporozoite Rate

Mosquito infectivity was estimated by calculating the sporozoite rate; i.e., the proportion of mosquitoes in a population with sporozoite in their salivary glands. A total of 2,256 *Anopheles* vectors were screened for the presence of sporozoite using ELISA. *An. Gambiae* complex (*An. arabiensis*, *An. merus*, and *An. gambiae* s.s.) constituted about 85% of the total number of the samples examined. The remaining 15% included other species, such as *An. rivulorum*, *An. leesonii*, *An. funestus* s.s., *An. parensis*, *An. quadriannulatus*, and non-amplified mosquitoes. No sporozoite was detected from samples collected in Pemba, which corresponds to fewer local malaria cases.

Indoor resting density of malaria vectors

This variable was not determined because of the small number of vectors collected within households.

3.2 Monitoring of insecticide resistance in malaria vectors in Zanzibar

This activity was carried out by ZAMEP with the main objective to monitor insecticide resistance among malaria vectors to selected insecticides in 8 sentinel sites in Zanzibar. The specific objectives of these monitoring activities were to determine the following:

1. Susceptibility status of *An. gambiae* s.l. to deltamethrin (0.05%), permethrin (0.75%), alphacypermethrin (0.05%), bendiocarb (0.1%), and pirimiphos-methyl (0.25%);
3. Resistance intensity in *An. gambiae* s.l. to permethrin and alphacypermethrin;
4. The involvement of mixed function oxidases in the observed phenotypic resistance by conducting PBO synergist bioassays in selected sentinel sites with pyrethroid resistance; and
6. Resistance mechanisms using PCR.

3.2.1 Methods

Study design

This was a cross-sectional survey conducted between March and June 2019 in eight sentinel sites of Zanzibar: four sites in Pemba and four in Unguja.

Context and sites selection

The criteria for selection of sentinel districts for insecticide resistance monitoring was similar to those used for Mainland Tanzania. The list of sentinel sites is shown in **Table 5**.

Table 6. The 2019 sentinel sites for insecticide resistance monitoring in Zanzibar

S/N	Region	Site	S/N	Region	Site
Pemba			Unguja		
1	Micheweni	Tumbe	5	Magharibi	Mwera
2	Wete	Bopwe	6	North B	Donge
3	Mkoani	Wambaa	7	North A	Bumbwini
4	Chake Chake	Uwandani	8	Kati	Cheju

Mosquito collections in the field

The adult mosquitoes for testing were reared from larvae collected in selected sentinel sites. The detailed collection and rearing procedure are similar to the techniques outlined earlier for Mainland Tanzania.

WHO insecticide susceptibility and resistance intensity testing

Susceptibility tests were carried out using the WHO's test kits for adult mosquitoes (WHO, 2016) as highlighted in the Mainland Tanzania section above. Insecticide resistance intensity testing was also carried out using WHO standard protocols for this assay.

PBO synergist bioassays

PBO synergist tests were conducted at sentinel sites where mosquitoes were found to be resistant to permethrin or deltamethrin or alphacypermethrin to determine the involvement of mixed function oxidases in the observed phenotypic resistance. The methodology described in Mainland Tanzania was also used in Zanzibar.

Laboratory analyses

Identification of *An. gambiae* s.l. and detection of target site resistance mechanisms

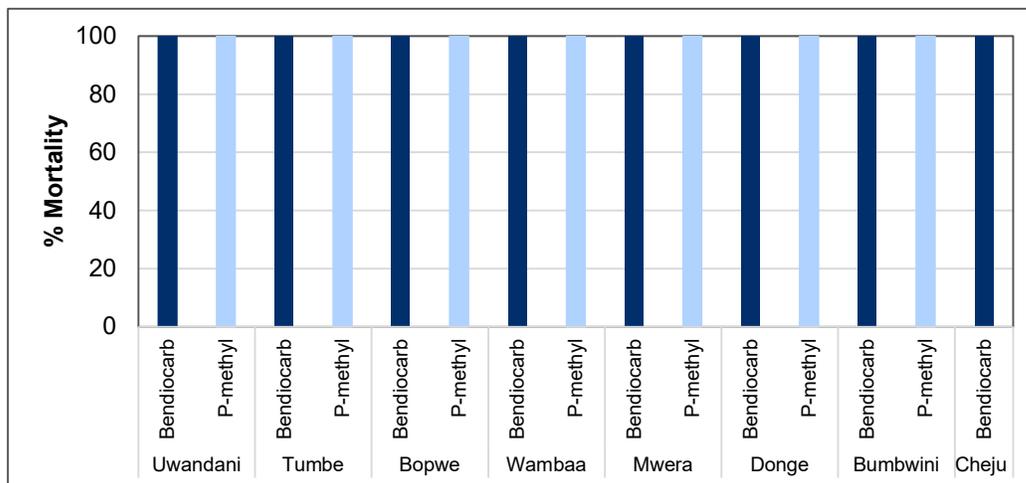
For each sentinel site, genomic DNA from 40 to 130 female adult mosquitoes was individually extracted using the method described by Collins et al. (1987). Sibling species of the *An. gambiae* s.l. were identified using the standard PCR method of Wilkins et al. (2006). Target site mutations were screened using standard PCR techniques described by Huynh et al. (2007).

3.2.2 Results

Susceptibility tests of *An. gambiae* complex

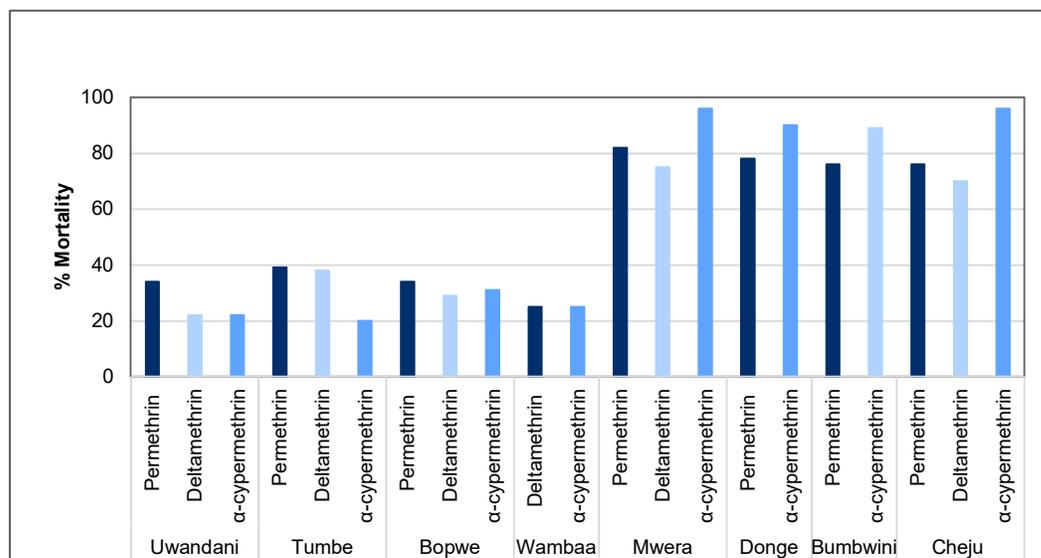
The results of the WHO susceptibility tests indicated that *An. gambiae* s.l. from both sprayed and non-sprayed sentinel sites in Pemba and Unguja were fully susceptible to pirimiphos-methyl and bendiocarb. However, the vectors were resistant to permethrin, deltamethrin, and alphacypermethrin across all tested sites (Error! Reference source not found. and Error! Reference source not found.).

Figure 27. Susceptibility status of malaria vectors to bendiocarb and pirimiphos-methyl at the diagnostic dose, Zanzibar



Note: In Cheju, P-methyl was not tested because there was an inadequate number of mosquitoes.

Figure 15. Susceptibility test results of malaria vectors to pyrethroids at the diagnostic dose, Zanzibar

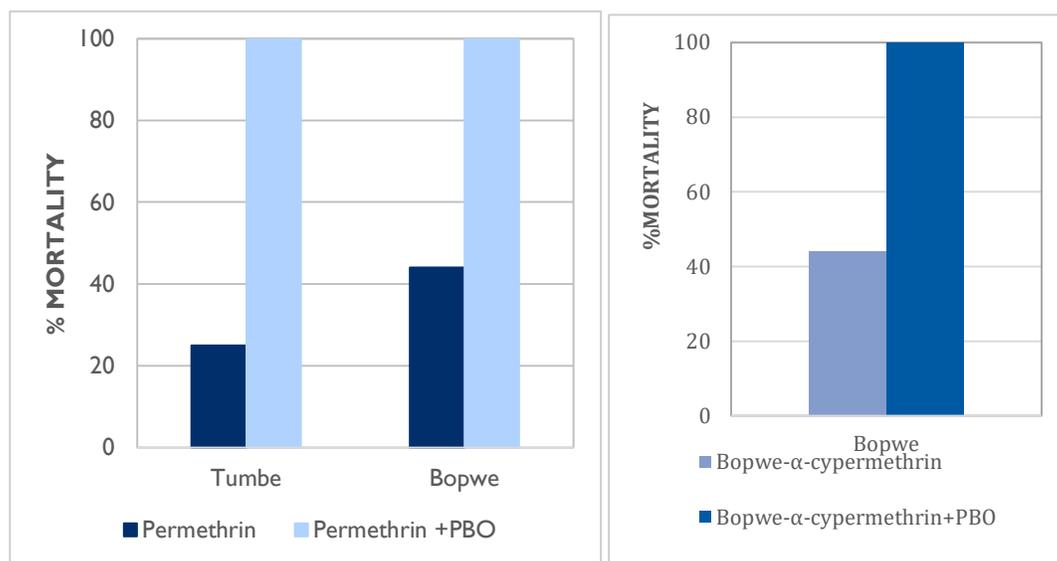


Note: In Donge and Bumbwini, deltamethrin was not tested because there was an inadequate number of mosquitoes.

Synergist tests with PBO

Complete restoration of susceptibility to permethrin and alphacypermethrin (100% mortality) after exposing mosquitoes to PBO implies the existence of a monooxygenase-based resistance mechanism in the vector population (Error! Reference source not found.). However, this test was not conducted in Unguja because of inadequate mosquito samples.

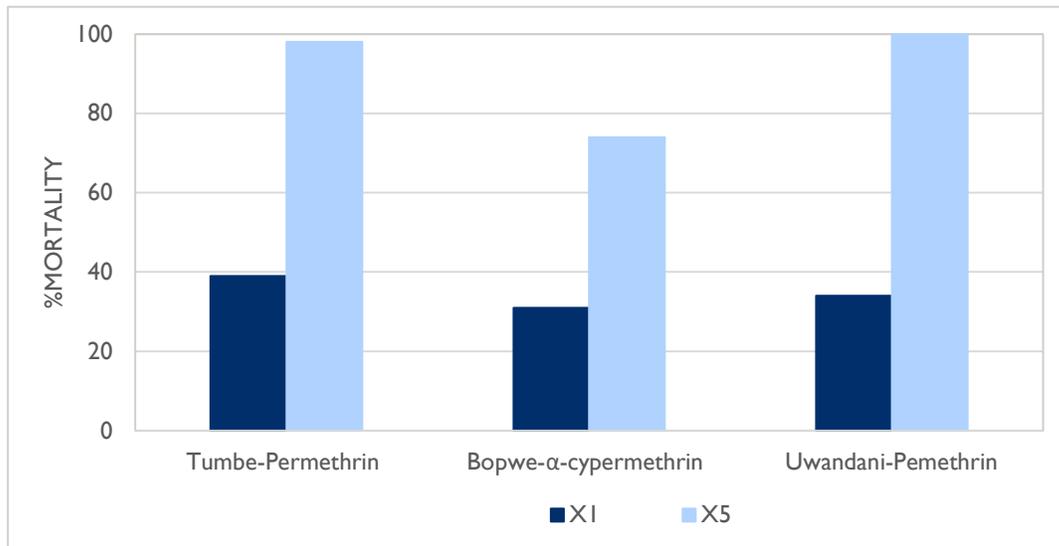
Figure 16. Synergist bioassay tests with PBO, Pemba



Intensity of pyrethroid resistance

The intensity of pyrethroid resistance to *An. gambiae* complex is not homogeneous across sentinel sites in Pemba, ranging between low and high values (Error! Reference source not found.). This was not tested in Unguja because of inadequate mosquito samples.

Figure 30. Intensity of pyrethroid resistance in Pemba



Mechanism of pyrethroid resistance to *An. arabiensis*

Target site resistance

The 350 mosquitoes collected from sites in Pemba that survived in the insecticide resistance assays were subjected to *kdr* testing. Priority for *kdr* screening was given to mosquito samples from Pemba where pyrethroid resistance is stronger as compared with Unguja. None of the samples were found to have either the east or west *kdr* gene mutation.

4. General Discussion and Recommendations

4.1 Malaria vector bionomics and IRS quality monitoring

Mainland Tanzania: At all Lake Zone sites where IRS was implemented, clothianidin (SumiShield 50WG) remained efficacious on several types of sprayed wall surfaces for 4 months post-IRS operations. Between 5 and 10 months post-IRS, the residual efficacy of most of the surfaces declined. Mud surfaces held the insecticide longer likely because of their rough texture when compared with other surfaces. Nonetheless, all surfaces recorded an average of greater than 80% mortality in the 10-month post-spray survey.

The population density of malaria vectors was low in sprayed districts during the reporting period with the exception of the Chato and Kakonko districts where relatively high vector densities were observed possibly due to a short rainfall period. The mean sporozoite rate was lower in sprayed sites, compared with non-sprayed sentinel sites. *An. gambiae* s.l. was the predominant vector species, caught in both sprayed and unsprayed sites. Furthermore, *An. funestus* s.s. was found in unsprayed sites and in one sprayed site—Kakonko District. Overall, the highest sporozoite rate was observed in the non-sprayed districts.

Assessment of vector biting behavior demonstrated that there is considerable outdoor biting risk in both sprayed and unsprayed sites. Blood-meal analysis indicates that *An. arabiensis* is the main vector in 7 out of the 11 sites, feeding both on human and animals and demonstrating exophilic behavior.

IRS was also found to reduce the longevity of *Anopheles* mosquitoes; future entomological surveillance efforts will need to carefully examine and confirm this finding. Currently, these analyses are conducted by community mosquito collectors or district vector control officers who may require more training and additional quality checks to ensure the reliability of parity data. Also, the interval of assessing parous rates before IRS (end of September to end of October 2018) was very short; 54his interval may need to be extended to determine whether reported trends are accurate. Finally, dissected mosquitoes should be identified to the species level to determine whether parous rates are different by species.

In conclusion, results reported here indicate that clothianidin had a prolonged IRS residual efficacy 5–8 months post-IRS operations and that IRS operations using clothianidin have been successful in keeping vector densities relatively low, which has resulted in reduced sporozoite rates. From the data collected, there seems to be a slow gradual shift in vector species because of the increased appearance of minor vectors, such as *An. coustani*, *An. pharoensis*, and *An. rufipes*.

In Zanzibar where pirimiphos-methyl was used for IRS, its residual efficacy on cement, oil and water paint, lime wash, stone blocks, and mud walls lasted up to 5 months with complete mortality of susceptible *An. gambiae* s.s. The insecticide remained effective for up to 7 months with a gradual decrease of its residual efficacy beginning at the eighth month. By 8 months, the average residual effect of pirimiphos-methyl remained much better on cement and both painted walls (mortality $\geq 75\%$) than other walls types (mortality $< 75\%$). Pirimiphos-methyl has been used for IRS in Zanzibar for 6 consecutive years. While pirimiphos-methyl is still effective for IRS, after 6 years (2013 to 2019) of use, we might consider new insecticides such as clothianidin (Sumishield) and clothianidin + deltamethrin (Fudora Fusion) as part of a pro-active insecticide

resistance management strategy for Zanzibar. The predominant vector species were *An. arabiensis* in both Pemba and Unguja. However, in previous years *An. gambiae* s.s. contributed to a higher percentage (68%) of the *An. gambiae* complex mosquitoes collected in some parts of Unguja. The reduction of the *An. gambiae* s.s. to only 1% in Unguja might be attributed to the effectiveness of IRS as IRS tends to be more effective against malaria vectors that are endophilic and anthropophilic like *An. gambiae* s.s. *Anopheles arabiensis* exhibited significantly higher outdoor biting behavior in both Pemba and Unguja. Their outdoor biting density was higher earlier in the night (7 pm–10 pm) when people are active outdoors and maintained at a lower level throughout the night *An. arabiensis* is known to feed on both indoors and outdoors, on humans and non-human hosts and can rest outdoors. This information suggests that after effective IRS, malaria vectors in Zanzibar are mostly feeding outdoors. This might have an implication in outdoor malaria transmission.

4.2 Insecticide Resistance Monitoring

The survey in **Mainland Tanzania** and **Zanzibar** showed a wide distribution of and high resistance to all pyrethroids tested (i.e., permethrin, alphacypermethrin, and deltamethrin). Furthermore, there was widespread susceptibility to carbamate and organophosphate insecticides with full susceptibility to clothianidin insecticide across surveyed sentinel districts. High resistance to pyrethroids permethrin, alphacypermethrin, and deltamethrin among *An. gambiae* s.l. mosquitoes was observed. Mosquitoes were fully susceptible to both permethrin and deltamethrin only in five districts: Bariadi, Kilosa, Meatu, Nyasa, and Shinyanga.

The high level of permethrin, alphacypermethrin, and deltamethrin resistance observed may be attributed to insecticide pressure created by the cumulative effect of long-term use of LLINs, which have been scaled-up in Mainland Tanzania and Zanzibar since 2010. Most of the LLINs used were Olyset® nets treated with permethrin (NBS, 2016) and PERMANENT™ treated with deltamethrin. The presence of millions of pyrethroid-treated bed nets in the field exposes mosquitoes to pyrethroids and therefore contributes to the survey's observed insecticide resistance. Similarly, extensive use of pyrethroids in agriculture might also contribute to the observed insecticide resistance pattern.

These results clearly indicate that pyrethroid resistance intensity is high in some sites and is increasing. Malaria vectors were still resistant to permethrin (mortality rate <90%) at 10× the diagnostic concentration in Kibondo and Ukerewe. They also showed high resistance intensity to deltamethrin in Misenyi and Rorya (mortality rate <90%). Presence of such high resistance intensity may result in LLIN programmatic failure (WHO, 2016). These four districts require close follow-up on the progress of observed resistance and effects on malaria transmission.

Resistance to bendiocarb was also observed in two districts: Biharamulo and Misenyi. Likewise, resistance to pirimiphos-methyl was detected in two districts: Biharamulo and Kilosa. The bendiocarb and pirimiphos-methyl resistances in Biharamulo, Kilosa, and Misenyi are most likely due to insecticide pressure created by their previous agricultural use in the area. Biharamulo, located in the Kagera region, is the only district that has indicated resistance to all four classes of insecticides: pyrethroids (deltamethrin and permethrin), carbamates (bendiocarb), organochlorines (DDT), and organophosphate (pirimiphos-methyl). As such, deployment of LLINs treated with a pyrethroid insecticide

and the synergist PBO, coupled with IRS using clothianidin, is highly recommended in districts to manage escalated resistance.

The tests also revealed that all local malaria vectors were fully susceptible to clothianidin in all seven sentinel districts of Mainland Tanzania. *Anopheles* mosquitoes showed 100% mortality indicating that clothianidin is effective.

Analysis for resistance mechanisms using synergist tests with PBO indicates that there may be more than one insecticide resistance mechanism contributing to the resistance observed at the sentinel sites. Full restoration of susceptibility after pre-exposure to PBO was observed against permethrin, alphacypermethrin, and deltamethrin in Mainland Tanzania and Zanzibar, with the exception of Ukerewe and Misenyi (for permethrin) and Biharamulo and Misenyi (for deltamethrin). These results suggest that insecticide resistance in the *An. gambiae* population is mainly mediated by oxidase (cytochrome P450)-based metabolic resistance with a minor contribution from other mechanisms.

Biochemical tests revealed the occurrence of both target site mutations (*kdr* east [L1014S]) and metabolic (cytochrome P450) resistance mechanisms in the sentinel districts in the Mainland. Only metabolic (cytochrome P450) resistance was recorded in Zanzibar.

Kdr-east [L1014S] mutation was detected in mosquitoes from more than half (14 of 22) of the sentinel districts with allelic frequencies ranging from 2% in Nachingwea and Geita to close to 90% in Kibondo. This shows that the *kdr* gene mutation intensity is increasing and spreading widely geographically. The increasing resistance trends indicate that selection pressure is still not adequately contained; more emphasis is needed to ensure resistance management. Likewise, the mean levels of oxidase enzymes were significantly higher in mosquitoes from almost all resistant sentinel districts, which shows the existence of the oxidase type of metabolic resistance. This suggests cytochrome P450 has a, likely, significant role in the observed pyrethroid resistance phenotype, which malaria control interventions such as LLINs with PBO may be more effective for malaria vector control in metabolic resistant areas.

About 52% and 82% of the sites in Mainland Tanzania and Zanzibar, respectively were dominated by *An. arabiensis*. This observation may be a strong indication that residual malaria transmission in some of the districts may be associated with the predominance of *An. arabiensis*, which may contribute to high malaria prevalence rates in the surveyed sites. The presence of outdoor biting *An. arabiensis*, may result in outdoor residual malaria transmission as described in some parts of Tanzania (Milali et al., 2017). Ecologically, *An. arabiensis* has a wide range of behavior patterns, which makes it more difficult to control, as compared with *An. gambiae* s.s. The increasing dominance of *An. arabiensis*, as indicated in this study, has significant implications in malaria vector control in Tanzania as it may indicate the need additional outdoor interventions.

4.3 Recommendations

1. Continue with longitudinal entomological monitoring to provide real-time informed evidence for malaria vector control in Mainland Tanzania and Zanzibar.
2. Intensify entomological monitoring in the event of an active foci investigation in Zanzibar.

3. In some parts of Mainland Tanzania and Zanzibar, *An. funestus* contributes a significant number of the malaria vector population. It is therefore important to expand the insecticide resistance monitoring to include *An. funestus*.
4. Expand the intensity of insecticide resistance testing of local malaria vectors. This will help link the observed phenotypic resistance and performance of vector control tools in the field. This information can then be used to inform operational decisions, such as changing the insecticide for IRS or the introducing a non-pyrethroid for IRS in areas with LLINs as the main intervention.
5. Expand the synergist assays in other pyrethroid resistance areas during insecticide resistance monitoring activities to help assess the involvement of oxidase-based metabolic resistance mechanisms in the production of resistance phenotypes.
6. In the presence of insecticide resistance, while researchers develop new tools to manage resistance, integrated vector management (i.e., environmental management) may have the most substantial impact on malaria transmission. The integrated vector management approach should be encouraged and appropriately deployed depending on specific local settings, particularly targeting areas with high malaria transmission, resistance, and reduced susceptibility to some insecticides of public health importance.
7. LLINs treated with a pyrethroid insecticide and the synergist PBO should be deployed in areas with metabolic (cytochrome P450) resistance mechanisms.

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Annexes

Annex I. Cone wall bioassay tests: percentage mortality obtained for female *Anopheles (An.) gambiae sensu stricto (s.s.)* (Kisumu strain) exposed on sprayed surface

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	November	Mud	2.5	100	100	100	100	100	100
Chato	November	Cement	0	100	100	100	100	100	100
Chato	November	Painted	0	100	100	100	100	100	100
Chato	November	Whitewash	0	100	100	100	100	100	100
Chato	November	Burnt brick	0	100	100	100	100	100	100
Misenyi	November	Mud	0	100	100	100	100	100	100
Misenyi	November	Cement	0	100	100	100	100	100	100
Misenyi	November	Painted	0	100	100	100	100	100	100
Misenyi	November	Whitewash	2.5	100	100	100	100	100	100
Misenyi	November	Burnt brick	0	100	100	100	100	100	100
Bukoba Rural	November	Mud	0	100	100	100	100	100	100
Bukoba Rural	November	Cement	0	100	100	100	100	100	100
Bukoba Rural	November	Painted	0	100	100	100	100	100	100
Bukoba Rural	November	Whitewash	0	100	100	100	100	100	100
Bukoba Rural	November	Burnt brick	0	100	100	100	100	100	100
Ngara	November	Mud	0	100	100	100	100	100	100
Ngara	November	Cement	0	100	100	100	100	100	100
Ngara	November	Painted	0	95	100	100	100	100	100
Ngara	November	Whitewash	2.5	100	100	100	100	100	100
Ngara	November	Burnt brick	0	100	100	100	100	100	100
Kakonko	November	Mud	10	100	100	100	100	100	100
Kakonko	November	Cement	0	100	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Kakonko	November	Painted	2.5	100	100	100	100	100	100
Kakonko	November	Whitewash	7.5	100	100	100	100	100	100
Kakonko	November	Burnt brick	0	100	100	100	100	100	100
Nyang'hwale	November	Mud	7.5	100	100	100	100	100	100
Nyang'hwale	November	Cement	10	100	100	100	100	100	100
Nyang'hwale	November	Painted	2.5	95	100	100	100	100	100
Nyang'hwale	November	Whitewash	5	100	100	100	100	100	100
Nyang'hwale	November	Burnt brick	5	100	100	100	100	100	100
Buchosa	November	Mud	17.5	95	100	100	100	100	100
Buchosa	November	Cement	15	100	100	100	100	100	100
Buchosa	November	Painted	0	97.5	100	100	100	100	100
Buchosa	November	Whitewash	40	100	100	100	100	100	100
Buchosa	November	Burnt brick	10	100	100	100	100	100	100
Chato	December	Mud	95	100	100	100	100	100	100
Chato	December	Cement	100	100	100	100	100	100	100
Chato	December	Painted	97.5	100	100	100	100	100	100
Chato	December	Whitewash	90	100	100	100	100	100	100
Chato	December	Burnt brick	100	100	100	100	100	100	100
Misenyi	December	Mud	100	100	100	100	100	100	100
Misenyi	December	Cement	90	100	100	100	100	100	100
Misenyi	December	Painted	57.5	92.5	100	100	100	100	100
Misenyi	December	Whitewash	87.5	100	100	100	100	100	100
Misenyi	December	Burnt brick	77.5	100	100	100	100	100	100
Bukoba Rural	December	Mud	65	100	100	100	100	100	100
Bukoba Rural	December	Cement	37.5	100	100	100	100	100	100
Bukoba Rural	December	Painted	60	100	100	100	100	100	100
Bukoba Rural	December	Whitewash	47.5	100	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Bukoba Rural	December	Burnt brick	25	100	100	100	100	100	100
Ngara	December	Mud	67.5	92.5	100	100	100	100	100
Ngara	December	Cement	90	100	100	100	100	100	100
Ngara	December	Painted	92.5	100	100	100	100	100	100
Ngara	December	Whitewash	95	100	100	100	100	100	100
Ngara	December	Burnt brick	97.5	100	100	100	100	100	100
Kakonko	December	Mud	92.5	100	100	100	100	100	100
Kakonko	December	Cement	50	100	100	100	100	100	100
Kakonko	December	Painted	72.5	100	100	100	100	100	100
Kakonko	December	Whitewash	70	100	100	100	100	100	100
Kakonko	December	Burnt brick	55	100	100	100	100	100	100
Nyang'hwale	December	Mud	97.5	100	100	100	100	100	100
Nyang'hwale	December	Cement	90	100	100	100	100	100	100
Nyang'hwale	December	Painted	77.5	100	100	100	100	100	100
Nyang'hwale	December	Whitewash	97.5	100	100	100	100	100	100
Nyang'hwale	December	Burnt brick	90	100	100	100	100	100	100
Buchosa	December	Mud	97.5	100	100	100	100	100	100
Buchosa	December	Cement	100	100	100	100	100	100	100
Buchosa	December	Painted	97.5	100	100	100	100	100	100
Buchosa	December	Whitewash	97.5	100	100	100	100	100	100
Buchosa	December	Burnt brick	75	100	100	100	100	100	100
Chato	January	Mud	20	100	100	100	100	100	100
Chato	January	Cement	30	95	100	100	100	100	100
Chato	January	Painted	20	100	100	100	100	100	100
Chato	January	Whitewash	17.5	100	100	100	100	100	100
Chato	January	Burnt brick	22.5	97.5	100	100	100	100	100
Misenyi	January	Mud	12.5	67.5	90	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Misenyi	January	Cement	0	95	100	100	100	100	100
Misenyi	January	Painted	25	100	100	100	100	100	100
Misenyi	January	Whitewash	17.5	100	100	100	100	100	100
Misenyi	January	Burnt brick	22.5	87.5	92.5	100	100	100	100
Bukoba Rural	January	Mud	25	92.5	95	100	100	100	100
Bukoba Rural	January	Cement	22.5	100	100	100	100	100	100
Bukoba Rural	January	Painted	27.5	100	100	100	100	100	100
Bukoba Rural	January	Whitewash	15	100	100	100	100	100	100
Bukoba Rural	January	Burnt brick	27.5	100	100	100	100	100	100
Ngara	January	Mud	50	97.5	100	100	100	100	100
Ngara	January	Cement	45	100	100	100	100	100	100
Ngara	January	Painted	90	100	100	100	100	100	100
Ngara	January	Whitewash	25	100	100	100	100	100	100
Ngara	January	Burnt brick	37.5	97.5	100	100	100	100	100
Kakonko	January	Mud	65	100	100	100	100	100	100
Kakonko	January	Cement	42.5	97.5	100	100	100	100	100
Kakonko	January	Painted	45	97.5	100	100	100	100	100
Kakonko	January	Whitewash	40	100	100	100	100	100	100
Kakonko	January	Burnt brick	70	95	100	100	100	100	100
Nyang'hwale	January	Mud	60	100	100	100	100	100	100
Nyang'hwale	January	Cement	35	100	100	100	100	100	100
Nyang'hwale	January	Painted	30	100	100	100	100	100	100
Nyang'hwale	January	Whitewash	72.5	100	100	100	100	100	100
Nyang'hwale	January	Burnt brick	45	100	100	100	100	100	100
Buchosa	January	Mud	50	100	100	100	100	100	100
Buchosa	January	Cement	22.5	95	100	100	100	100	100
Buchosa	January	Painted	27.5	92.5	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Buchosa	January	Whitewash	60	100	100	100	100	100	100
Buchosa	January	Burnt brick	47.5	92.5	100	100	100	100	100
Chato	February	Mud	85	97.5	97.5	100	100	100	100
Chato	February	Cement	77.5	100	100	100	100	100	100
Chato	February	Painted	87.5	97.5	100	100	100	100	100
Chato	February	Whitewash	92.5	100	100	100	100	100	100
Chato	February	Burnt brick	92.5	100	100	100	100	100	100
Misenyi	February	Mud	55	100	100	100	100	100	100
Misenyi	February	Cement	27.5	100	100	100	100	100	100
Misenyi	February	Painted	30	100	100	100	100	100	100
Misenyi	February	Whitewash	30	100	100	100	100	100	100
Misenyi	February	Burnt brick	27.5	97.5	100	100	100	100	100
Bukoba Rural	February	Mud	12.5	87.5	100	100	100	100	100
Bukoba Rural	February	Cement	17.5	77.5	100	100	100	100	100
Bukoba Rural	February	Painted	45	67.5	77.5	95	100	100	100
Bukoba Rural	February	Whitewash	10	100	100	100	100	100	100
Bukoba Rural	February	Burnt brick	60	100	100	100	100	100	100
Ngara	February	Mud	22.5	100	100	100	100	100	100
Ngara	February	Cement	17.5	100	100	100	100	100	100
Ngara	February	Painted	42.5	100	100	100	100	100	100
Ngara	February	Whitewash	17.5	100	100	100	100	100	100
Ngara	February	Burnt brick	15	100	100	100	100	100	100
Kakonko	February	Mud	30	75	95	100	100	100	100
Kakonko	February	Cement	47.5	92.5	97.5	100	100	100	100
Kakonko	February	Painted	30	65	87.5	100	100	100	100
Kakonko	February	Whitewash	20	82.5	100	100	100	100	100
Kakonko	February	Burnt brick	42.5	92.5	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Nyang'hwale	February	Mud	25	100	100	100	100	100	100
Nyang'hwale	February	Cement	22.5	100	100	100	100	100	100
Nyang'hwale	February	Painted	50	100	100	100	100	100	100
Nyang'hwale	February	Whitewash	22.5	100	100	100	100	100	100
Nyang'hwale	February	Burnt brick	70	87.5	100	100	100	100	100
Buchosa	February	Mud	77.5	87.5	95	100	100	100	100
Buchosa	February	Cement	75	100	100	100	100	100	100
Buchosa	February	Painted	72.5	82.5	92.5	100	100	100	100
Buchosa	February	Whitewash	75	92.5	100	100	100	100	100
Buchosa	February	Burnt brick	92.5	100	100	100	100	100	100
Chato	March	Mud	57.5	75	90	100	100	100	100
Chato	March	Cement	67.5	70	82.5	100	100	100	100
Chato	March	Painted	82.5	80	92.5	100	100	100	100
Chato	March	Whitewash	42.5	90	97.5	100	100	100	100
Chato	March	Burnt brick	65	90	100	100	100	100	100
Misenyi	March	Mud	30	55	72.5	85	87.5	97.5	100
Misenyi	March	Cement	22.5	67.5	72.5	80	87.5	97.5	100
Misenyi	March	Painted	22.5	47.5	65	72.5	77.5	97.5	100
Misenyi	March	Whitewash	25	45	67.5	80	90	97.5	100
Misenyi	March	Burnt brick	52.5	67.5	80	87.5	87.5	100	100
Bukoba Rural	March	Mud	25	52.5	70	82.5	97.5	100	100
Bukoba Rural	March	Cement	30	50	70	87.5	100	100	100
Bukoba Rural	March	Painted	37.5	62.5	77.5	80	97.5	100	100
Bukoba Rural	March	Whitewash	27.5	75	92.5	93.3	100	100	100
Bukoba Rural	March	Burnt brick	37.5	75	87.5	90	100	100	100
Ngara	March	Mud	30	47.5	57.5	72.5	100	100	100
Ngara	March	Cement	20	40	50	80	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Ngara	March	Painted	22.5	40	57.5	70	100	100	100
Ngara	March	Whitewash	12.5	52.5	60	70	100	100	100
Ngara	March	Burnt brick	30	57.5	72.5	72.5	100	100	100
Kakonko	March	Mud	32.5	50	62.5	72.5	90	100	100
Kakonko	March	Cement	40	57.5	70	95	100	100	100
Kakonko	March	Painted	40	55	67.5	80	90	100	100
Kakonko	March	Whitewash	37.5	52.5	67.5	85	92.5	100	100
Kakonko	March	Burnt brick	42.5	62.5	72.5	87.5	95	100	100
Nyang'hwale	March	Mud	17.5	35	52.5	77.5	95	100	100
Nyang'hwale	March	Cement	5	47.5	62.5	77.5	95	100	100
Nyang'hwale	March	Painted	27.5	47.5	57.5	85	100	100	100
Nyang'hwale	March	Whitewash	30	55	65	82.5	100	100	100
Nyang'hwale	March	Burnt brick	22.5	52.5	65	77.5	95	100	100
Buchosa	March	Mud	17.5	52.5	65	95	100	100	100
Buchosa	March	Cement	27.5	50	72.5	77.5	100	100	100
Buchosa	March	Painted	20	60	77.5	90	100	100	100
Buchosa	March	Whitewash	22.5	50	70	90	100	100	100
Buchosa	March	Burnt brick	42.5	60	70	87.5	100	100	100
Chato	April	Mud	30	45	67.5	92.5	100	100	100
Chato	April	Cement	27.5	37.5	55	85	100	100	100
Chato	April	Painted	27.5	30	45	95	100	100	100
Chato	April	Whitewash	17.5	52.5	45	92.5	100	100	100
Chato	April	Burnt brick	45	60	67.5	87.5	100	100	100
Misenyi	April	Mud	15	27.5	55	77.5	100	100	100
Misenyi	April	Cement	15	32.5	62.5	87.5	100	100	100
Misenyi	April	Painted	17.5	30	60	80	100	100	100
Misenyi	April	Whitewash	12.5	32.5	47.5	85	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Misenyi	April	Burnt brick	17.5	42.5	67.5	85	100	100	100
Bukoba Rural	April	Mud	22.5	37.5	55	82.5	100	100	100
Bukoba Rural	April	Cement	12.5	27.5	50	65	97.5	100	100
Bukoba Rural	April	Painted	12.5	35	60	85	100	100	100
Bukoba Rural	April	Whitewash	17.5	45	62.5	95	100	100	100
Bukoba Rural	April	Burnt brick	17.5	35	52.5	75	100	100	100
Ngara	April	Mud	10	52.5	72.5	82.5	100	100	100
Ngara	April	Cement	40	70	80	100	100	100	100
Ngara	April	Painted	45	65	70	85	100	100	100
Ngara	April	Whitewash	27.5	52.5	85	100	100	100	100
Ngara	April	Burnt brick	22.5	55	100	100	100	100	100
Kakonko	April	Mud	12.5	40	65	92.5	100	100	100
Kakonko	April	Cement	52.5	52.5	95	97.5	100	100	100
Kakonko	April	Painted	15	70	100	100	100	100	100
Kakonko	April	Whitewash	17.5	77.5	85	100	100	100	100
Kakonko	April	Burnt brick	22.5	80	82.5	97.5	100	100	100
Nyang'hwale	April	Mud	20	47.5	87.5	100	100	100	100
Nyang'hwale	April	Cement	17.5	45	100	100	100	100	100
Nyang'hwale	April	Painted	10	57.5	100	100	100	100	100
Nyang'hwale	April	Whitewash	27.5	57.5	100	100	100	100	100
Nyang'hwale	April	Burnt brick	37.5	27.5	100	100	100	100	100
Buchosa	April	Mud	30	42.5	65	95	100	100	100
Buchosa	April	Cement	22.5	67.5	90	100	100	100	100
Buchosa	April	Painted	35	72.5	85	100	100	100	100
Buchosa	April	Whitewash	35	75	82.5	100	100	100	100
Buchosa	April	Burnt brick	27.5	77.5	82.5	100	100	100	100
Chato	May	Mud	20	42.5	62.5	75	90	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	May	Cement	17.5	27.5	50	62.5	95	100	100
Chato	May	Painted	20	30	37.5	45	82.5	100	100
Chato	May	Whitewash	17.5	22.5	30	40	92.5	100	100
Chato	May	Burnt brick	45	35	47.5	62.5	82.5	100	100
Misenyi	May	Mud	7.5	17.5	27.5	60	77.5	100	100
Misenyi	May	Cement	5	35	55	77.5	90	100	100
Misenyi	May	Painted	10	42.5	67.5	80	100	100	100
Misenyi	May	Whitewash	22.5	40	50	70	97.5	100	100
Misenyi	May	Burnt brick	17.5	27.5	47.5	72.5	92.5	100	100
Bukoba Rural	May	Mud	15	17.5	32.5	55	87.5	100	100
Bukoba Rural	May	Cement	10	12.5	30	52.5	80	100	100
Bukoba Rural	May	Painted	15	32.5	37.5	60	90	100	100
Bukoba Rural	May	Whitewash	12.5	25	30	47.5	85	100	100
Bukoba Rural	May	Burnt brick	10	17.5	37.5	52.5	85	100	100
Ngara	May	Mud	10	55	100	100	100	100	100
Ngara	May	Cement	17.5	75	95	100	100	100	100
Ngara	May	Painted	22.5	77.5	97.5	97.5	100	100	100
Ngara	May	Whitewash	10	47.5	95	92.5	100	100	100
Ngara	May	Burnt brick	10	57.5	100	100	100	100	100
Kakonko	May	Mud	25	55	67.5	92.5	100	100	100
Kakonko	May	Cement	20	52.5	72.5	82.5	97.5	100	100
Kakonko	May	Painted	2.5	55	70	87.5	95	100	100
Kakonko	May	Whitewash	15	35	52.5	65	82.5	100	100
Kakonko	May	Burnt brick	7.5	60	75	82.5	100	100	100
Nyang'hwale	May	Mud	12.5	47.5	62.5	100	100	100	100
Nyang'hwale	May	Cement	7.5	32.5	55	100	100	100	100
Nyang'hwale	May	Painted	12.5	52.5	75	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Nyang'hwale	May	Whitewash	15	50	75	100	100	100	100
Nyang'hwale	May	Burnt brick	7.5	32.5	87.5	100	100	100	100
Buchosa	May	Mud	17.5	55	65	97.5	100	100	100
Buchosa	May	Cement	30	57.5	77.5	95	100	100	100
Buchosa	May	Painted	35	57.5	95	100	100	100	100
Buchosa	May	Whitewash	20	47.5	90	97.5	100	100	100
Buchosa	May	Burnt brick	35	52.5	85	95	100	100	100
Chato	June	Mud	30	40	50	77.5	85	92.5	100
Chato	June	Cement	15	27.5	60	67.5	87.5	92.5	100
Chato	June	Painted	20	40	47.5	45	77.5	92.5	100
Chato	June	Whitewash	25	45	45	40	80	95	100
Chato	June	Burnt brick	27.5	30	47.5	55	75	95	100
Misenyi	June	Mud	12.5	15	25	52.5	72.5	90	100
Misenyi	June	Cement	10	15	40	67.5	75	90	100
Misenyi	June	Painted	7.5	17.5	50	67.5	85	92.5	100
Misenyi	June	Whitewash	10	17.5	42.5	62.5	87.5	87.5	100
Misenyi	June	Burnt brick	22.5	22.5	42.5	62.5	82.5	92.5	100
Bukoba Rural	June	Mud	15	17.5	30	50	80	90	100
Bukoba Rural	June	Cement	7.5	10	30	47.5	80	90	100
Bukoba Rural	June	Painted	25	17.5	37.5	52.5	90	92.5	100
Bukoba Rural	June	Whitewash	12.5	12.5	30	45	77.5	97.5	100
Bukoba Rural	June	Burnt brick	17.5	20	37.5	52.5	72.5	97.5	100
Ngara	June	Mud	10	30	62.5	80	92.5	100	100
Ngara	June	Cement	5	22.5	57.5	75	87.5	92.5	100
Ngara	June	Painted	10	35	50	77.5	92.5	95	100
Ngara	June	Whitewash	20	40	57.5	77.5	87.5	92.5	100
Ngara	June	Burnt brick	12.5	22.5	47.5	67.5	92.5	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Kakonko	June	Mud	10	32.5	45	70	82.5	87.5	100
Kakonko	June	Cement	12.5	32.5	55	70	87.5	95	100
Kakonko	June	Painted	10	30	60	80	95	97.5	100
Kakonko	June	Whitewash	12.5	30	57.5	65	92.5	100	100
Kakonko	June	Burnt brick	10	25	52.5	72.5	82.5	95	100
Nyang'hwale	June	Mud	5	20	50	67.5	87.5	92.5	100
Nyang'hwale	June	Cement	15	27.5	52.5	67.5	82.5	95	100
Nyang'hwale	June	Painted	10	32.5	55	72.5	90	95	100
Nyang'hwale	June	Whitewash	17.5	25	42.5	65	80	90	100
Nyang'hwale	June	Burnt brick	17.5	30	60	72.5	85	95	100
Buchosa	June	Mud	12.5	35	65	77.5	82.5	95	100
Buchosa	June	Cement	7.5	30	62.5	80	92.5	100	100
Buchosa	June	Painted	5	30	52.5	75	85	90	100
Buchosa	June	Whitewash	15	25	52.5	77.5	90	100	100
Buchosa	June	Burnt brick	15	27.5	72.5	85	92.5	97.5	100
Chato	July	Mud	10	17.5	40	60	80	82.5	95
Chato	July	Cement	15	22.5	45	60	77.5	90	97.5
Chato	July	Painted	10	20	40	57.5	70	85	97.5
Chato	July	Whitewash	12.5	20	42.5	60	70	87.5	92.5
Chato	July	Burnt brick	10	25	45	60	75	87.5	97.5
Misenyi	July	Mud	15	22.5	37.5	57.5	72.5	82.5	100
Misenyi	July	Cement	17.5	27.5	45	52.5	75	85	97.5
Misenyi	July	Painted	7.5	12.5	22.5	35	60	72.5	87.5
Misenyi	July	Whitewash	22.5	27.5	47.5	57.5	77.5	87.5	97.5
Misenyi	July	Burnt brick	10	12.5	32.5	47.5	65	80	90
Bukoba Rural	July	Mud	7.5	25	45	65	82.5	87.5	97.5
Bukoba Rural	July	Cement	10	22.5	40	57.5	75	90	97.5

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Bukoba Rural	July	Painted	5	10	35	57.5	75	80	90
Bukoba Rural	July	Whitewash	2.5	12.5	35	50	70	82.5	92.5
Bukoba Rural	July	Burnt brick	10	12.5	35	60	67.5	85	90
Ngara	July	Mud	7.5	17.5	40	65	77.5	82.5	92.5
Ngara	July	Cement	10	25	40	60	82.5	90	100
Ngara	July	Painted	10	25	40	60	77.5	87.5	95
Ngara	July	Whitewash	12.5	20	42.5	62.5	75	90	92.5
Ngara	July	Burnt brick	10	20	42.5	67.5	82.5	95	100
Kakonko	July	Mud	15	32.5	62.5	75	80	92.5	15
Kakonko	July	Cement	20	40	65	80	90	100	20
Kakonko	July	Painted	20	35	52.5	72.5	87.5	92.5	20
Kakonko	July	Whitewash	20	37.5	55	77.5	82.5	92.5	20
Kakonko	July	Burnt brick	17.5	42.5	57.5	72.5	87.5	97.5	17.5
Nyang'hwale	July	Mud	5	20	45	67.5	75	85	95
Nyang'hwale	July	Cement	7.5	17.5	45	57.5	77.5	87.5	97.5
Nyang'hwale	July	Painted	10	20	40	60	77.5	85	95
Nyang'hwale	July	Whitewash	12.5	30	45	60	72.5	87.5	92.5
Nyang'hwale	July	Burnt brick	10	25	47.5	67.5	82.5	90	97.5
Buchosa	July	Mud	15	25	45	62.5	70	82.5	92.5
Buchosa	July	Cement	5	12.5	30	52.5	77.5	87.5	100
Buchosa	July	Painted	2.5	10	30	47.5	67.5	85	95
Buchosa	July	Whitewash	7.5	17.5	35	55	67.5	77.5	92.5
Buchosa	July	Burnt brick	10	20	37.5	60	75	90	97.5
Chato	August	Mud	7.5	15	30	42.5	60	70	82.5
Chato	August	Cement	2.5	15	22.5	40	62.5	75	85
Chato	August	Painted	5	10	27.5	45	57.5	77.5	85
Chato	August	Whitewash	2.5	12.5	30	42.5	52.5	72.5	80

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	August	Burnt brick	10	27.5	42.5	52.5	67.5	80	85
Misenyi	August	Mud	7.5	15	35	45	62.5	80	85
Misenyi	August	Cement	10	20	35	50	62.5	77.5	82.5
Misenyi	August	Painted	5	10	27.5	40	57.5	70	80
Misenyi	August	Whitewash	2.5	12.5	32.5	37.5	57.5	77.5	87.5
Misenyi	August	Burnt brick	10	12.5	35	47.5	62.5	80	85
Bukoba Rural	August	Mud	5	20	35	47.5	65	70	87.5
Bukoba Rural	August	Cement	5	12.5	27.5	40	50	77.5	85
Bukoba Rural	August	Painted	5	7.5	20	35	60	72.5	82.5
Bukoba Rural	August	Whitewash	10	20	30	42.5	60	75	82.5
Bukoba Rural	August	Burnt brick	5	10	25	42.5	50	67.5	85
Ngara	August	Mud	7.5	15	40	60	75	82.5	85
Ngara	August	Cement	10	22.5	35	52.5	62.5	75	85
Ngara	August	Painted	5	10	27.5	50	65	70	82.5
Ngara	August	Whitewash	2.5	12.5	32.5	42.5	65	75	85
Ngara	August	Burnt brick	10	12.5	30	45	62.5	77.5	82.5
Kakonko	August	Mud	7.5	22.5	42.5	60	72.5	82.5	87.5
Kakonko	August	Cement	7.5	15	27.5	47.5	62.5	75	82.5
Kakonko	August	Painted	5	15	30	50	65	77.5	85
Kakonko	August	Whitewash	2.5	12.5	35	50	65	75	85
Kakonko	August	Burnt brick	10	12.5	27.5	50	65	75	82.5
Nyang'hwale	August	Mud	5	20	35	52.5	67.5	77.5	85
Nyang'hwale	August	Cement	7.5	22.5	35	52.5	65	80	82.5
Nyang'hwale	August	Painted	5	10	25	40	50	72.5	82.5
Nyang'hwale	August	Whitewash	2.5	12.5	27.5	42.5	65	72.5	82.5
Nyang'hwale	August	Burnt brick	10	12.5	30	50	62.5	75	85
Buchosa	August	Mud	5	15	30	42.5	55	77.5	87.5

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Buchosa	August	Cement	7.5	20	37.5	50	67.5	75	82.5
Buchosa	August	Painted	10	15	35	47.5	60	72.5	85
Buchosa	August	Whitewash	5	10	30	40	52.5	75	80
Buchosa	August	Burnt brick	10	20	35	45	60	77.5	85

Annex 2. Fumigant bioassay tests: percentage mortality obtained for female *Anopheles (An.) gambiae sensu stricto (s.s.)* (Kisumu strain) exposed on sprayed surfaces

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	November	Mud	0	90	100	100	100	100	100
Chato	November	Cement	0	100	100	100	100	100	100
Chato	November	Painted	0	85	100	100	100	100	100
Chato	November	Whitewash	0	100	100	100	100	100	100
Chato	November	Burnt brick	0	80	100	100	100	100	100
Misenyi	November	Mud	5	100	100	100	100	100	100
Misenyi	November	Cement	0	100	100	100	100	100	100
Misenyi	November	Painted	10	100	100	100	100	100	100
Misenyi	November	Whitewash	0	100	100	100	100	100	100
Misenyi	November	Burnt brick	0	100	100	100	100	100	100
Bukoba Rural	November	Mud	0	100	100	100	100	100	100
Bukoba Rural	November	Cement	0	100	100	100	100	100	100
Bukoba Rural	November	Painted	0	95	100	100	100	100	100
Bukoba Rural	November	Whitewash	0	100	100	100	100	100	100
Bukoba Rural	November	Burnt brick	10	100	100	100	100	100	100
Ngara	November	Mud	5	100	100	100	100	100	100
Ngara	November	Cement	0	100	100	100	100	100	100
Ngara	November	Painted	5	75	100	100	100	100	100
Ngara	November	Whitewash	0	90	100	100	100	100	100
Ngara	November	Burnt brick	0	85	100	100	100	100	100
Kakonko	November	Mud	0	100	100	100	100	100	100
Kakonko	November	Cement	0	100	100	100	100	100	100
Kakonko	November	Painted	0	100	100	100	100	100	100
Kakonko	November	Whitewash	0	50	90	100	100	100	100
Kakonko	November	Burnt brick	5	100	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Nyang'hwale	November	Mud	0	50	60	100	100	100	100
Nyang'hwale	November	Cement	0	100	100	100	100	100	100
Nyang'hwale	November	Painted	0	90	95	100	100	100	100
Nyang'hwale	November	Whitewash	0	100	100	100	100	100	100
Nyang'hwale	November	Burnt brick	20	20	30	100	100	100	100
Buchosa	November	Mud	5	100	100	100	100	100	100
Buchosa	November	Cement	70	100	100	100	100	100	100
Buchosa	November	Painted	0	100	100	100	100	100	100
Buchosa	November	Whitewash	10	100	100	100	100	100	100
Buchosa	November	Burnt brick	10	100	100	100	100	100	100
Chato	December	Mud	85	100	100	100	100	100	100
Chato	December	Cement	90	100	100	100	100	100	100
Chato	December	Painted	100	100	100	100	100	100	100
Chato	December	Whitewash	100	100	100	100	100	100	100
Chato	December	Burnt brick	100	100	100	100	100	100	100
Misenyi	December	Mud	85	100	100	100	100	100	100
Misenyi	December	Cement	80	100	100	100	100	100	100
Misenyi	December	Painted	50	80	90	100	100	100	100
Misenyi	December	Whitewash	60	70	100	100	100	100	100
Misenyi	December	Burnt brick	100	100	100	100	100	100	100
Bukoba Rural	December	Mud	20	100	100	100	100	100	100
Bukoba Rural	December	Cement	0	100	100	100	100	100	100
Bukoba Rural	December	Painted	20	100	100	100	100	100	100
Bukoba Rural	December	Whitewash	10	100	100	100	100	100	100
Bukoba Rural	December	Burnt brick	0	100	100	100	100	100	100
Ngara	December	Mud	20	40	80	100	100	100	100
Ngara	December	Cement	80	100	100	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Ngara	December	Painted	50	100	100	100	100	100	100
Ngara	December	Whitewash	60	90	100	100	100	100	100
Ngara	December	Burnt brick	80	100	100	100	100	100	100
Kakonko	December	Mud	80	100	100	100	100	100	100
Kakonko	December	Cement	5	100	100	100	100	100	100
Kakonko	December	Painted	70	100	100	100	100	100	100
Kakonko	December	Whitewash	10	100	100	100	100	100	100
Kakonko	December	Burnt brick	0	100	100	100	100	100	100
Nyang'hwale	December	Mud	40	100	100	100	100	100	100
Nyang'hwale	December	Cement	50	95	100	100	100	100	100
Nyang'hwale	December	Painted	50	80	100	100	100	100	100
Nyang'hwale	December	Whitewash	80	100	100	100	100	100	100
Nyang'hwale	December	Burnt brick	70	100	100	100	100	100	100
Buchosa	December	Mud	70	100	100	100	100	100	100
Buchosa	December	Cement	95	100	100	100	100	100	100
Buchosa	December	Painted	90	100	100	100	100	100	100
Buchosa	December	Whitewash	40	100	100	100	100	100	100
Buchosa	December	Burnt brick	100	100	100	100	100	100	100
Chato	January	Mud	30	30	90	100	100	100	100
Chato	January	Cement	40	50	80	100	100	100	100
Chato	January	Painted	30	90	100	100	100	100	100
Chato	January	Whitewash	10	70	90	100	100	100	100
Chato	January	Burnt brick	50	100	100	100	100	100	100
Misenyi	January	Mud	30	80	100	100	100	100	100
Misenyi	January	Cement	50	70	80	100	100	100	100
Misenyi	January	Painted	20	100	100	100	100	100	100
Misenyi	January	Whitewash	70	70	90	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Misenyi	January	Burnt brick	80	70	100	100	100	100	100
Bukoba Rural	January	Mud	20	100	100	100	100	100	100
Bukoba Rural	January	Cement	50	100	100	100	100	100	100
Bukoba Rural	January	Painted	70	90	100	100	100	100	100
Bukoba Rural	January	Whitewash	10	80	90	100	100	100	100
Bukoba Rural	January	Burnt brick	70	90	100	100	100	100	100
Ngara	January	Mud	10	50	60	100	100	100	100
Ngara	January	Cement	20	60	80	100	100	100	100
Ngara	January	Painted	40	10	90	100	100	100	100
Ngara	January	Whitewash	10	70	80	100	100	100	100
Ngara	January	Burnt brick	50	30	100	100	100	100	100
Kakonko	January	Mud	20	30	70	100	100	100	100
Kakonko	January	Cement	50	100	100	100	100	100	100
Kakonko	January	Painted	50	100	100	100	100	100	100
Kakonko	January	Whitewash	40	100	100	100	100	100	100
Kakonko	January	Burnt brick	20	100	100	100	100	100	100
Nyang'hwale	January	Mud	10	80	90	90	100	100	100
Nyang'hwale	January	Cement	40	40	100	100	100	100	100
Nyang'hwale	January	Painted	10	50	100	100	100	100	100
Nyang'hwale	January	Whitewash	50	60	100	100	100	100	100
Nyang'hwale	January	Burnt brick	10	40	90	100	100	100	100
Buchosa	January	Mud	20	100	100	100	100	100	100
Buchosa	January	Cement	20	90	100	100	100	100	100
Buchosa	January	Painted	10	30	80	100	100	100	100
Buchosa	January	Whitewash	30	50	100	100	100	100	100
Buchosa	January	Burnt brick	10	100	100	100	100	100	100
Chato	February	Mud	0	80	90	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	February	Cement	40	100	100	100	100	100	100
Chato	February	Painted	0	40	90	100	100	100	100
Chato	February	Whitewash	0	0	90	100	100	100	100
Chato	February	Burnt brick	40	100	100	100	100	100	100
Misenyi	February	Mud	20	100	100	100	100	100	100
Misenyi	February	Cement	60	100	100	100	100	100	100
Misenyi	February	Painted	50	100	100	100	100	100	100
Misenyi	February	Whitewash	80	100	100	100	100	100	100
Misenyi	February	Burnt brick	40	100	100	100	100	100	100
Bukoba Rural	February	Mud	30	90	100	100	100	100	100
Bukoba Rural	February	Cement	0	100	100	100	100	100	100
Bukoba Rural	February	Painted	10	0	0	0	100	100	100
Bukoba Rural	February	Whitewash	0	100	100	100	100	100	100
Bukoba Rural	February	Burnt brick	0	100	100	100	100	100	100
Ngara	February	Mud	0	100	100	100	100	100	100
Ngara	February	Cement	10	100	100	100	100	100	100
Ngara	February	Painted	10	100	100	100	100	100	100
Ngara	February	Whitewash	90	100	100	100	100	100	100
Ngara	February	Burnt brick	10	80	100	100	100	100	100
Kakonko	February	Mud	0	100	100	100	100	100	100
Kakonko	February	Cement	0	50	70	70	100	100	100
Kakonko	February	Painted	0	80	90	100	100	100	100
Kakonko	February	Whitewash	0	0	0	0	0	60	100
Kakonko	February	Burnt brick	10	50	60	100	100	100	100
Nyang'hwale	February	Mud	10	100	100	100	100	100	100
Nyang'hwale	February	Cement	0	100	100	100	100	100	100
Nyang'hwale	February	Painted	10	40	60	90	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Nyang'hwale	February	Whitewash	40	70	100	100	100	100	100
Nyang'hwale	February	Burnt brick	30	60	80	100	100	100	100
Buchosa	February	Mud	100	70	90	90	100	100	100
Buchosa	February	Cement	70	90	100	100	100	100	100
Buchosa	February	Painted	10	70	80	100	100	100	100
Buchosa	February	Whitewash	60	50	70	80	100	100	100
Buchosa	February	Burnt brick	90	100	100	100	100	100	100
Chato	March	Mud	30	50	60	70	90	100	100
Chato	March	Cement	20	60	70	80	90	100	100
Chato	March	Painted	30	50	50	50	70	80	100
Chato	March	Whitewash	10	30	60	70	90	100	100
Chato	March	Burnt brick	30	60	80	90	100	100	100
Misenyi	March	Mud	30	60	90	90	100	100	100
Misenyi	March	Cement	40	70	80	90	90	100	100
Misenyi	March	Painted	30	70	80	90	90	100	100
Misenyi	March	Whitewash	10	60	70	80	90	100	100
Misenyi	March	Burnt brick	10	40	60	70	80	100	100
Bukoba Rural	March	Mud	30	40	50	70	80	100	100
Bukoba Rural	March	Cement	50	50	60	70	70	90	100
Bukoba Rural	March	Painted	30	40	60	70	80	90	100
Bukoba Rural	March	Whitewash	10	20	30	40	70	90	100
Bukoba Rural	March	Burnt brick	20	50	90	100	100	100	100
Ngara	March	Mud	30	40	50	60	80	100	100
Ngara	March	Cement	20	40	60	60	90	100	100
Ngara	March	Painted	10	40	50	50	80	100	100
Ngara	March	Whitewash	20	30	40	40	70	100	100
Ngara	March	Burnt brick	10	20	30	30	60	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Kakonko	March	Mud	20	30	50	60	80	100	100
Kakonko	March	Cement	20	30	50	60	70	100	100
Kakonko	March	Painted	30	40	50	70	90	100	100
Kakonko	March	Whitewash	10	30	50	70	100	100	100
Kakonko	March	Burnt brick	10	50	60	60	90	100	100
Nyang'hwale	March	Mud	20	50	60	70	90	100	100
Nyang'hwale	March	Cement	10	30	40	60	100	100	100
Nyang'hwale	March	Painted	20	50	60	70	90	100	100
Nyang'hwale	March	Whitewash	30	50	70	80	100	100	100
Nyang'hwale	March	Burnt brick	40	60	70	80	100	100	100
Buchosa	March	Mud	30	40	50	90	90	100	100
Buchosa	March	Cement	20	40	60	80	90	100	100
Buchosa	March	Painted	40	30	50	60	70	100	100
Buchosa	March	Whitewash	10	60	70	80	90	100	100
Buchosa	March	Burnt brick	40	50	70	80	90	100	100
Chato	April	Mud	10	20	30	50	80	100	100
Chato	April	Cement	10	50	60	60	100	100	100
Chato	April	Painted	10	30	40	50	100	100	100
Chato	April	Whitewash	20	30	40	40	100	100	100
Chato	April	Burnt brick	10	20	30	40	100	100	100
Misenyi	April	Mud	30	80	80	90	100	100	100
Misenyi	April	Cement	30	40	70	80	100	100	100
Misenyi	April	Painted	0	0	60	80	100	100	100
Misenyi	April	Whitewash	20	50	80	90	100	100	100
Misenyi	April	Burnt brick	10	40	60	100	100	100	100
Bukoba Rural	April	Mud	20	30	60	80	100	100	100
Bukoba Rural	April	Cement	10	20	40	70	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Bukoba Rural	April	Painted	0	10	40	70	100	100	100
Bukoba Rural	April	Whitewash	20	60	80	90	100	100	100
Bukoba Rural	April	Burnt brick	10	20	60	70	100	100	100
Ngara	April	Mud	0	80	90	100	100	100	100
Ngara	April	Cement	0	80	80	100	100	100	100
Ngara	April	Painted	20	90	100	100	100	100	100
Ngara	April	Whitewash	20	80	100	100	100	100	100
Ngara	April	Burnt brick	0	70	80	100	100	100	100
Kakonko	April	Mud	0	100	100	100	100	100	100
Kakonko	April	Cement	10	100	100	100	100	100	100
Kakonko	April	Painted	10	100	100	100	100	100	100
Kakonko	April	Whitewash	10	100	100	100	100	100	100
Kakonko	April	Burnt brick	0	100	100	100	100	100	100
Nyang'hwale	April	Mud	10	20	80	100	100	100	100
Nyang'hwale	April	Cement	0	40	60	100	100	100	100
Nyang'hwale	April	Painted	0	50	70	100	100	100	100
Nyang'hwale	April	Whitewash	10	60	60	100	100	100	100
Nyang'hwale	April	Burnt brick	10	40	50	100	100	100	100
Buchosa	April	Mud	0	50	100	100	100	100	100
Buchosa	April	Cement	10	50	90	100	100	100	100
Buchosa	April	Painted	0	0	90	100	100	100	100
Buchosa	April	Whitewash	0	60	100	100	100	100	100
Buchosa	April	Burnt brick	0	40	80	100	100	100	100
Chato	May	Mud	10	30	50	70	80	100	100
Chato	May	Cement	10	10	20	40	50	100	100
Chato	May	Painted	0	20	30	20	30	100	100
Chato	May	Whitewash	30	50	60	70	80	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Chato	May	Burnt brick	20	30	40	50	60	100	100
Misenyi	May	Mud	10	20	30	50	60	100	
Misenyi	May	Cement	10	20	30	70	80	100	100
Misenyi	May	Painted	0	30	40	50	70	100	100
Misenyi	May	Whitewash	0	30	50	80	80	100	100
Misenyi	May	Burnt brick	0	10	20	50	80	100	100
Bukoba Rural	May	Mud	0	0	0	40	70	100	100
Bukoba Rural	May	Cement	10	0	0	40	60	100	100
Bukoba Rural	May	Painted	0	20	40	80	90	100	100
Bukoba Rural	May	Whitewash	0	10	40	50	60	100	100
Bukoba Rural	May	Burnt brick	30	30	40	40	50	90	100
Ngara	May	Mud	0	90	100	100	100	100	100
Ngara	May	Cement	10	70	90	100	100	100	100
Ngara	May	Painted	0	30	60	100	100	100	100
Ngara	May	Whitewash	10	60	80	100	100	100	100
Ngara	May	Burnt brick	10	80	90	100	100	100	100
Kakonko	May	Mud	0	100	100	100	100	100	100
Kakonko	May	Cement	0	90	100	100	100	100	100
Kakonko	May	Painted	20	100	100	100	100	100	100
Kakonko	May	Whitewash	10	100	100	100	100	100	100
Kakonko	May	Burnt brick	0	100	100	100	100	100	100
Nyang'hwale	May	Mud	20	30	40	100	100	100	100
Nyang'hwale	May	Cement	0	0	30	70	100	100	100
Nyang'hwale	May	Painted	20	40	60	100	100	100	100
Nyang'hwale	May	Whitewash	0	70	90	100	100	100	100
Nyang'hwale	May	Burnt brick	10	50	80	90	100	100	100
Buchosa	May	Mud	10	90	90	100	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Buchosa	May	Cement	10	80	90	100	100	100	100
Buchosa	May	Painted	20	60	80	100	100	100	100
Buchosa	May	Whitewash	20	60	80	100	100	100	100
Buchosa	May	Burnt brick	30	50	70	80	100	100	100
Chato	June	Mud	0	20	40	60	60	90	100
Chato	June	Cement	0	10	20	40	50	80	100
Chato	June	Painted	10	10	20	20	30	90	100
Chato	June	Whitewash	20	20	30	50	70	90	100
Chato	June	Burnt brick	10	20	40	40	60	100	100
Misenyi	June	Mud	0	10	20	40	60	70	100
Misenyi	June	Cement	10	20	30	40	70	90	100
Misenyi	June	Painted	0	20	30	50	70	80	100
Misenyi	June	Whitewash	0	20	30	60	80	90	100
Misenyi	June	Burnt brick	0	10	20	40	60	80	100
Bukoba Rural	June	Mud	0	0	0	40	70	90	100
Bukoba Rural	June	Cement	10	0	0	40	60	90	100
Bukoba Rural	June	Painted	0	10	40	80	90	100	100
Bukoba Rural	June	Whitewash	0	10	40	50	60	90	100
Bukoba Rural	June	Burnt brick	10	20	40	40	50	90	100
Ngara	June	Mud	0	10	30	70	80	90	100
Ngara	June	Cement	10	30	50	70	70	80	100
Ngara	June	Painted	0	20	40	60	80	100	100
Ngara	June	Whitewash	10	10	50	70	100	90	100
Ngara	June	Burnt brick	10	20	40	50	70	80	100
Kakonko	June	Mud	0	20	50	80	90	90	100
Kakonko	June	Cement	0	30	30	50	80	100	100
Kakonko	June	Painted	10	40	60	80	100	100	100

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Kakonko	June	Whitewash	10	10	50	80	80	100	100
Chato	July	Mud	10	10	50	60	80	90	100
Chato	July	Cement	10	20	30	50	80	80	90
Chato	July	Painted	0	10	40	40	70	80	100
Chato	July	Whitewash	0	10	20	50	60	80	90
Chato	July	Burnt brick	0	20	20	40	60	80	80
Misenyi	July	Mud	0	10	10	40	60	60	80
Misenyi	July	Cement	0	0	20	40	80	90	100
Misenyi	July	Painted	0	20	20	50	70	80	100
Misenyi	July	Whitewash	0	0	10	20	50	70	70
Misenyi	July	Burnt brick	0	10	20	40	80	80	100
Bukoba Rural	July	Mud	0	10	40	50	80	90	100
Bukoba Rural	July	Cement	0	20	30	60	80	80	90
Bukoba Rural	July	Painted	10	10	50	50	70	80	80
Bukoba Rural	July	Whitewash	0	10	40	60	80	90	100
Bukoba Rural	July	Burnt brick	0	20	30	70	70	80	100
Ngara	July	Mud	0	20	50	70	80	90	100
Ngara	July	Cement	0	20	30	50	70	80	90
Ngara	July	Painted	10	20	40	60	80	90	100
Ngara	July	Whitewash	0	10	30	50	70	80	100
Ngara	July	Burnt brick	0	30	50	70	80	90	100
Kakonko	July	Mud	10	10	30	50	60	80	80
Kakonko	July	Cement	20	30	30	60	70	70	90
Kakonko	July	Painted	10	20	30	60	80	90	90
Kakonko	July	Whitewash	10	30	40	60	70	90	100
Kakonko	July	Burnt brick	0	0	20	50	80	80	100
Nyang'hwale	July	Mud	0	10	20	40	60	80	90

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Nyang'hwale	July	Cement	0	20	30	50	70	90	100
Nyang'hwale	July	Painted	10	20	50	70	80	80	90
Nyang'hwale	July	Whitewash	0	10	40	60	80	90	100
Nyang'hwale	July	Burnt brick	0	20	40	60	70	80	90
Buchosa	July	Mud	0	20	40	70	80	80	90
Buchosa	July	Cement	0	10	30	50	70	90	100
Buchosa	July	Painted	10	10	40	60	80	80	90
Buchosa	July	Whitewash	10	20	50	60	70	80	80
Buchosa	July	Burnt brick	0	20	40	50	60	60	80
Chato	August	Mud	0	10	40	50	60	60	0
Chato	August	Cement	0	20	30	50	50	70	0
Chato	August	Painted	0	10	30	40	60	80	0
Chato	August	Whitewash	10	10	20	30	50	60	10
Chato	August	Burnt brick	10	20	30	40	40	70	10
Misenyi	August	Mud	10	30	40	50	60	80	10
Misenyi	August	Cement	10	20	30	60	70	80	10
Misenyi	August	Painted	20	40	50	50	60	70	20
Misenyi	August	Whitewash	10	10	20	40	60	70	10
Misenyi	August	Burnt brick	10	20	20	30	60	80	10
Bukoba Rural	August	Mud	10	10	20	40	40	50	80
Bukoba Rural	August	Cement	10	30	40	60	60	70	70
Bukoba Rural	August	Painted	0	10	30	50	70	80	90
Bukoba Rural	August	Whitewash	10	10	20	50	80	80	80
Bukoba Rural	August	Burnt brick	0	20	30	40	60	60	90
Ngara	August	Mud	0	10	30	50	70	80	90
Ngara	August	Cement	0	20	40	60	80	80	90
Ngara	August	Painted	10	10	30	50	70	80	80

District	Month	Type of Wall Surface	Knock Down Percentage After 60 Minutes	Mortality Percentage					
				Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Ngara	August	Whitewash	0	10	20	50	80	80	90
Ngara	August	Burnt brick	0	20	30	50	70	80	80
Kakonko	August	Mud	0	10	20	40	60	70	80
Kakonko	August	Cement	0	20	30	50	50	70	90
Kakonko	August	Painted	10	10	20	40	60	80	80
Kakonko	August	Whitewash	0	10	20	30	50	70	80
Kakonko	August	Burnt brick	0	20	30	40	70	80	90
Nyang'hwale	July	Mud	10	10	40	50	70	70	80
Nyang'hwale	July	Cement	0	10	30	60	70	80	90
Nyang'hwale	July	Painted	0	10	50	50	60	70	80
Nyang'hwale	July	Whitewash	10	10	30	50	70	80	80
Nyang'hwale	July	Burnt brick	10	20	30	60	80	80	90
Buchosa	July	Mud	0	10	10	30	50	80	90
Buchosa	July	Cement	0	20	30	60	80	80	90
Buchosa	July	Painted	10	10	30	50	70	80	80
Buchosa	July	Whitewash	0	10	40	40	50	60	70
Buchosa	July	Burnt brick	0	10	30	70	70	80	90

Annex 3. Summary of all samples tested and identified by enzyme-linked immunosorbent assay (ELISA) for blood meal source, October 2018 through September 2019

District Sentinel Site	Species	Number Tested	Blood Meal Sources					Unidentified Meal
			Human	Cow or Goat	Dog	Mixed (Human Animal)	Mixed (Animal Animal)	
Sprayed Sites								
Buchosa	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	13	6	0	0	4	3	0
	<i>An. funestus</i> s.s.	4	2	0	0	2	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	6	1	0	0	3	2	0
Nyang'hwale	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	4	2	0	0	2	0	0
	<i>An. funestus</i> s.s.	13	6	0	0	6	0	1
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	1	0	0	0	1	0	0
Chato	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	100	0	3	0	31	66	0
	<i>An. funestus</i> s.s.	2	0	0	0	0	2	0
	<i>An. parensis</i>	7	0	1	0	2	4	0
	Unidentified	77	0	4	0	20	53	0
Bukoba Rural	<i>An. gambiae</i> s.s.	1	0	0	0	0	1	0
	<i>An. arabiensis</i>	23	2	0	0	17	4	0
	<i>An. funestus</i> s.s.	3	0	0	0	2	1	0
	<i>An. parensis</i>	1	0	0	0	1	0	0
	Unidentified	63	0	4	0	25	34	0
Misenyi	<i>An. gambiae</i> s.s.	1	1	0	0	0	0	0
	<i>An. arabiensis</i>	1	1	0	0	0	0	0
	<i>An. funestus</i> s.s.	3	2	0	0	1	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	17	0	3	0	1	13	0
Ngara	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	0	0	0	0	0	0	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	0	0	0	0	0	0	0

District Sentinel Site	Species	Number Tested	Blood Meal Sources					
			Human	Cow or Goat	Dog	Mixed (Human Animal)	Mixed (Animal Animal)	Unidentified Meal
Kakonko	<i>An. gambiae</i> s.s.	67	44	0	0	23	0	0
	<i>An. arabiensis</i>	313	122	1	0	190	0	0
	<i>An. funestus</i> s.s.	136	40	0	0	95	1	0
	<i>An. parensis</i>	1	0	1	0	0	0	0
	Unidentified	56	3	0	0	51	0	2
TOTAL	<i>An. gambiae</i> s.s.	69	45	0	0	23	1	0
	<i>An. arabiensis</i>	454	133	4	0	244	73	0
	<i>An. funestus</i> s.s.	161	50	0	0	106	4	1
	<i>An. parensis</i>	9	0	2	0	3	4	0
	Unidentified	220	4	11	0	101	102	2
Control (Unsprayed) Sites								
Magu	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	0	0	0	0	0	0	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	0	0	0	0	0	0	0
Bukombe	<i>An. gambiae</i> s.s.	2	0	0	0	2	0	0
	<i>An. arabiensis</i>	45	29	0	0	13	0	3
	<i>An. funestus</i> s.s.	11	8	0	0	3	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	13	4	0	0	8	1	0
Biharamulo	<i>An. gambiae</i> s.s.	3	2	1	0	0	0	0
	<i>An. arabiensis</i>	23	2	8	0	13	0	0
	<i>An. funestus</i> s.s.	14	0	1	0	13	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	36	2	11	0	33	0	0
Kibondo	<i>An. gambiae</i> s.s.	0	0	0	0	0	0	0
	<i>An. arabiensis</i>	1	0	0	0	1	0	0
	<i>An. funestus</i> s.s.	0	0	0	0	0	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	0	0	0	0	0	0	0
Total	<i>An. gambiae</i> s.s.	5	2	1	0	2	0	0
	<i>An. arabiensis</i>	69	31	8	0	27	0	3

District Sentinel Site	Species	Number Tested	Blood Meal Sources					
			Human	Cow or Goat	Dog	Mixed (Human Animal)	Mixed (Animal Animal)	Unidentified Meal
	<i>An. funestus</i> s.s.	25	8	1	0	16	0	0
	<i>An. parensis</i>	0	0	0	0	0	0	0
	Unidentified	49	6	11	0	41	1	0

Note: *An.* = *Anopheles*; s.s. = sensu stricto.