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PMI VECTORLINK MOZAMBIQUE ENTOMOLOGICAL MONITORING ANNUAL REPORT AUGUST 2020 – JUNE 2021

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

Ace-1	Acetylcholinesterase 1 gene
b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
COVID-19	Coronavirus Disease 2019
DDT	Dichlorodiphenyltrichloroethane
INS	National Institute of Health (*Instituto Nacional de Saúde)*
IRS	Indoor Residual Spray
kdr	knockdown resistance gene
m/t/n	Mosquitoes per Trap per Night
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
SOP	Standard Operating Procedure
USAID	United States Agency for International Development
WHO	World Health Organization

EXECUTIVE SUMMARY

Indoor residual spraying (IRS) and insecticide-treated nets remain the primary mosquito vector control interventions in many parts of the world, including sub-Saharan Africa, where malaria continues to be a major public health concern.

Abt Associates implemented the U.S. President's Malaria Initiative VectorLink Project in Mozambique from July 1, 2020, to June 30, 2021. In the 2020 spray campaign, carried out from October 20 to November 30, 2020, VectorLink Mozambique conducted IRS with SumiShield 50WG (a neonicotinoid with clothianidin as active ingredient) in Molumbo, Mopeia, and Morrumbala, and with Fludora Fusion (a neonicotinoid with a mixture of clothianidin and a pyrethroid deltamethrin) in Maganja da Costa and Milange districts, in Zambezia Province. Monthly entomological monitoring was performed in three intervention districts (Maganja da Costa, Milange, and Mopeia) and one control district (Lugela), which had not received IRS. Surveillance employed two collections techniques: Prokopack aspirator and Centers for Disease Control Prevention (CDC) light trap. Cone wall bioassays were conducted to monitor the spray quality and residual life of insecticides sprayed in Maganja da Costa, Milange, and Mopeia districts. Annual insecticide susceptibility tests were carried out in all five sprayed districts (Maganja da Costa, Milange, Molumbo, Mopeia and Morrumbala) and one control district (Lugela).

In Nampula Province, the Government of Mozambique, with support from the Global Fund, conducted IRS using Fludora Fusion in the districts of Meconta, Murrupula, Nampula, Erati, and Ribaue, and SumiShield 50WG in the districts of Angoche, Monapo, and Nacala. VectorLink Mozambique performed monthly entomological monitoring using Prokopack aspirators and CDC light traps in two intervention districts (Nampula and Monapo) and Erati, the control district for Nampula Province, until September 2020. In October 2020, the project replaced Erati and made Mogovolas a control district, after Erati became included as an intervention district in the 2020 spray campaign following a decision by the Government, and Monapo was dropped as an intervention site. Cone wall bioassays were conducted in the sprayed districts of Nampula and Erati. Annual insecticide susceptibility tests were carried out in the same two sprayed districts and in the control district (Mogovolas).

Mosquito collections using the two methods described above demonstrated the presence of highly diverse species composition of anophelines, which included the two main vectors *Anopheles funestus* s.l. and *An. gambiae* s.l., and other potential vectors and non-vectors such as *An. coustani*, *An. pharoensis*, *An. pretoriensis*, *An. tenebrosus*, *An. caliginosus, An. squamosus, An. rufipes,* and *An. maculipalpis*. Our findings highlight high levels of heterogeneity and diversity in mosquito vector species composition and behavior in the monitored areas.

In general, post-IRS *An. funestus* s.l. indoor resting densities were suppressed compared with pre-IRS (August to October) densities in Zambezia Province. There was also a decrease in the indoor resting densities of *An. funestus* s.l. following the IRS campaign in October only in Erati district in Nampula Province. *An. gambiae* s.l. densities appeared to increase slightly, most likely because of the rapid build-up of breeding habitats due to the rain during the post-IRS period. However, the indoor resting densities of *An. gambiae* s.l. were generally low at most sentinel sites.

Malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. were collected both indoors and outdoors with the CDC light traps. *An. funestus* s.l. tended to bite predominantly indoors. Biting activity seemed to follow human sleeping patterns, with peak indoor biting activity starting at around 10–11 pm and mid-night and extending into the morning hours, mainly between 1 am and 4 am. This kind of vector behavior makes indoor based vector control interventions (both IRS and insecticide-treated nets) suitable for the control of malaria vectors in the areas.

Quality of IRS, assessed by cone wall bioassays, showed no underdosing of insecticides applied by the spray teams in all districts. The insecticide decay rate assessment showed that SumiShield 50WG and Fludora Fusion

lasted at least ten to eleven months. This is similar to the period (9 and 9–10 months) of residual effect of SumiShield 50WG and Fludora Fusion, respectively, reported in the last two years in Mozambique.

Results of insecticide susceptibility tests showed that local vectors are fully susceptible to pirimiphos-methyl, chlorfenapyr, clothianidin, and bendiocarb (except at Maganja da Costa and Nampula districts, where resistance to bendiocarb was observed). Assays for pyrethroids (deltamethrin, permethrin, and alpha-cypermethrin) again revealed widespread vector resistance to pyrethroids. This finding demonstrates that the current insecticide resistance profile of the mosquito populations tested poses a major threat for tools dependent on pyrethroid insecticides and therefore the importance of continued use of non-pyrethroid insecticides for IRS. Synergist assays with piperonyl butoxide (PBO) demonstrated full restoration of vector susceptibility to pyrethroids (deltamethrin, lambda-cyhalothrin, alpha-cypermethrin, and permethrin) at most of the sites in Zambezia, indicating that PBO nets are a viable option for vector control to overcome the observed pyrethroid resistance in Zambezia. However, PBO did not restore vector's susceptibility to pyrethroid insecticides in Nampula Province, and the continued use of IRS with next generation insecticides is recommended for malaria vector control in the area.

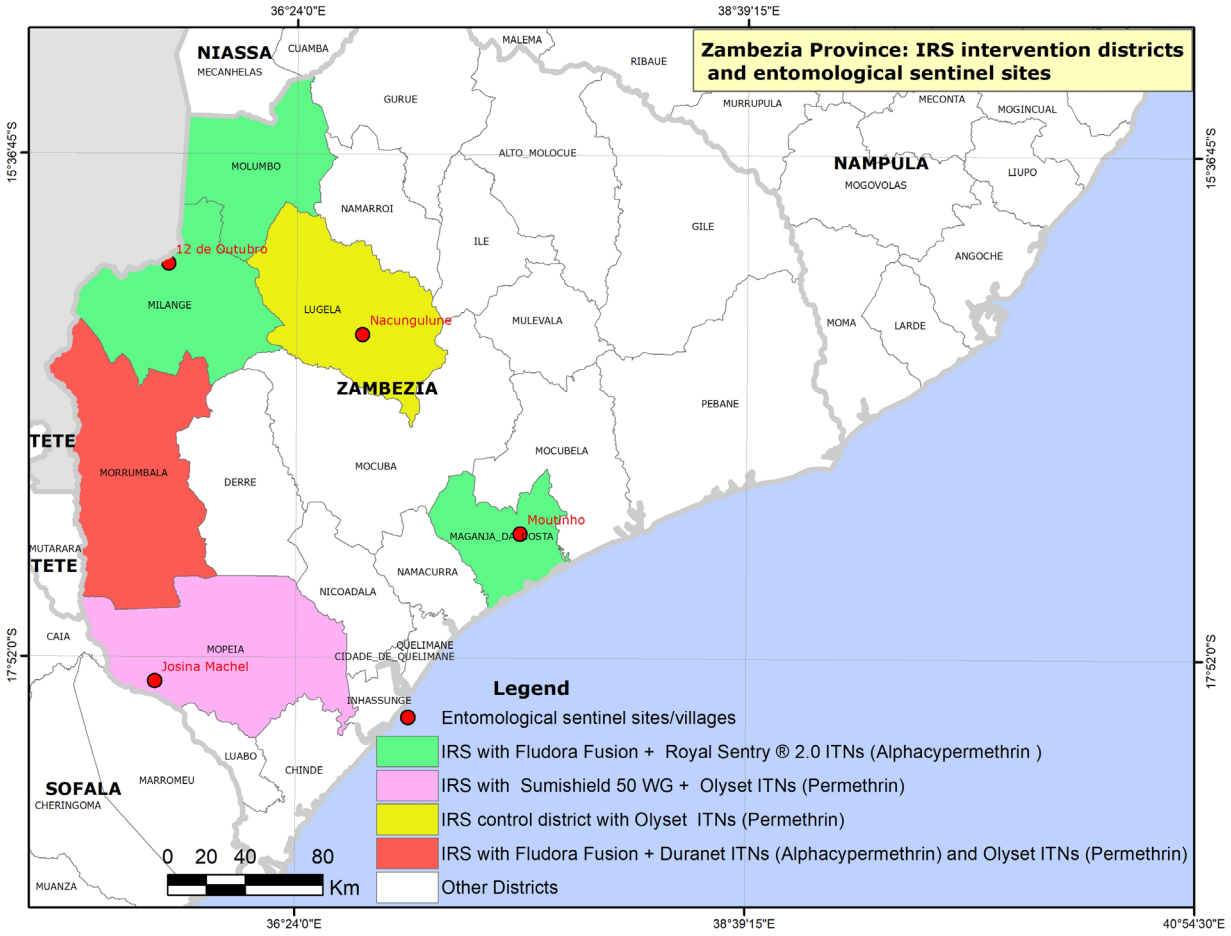
I. INTRODUCTION

Through support from the U.S. President's Malaria Initiative (PMI), Zambezia Province implemented six spray rounds of indoor residual spraying (IRS) under the PMI Africa Indoor Residual Spraying (AIRS) Project (2011–2017). Between 2018 and 2020, Zambezia implemented three spray campaigns under the PMI VectorLink Project. During the 2020 spray campaign, PMI VectorLink Mozambique conducted IRS in five target districts (Maganja da Costa, Milange, Molumbo, Mopeia, and Morrumbala). VectorLink Mozambique also carried out entomological monitoring activities in Zambezia and supported the country's National Malaria Control Program's entomological activities in Nampula and five other provinces in the North and Central regions of the country to enhance in-country capacity for entomological monitoring. Having entomological monitoring data that supplement epidemiological data is essential to properly target vector control interventions; evaluate the susceptibility level of the local vectors to different insecticides and determine the underlying mechanisms; inform selection of insecticides; ensure the quality of spraying; monitor the impact of IRS and insecticide-treated nets on vector density, behavior, and composition; and monitor the residual life of different insecticides on different types of wall surfaces. This entomological monitoring annual report normally covers the period from July 1, 2020, to June 30, 2021. However, the data in this report cover the period from August 1, 2020, to June 30, 2021, because data could not be collected during the month of July 2020 due to restrictions related to the novel coronavirus disease 2019 (COVID-19) pandemic. On March 11, 2020, the World Health Organization (WHO) declared the global COVID-19 situation as pandemic. PMI and WHO issued guidance limiting the implementation of entomological monitoring activities on March 25 and April 10, respectively. In accordance with that guidance and based on guidance from the National Malaria Control Program, VectorLink put on hold all of its field entomological monitoring activities (cone wall bioassay for monitoring the decay rate of insecticides sprayed; Prokopack aspirator and CDC light trap collections for longitudinal monitoring; and adult *Anopheles funestus* s.l. collections with Prokopack for susceptibility tests between April and July 2020.

Entomological monitoring was conducted in three IRS intervention districts in Zambezia Province: Maganja da Costa, Milange, and Mopeia. Unsprayed Lugela district was used as a control district (Figure 1A).

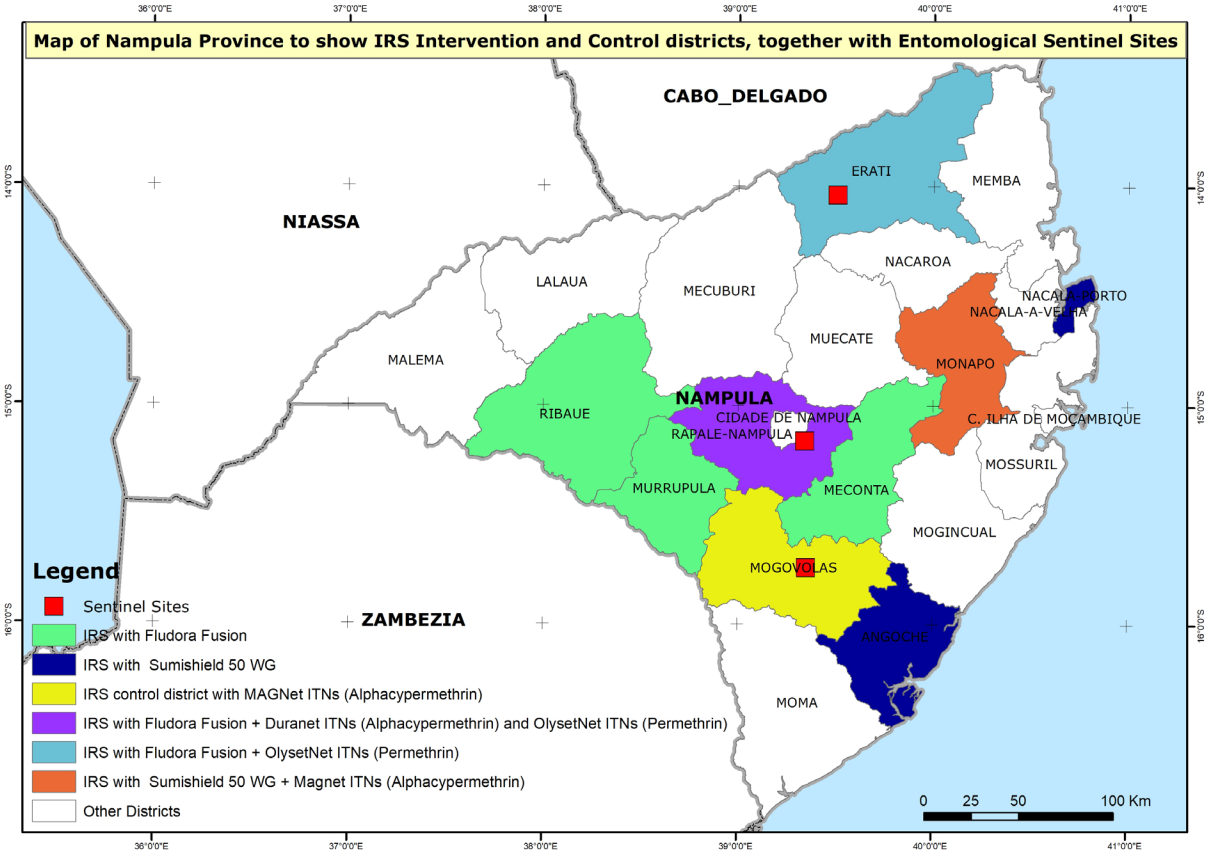
For susceptibility tests, Prokopack aspirators were used in all districts to collect adult *An. funestus* s.l., and larval collections were conducted to collect *An. gambiae* s.l. mosquitoes. The susceptibility tests were conducted in two different seasons to ensure enough mosquitoes of each complex were collected at their peak abundance: *An. funestus* s.l. is found mostly from June to September (dry season), while *An. gambiae* s.l. is found mostly from January to April (rainy season).

FIGURE 1A. ZAMBEZIA PROVINCE IRS INTERVENTION, CONTROL DISTRICTS, ENTOMOLOGICAL SENTINEL SITES, INSECTICIDE SPRAYED AND ITNS DISTRIBUTED.



The Government of Mozambique, through the National Malaria Control Program, conducted IRS in eight districts of Nampula Province: Angoche, Meconta, Monapo, Murrupula, Nacala, Nampula, Erati, and Ribaue. VectorLink Mozambique provided technical support to the province for the implementation of entomological surveillance in two IRS intervention districts, Monapo and Nampula, as well as in the control district of Erati until September 2020. In October 2020, the control district Erati became an intervention district, and it was replaced with Mogovolas as a control district. Monapo was dropped as an entomological surveillance district. The map of Nampula in Figure 1B shows the province's IRS intervention and control districts, together with the entomological sentinel sites.

FIGURE 1B: NAMPULA PROVINCE IRS INTERVENTION CONTROL DISTRICTS, ENTOMOLOGICAL SENTINEL SITES, INSECTICIDES SPRAYED AND ITNS DISTRIBUTED



2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Data were collected from August 2020 through June 2021, using Prokopack aspirator and CDC light trap collections. Data were not collected in July 2020 due to travel restrictions related to the COVID-19 pandemic.

2.1.1 PROKOPACK COLLECTIONS

The Prokopack method was used to collect mosquitoes to determine indoor resting density (number of mosquitoes collected per room per day)[1] of malaria vectors at sentinel sites in selected IRS intervention and control districts in Zambezia and Nampula provinces. In Zambezia, Prokopack collections were conducted in the intervention districts of Maganja da Costa (Motinho and Mecia villages), Milange (12 de Outubro and 3 de Fevereiro villages), and Mopeia (Josina Machel and Eduardo Mondlane villages), and in the control district of Lugela (Nhacungulune and Dabane villages). In Nampula, Prokopack collections were conducted in the intervention districts of Erati (Intuto and Mualangonha villages) and Nampula (Nawithipele and Murrapaniua villages) and the control district of Mogovolas (Meluli B and Nanhupo Rio villages). Five houses in each of two villages in each district were selected for Prokopack collections, totalling 10 houses per district. Prokopack collections were conducted from 6 am to 8 am, once per month over two consecutive days in each district. The same houses were visited each month. Data were collected in five houses per day per district. The first collection was conducted two months prior to the IRS campaign, and collection continued during and after the campaign. In each house, one sleeping room was used for Prokopack collection. The Prokopack used a sealed, lead acid, rechargeable 12-volt battery. One team member entered the room and connected the aspirator to the battery terminals. After fitting the collection cup, the mosquitoes were aspirated systematically, starting from the door, moving on to the walls and furniture and then under beds and tables, and finishing with the roof or ceiling. Live mosquitoes in the cups were transferred first to small cages and then to paper cups. The mosquitoes were killed with chloroform, counted, and their abdominal stage was recorded on the form. They were then placed in a petri dish for morphological identification and thereafter preserved in 1.5 ml Eppendorf tubes containing silica gel for further identification using the Polymerase Chain Reaction (PCR) technique. Prokopack collections were conducted based on Standard Operating Procedure (SOP) 11/01[2]. Samples collected by this method during the August 2020–June 2021 period were sent for PCR identification of species and blood meal sources, and enzyme-linked immunosorbent assay (ELISA) for detection of sporozoite infection at the National Institute of Health (*Instituto Nacional de Saúde*, INS) laboratory.

2.1.2 CDC LIGHT TRAP

Human-baited CDC light traps collections were used as proxy to human landing catches to primarily collect the host-seeking population to estimate the biting rates. The number of host-seeking mosquitoes caught by light traps can be used to estimate the human biting rates. In this case the person protected under net serve as equivalent to the person who manually aspirates mosquitoes landing on legs in the human landing catches. In Zambezia Province, CDC light traps were installed in four houses in the same villages listed for prokopack collections in three intervention districts (Maganja da Costa, Milange, and Mopeia), as well as in the control district of Lugela. Likewise, in Nampula Province, CDC light traps were installed in four houses in the two intervention districts of Erati (Intuto village) and Nampula (Nawithipele village) and the control district of Mogovolas (Meluli B village). Each month, the traps were set over three consecutive nights, from 6 pm to 6 am, for a total of 12 trap nights per month for each district.

[1] In Mozambique, most houses do not have partitions. The number of houses is same as number of rooms in a house.
[2] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

The traps were set up inside the house in the bedroom, at the foot of a bed with humans sleeping under treated bed nets, about 1.5 m above the floor. The outdoor CDC light traps were set up in a similar manner to the indoor one about 10 m away from the house. The collectors exchanged positions, indoors and outdoors, every collection hour. Trapped mosquitoes were transferred into paper cups covered with non-treated net material during each hour of changing positions. After each night of collection, chloroform was used to kill the mosquitoes in the paper cups, and the mosquitoes were identified morphologically and preserved in 1.5 ml Eppendorf tubes for future species identification, and detection of blood meal sources and sporozoite infections. The same houses were used each month for the CDC light trap collections using SOP 01/01.

2.2 IRS Quality Assays and Insecticide Decay Rate Monitoring

Standard WHO cone bioassay tests were performed in Maganja da Costa (Motinho and Mecia villages), Milange (12 de Outubro and 3 de Fevereiro villages), and Mopeia (Josina Machel and Zona Verde villages) from October 2020 through August 2021 to evaluate spray quality and residual efficacy of the insecticides used during the 2020 spray campaign. In Nampula Province, wall bioassays were conducted in Nampula (Nawithipele and Murrapaniua villages) and Erati (Intuto and Mualangonha villages) districts from October 2020 through June 2021. In both provinces, wall bioassays were conducted 24 hours after spraying and then monthly until mortality rate was less than 80% for two consecutive months or a month before the start of the new IRS campaign. The data presented in this report is for the period up to August 2021.

In each village, five houses were randomly selected. Cones lined with self-adhesive tape were fixed on sprayed walls in either the living room or bedroom for the assays. The cones were placed at heights of 0.5 m, 1.0 m, and 1.5 m above the floor. The same houses were used each month. The control cone was affixed to a wall lined with a paperboard with adhesive in an unsprayed house or in the shade of a tree in the yard away from the sprayed house to avoid any potential airborne effect. Two- to five-day-old female *An. arabiensis* KGB strain mosquitoes were introduced into the plastic cones in batches of 10 and left exposed on the sprayed surface for 30 minutes at different heights. The number of mosquitoes knocked down at the 30th minute was recorded. At the end of the 30-minute exposure period, the mosquitoes were carefully collected and transferred to paper cups and provided with 10% sugar solution soaked on cotton wool pads placed on top of the paper cups covered with net. Dead and live mosquitoes were counted at 24-hour interval up to five days for both Fludora Fusion and SumiShield 50WG exposed mosquitoes, and the percentage mortality was calculated in the replicates for each house and recorded according to the WHO protocol. When control mortality is between 5 to 20% observed, mortality was corrected using Abbott formula.

Tests for the airborne effect were conducted with mosquitoes placed inside of a small cage and hung 10 cm away from the sprayed wall surface for 30 minutes at a height of 1.5 m above the floor. The mosquitoes were transferred into clean paper cups that were kept for holding periods up to five days. Dead and live mosquitoes were counted after each 24 hours up to five days and mortality recorded as described above, SOP 009/01.

2.3 Vector Susceptibility Testing

From August to October 2020, unfed adult (not blood fed) *An. funestus* s.l. mosquitoes, which are difficult to find at the immature stage, were collected using Prokopack aspirators and directly used for susceptibility testing in Zambezia Province (Maganja da Costa, Milange, Molumbo, Mopeia, and Morrumbala), and Nampula Province (Nampula, Erati, and Mogovolas). Immature *An. gambiae* s.l. malaria vectors were collected from different larval habitats in Zambezia (Maganja da Costa, Milange, Molumbo, Mopeia, Morrumbala, and Lugela districts) and Nampula (Nampula, Erati, and Mogovolas) from January to April 2021, and reared to adults for susceptibility tests.

Field-collected larvae of *An. gambiae* s.l. were reared in the insectary to adult stage. Batches of 25 females, sugar-fed and aged from three to five days in four replicates, were subsequently subjected to the WHO tube tests following the standard WHO protocol (WHO, 2016). Insecticides used (clothianidin) and to be used pirimiphos-methyl and bendiocarb) for IRS and on nets (permethrin and alphacypermethrin) distributed were prioritize the insecticides for susceptibility tests on each of districts. The mosquitoes were exposed to pirimiphos-methyl 0.25%, deltamethrin 0.05%, permethrin 0.75%, alpha-cypermethrin 0.05%, lambda-

cyhalothrin 0.05%, bendiocarb 0.1%, and clothianidin 2%. Chlorfenapyr (100µg/bottle) was also tested following the CDC bottle assay tests procedure. Knockdown was scored at 60 minutes immediately after the exposure period, at which time all mosquitoes were gently transferred to holding tubes and paper cups. Mortality was recorded at 24 hours after exposure and followed up to three or seven days for chlorfenapyr and clothianidin, respectively. Where control mortality scored higher than 5% but below 20%, Abbott's correction was applied to correct test mortalities and those above 20% led to tests being discarded (Abbott 1925). Susceptibility levels of *An. gambiae* s.l. were evaluated based on WHO criteria (WHO 2016) and SOP06/01.

Intensity assays were not conducted, since priority was given to synergist assays to investigate the resistance mechanism involved. The synergist assays were conducted using mosquitoes reared from field-collected larvae. Four bioassay exposures were done as follows: In the first group of replicates, the mosquitoes were exposed to the insecticide only (alpha-cypermethrin, permethrin, lambda-cyhalothrin, and deltamethrin); the second group was exposed to 4% piperonyl butoxide (PBO) only, the third group to 4% PBO followed by insecticide, and the last group was exposed to the solvent (control). All replicates were exposed for 60 minutes, and mortality was recorded 24 hours after exposure, according to the WHO (2016) protocol. This process was repeated three times based on the standard procedure.

All the above susceptibility tests were conducted under the recommended optimal conditions, at temperatures around 27°C +-2°C and 70–80% relative humidity. Similar to other collections, a portion of samples from these tests were sent to the INS (Instituto Nacional de Saúde) laboratory for PCR assays to identify sibling species and the presence of knockdown (*kdr)* and acetylcolinesterase-1 (*Ace-1*) genes.

2.4 Statistical Tests

The average number of mosquitoes collected by the CDC light trap method was calculated. To compare mean indoor and outdoor biting rates, Chi-square tests were used, and *P* values less than 0.05 were considered significant.

2.5 Molecular Analyses

A total of 1,244 mosquitoes from Zambezia and 1,151 from Nampula province (around 10% of all collections) morphologically identified as *An. funestus* s.l. and *An. gambiae* s.l. were sent to the INS laboratory to be analyzed by PCR. Because the reagents were not received until August 2021, the samples could not be analyzed in time for data to be included in this report. Results now are expected around mid-October. An addendum to this report will be prepared when the full results are available.

3. RESULTS: ZAMBEZIA

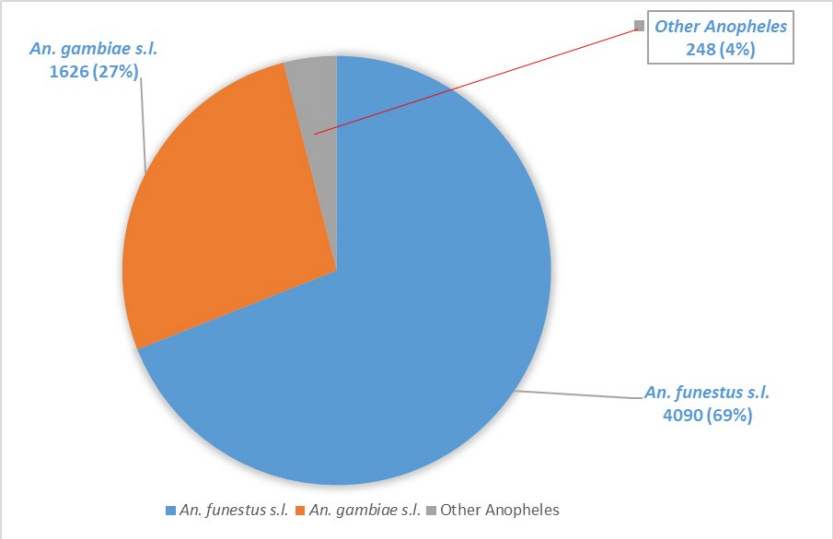
3.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT METHODS

Using the two collection methods (Prokopack and CDC light trap), 5,964 anopheline mosquitoes belonging to 10 different species and species complexes were collected in the intervention districts of Zambezia Province (Maganja da Costa, Milange, and Mopeia, and the control district of Lugela). The anophelines included *An. funestus* s.l., *An. gambiae* s.l., *An. coustani*, *An. pharoensis*, *An. pretoriensis*, *An. tenebrosus*, *An. caliginosus, An. maculipalpis, An. rufipes,* and *An. squamosus*. Table 1 and Figure 2 summarize the number of mosquitoes collected, by species and site. *An. funestus* s.l. was the most abundant anopheline collected, accounting for 69% of all collections, followed by *An. gambiae* s.l. at 27% and other anophelines at 4%.

TABLE 1. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN ZAMBEZIA PROVINCE USING BOTH COLLECTION METHODS

Species Collected	Maganja da Cosa	Milange	Mopeia	Lugela	Total per Species
An. funestus s.l.	689	646	1,244	1,511	4,090
An. gambiae s.l.	881	432	69	244	1,626
An. pharoensis	0	0	74	0	74
An. tenebrosus	4	11	90	3	108
An. pretoriensis	3	0	6	0	9
An. coustani	2	1	2	5	10
An. maculipalpis	0	11	0	12	23
An. rufipes	0	13	0	0	13
An. squamosus	0	1	8	0	9
An. caliginosus	0	0	2	0	2
Total	1,579	1,115	1,495	1,775	5,964

FIGURE 2. SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES FOR THE FOUR DISTRICTS IN ZAMBEZIA USING BOTH COLLECTION METHODS



3.1.1 PROKOPACK COLLECTIONS

Prokopack collections yielded a total of 692 *Anopheles* mosquitos (Table 2). Based on morphological identification, 643 of these belonged to *An. funestus* s.l. (92.9%) and 43 to *An. gambiae* s.l. (6.2%). Other species collected by this method were four (0.57%) *An. pharoensis* and two (0.28%) *An. tenebrosus*.

TABLE 2. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN ZAMBEZIA PROVINCE USING PROKOPACK ASPIRATORS

Mosquito Species /District	Maganja da Costa	Milange	Mopeia	Lugela	Total
An. funestus s.l.	42	75	248	278	643
An. gambiae s.l.	13	14	2	14	43
An. pharoensis	0	0	4	0	4
An. tenebrosus	0	0	2	0	2
Total	55	89	256	292	692

The indoor resting density of *An. funestus* s.l. estimated from Prokopack collections was relatively low in all intervention districts, mainly after IRS. The highest density of *An. funestus* s.l. was observed in the pre-spray season, in October 2020, at 8.4 mosquitoes per room per day at the Mopeia intervention site (Figure 3A). The pattern of *An. gambiae* s.l. resting densities was similar to that of *An. funestus* s.l., with the highest density observed in the pre-spray season (in September 2020) at Maganja da Costa, at 0.8 mosquito per room per day (Figure 3B). Even with similar pattern between *An. funestus* s.l. and *An. gambiae* s.l., the project collected 3.23, 5.35, 124 and 19.8 times more *An. funestus* s.l., than *An. gambiae* s.l., in Maganja da Costa, Milange, Mopeia and Lugela respectively.

In Mopeia, densities of *An. funestus* s.l. dropped immediately after IRS (from October to November) from 8.4 to 0.2 mosquitoes per room per day. In Lugela (control site), the indoor resting densities of *An. funestus* s.l. were about the same, 3.8 mosquitoes per room per day, before and after IRS. Although Lugela was not sprayed, a decrease was noted during the IRS month, but the density increased immediately after the spray period, peaked in December (at 5.5 mosquitoes per room per day), and then dropped again, reaching close to zero in March and May (Figure 3A). The *An. gambiae* s.l. indoor resting densities pattern fluctuated but remaining below 1.0 mosquito per room per day in the intervention and control districts throughout the monitoring season. In Maganja da Costa, the density of *An. gambiae* s.l. from its peak observed in September 2020 (0.8 mosquito per room per day) fell substantially in the post-intervention season (Figure 3B).

FIGURE 3. MEAN INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE FOUR DISTRICTS IN ZAMBEZIA BEFORE AND AFTER IRS INTERVENTION, AS ESTIMATED FROM THE PROKOPACK COLLECTIONS

Figure 3A. *An. funestus* s.l.

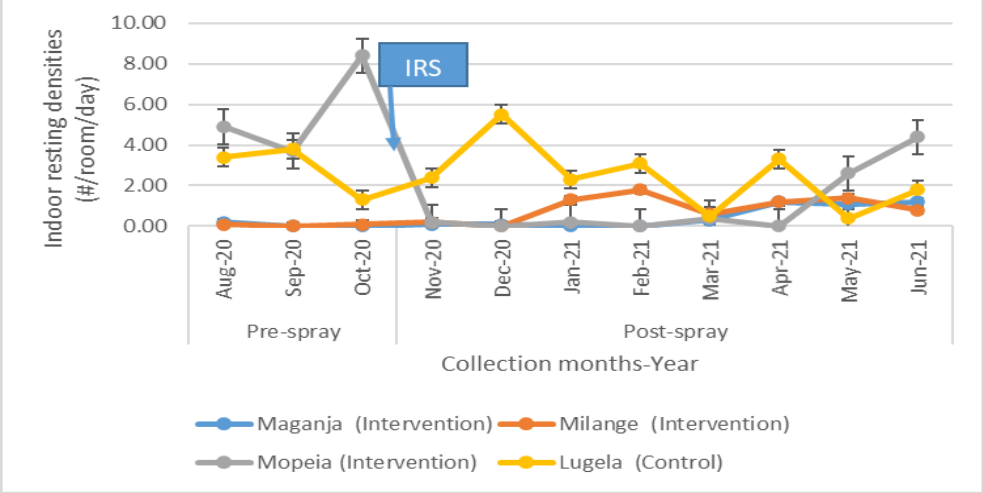
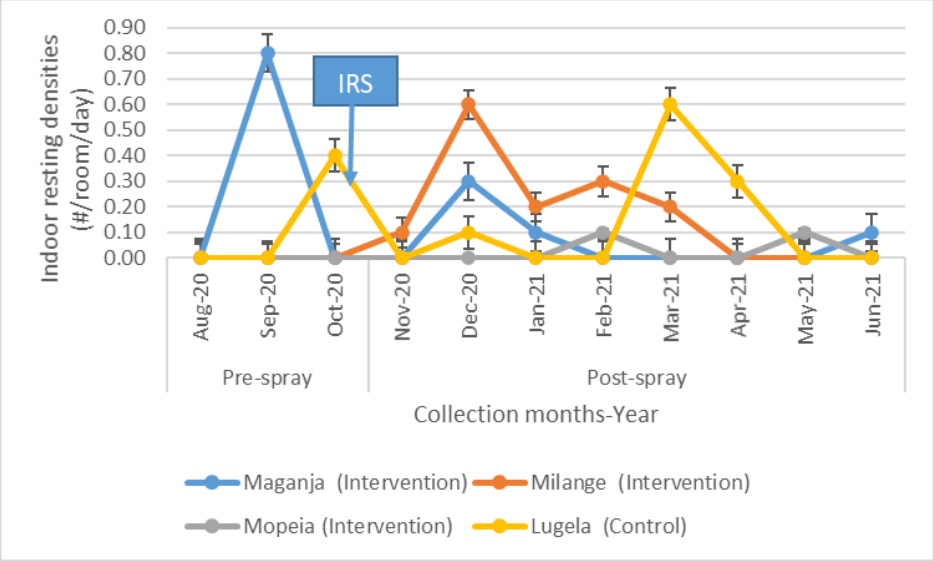


Figure 3B. *An. gambiae* s.l.



3.1.2 CDC Light Trap Collections

The CDC light trap collections yielded a total of 5,272 *Anopheles* mosquitoes from the intervention districts (Maganja da Costa, Milange, and Mopeia) and the control district (Lugela) in Zambezia Province. Morphological identification showed that 3,447 (65.38 %) were *An. funestus* s.l., 1,583 (30.03 %) *An. gambiae* s.l., 106 (2.01 %) *An. tenebrosus,* 10 (0.19 %) *An. coustani,* 23 (0.44 %) *An. maculipalpis*, 9 (0.17 %) *An. pretoriensis*, 13 (0.25 %) *An. rufipes*, 9 (0.17 %) *An. squamosus*, 2 (0.04 %) *An. caliginosus,* and 70 (1.33 %) *An. pharoensis*. The highest proportion of the total collected *An. funestus* s.l. (the predominant species in most areas) was from the Lugela control site (35.77%), followed by Mopeia (28.89 %), Maganja da Costa (18.76 %), and Milange (16.56%) intervention districts. Table 3 summarizes the total number of mosquitoes collected per species per district and the respective percentages of total *Anopheles* mosquitoes collected in each district using the CDC light trap collection method.

Table 3. Number of Mosquitoes Collected by Species and by District in Zambezia Province Using CDC Light Traps

	An. funestus s.l.	*An. gambiae* s.l.	*An. coustani*	*An. maculipalpis*	*An. tenebrosus*	*An. pretoriensis*	*An. rufipes*	*An. squamosus*	*An. caliginosus*	*An. pharoensis*	Total/district
Maganja da Costa	647	868	2	0	4	3	0	0	0	0	1524 (28.91)
Milange	571	418	1	11	11	0	13	1	0	0	1026 (19.46)
Mopeia	996	67	2	0	88	6	0	8	2	70	1239 (23.50)
Lugela	1233	230	5	12	3	0	0	0	0	0	1483 (28.13)
Total collected	3447 (65.38)	1583 (30.03)	10 (0.19)	23 (0.44)	106 (2.01)	9 (0.17)	13 (0.25)	9 (0.17)	2 (0.04)	70 (1.33)	

Note: Numbers in brackets are in percentage

Table A1 in the annex shows that, in terms of mean collections per trap per night, *An. funestus* s.l., at 9.34 mosquitoes per trap per night (m/t/n) over the 11 collection months, was most abundant in Lugela (control), followed by 7.55 m /t /n in Mopeia.

Figure 4A shows that before IRS (in August 2020), *An. funestus* s.l. indoor trap densities in Mopeia and Maganja da Costa were around 16.42 m/t/n and 9.83 m/t/n, respectively, and less than 1.0 in Milange. The densities were less than 1.0 after IRS in Maganja da Costa and Mopeia; in Milange, there was a slight increase (to 7.75 m/t/n) observed in January and a larger increase in April (to 9.42 m/t/n). This increase of *An. funestus* s.l. was not expected due to the rainy season during which the area has a higher abundance of *An. gambiae* s.l. . In Maganja da Costa, density was 10 m/t/n in June, about eight months after spraying. This shows that immediately after IRS, there was a reduction in the densities of *An. funestus* s.l. mosquitoes.

Figure 4B shows that *An. gambiae* s.l. indoor density was low (less than 2.33 m/t/n) before IRS. This is expected due to the seasonality of the species where the densities are low during the dry season. In Milange, the densities remained low after IRS until March, when density reached 11.67 m/t/n. For Maganja da Costa, Mopeia, and Lugela, density also increased in March, but to less than 3.5 m/t/n.

Maganja da Costa was the only district where density increased one month after spray, in December, reaching 20.33 m/t/n. This increase could be related to the pattern of the rainy season in the area. The district is a coastal area with potential flooding after heavy rains that could create favorable breeding habitats causing a sharp increase in mosquito population.

FIGURE 4. INDOOR CDC LIGHT TRAP DENSITY PER TRAP PER NIGHT IN MAGANJA DA COSTA, MILANGE, MOPEIA, AND LUGELA DISTRICTS

Figure 4A. *An. funestus* s.l.

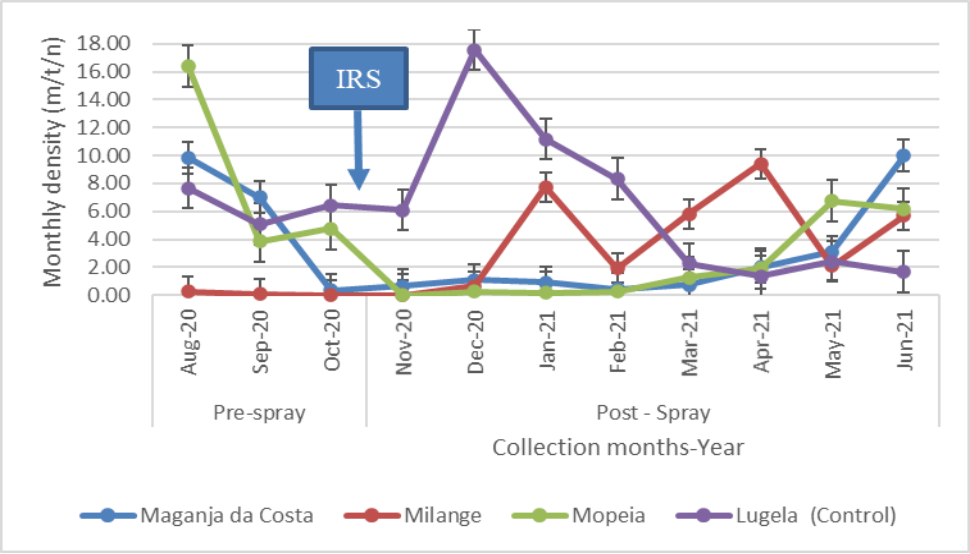
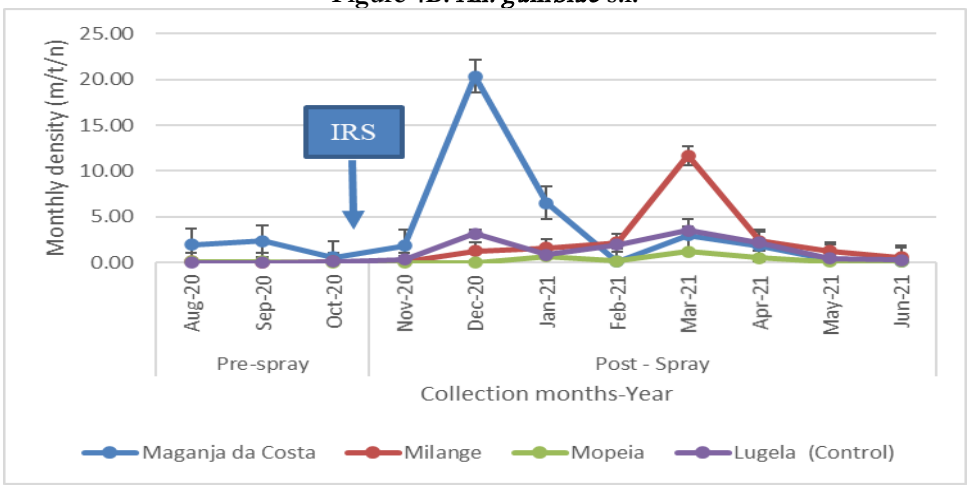


Figure 4B. *An. gambiae* s.l.



Outdoor CDC light trap density per trap per night showed varying trends in the four districts (Figure 5A). In Milange, the pre-spray season density of *An. funestus* s.l. was zero while in Maganja da Costa and Lugela, density was below 7.0 m/t/n. Mopeia had the highest pre-spray density, 15.17 m/t/n, in August 2020. After IRS, from November to March, the densities of *An. funestus* s.l. remained below 1.83 m/t/n in Maganja da Costa, Milange, and Mopeia. In Lugela, densities were higher from November to February, with a peak of 8.25 m/t/n in December. Starting in April, the outdoor densities of *An. funestus* s.l. increased in all the intervention districts, while in Lugela (control), density was near zero.

The outdoor densities of *An. gambiae* s.l. collected by CDC light trap, plotted in Figure 5B, show that Maganja da Costa was the only district where the species was found in the pre-spray season, with a density of 4.58 m/t/n in September; in other districts, outdoor density was near zero. After a dip in October and November, density of *An. gambiae* s.l. peaked at 19.67 m/t/n in Maganja da Costa in December. Density also increased, to 8.17 m/t/n, in Milange in March. Comparing the peak densities from Maganja da Costa in September before IRS and in December after IRS, there is a significant difference between these two points (X^2 =9.42 and p=0.002). The increase of *An. gambiae* s.l. in December could be attributed to an increase in breeding sites at the start of the rainy season.

FIGURE 5. OUTDOOR CDC LIGHT TRAP DENSITY PER TRAP PER NIGHT IN MAGANJA DA COSTA, MILANGE, MOPEIA, AND LUGELA DISTRICTS

Figure 5A. *An. funestus* s.l.

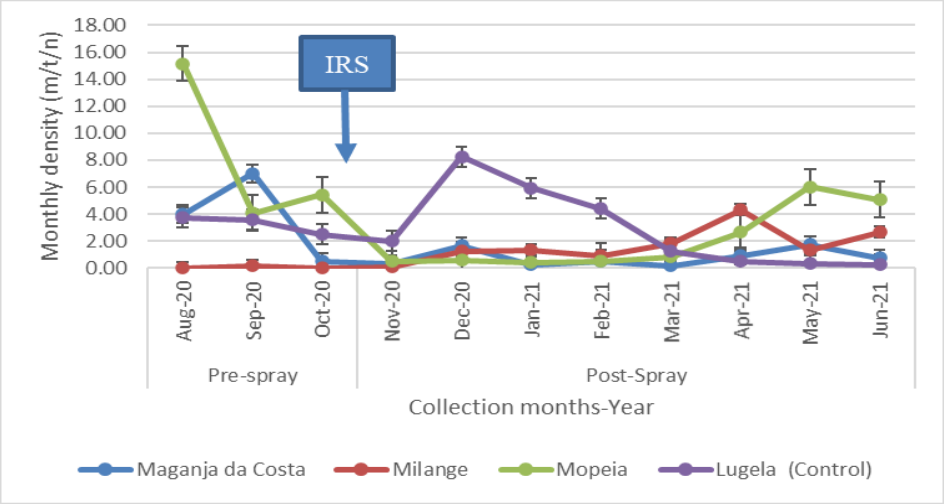
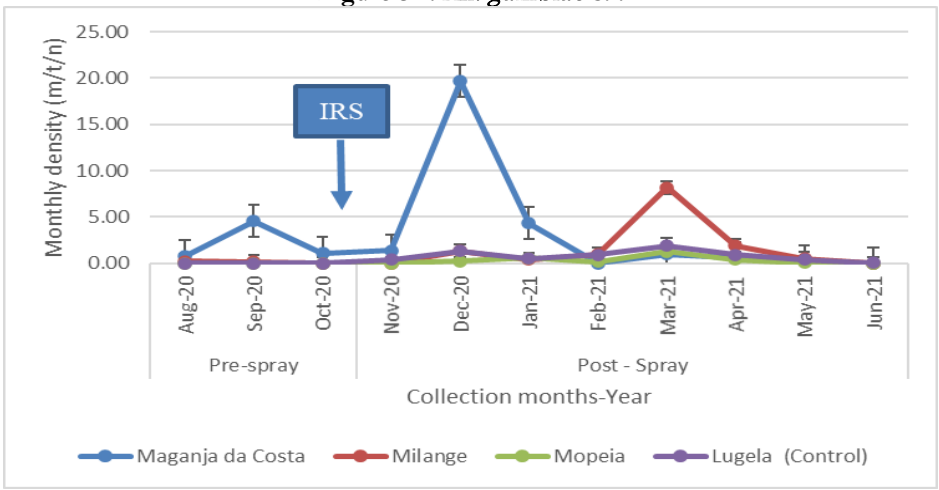


Figure 5B. *An. gambiae* s.l.



3.1.3 BITING TIME AND LOCATION BASED ON CDC LIGHT TRAP COLLECTIONS

Table 4 shows the mean indoor and outdoor vector biting rates for *An. funestus* s.l. and *An. gambiae* s.l. before and after spraying. The indoor biting rate of *An. funestus* s.l. decreased after IRS, in Maganja da Costa from 5.72 to 2.36 bites/per/night (b/p/n) and in Mopeia from 8.33 to 2.09 b/p/n. In Milange, however, mean indoor biting increased, from 0.11 to 4.17 b/p/n. The mean indoor biting rate in Lugela (control) was similar in both the pre- and post-intervention periods (6.39 vs 6.35 b/p/n). Mean outdoor biting rates were similar, tending to decrease, in Maganja da Costa, Mopeia, and Lugela, whereas there was an increasing trend for Milange, though the biting rate was generally low. For *An. gambiae* s.l., indoor and outdoor mean biting rates for all districts increased in the post-spray rainy season relative to the pre-spray season. Usually, the post-spray period is followed by rain and the potential breeding sites for *An. gambiae* s.l., increases and consequently the population of *An. gambiae* s.l., could increase.

TABLE 4. INDOOR AND OUTDOOR MEAN BITING RATE FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L., ESTIMATED USING CDC LIGHT TRAPS FROM ALL COLLECTION ROUNDS, BY DISTRICT, BEFORE AND AFTER SPRAYING

District	*An. funestus* s.l. (b/p/n)				*An. gambiae* s.l. (b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Maganja	5.72	2.36	3.83	0.79	1.61	4.24	2.14	3.40
Milange	0.11	4.17	0.06	1.72	0.06	2.61	0.14	1.67
Mopeia	8.33	2.09	8.22	2.07	0.06	0.33	0.00	0.34
Lugela*	6.39	6.35	3.28	2.86	0.03	1.57	0.00	0.81

*Unsprayed control District.

Table 5 shows no significant difference between the total numbers of *An. funestus* s.l. samples collected indoors and outdoors ($p>0.05$) in Mopeia. However, a significant difference was observed in Maganja da Costa, Milange, and Lugela (control), ($p<0.05$), where *An. funestus* s.l. showed endophagic tendencies. Similarly, there was no significant difference between the numbers of *An. gambiae* s.l. collected indoors and outdoors in Mopeia ($p>0.05$), whereas the vector was collected more indoors than outdoors ($p<0.05$) in Maganja da Costa, Milange, and Lugela (control) showing endophagic tendencies.

TABLE 5. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAES*.L. COLLECTED BY CDC LIGHT TRAP COLLECTION IN FOUR DISTRICTS OF ZAMBEZIA PROVINCE

District	*An. funestus* s.l. # Collected indoors	# Collected outdoors	X^2	*p-value*	*An. gambiae* s.l. # Collected indoors	# Collected outdoors	X^2	*p-value*
Maganja da Costa	433	214	74.13	$7.3x10^{-18}$*	465	403	4.43	0.03*
Milange	404	167	98.37	$3.4X10^{-23}$*	253	165	18.53	$1.68x10^{-5}$*
Mopeia	501	495	0.04	0.84	34	33	0.01	0.9
Lugela (Control)	840	393	162.05	$4.04x10^{-37}$*	152	78	23.81	$1.06x10^{-6}$*

*Differences in total indoor/outdoor collection are statistically significant at 0.05 level.

The indoor and outdoor overnight biting patterns of *An. funestus* s.l. are depicted in Figures 6A and 6B, respectively. Indoor and outdoor biting activity appeared to follow almost the same pattern in each district. In Lugela (control), indoor biting activity remained consistently above 3.17 bites per person per hour (b/p/h) for

most of the night, starting at 7–8 pm and lasting to 4–5 am, with peak biting activity of 9.08 b/p/h observed at 11 pm–12 am before decreasing to 1.42 b/p/h at 5–6 am. In Milange, the highest hourly biting, 5.17 b/p/h, was observed at 2–3 am. Maganja da Costa and Mopeia had almost the same peak biting rates, 5.17 b/p/h and 5.08 b/p/h, at 2–3 am and 12–1am, respectively.

Mopeia was the one district where the highest outdoor biting activity was observed in the early part of the night, although the highest peak biting rate, 5.08 b/p/h, was observed late at night, around 2–3 am, when most community members were sleeping.

Similarly, a high outdoor biting rate was observed in Lugela, with the peak biting, 5.58 b/p/h, at 1–2 am. In Maganja da Costa and Milange, outdoor biting followed the same pattern with the exception of the early night, 6–7 pm and 8–9 pm, where Maganja da Costa had a high biting rate. Both districts had their peak biting from 11 pm to 12 am (midnight), at 2.50 b/p/h. The general observation here is that *An. funestus* s.l. fed actively mainly during late night, both indoors and outdoors.

FIGURE 6. HOURLY BITING RATE OF *AN. FUNESTUS* S.L. IN FOUR DISTRICTS OF ZAMBEZIA DETERMINED THROUGH CDC LIGHT TRAP

Figure 6A. *An. funestus* s.l. Indoor

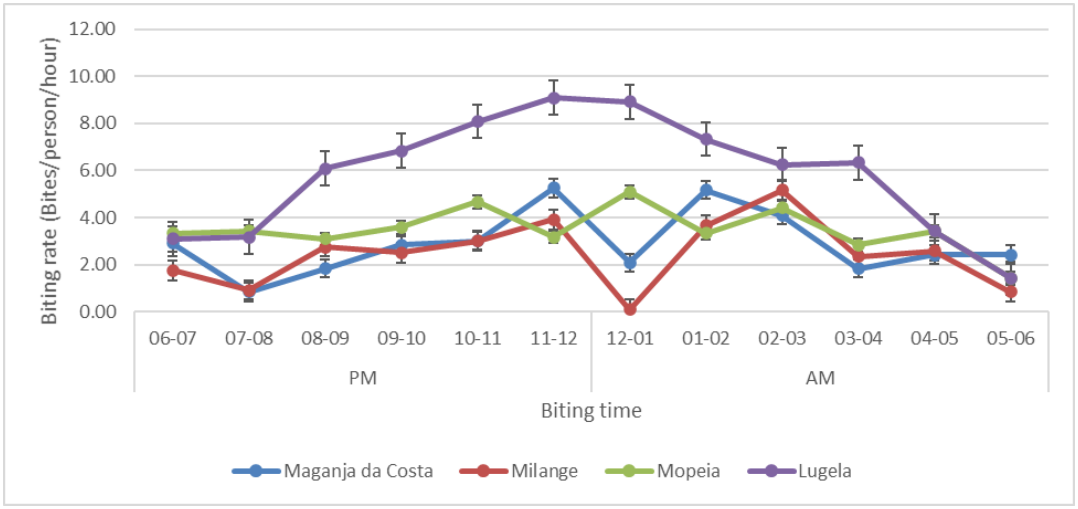
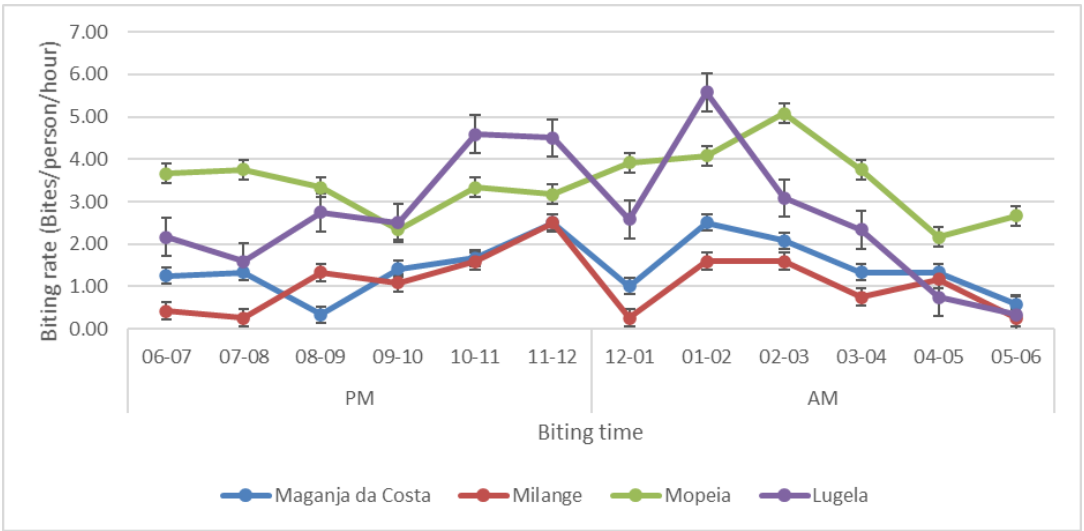


Figure 6B. *An. funestus* s.l. Outdoor



The indoor and outdoor hourly biting activities for *An. gambiae* s.l. are shown in Figures 6C and 6D, respectively.

The indoor biting activity remained below 2.67 b/p/h from 6 pm to 6 am in Milange, Mopeia, and Lugela (control), while in Maganja da Costa, the indoor biting per person per hour was higher than in the other districts from 6 pm to 2 am, with peak biting activity, 6.42 b/p/h, observed at 1–2 am. This indicates that *An. gambiae* s.l. bites mainly while people are sleeping. In Lugela (control), the indoor biting rate was fairly uniform throughout the night.

An. gambiae s.l. outdoor biting shows different trends from indoor biting in each district. Lugela (control) district displayed the highest outdoor biting activity, followed by Maganja da Costa, then Milange, and finally Mopeia with the least biting. In Lugela, the peaks were bimodal for outdoor biting, at 5.75 b/p/h at 10–11 pm and 5.92 b/p/h at 1–2 am, respectively. In Maganja da Costa, Milange, and Mopeia, peak biting was 4.0 b/p/h at 1-2am, 1.83 b/p/h at 11–12pm, and 0.75 b/p/h at 10–11pm, respectively.

Figure 6C. *An. gambiae* s.l. Indoor

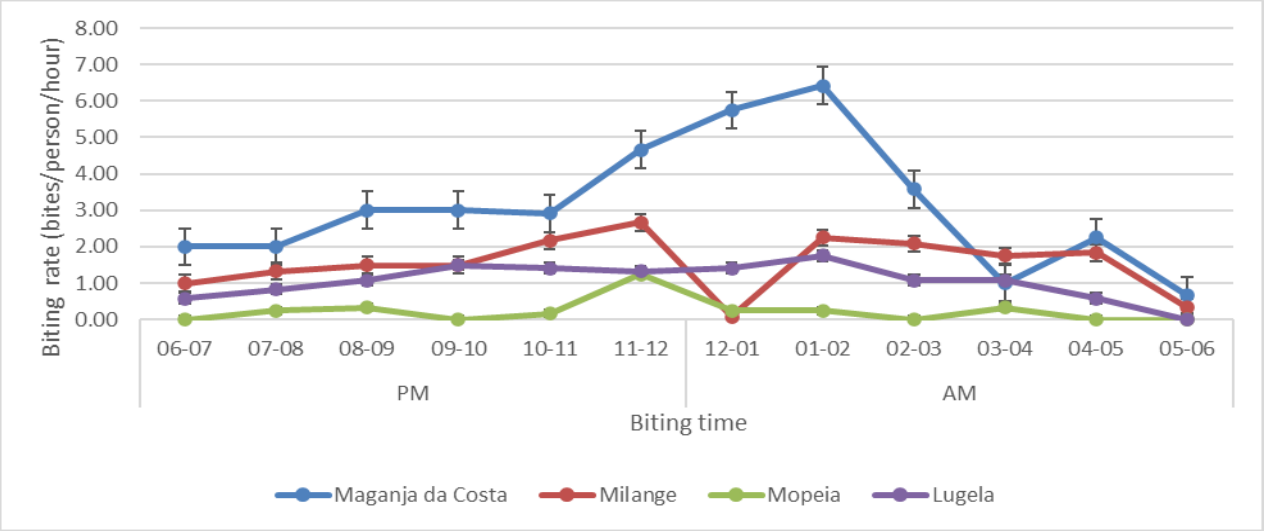
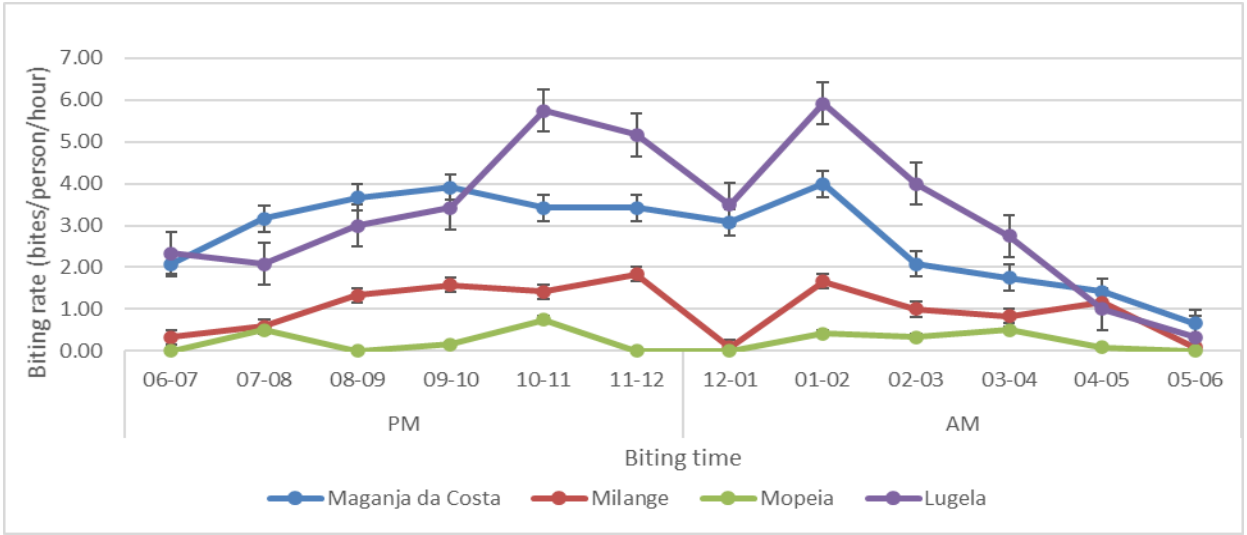


Figure 6D. *An. gambiae* s.l. Outdoor



3.1.4 Molecular Analysis

A total of 1,244 mosquitoes (about 10% of all collections) morphologically identified as *An. funestus* s.l. and *An. gambiae* s.l. were sent to the INS laboratory to be analyzed using PCR; due to the late delivery (in August 2021) of the reagents, the samples could not be processed in time for the data to be included in this report. The analysis will be done now that laboratory has received the reagents.

3.2 Cone Wall Bioassays

Monthly assays were performed to monitor the insecticide decay rate on various types of wall surfaces. Results of the quality assurance, decay rate monitoring and airborne effect of SumiShield 50WG (clothianidin (50% w/w) active ingredient) in Mopeia and Fludora Fusion (a combination of clothianidin (500 g/kg) and deltamethrin (62.5 g/kg) active ingredients) in Maganja da Costa and Milange districts are shown below in Sections 3.2.1 to 3.2.3.

3.2.1 Quality of Spraying

For SumiShield 50WG, mortality was scored at 93% in all houses tested with cone wall bioassays one day (24 hours) after spraying (T_0), and it was 100% two days (48 hours) after spraying (Figure 7).

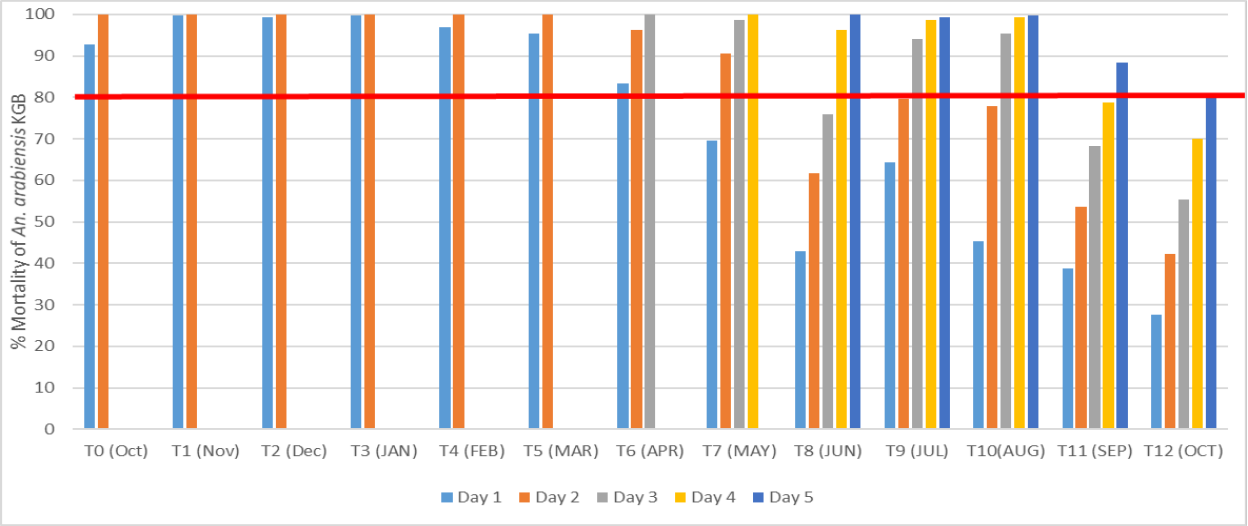
For Fludora Fusion, mortality was scored at 99.3% one day after spraying in Maganja da Costa; the final scored mortality, at day 2 (48 hours), was 100%. For Milange, the scored mortality was 100% at day 1 (24 hours).

3.2.2 Insecticide Decay Rate

SumiShield 50WG Decay Rate

Baseline cone wall bioassays for assessing SumiShield 50WG spray quality and subsequent monitoring of the insecticide's decay rate was conducted in Josina Machel and Zona Verde villages in Mopeia. Baseline (T0) was conducted in October 2020 and elicited 100% mortality by day 2 after exposure (Figure 7). Subsequent monthly cone bioassays resulted in 99% mortality. It was also noted that scores of 100% were observed up to eight months after IRS and between days 2 and 5. There was a notable increase in the number of days to reach 100% mortality, from three to five days, from month 6 to month 10. This is presumably due to the decreasing efficacy of the insecticide deposits on the sprayed surface over time. These results show that SumiShield 50WG remained efficacious at least 10 to 11 months post-spray.

Figure 7: Spray Quality Assessment and Residual Bioefficacy of SumiShield 50WG (Clothianidin 50WG) in Mopeia



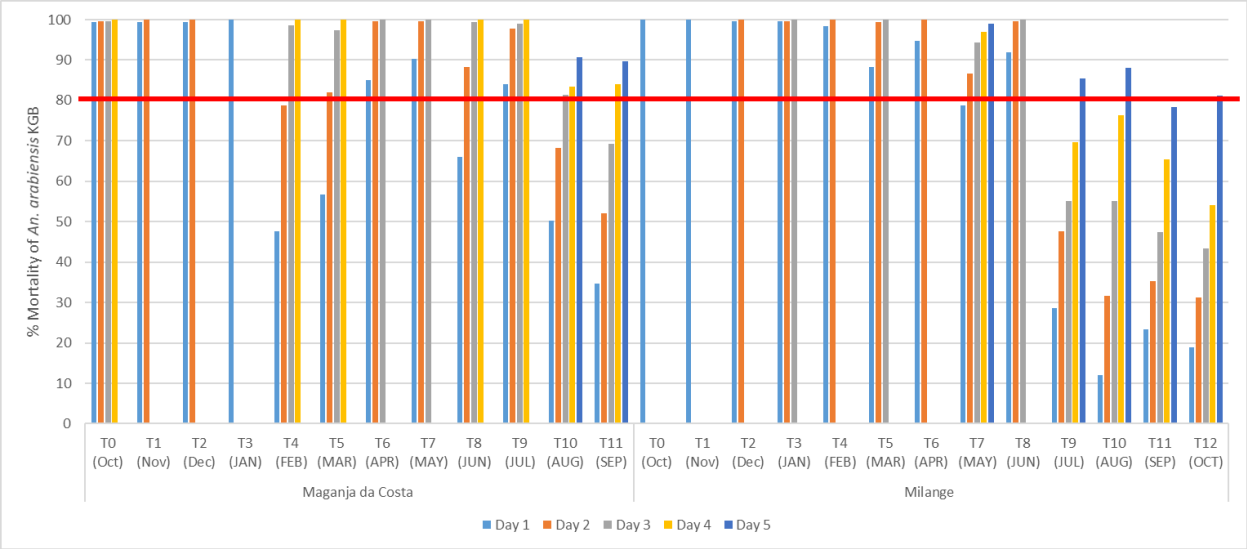
Red horizontal line indicates the 80% mortality cut-off point.

FLUDORA FUSION DECAY RATE

Baseline cone wall bioassays for assessing Fludora Fusion IRS quality and subsequent monitoring of its decay rate were conducted in Maganja da Costa and Milange.

Cone bioassays data showed high mortality (100%) up to month 9 (July) and month 8 (June) in Maganja da Costa and Milange, respectively. In Maganja da Costa, mortality remained 100% through month 9 and dropped to 90% in month 10 and 89.67 % in month 11. In Milange, it dropped to 85.3% on day 5 of follow-up in month 9, and in month 10 it remained at 88% and dropped to 78% in month 11 and 81% in month 12, indicating a clear decrease in residual efficacy (Figure 8).

FIGURE 8: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF FLUDORA FUSION (CLOTHIANIDIN AND DELTAMETHRIN) IN MILANGE AND MAGANJA DA COSTA



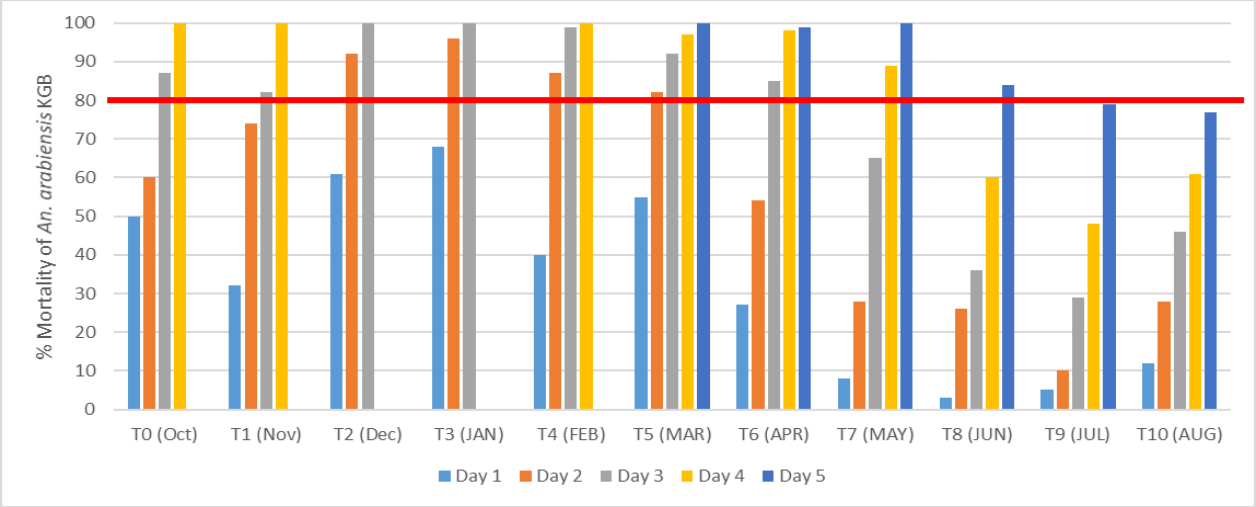
Red horizontal line indicates the 80% mortality cut-off point.

3.2.3 THE AIRBORNE EFFECT

Figure 9 (A and B) illustrates bioassay data about the airborne effect of the insecticides in Mopeia (Figure 9A) and Maganja da Costa and Milange (Figure 9B). The airborne fumigant effect of SumiShield 50WG was high (100% mortality) in Mopeia up to month 7, after which it started dropping. At month 9 and 10, it was below the cut-off point of 80%. The effect of Fludora Fusion in Maganja da Costa was high until month 8 with 94% mortality, after which it dropped to below 80%. In Milange, the fumigant effect remained high with 100% mortality up to month 6, then dropped to below 80% in month 7. In month 8, mortality increased to 91% due to the airborne effect, but dropped to 1% on month 9 and 10, an unusual situation.

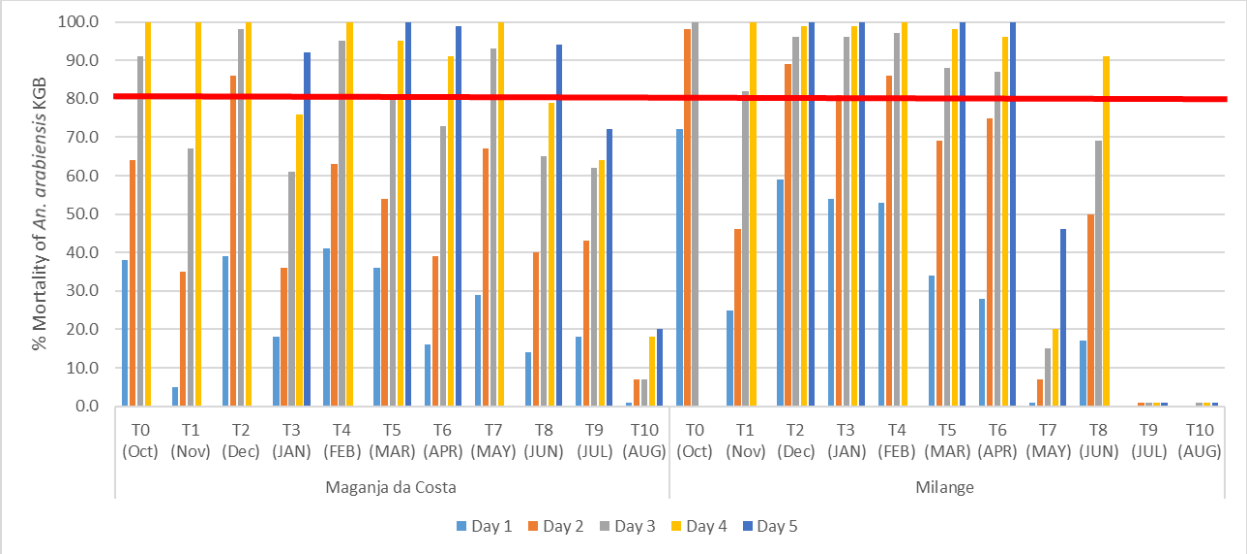
FIGURE 9. PERCENT MORTALITY OF *AN. ARABIENSIS* KGB SUSCEPTIBLE STRAIN ON AIRBORNE FUMIGANT EFFECT TEST AGAINST SUMISHIELD 50WG IN MOPEIA AND FLUDORA FUSION IN MILANGE AND MAGANJA DA COSTA

Figure 9 A. SumiShield 50WG in Mopeia



Red horizontal line indicates the 80% mortality cut-off point.

Figure 9 B. Fludora Fusion in Milange and Maganja da Costa



Red horizontal line indicates the 80% mortality cut-off point.

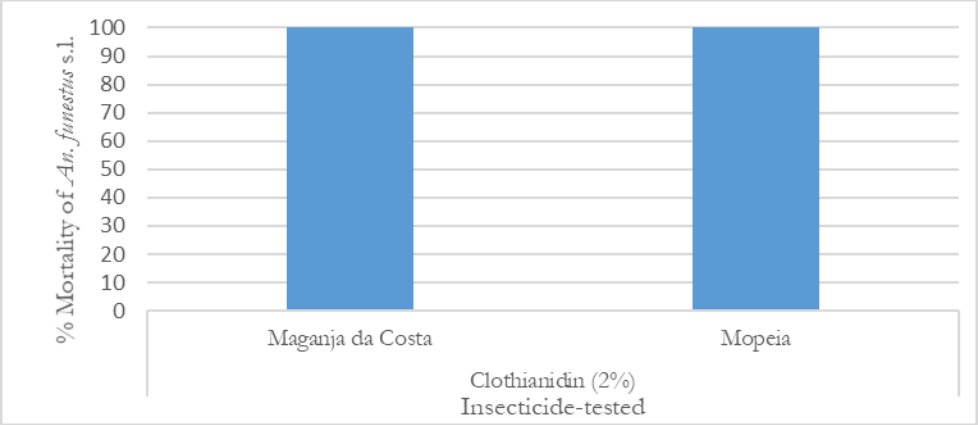
3.3 INSECTICIDE SUSCEPTIBILITY TESTS

Susceptibility tests against *An. funestus* s.l. and *An. gambiae* s.l. were conducted in Lugela, Maganja da Costa, Milange, Molumbo, Mopeia, and Morrumbala, by exposing *An. funestus* s.l. to clothianidin 2% and *An. gambiae* s.l. to diagnostic dosages of alpha-cypermethrin (0.05%), deltamethrin (0.05%), lambda-cyhalothrin (0.05%), permethrin (0.75%), pirimiphos-methyl (0.25%), clothianidin (2%), chlorfenapyr (100 µg/bottle), and bendiocarb (0.1%). More tests were conducted to explore the role of synergist on pyrethroids using PBO. These assays were conducted on alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, and permethrin.

In each district, the insecticide used to perform the tests were prioritized according to the insecticides that are planned to be used in the upcoming IRS campaign as well as the insecticide used on ITNs already distributed in each of the intervention districts and the control district

The mortality results presented in Figure 10 show that *An. funestus* s.l. was fully susceptible to clothianidin 2% in Maganja da Costa and Mopeia. Few *An. funestus* s.l. were collected and no other insecticide was tested against this population.

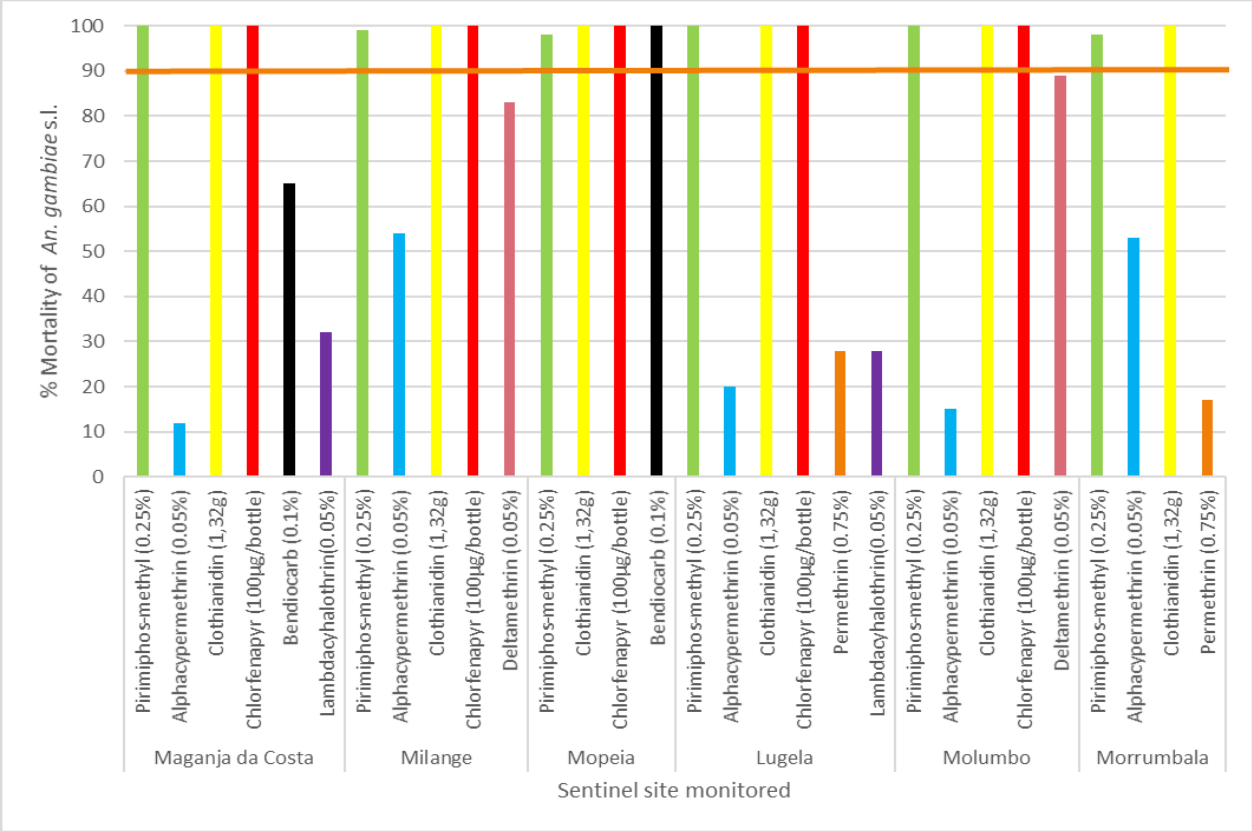
FIGURE 10. PERCENTAGE MORTALITY OF ADULT *AN. FUNESTUS* S.L. COLLECTED BY PROKOPACK FROM THE FIELD EXPOSED TO CLOTHIANIDIN 2% AT SEVEN-DAY HOLDING PERIOD



Susceptibility tests conducted with *An. gambiae* s.l. samples collected from larvae and reared to adult, results of which are presented in Figure 11, showed that this species was fully susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr in all districts where the insecticides were tested. Resistance to alpha-cypermethrin (0.05%) was observed in all districts where the tests were conducted.[3] Resistance to lambda-cyhalothrin (0.05%) was detected in Maganja da Costa and Lugela, while resistance to bendiocarb (0.1%) was detected only in Maganja da Costa. Full susceptibility of *An. gambiae* s.l. was observed to all insecticides tested in Mopeia. Resistance to deltamethrin (0.05%) was observed in Milange and Molumbo, while resistance to permethrin (0.75%) was observed in Lugela and Morumbala. *An. gambiae* s.l. was resistant to all pyrethroids tested at all sites. Some insecticides were not tested in certain districts where too few larvae were collected to cover all insecticides.

[3] Some insecticides were not tested in certain districts due to low number of larvae collected.

FIGURE 11. PERCENTAGE MORTALITY OF ADULT *AN. GAMBIAE* S.L. RAISED FROM LARVAL COLLECTIONS EXPOSED TO A RANGE OF INSECTICIDES AT RESPECTIVE DIAGNOSTIC CONCENTRATIONS AND HOLDING PERIODS



Red horizontal line indicates the 98% mortality cut-off point for susceptibility.

Note: clothianidin and chlorfenapyr holding periods were up to three and seven days, respectively. The mortality for other insecticides was observed at a 24-hour holding period.

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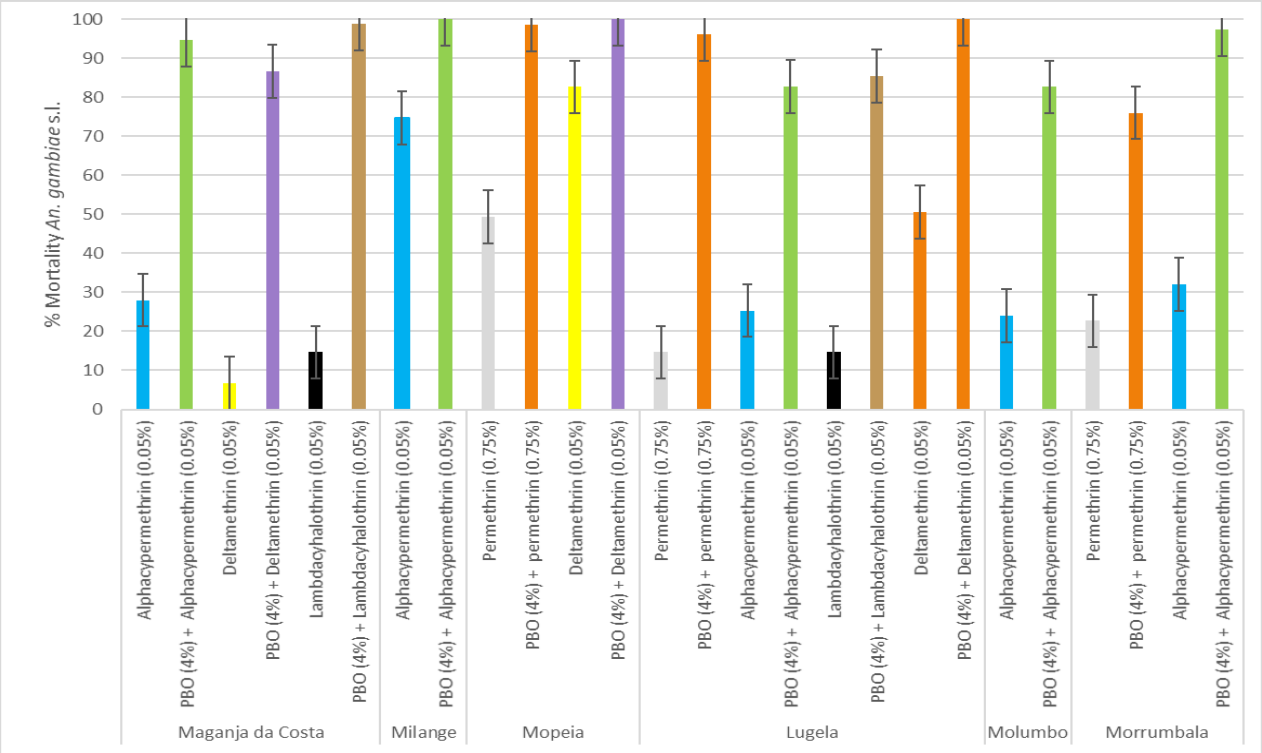
3.3.1 SYNERGIST ASSAYS USING WHO TUBE TESTS ON *AN. GAMBIAE* S.L.

Figure 12 depicts the results of synergist (PBO) assays on *An. gambiae* s.l. from Maganja da Costa, Milange, Mopeia, Lugela, Molumbo, and Morrumbala. In Milange, the synergist restored susceptibility to alpha-cypermethrin to 100%. It partially restored susceptibility to alpha-cypermethrin at 94.66%, 82.66%, 82.60%, and 97.33% in Maganja da Costa, Lugela, Molumbo, and Morrumbala, respectively. In Mopeia and Lugela, susceptibility was fully restored to deltamethrin; it was partially restored to 86.66% in Maganja da Costa.

Susceptibility to permethrin was restored to 99% in Mopeia, and partially to 96% in Lugela and 76% in Morrumbala.

Synergist assays with lambda-cyhalothrin were conducted only in Maganja da Costa and Lugela, where susceptibility was restored to 98.66%, and partially to 85.33% in the respective sites. This indicates involvement and partial involvement of monooxygenases as the mechanism of resistance. Additionally, the fact that susceptibility to most pyrethroids was not restored to 100% in most districts might indicate that other mechanisms are involved.

FIGURE 12. SYNERGIST ASSAY MORTALITY RESULTS IN *AN. GAMBIAE* S.L. FROM SIX INTERVENTION DISTRICTS



4. RESULTS: NAMPULA PROVINCE

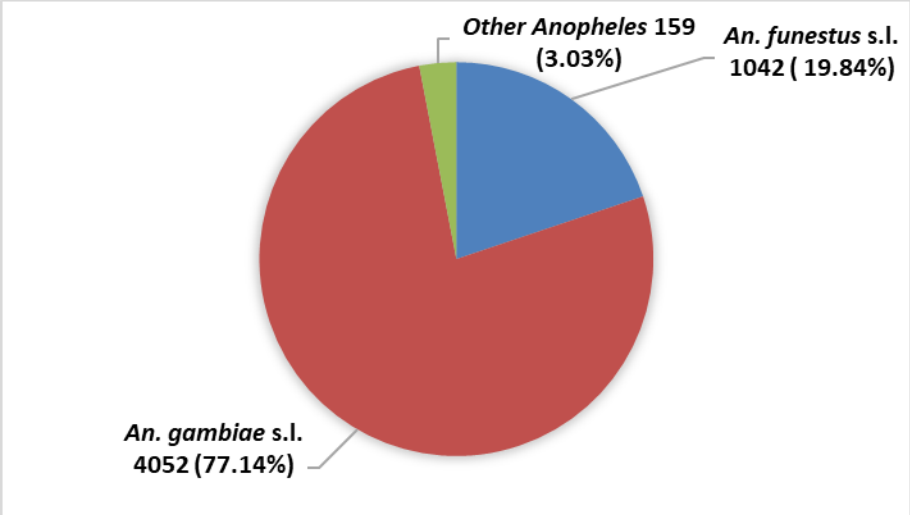
4.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT METHODS

Using the two collection methods (Prokopack and CDC light traps indoors and outdoors), 5,252 anopheline mosquitoes belonging to six different species and species complexes were collected: *An. funestus* s.l., *An. gambiae* s.l., *An. pretoriensis*, *An. rufipes*, *An. tenebrosus*, and *An. maculipalpis*. Table 6 and Figure 13 lay out the number of mosquitoes collected by species and site. *An. gambiae* s.l. was the most abundant anopheline, accounting for 77.14% of all collections, followed by *An. funestus* s.l. at 19.84%, and the other anophelines at 3.03%.

TABLE 6. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN NAMPULA PROVINCE USING BOTH COLLECTION METHODS

Species Collected	Monapo[4]	Nampula	Erati	Mogovolas	Total per Species
An. funestus s.l.	4	631	167	240	**1,042**
An. gambiae s.l.	9	1,392	1,744	907	**4,052**
An. pretoriensis	1	6	43	4	**54**
An. rufipes	0	51	21	20	**92**
An. tenebrosus	0	1	1	0	**2**
An. maculipalpis	0	8	3	0	**11**
Total	**14**	**2,089**	**1,978**	**1,171**	**5,252**

FIGURE 13. SPECIES COMPOSITION OF *ANOPHELES* MOSQUITOES FOR ALL SITES IN NAMPULA PROVINCE



A total of 4,052 *An. gambiae* s.l. mosquitoes were collected, 122 using Prokopack (3.01%) and 3,930 using CDC light traps indoor and outdoor (96.99%). A total of 1,042 *An. funestus* s.l. were collected, including 71 from Prokopack (6.81%) and 971 using CDC light traps indoor and outdoor (93.19%) collections.

[4] Monapo was dropped as monitoring site in October 2020

4.1.1 PROKOPACK COLLECTIONS

Prokopack collections yielded a total of 198 *Anopheles* mosquitoes (Table 7). Based on morphological identification, 122 of these belonged to *An. gambiae* s.l. (61.62%), 71 to *An. funestus* s.l. (35.86%), 1 *An. pretoriensis* (0.51%), 1 *An. rufipes* (0.51%), and 3 *An. maculipalpis* (1.52%).

TABLE 7. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN NAMPULA PROVINCE USING PROKOPACK ASPIRATORS

Mosquito Species/District	Monapo	Nampula	Erati	Mogovolas	Total
An. funestus s.l.	0	47	6	18	**71**
An. gambiae s.l.	1	38	69	14	**122**
An. pretoriensis	1	0	0	0	**1**
An. rufipes	0	1	0	0	**1**
An. maculipalpis	0	3	0	0	**3**
Total	**2**	**89**	**75**	**32**	**198**

Over the monitoring period, the *An. funestus* s.l. resting densities were consistently low (<1 mosquito/ room/day) in all districts over most months. The highest mean indoor resting density of *An. gambiae* s.l., estimated at 0.5 mosquitoes per room per day, was observed in Nampula in February 2021 and at 0.45 mosquitoes per room per day in Mogovolas in June 2021.

In Nampula, indoor resting density declined from the peak of 0.50 *An. funestus* s.l./ room/day in February to 0.15 *An. funestus* s.l./room/day in March, and then it increased through June 2021 (Figure 14A). There was a decline in the indoor resting density in Erati, from 0.25 *An. funestus* s.l./room/day in March to 0.0 *An. funestus* s.l./room/day from April to June. Resting density in Mogovolas increased from 0.0 *An. funestus* s.l./room/day (October to February) to 0.45 *An. funestus* s.l./room/day in June 2021. Indoor resting density of *An. gambiae* s.l. was less than 1.0 mosquito/room/day in the intervention and control districts throughout the monitoring season, except for Erati in March 2021. The highest densities observed were in March 2021 for Erati (1.25 *An. gambiae* s.l./room/day) and Nampula (0.85 *An. gambiae* s.l./room/day), and in May 2021 in Mogovolas (0.30 *An. gambiae* s.l./room/day (Figure 14B). This was after IRS with Fludora Fusion in both Nampula and Erati districts.

FIGURE 14. MEAN INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE FOUR DISTRICTS IN NAMPULA PROVINCE BEFORE AND AFTER IRS INTERVENTION AS ESTIMATED FROM THE PROKOPACK COLLECTIONS

Figure 14A: *An. funestus* s.l.

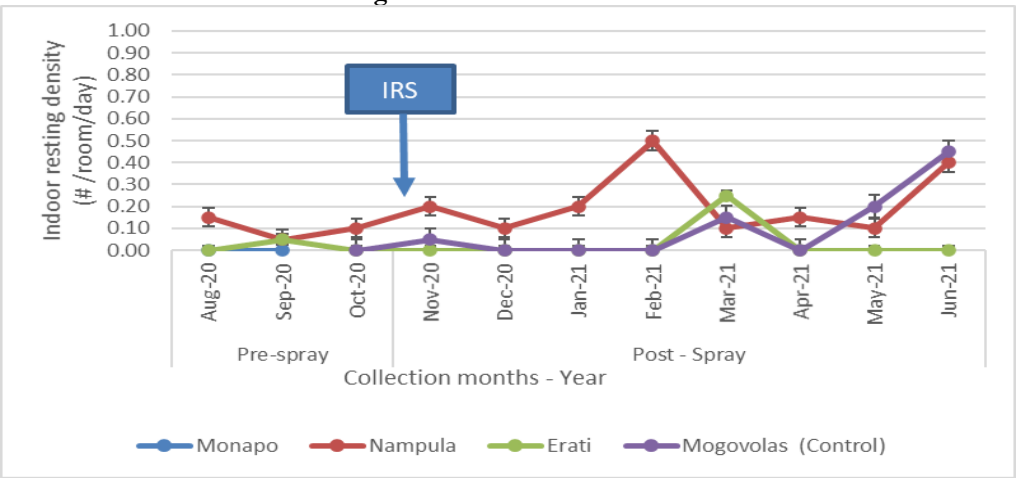
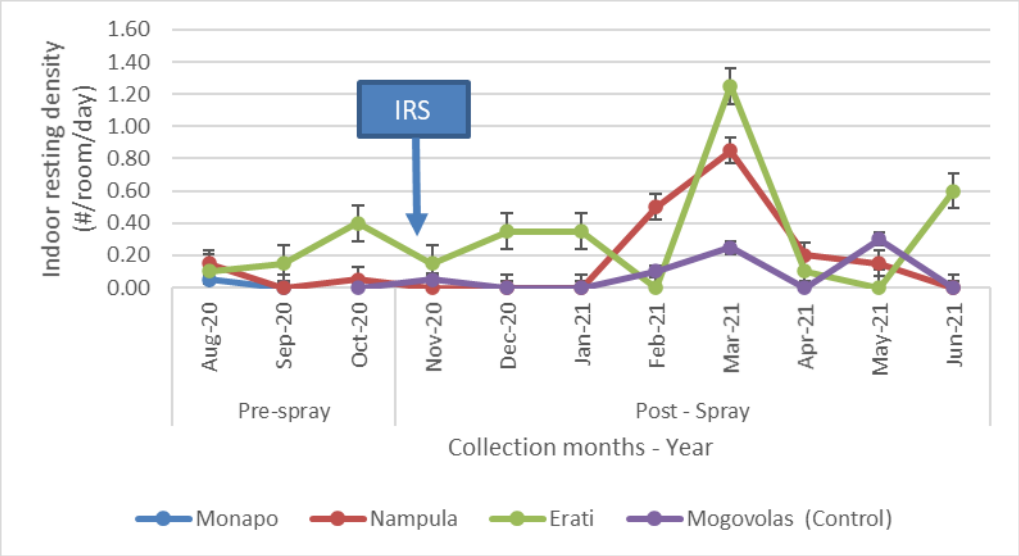


Figure 14B: *An. gambiae* s.l.



4.1.2 CDC Light Trap Collections

The CDC light trap collections yielded a total of 5,054 *Anopheles* mosquitoes from three intervention districts (Monapo, Nampula, and Erati) and the control district (Mogovolas). Morphological identification of the mosquitoes revealed that 971 (19.21 %) were *An. funestus* s.l., 3,930 (77.76 %) *An. gambiae* s.l., 91 (1.80 %) *An. rufipes*, 53 (1.05 %) *An. pretoriensis*, 8 (0.15 %) *An. maculipalpis*, and 1 (0.02%) *An. tenebrosus*. Nampula and Erati (intervention) districts had the highest percentages of all *Anopheles* collected, with 39.57% and 37.65%, respectively. The highest proportion of the total collected *An. gambiae* s.l. (the predominant species collected across all sites) was from Erati (42.62%) followed by Nampula (34.45%), Mogovolas (22.72%), and Monapo (0.20%). Table 8 summarizes the total number of mosquitoes collected per species per district and the respective percentages and the number of mosquito species collected in each district using CDC light traps.

Table 8. Number of Mosquitoes Collected by Species and by District in Nampula Province Using CDC Light Traps

	An. funestus s.l.	*An. gambiae* s.l.	*An. maculipalpis*	*An. tenebrosus*	*An. pretoriensis*	*An. rufipes*	Total/district
Monapo	4	8	0	0	0	0	**12 (0.23**
Nampula	584	1354	5	1	6	50	**2,000 (39.57)**
Erati	161	1675	3	0	43	21	**1,903 (37.65)**
Mogovolas	222	893	0	0	4	20	**1,139 (22.54)**
Total collected	**971 (19.21)**	**3,930 (77.76)**	**8 (0.15)**	**1 (0.02)**	**53 (1.05)**	**91 (1.80)**	**5,054 (100)**

As shown in Annex A, Table A2, over the reporting period, *An. funestus* s.l. was most abundant in Nampula (mean collection of 4.42 m/t/n), followed by Mogovolas (mean collection of 2.06 m/t/n). *An. gambiae* s.l. was the most abundant species in Erati (12.69 m/t/n) and Nampula (10.48 m/t/n). Monapo had the lowest mean collection for both *An. funestus* s.l. (0.17 m/t/n) and *An. gambiae* s.l. (0.33 m/t/n). The highest densities of *An. funestus* and *An. gambiae* s.l. were recorded in June and March, respectively, both in Nampula district.

Figure 15A shows that before IRS, in August 2020, *An. funestus* s.l. indoor densities in Erati and Nampula districts were 4.42 m/t./n and 3.17 m/t/n, respectively, and near zero in Monapo. In Erati, the densities dropped close to zero in September and remained below 1.0 after IRS with a slight increase (to 1.25 m/t/n) observed in April and then a decline in the next two months (to 0.08 m/t/n in June). In Mogovolas in May,

density rose sharply to 3.85 m/t/n followed by a decline to 1.58 m/t/n in June. Nampula district showed the highest densities relative to the other districts through the reporting period with densities remaining below 4.0 m/t/n until March. The highest density in Nampula might be associated with the availability of more favorable breeding sites in the area to this species. A steady rise was observed from 0.57 m/t/n in March to 7.50 m/t/n in June. The overall trend indicates that after IRS, densities of *An. funestus* s.l. tended to decrease until April, after about six months after IRS.

Figure 15B shows the *An. gambiae* s.l. indoor densities were 8.42 m/t/n in Erati and 4.17 m/t/n in Nampula district just before IRS. Density remained low after IRS and increased in February in Nampula, when it reached 19.25 m/t/n. It reached 15.58 m/t/n in Erati in March, and 9.50 m/t/n in Mogovolas in April. This increase observed in both the interventions and control districts might be attributable to the rainy season.

FIGURE 15. MEAN INDOOR RESTING DENSITIES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THE FOUR DISTRICTS IN NAMPULA PROVINCE BEFORE AND AFTER IRS INTERVENTION AS ESTIMATED FROM THE CDC LIGHT TRAP COLLECTIONS

Figure 15A: *An. funestus* s.l.

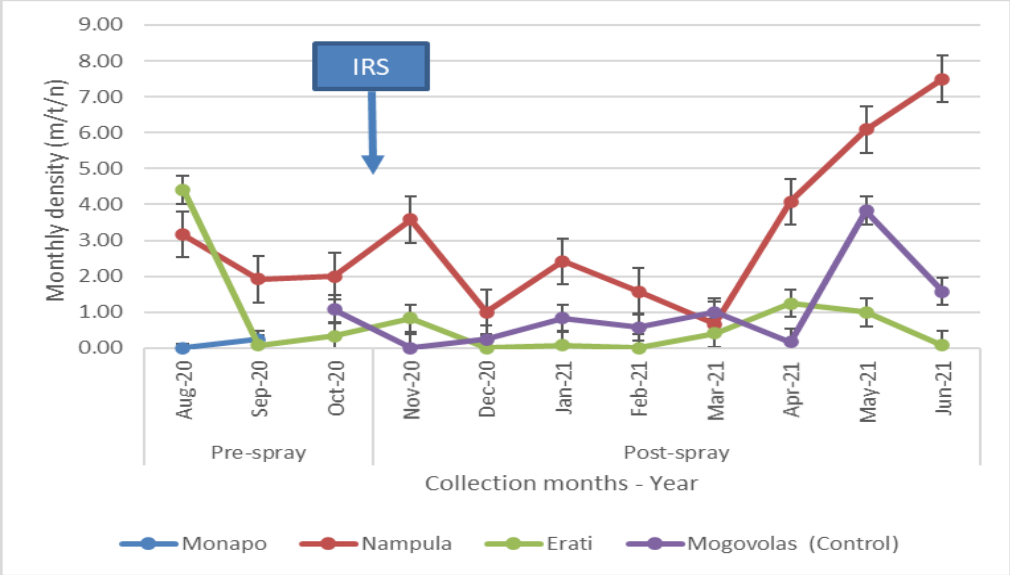
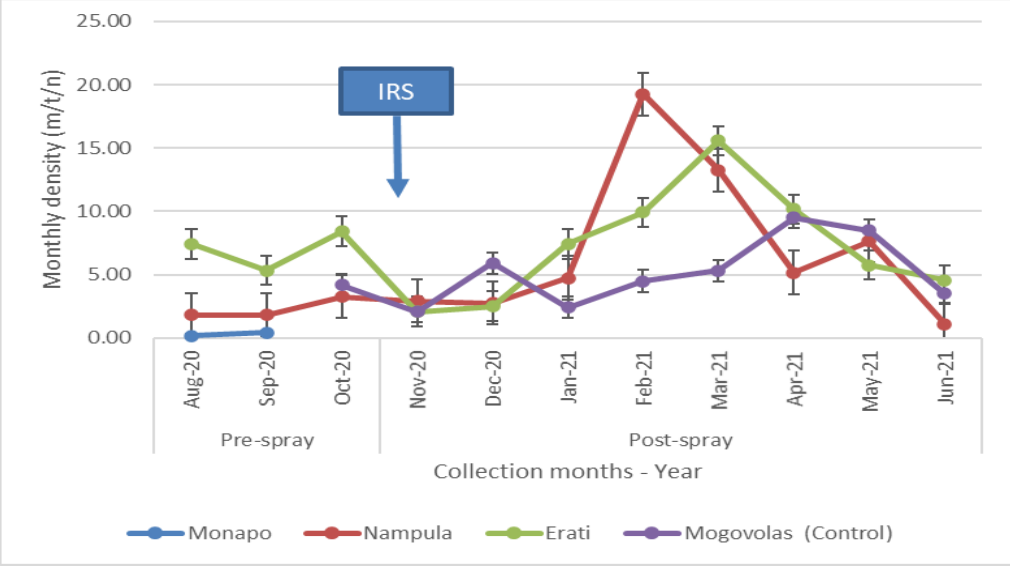


Figure 15B: *An. gambiae* s.l.



The outdoor densities of *An. funestus* s.l. in Monapo in the pre-spray season was close to zero, and in Erati and Nampula the densities were below 2.08 m/t/n (Figure 16A). After IRS, from November to April, the monthly densities of *An. funestus* s.l. were below 1.17 m/t/n in Mogovolas. The outdoor densities of *An. funestus* s.l. increased in April and May at Nampula and Mogovolas sites. The outdoor densities of *An. gambiae* s.l. before IRS show that Erati had average densities of 7.58 m/t/n, and around zero in the other districts (Figure 16B). However, in March, the densities of *An. gambiae* s.l., increased to 21.00 m/t/n in Nampula and 13.50 m/t/n in Erati, and 5.58 m/t/n in Mogovolas. A comparison between the peak densities from Erati in October before IRS and in March after IRS shows no significant difference between those two points (X^2 =1.66 and p=0.197). The relative increase of *An. gambiae* s.l. in March is most likely related to the rainy season.

FIGURE 16. OUTDOOR LIGHT TRAP DENSITY PER TRAP PER NIGHT IN THE FOUR DISTRICTS IN NAMPULA PROVINCE

Figure 16A: *An. funestus* s.l.

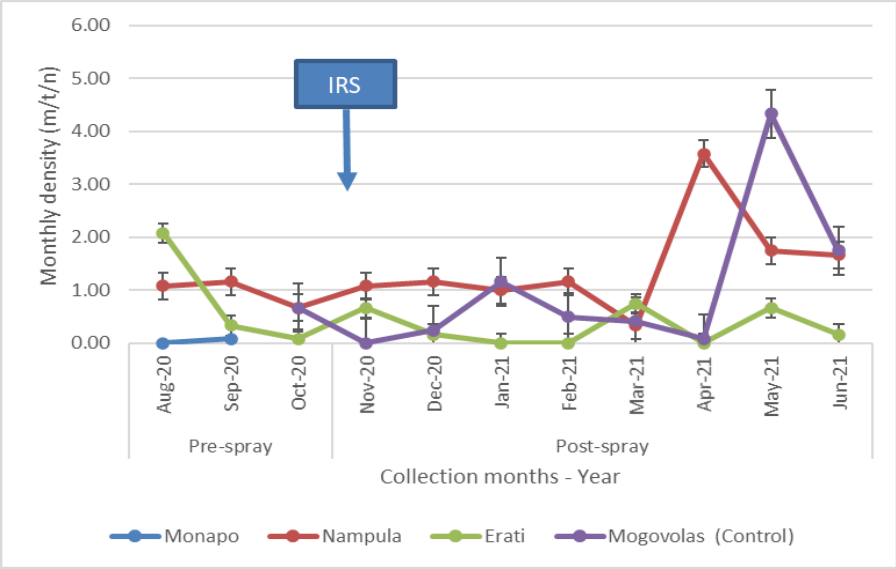
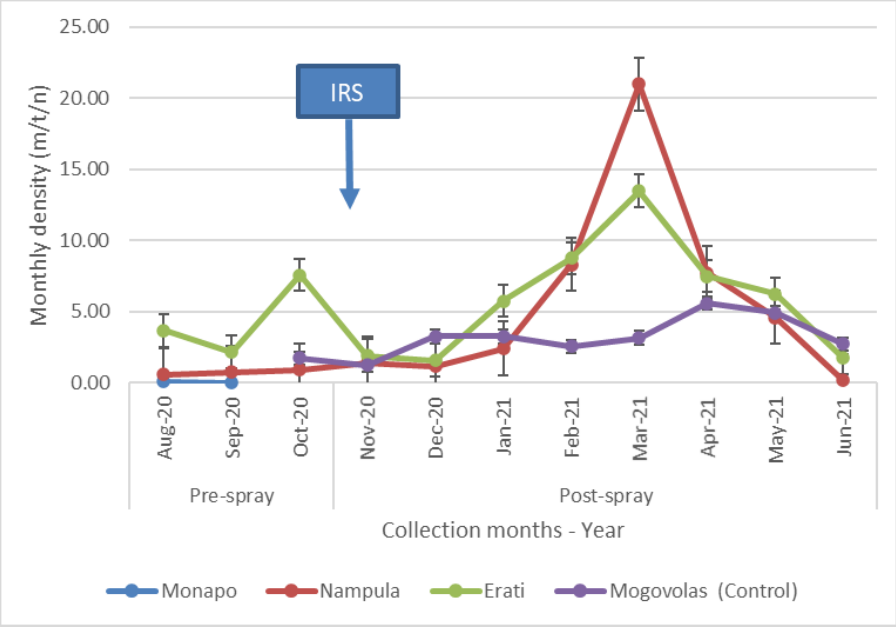


Figure 16B: *An. gambiae* s.l.



4.1.3 BITING TIME AND LOCATION BASED ON CDC LIGHT TRAP COLLECTIONS

A total of 5,054 *Anopheles* mosquitoes were collected using the CDC light trap indoor and outdoor technique from August 2020 to June 2021. The species identified morphologically from this collection belonged to *An. gambiae* s.l. (3,930), *An. funestus* s.l. (971), *An. rufipes* (91), *An. pretoriensis* (53), *An. maculipalpis* (8), and *An. tenebrosus* (1). Nampula was the district with the highest diversity of *Anopheles* mosquitoes collected.

Table 9 shows no significant difference between the total numbers of *An. funestus* s.l. samples collected indoors and outdoors ($p>0.05$) in Monapo and Mogovolas (control). In Nampula and Erati districts, however, where *An. funestus* s.l. showed more endophagic tendencies, the differences were significant ($p<0.05$).

Significantly higher numbers of *An. gambiae* s.l. samples were collected indoors as compared to outdoors ($p<0.05$) in Monapo, Nampula, Erati, and Mogovolas (control) sites.

TABLE 9. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC LIGHT TRAP INDOORS AND OUTDOORS IN FOUR DISTRICTS OF NAMPULA PROVINCE

District	*An. funestus* s.l.				*An. gambiae* s.l.			
	# Collected indoors	# Collected outdoors	X^2	p-value	# Collected indoors	# Collected outdoors	X^2	p-value
Monapo	3	1	1.000	0.317	7	1	4.500	0.034*
Nampula	408	176	92.164	0..000*	765	589	22.877	< 0.001*
Erati	102	59	11.484	<0.001*	950	725	30.224	< 0.001*
Mogovolas (Control)	112	100	0.679	0.410	551	342	48.915	< 0.001*

*Difference in mean indoor/outdoor biting rates is statistically significant at 0.05 level.

Table 10 summarizes the combined outdoor and indoor collections from the intervention and control districts, providing mean biting rates per night (b/p/n) for each species. The control district showed a similar overall biting rate (0.66 b/p/n) to that of the intervention districts (0.76 b/p/n). For *An. funestus* s.l. alone, a slightly higher biting rate (0.87 b/p/n) was observed in the intervention districts compared to the control district (0.77 b/p/n); however, the difference was not significant ($p>0.05$). For *An. gambiae* s.l. alone, the intervention districts also showed slightly higher biting rates (3.52 b/p/n) than the control district (3.10 b/p/n), but the difference was not significant ($p>0.05$).

TABLE 10. MOSQUITO SPECIES COLLECTED BY CDC LIGHT TRAP AND THEIR COMBINED OUTDOOR AND INDOOR MEAN BITING RATES IN INTERVENTION DISTRICTS OF NAMPULA AND MONAPO AND CONTROL DISTRICT OF ERATI

Species Collected	Intervention Area			Control Area		
	Total numbers collected	Total person nights	b/p/n	Total numbers collected	Total person nights	b/p/n
An. funestus s.l.	749	864	0.87	222	288	0.77
An. gambiae s.l.	3037	864	3.52	893	288	3.10
An. pretoriensis	49	864	0.06	4	288	0.01
An. tenebrosus	1	864	0.001	0	288	0
An. maculipalpis	8	864	0.009	0	288	0
An. rufipes	71	864	0.082	20	288	0.07
Total	**3,915**	**5,184**	**0.76**	**1,139**	**1,728**	**0.66**

Table 11 shows that, after IRS, the *An. funestus* s.l. indoor and outdoor biting rates increased in Nampula and decreased in Erati. The *An. gambiae* s.l. indoor and outdoor biting rates showed a notable increase after spraying in all sites. These observations were expected as they reflect the natural increase in vector populations after the rainy season. For *An. gambiae*s s.l. the observations were the same in previous year. For *An. funestus* s.l., observations were different. In 2018 - 2019 *An. funestus* s.l. decreased after IRS in all sites both indoors and outdoors, but in 2019 – 2020 *An. funestus* s.l. decreased after IRS both indoor and outdoor only in Monapo.

TABLE 11. INDOOR AND OUTDOOR MEAN BITING RATE FOR *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L., ESTIMATED USING CDC LIGHT TRAPS, BY DISTRICT, BEFORE AND AFTER SPRAYING

District	*An. funestus* s.l. (b/p/n)				*An. gambiae* s.l. (b/p/n)			
	Indoors		Outdoors		Indoors		Outdoors	
	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray	Pre-spray	Post-spray
Monapo	0.13	------	0.04	------	0.29	------	0.04	------
Nampula	2.54	3.21	1.13	1.38	1.83	6.68	0.67	5.31
Erati	2.25	0.44	1.21	0.28	6.38	7.38	2.92	6.06
Mogovolas*	------	1.04	------	1.02	------	5.22	------	3.17

*Unsprayed control district.

Figures 17A and 17B show the overnight biting pattern of *An. funestus* s.l. Both indoor and outdoor biting activities were relatively low during the evening hours of 6 pm–8 pm; after 8 pm, there was a steady increase in biting activity for several hours, both indoors and outdoors in Nampula and Mogovolas districts. Most indoor bites took place from 8 pm to 4 am, peaking at 5.0 b/p/h in Nampula at 1 am–2 am, at 1.33 b/p/h in Erati at 3 am–4 am, and in Mogovolas at 2 am–3 am. Most outdoor bites took place also between 8 pm and 1 am, peaking at 11 am–12 pm for Nampula (2.09 b/p/h), at 9 pm–10 pm and 11 am–12 pm (1.00 b/p/h) in Erati and at 8 pm–9 pm (1.42 b/p/h) in Mogovolas. The outdoor biting in Monapo remained low (<0.08 b/p/h) throughout the night, whereas in Nampula and Mogovolas biting continued actively until 6 am.

FIGURE 17. HOURLY BITING RATES OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. INDOORS AND OUTDOORS IN NAMPULA PROVINCE, AS DETERMINED THROUGH CDC LIGHT TRAPS

Figure 17A. *An. funestus* s.l. Indoor

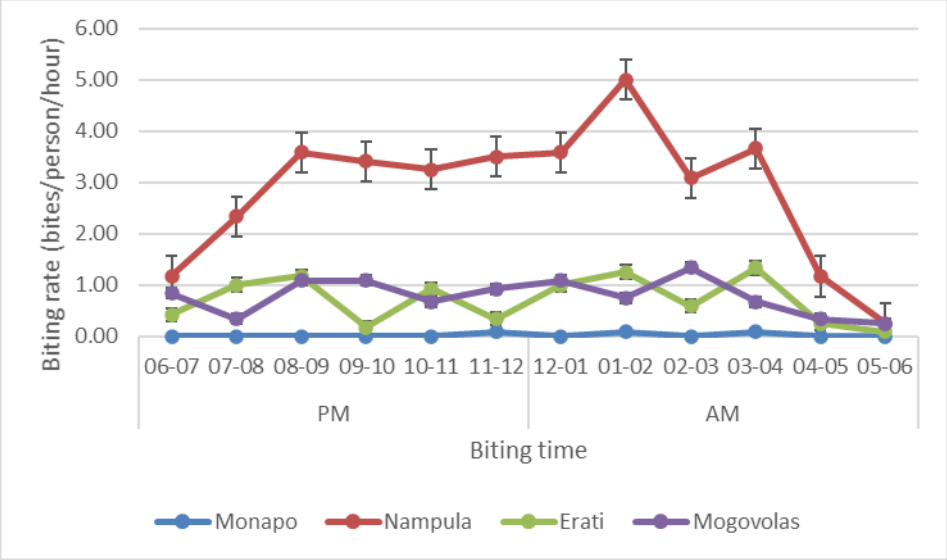
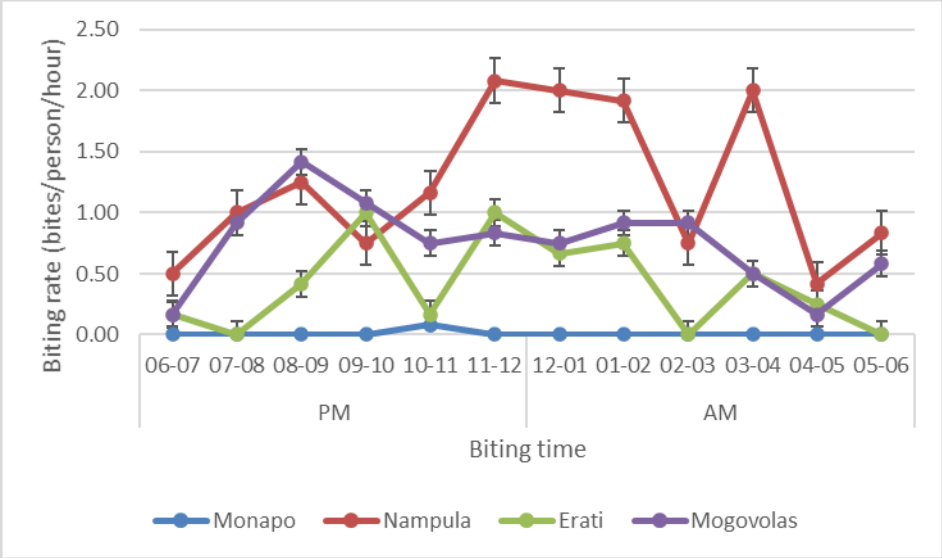


Figure 17B. *An. funestus* s.l. Outdoor



Figures 17C and 17D show the overnight biting pattern of *An. gambiae* s.l. indoors and outdoors. Both indoor and outdoor biting activities in Nampula, Erati, and Mogovolas started at a higher level than in Monapo (0.0 b/p/h). The *An. gambiae* s.l. bites took place between 6 pm and 6 am both indoors and outdoors in Nampula, Erati, and Mogovolas. Indoor bites peaked at 1 am–2 am in Erati (10.50 b/p/n) and Nampula (9.25 b/p/n), and at 11 pm–12 pm in Mogovolas (1.33 b/p/n). Outdoor bites peaked at 3 pm–4 pm for Erati (8.25 b/p/h), at 11 pm–12 pm for Nampula (7.0 b/p/h), and at 1 am–2 am for Mogovolas (4.25 b/p/h). Indoor and outdoor biting activity in Monapo continued at a low level through the night, at less than 1.0 b/p/h between 6 pm and 6 am.

Figure 17C. *An. gambiae* s.l. Indoor

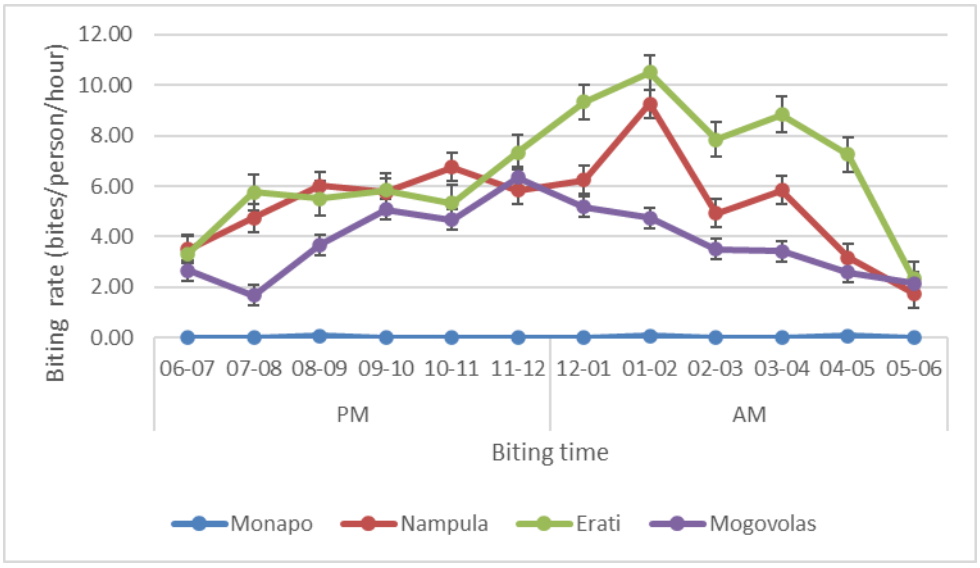
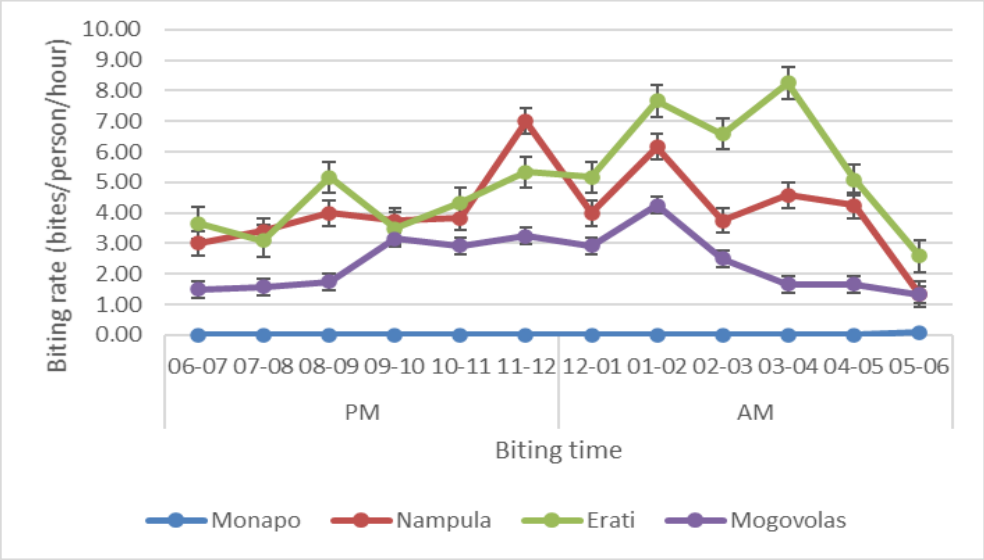


Figure 17D. *An. gambiae* s.l. Outdoor



4.2 CONE WALL BIOASSAYS

During spray operations in October 2020, cone wall bioassays were conducted to measure the quality of the spray, starting 24 hours after spray. Thereafter, monthly assays were performed to monitor the insecticide decay rate on various sprayed wall surfaces. Results of the quality assurance and decay rate monitoring of Fludora Fusion in Erati and Nampula districts are summarized in Figure 18.

4.2.1 QUALITY OF SPRAY

For Fludora Fusion (Erati and Nampula districts), mortality scored at T0 was 100% in all houses tested with cone wall bioassays 24 hours after spraying.

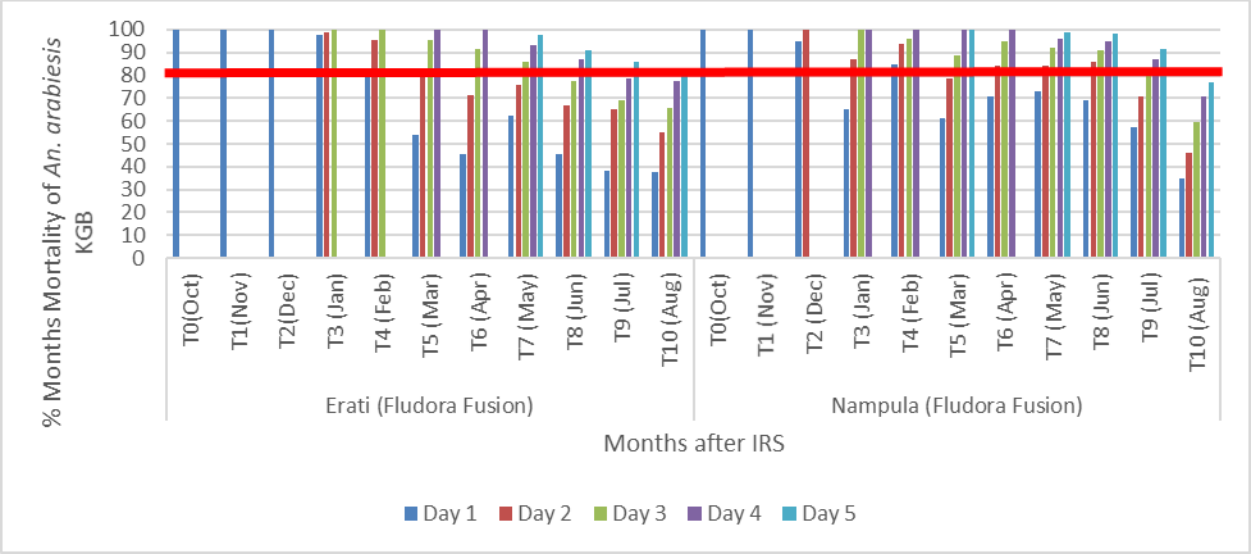
Bioassay results for assessing the quality of spraying exhibited high mortalities of 100% of female *An. arabiensis* KGB (susceptible) strain upon exposure to all three types of sprayed surfaces (cement, cob blocks, and mud). As expected, Fludora Fusion elicited high knockdowns at 30 minutes after exposure, and mosquitoes were observed to survive up to 24 hours after exposure, at which 100% mortality was recorded. The results obtained from these wall assays strongly suggest that the spraying was not underdosed, resulting in high 24-hour mortalities for Fludora Fusion.

4.2.2 INSECTICIDE DECAY RATE

FLUDORA FUSION DECAY RATE

Baseline cone wall bioassays for assessing Fludora Fusion IRS quality and subsequent monitoring of its decay rate were conducted in Erati district (Intuto and Mualangonha) and Nampula district (Nawithipele and Murrapaniua). Baseline (T0) was conducted in October 2020 and elicited a 100% mortality by day 1 post-exposure (Figure 18). Subsequent monthly cone bioassays resulted in more than 80% mortality at day 5 up to 10 months in Erati and nine months in Nampula. It was also noted that scores of 100% were observed up to six months after IRS. However, there was a notable increase in the number of days when 100% mortality was achieved, from three days during the first three months to four days by the fifth month in Erati. In Nampula, there was a notable increase in the number of days when 100% mortality was achieved from two days during the first two months to four days by the third month and up to five days by the fifth month. This is presumably due to the decreasing efficacy of the insecticide deposits on the sprayed surface. These results show that Fludora Fusion remained efficacious up to 10 months after spray.

FIGURE 18. RESULTS OF CONE WALL BIOASSAYS ON WALLS SPRAYED WITH FLUDORA FUSION IN ERATI AND NAMPULA DISTRICT

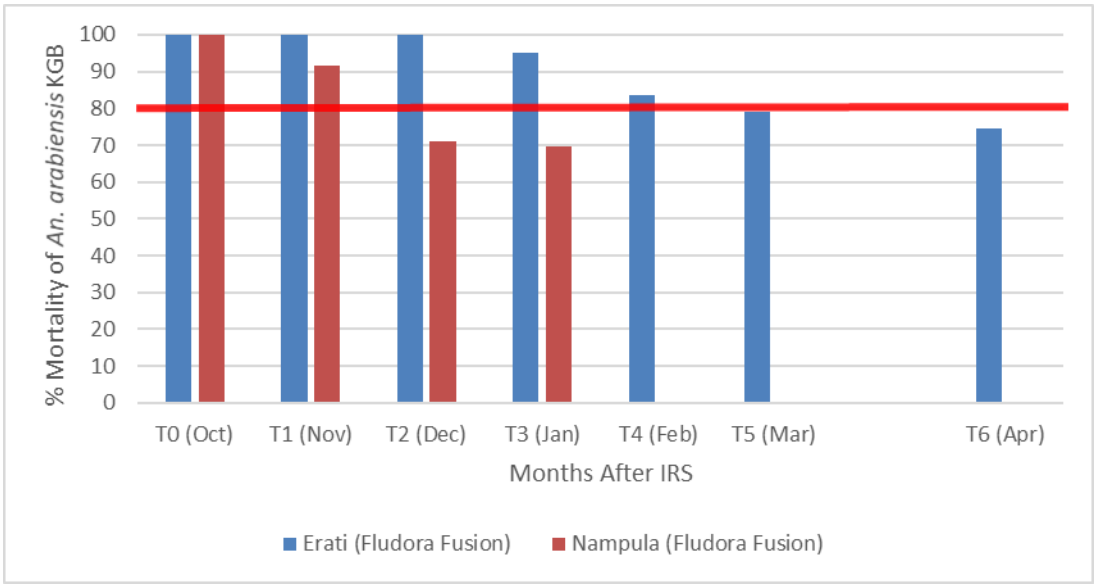


Red line indicates 80% mortality cut-off point.

4.2.3 THE AIRBORNE EFFECT

Figure 19 shows bioassay data to illustrate the airborne effect of the insecticides. The airborne fumigant effect of Fludora Fusion was found to be high in the first four months, then dropped to below 80% in Erati. In Nampula, the fumigant effect of Fludora Fusion dropped below 80% around two months after spray.

FIGURE 19. PERCENT MORTALITY OF *AN. ARABIENSIS* KGB SUSCEPTIBLE STRAIN ON AIRBORNE FUMIGANT EFFECT TEST AGAINST FLUDORA FUSION IN ERATI AND NAMPULA DISTRICTS



Red line indicates 80% mortality cut-off point.

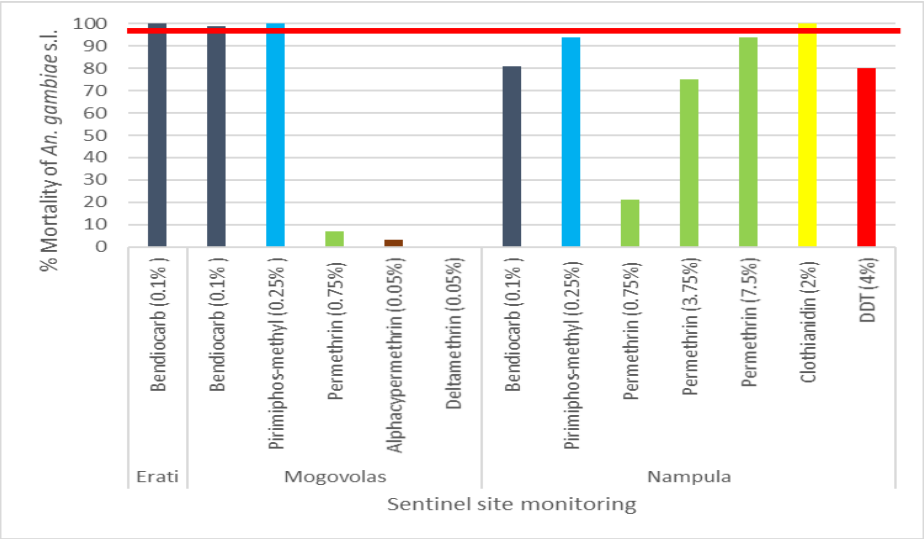
4.3 WHO SUSCEPTIBILITY TESTING

Susceptibility tests against *An. gambiae* s.l. were conducted from January through April 2021 in Erati, Nampulaand Mogovolas districts, by exposing the *An. gambiae* s.l. to diagnostic dosages of bendiocarb (0.1%), pirimiphos-methyl (0.25%), permethrin (0.75%), alpha-cypermethrin (0.05%), deltamethrin (0.05%), clothianidin (2%), and dichlorodiphenyltrichloroethane (DDT) (4%). . In prior year, in Nampula district, project conducted susceptibility testing using diagnostic concentration for all pyrethroids. This year, project begun more to assess the intensity of resistance to permethrin (5X), and permethrin (10X), and synergist assays were also conducted with PBO for permethrin, alpha-cypermethrin, and deltamethrin.

WHO susceptibility tests indicated that *An. gambiae* s.l. mosquitoes from Mogovolas and Erati districts were susceptible to bendiocarb (with mortalities ranging between 99% and 100%) and those from Nampula district were resistant to it (81% mortality). Possible resistance to pirimiphos-methyl (0.25%) was observed in *An. gambiae* s.l. from Nampula with 94% mortality, and fully susceptible in Erati with 100% mortality (Figure 20)

In Nampula district, *An. gambiae* s.l. was resistant to permethrin, and intensity assay results indicated that there was high intensity of resistance (75% mortality at 5x, and 94% mortality at 10x) and resistance to DDT (4%) with 80% mortality. The vector is fully susceptible to clothianidin (2%) in Nampula. Resistance was detected to all pyrethroids tested in Mogovolas (alpha-cypermethrin 0.05% (3% mortality), deltamethrin 0.05% (0% mortality), and permethrin 0.75% (7% mortality).

FIGURE 20. PERCENTAGE MORTALITY OF ADULT *AN. GAMBIAE* S.L. RAISED FROM LARVAL COLLECTIONS EXPOSED TO A RANGE OF INSECTICIDES AT RESPECTIVE DIAGNOSTIC CONCENTRATIONS AND HOLDING PERIODS

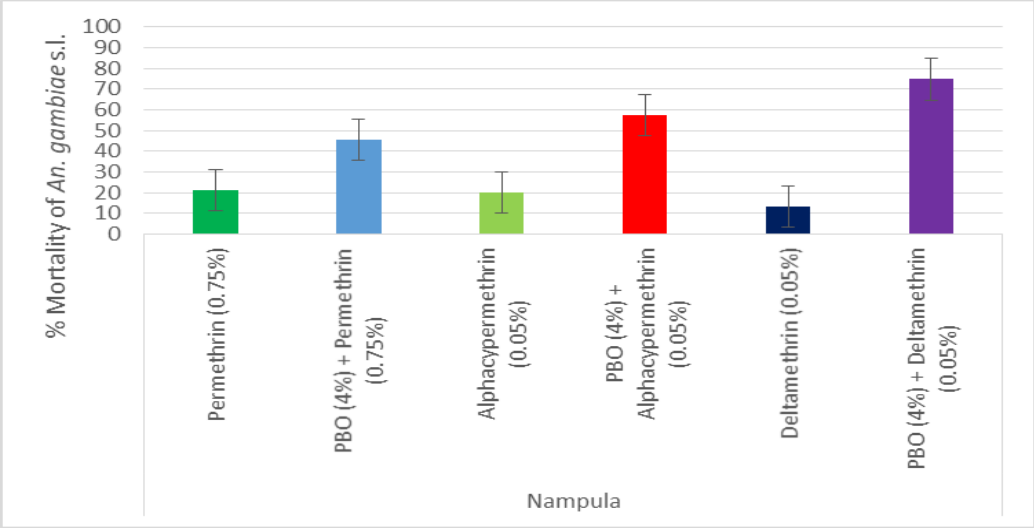


Note: Clothianidin holding periods was up to seven days; the mortality for other insecticides was at 24-hour holding period.
Red horizontal line indicates the 98% mortality cut-off point for susceptibility.

4.3.1 SYNERGIST ASSAYS USING WHO TUBE TESTS

Pre-exposure of *An. gambiae* s.l. to PBO seems to increase susceptibility to permethrin, alpha-cypermethrin, and deltamethrin, but did not restore full susceptibility (Figure 21). The mortality rates increased from 21.33% with permethrin alone to 45.33% with permethrin + PBO; from 20% with alpha-cypermethrin alone to 57.33% with alpha-cypermethrin + PBO; and from 13.33% with permethrin alone to 74.67% with permethrin + PBO. However, for all insecticides (permethrin, alpha-cypermethrin, and deltamethrin), mortality after pre-exposure to PBO was below 98%, indicating that monooxygenases are not the only form of metabolic resistance in the area. The pre-exposure of *An. gambiae* s.l. to PBO aimed to understand the involvement of monooxygenases in the resistance mechanism. Figure 21 shows that the pre-exposure to PBO resulted in only partial restoration of susceptibility to permethrin, alpha-cypermethrin, and deltamethrin in Nampula district.

FIGURE 21. MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO ALPHA-CYPERMETHRIN, DELTAMETHRIN, AND PERMETHRIN WITH AND WITHOUT PBO IN NAMPULA DISTRICT (JANUARY–MARCH 2021)



5. Discussion and Lessons Learned

5.1 Zambezia Province

The entomological surveillance conducted in Zambezia employed two main collection methods, Prokopack aspirators and CDC light traps, set next to humans sleeping under treated nets indoors and outdoors. Anophelines collected by both methods were identified using the morphological identification key, revealing the presence of 10 anopheline species, with *An. funestus* s.l. being the most abundant (69%), followed by *An. gambiae* s.l. (27%) and other species, which accounted for 4%. Together, the two dominant vectors constituted 96% of the anopheline population collected. Coincidentally, the two species are known to be the most efficient malaria vectors in Africa. The greatest diversity of anopheline species was collected using CDC light traps, with 10 species out of the total 10 collected, followed by Prokopack at four species.

Unlike the other three districts, in Maganja da Costa, *An. gambiae* s.l. was more abundant than *An. funestus* s.l. This is in addition to the previous annual report that indicated the emerging dominance of *An. gambiae* s.l. in Milange. It seems that the species abundance might have been shifting gradually in these areas from *An. funestus* s.l. to *An. gambiae* s.l., and there is a need to regularly monitor the evolving dynamics of the species composition in this area.

Low levels of indoor resting mosquitoes were recorded in both the intervention and control districts for one of the main malaria vector species, *An. gambiae* s.l., although more *An. funestus* s.l. than *An. gambiae* s.l. were recorded in most districts. However, we have a gap in knowledge as we have not done outdoor resting collections to determine that there is a tendency for outdoor resting. But recently our teams started outdoor resting collections from pit-shelters that will provide more insight on resting behavior of the malaria vectors in the areas. Monthly indoor resting patterns show *An. funestus* s.l. to be more abundant in Lugela (control) and Mopeia and mostly during the dry season, between August and October, with a peak in October and again around December in Lugela at the beginning of the rainy season. This is an indication of more prolonged abundance of this species, perhaps at the beginning of the rainy season. *An. gambiae* s.l. was found relatively more frequently during the rainy season, with intermittent peaks in different districts between October, December, and March. However, *An. funestus* s.l. seems not strictly associated with the rainy season.

Our findings show that IRS has contributed to the reduction of the indoor resting density and biting rates of *An. funestus* s.l. in at least two districts. In Maganja da Costa, a reduction in the biting rate was observed, which fell by half in the post-spray period as compared to pre-spray period. The most notable reduction of *An. funestus* s.l. indoor biting activities was observed in Mopeia in the post-spray season compared to the pre-spray season. The biting rate in the control remains almost the same during the pre- and post-intervention seasons. This may indicate potential effect of IRS on *An. funestus* s.l. However, for the *An. gambiae* s.l., there was an increase in biting rates at all sentinel sites. This might have been associated with the rainfall patterns and availability of suitable breeding habitats of *An. gambiae* s.l. during the rainy seasons in those areas.

The biting patterns for both *An. funes*tus s.l. and *An. gambia*e s.l. show that most biting occurs around midnight and in the early morning hours when most people are expected to be sleeping in the houses under a treated net. This finding shows the potential for sprayed houses and treated nets in protecting communities against infective bites from the two major vectors.

The molecular results are not yet available due to the delay in delivery of the reagents. The results are expected around mid-October or in November. The project will produce an addendum report with those results in December.

The quality of IRS assessed by cone wall bioassays showed that spray teams did not underdose the spraying in all districts, demonstrating appreciable skills in consistent, uniform application of the insecticides across districts. Subsequent monthly cone wall bioassays to monitor insecticide decay rates found that SumiShield 50WG and Fludora Fusion remain effective at least ten to eleven months post-spray in all sprayed districts. This long period of residual efficacy is consistent with the observation made with the 2019-2020 bioassays. The airborne fumigant effect of SumiShield 50WG in Mopeia was found to be high for around seven months; the same was observed with Fludora Fusion, where the effect lasted for around eight months in Maganja da Costa and six months in Milange.

Insecticide susceptibility test results show that local vectors are fully susceptible to pirimiphos-methyl, chlorfenapyr, clothianidin, and bendiocarb. Assays for pyrethroids again revealed widespread *An. gambiae* s.l. resistance to these insecticides. Synergist assays with PBO demonstrated restoration of susceptibility in most of the sites, indicating involvement of oxidase-mediated resistance mechanisms. This shows the potential for PBO nets to effectively overcome the observed pyrethroid resistance threat in the area.

5.2 NAMPULA PROVINCE

A total of 5,252 anopheline mosquitoes were collected in surveyed districts of Nampula Province, using Prokopack and CDC light traps. The anopheline mosquitoes were found to belong to six species and species complexes: *An. gambiae* s.l., *An. funestus* s.l., *An. rufipes*, *An. pretoriensis*, *An. tenebrosus*, and *An. maculipalpis*. *An. gambiae* s.l. and *An. funestus* s.l. were the major vectors, making up 77.14% and 19.84%, respectively, of the mosquitoes caught.

Lower indoor resting densities of *An. funestus* s.l. were observed before IRS in all intervention and control districts. The densities recorded in Erati district (control) were similar to Nampula (intervention) for both *An. funestus* s.l. and *An. gambiae* s.l. before spraying, and Erati (intervention) and Mogovolas (control) after spraying. Collections using CDC light traps show that indoor and outdoor *An. funestus* s.l. densities in Erati and Mogovolas were lower than in Nampula district, but much higher than in Monapo. *An. gambiae* s.l. densities both indoor and outdoor was high in Nampula district. *An. gambiae* s.l. biting activity demonstrated an increase in both control (Mogovolas) and intervention (Erati and Nampula) districts. These findings could potentially be an outcome of seasonal abundance of vector species during the rainy season.

Although the peak outdoor biting time of *An. funestus* s.l. in Nampula district was between 11 pm and 12 pm, biting continued outdoors in the morning hours of 5 am to 6 am in Nampula and Mogovolas (control), when residents start to awake and go outdoors. This might not be a favorable situation for any indoor-based malaria vector control intervention. The result suggests extending the collection period in the morning at least up to 7 am to better understand the extent of daytime outdoor biting by this species in the area. A previous study in Senegal showed a behavioral change of *An. funestus* after introduction of insecticide-treated nets: It remained anthropophilic and endophilic but adopted diurnal feeding (Sougoufara et al. 2014); the study indicated that six times more *An. funestus* s.l. were captured in broad daylight than at night. Significant biting also occurred between 6 pm and 7 pm both indoors and outdoors by *An. gambiae* s.l. in Erati (intervention), Nampula (intervention), and Mogovolas (control) and continued until the morning hours of 5 am to 6 am. It is important to start collections at 5 pm to better understand potential exposure to mosquito bites in the early evening hours.

The quality of IRS assessed by cone wall bioassays showed that spray teams were able to achieve optimal insecticide application in all districts, demonstrating appreciable skills in consistent and uniform application of insecticides across districts. Subsequent monthly cone wall bioassays to monitor insecticide decay rates found that Fludora Fusion in Erati and Nampula were found to remain effective on sprayed walls at least for 10 months.

Based on the data collected so far, *An. gambiae* s.l. was resistant to DDT, bendiocarb, and pirimiphos-methyl in Nampula district. The National Malaria Control Program should not consider these insecticides for IRS in the area. *An. gambiae* s.l. remained susceptible to clothianidin in Nampula district, bendiocarb in Erati and Mogovolas districts, and pirimiphos-methyl in Mogovolas district. However, the vector is resistant to bendiocarb in Nampula. There is also high intensity of resistance to permethrin in Nampula. The synergist assays showed that PBO did not restore susceptibility to pyrethroid insecticides in the area. This might indicate

that PBO nets with permethrin, alpha-cypermethrin, and deltamethrin insecticides may not be an option for malaria vector control in those areas in the future. This signifies the importance of continued application of next generation insecticides with IRS.

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ANNEX A

TABLE A1. CDC LIGHT TRAP DATA FROM MONTHLY COLLECTIONS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN FOUR DISTRICTS OF ZAMBEZIA PROVINCE: MAGANJA DA COSTA, MILANGE, MOPEIA, AND LUGELA

Districts	Species	2020					2021						Total and annual average densities	
		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June		
Maganja	*An. funestus* s.l.	166	168	10	12	33	14	11	11	35	58	129	647	1515
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	13.83	14.00	0.83	1.00	2.75	1.17	0.92	0.92	2.92	4.83	10.75	3.92	
	An. gambiae s.l.	32	83	20	39	480	130	1	48	29	5	1	868	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	2.67	6.92	1.67	3.25	40.00	10.83	0.08	4.00	2.42	0.42	0.08	6.58	
Milange	*An. funestus* s.l.	3	3	0	1	23	109	34	92	165	41	100	571	989
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.25	0.25	0.00	0.08	1.92	9.08	2.83	7.67	13.75	3.42	8.33	4.33	
	An. gambiae s.l.	4	2	1	1	31	24	38	238	51	21	7	418	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.33	0.17	0.08	0.08	2.58	2.00	3.17	19.83	4.25	1.75	0.58	3.17	
Mopeia	*An. funestus* s.l.	379	95	122	6	10	7	9	25	55	153	135	996	1063
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	31.58	7.92	10.17	0.50	0.83	0.58	0.75	2.08	4.58	12.75	11.25	7.55	
	An. gambiae s.l.	1	1	0	0	3	15	4	29	11	2	1	67	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.08	0.08	0.00	0.00	0.25	1.25	0.33	2.42	0.92	0.17	0.08	0.51	
Lugela	*An. funestus* s.l.	137	104	107	97	310	205	153	42	22	33	23	1233	1463
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	11.42	8.67	8.92	8.08	25.83	17.08	12.75	3.50	1.83	2.75	1.92	9.34	
	An. gambiae s.l.	0	0	1	9	54	16	34	65	37	10	4	230	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.00	0.08	0.75	4.50	1.33	2.83	5.42	3.08	0.83	0.33	1.74	-*
Total		**722**	**456**	**261**	**165**	**944**	**520**	**284**	**550**	**405**	**323**	**400**	**5030**	

TABLE A2. CDC LIGHT TRAP DATA FOR MONTHLY COLLECTIONS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED IN NAMPULA PROVINCE

Districts	Species	2020					2021						Total & Average Densities/Month/Night	
		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June		
Monapo	*An. funestus* s.l.	0	4										**4**	**12**
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.33										**0.17**	
	An. gambiae s.l.	3	5										**8**	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.25	0.42										**0.33**	
Nampula	*An. funestus* s.l.	51	37	32	56	26	41	33	12	92	94	110	**584**	**1938**
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	4.25	3.08	2.67	4.67	2.17	3.42	2.75	1.00	7.67	7.83	9.17	**4.42**	
	An. gambiae s.l.	29	31	50	52	47	86	331	441	155	147	15	**1354**	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	2.42	2.58	4.17	4.33	3.92	7.17	27.58	36.75	12.92	12.25	1.25	**10.48**	
Erati	*An. funestus* s.l.	78	5	5	18	2	1	0	14	15	20	3	**161**	**1836**
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	6.5	0.42	0.42	1.5	0.17	0.08	0	1.17	1.25	1.67	0.25	**1.22**	
	An. gambiae s.l.	133	90	192	48	49	158	224	349	212	144	76	**1675**	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night	11.08	7.5	16	4	4.08	13.17	18.67	29.08	17.67	12	6.33	**12.69**	
Mogovolas	*An. funestus* s.l.			21	0	6	24	13	17	3	98	40	**222**	**1115**
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night			1.75	0	0.5	2	1.08	1.42	0.25	8.17	3.33	**2.06**	
	An. gambiae s.l.			71	40	110	68	85	102	181	161	75	**893**	
	Trap nights	12	12	12	12	12	12	12	12	12	12	12		
	Mean # Mosq/trap/night			5.92	3.33	9.17	5.67	7.08	8.5	15.08	13.42	6.25	**8.27**	
Total		**294**	**172**	**371**	**214**	**240**	**378**	**686**	**935**	**658**	**664**	**319**	**4,901**	

Note: The blacked out rows indicate that mosquito collections were not carried out due to changes on the sites.