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PMI VECTORLINK MOZAMBIQUE ENTOMOLOGICAL MONITORING ANNUAL REPORT AUGUST 2021 – JUNE 2022

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ACRONYMS

b/p/h	Bites per Person per Hour
b/p/n	Bites per Person per Night
CDC	Centers for Disease Control and Prevention
INS	National Institute of Health (*Instituto Nacional de Saúde)*
IRS	Indoor Residual Spray
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 implements the U.S. President's Malaria Initiative VectorLink Project in Mozambique. In 2021 the spray campaign was carried out from November 8 to December 23 in Zambezia Province. VectorLink Mozambique conducted IRS with Actellic 300CS (an organophosphate) in Molumbo, Milange, and Morrumbala districts and with bendiocarb (a carbamate) in Mopeia district. Monthly entomological monitoring was performed in three intervention districts (Molumbo, Milange, and Mopeia) and one control district (Lugela), which did not receive IRS. Surveillance employed three collections techniques: Prokopack aspirator, Centers for Disease Control and Prevention (CDC) light traps, and pit shelters traps. Cone wall bioassays were conducted to monitor the spray quality and residual life of insecticides sprayed in Molumbo, Milange, and Mopeia. Annual insecticide susceptibility tests were carried out in all four sprayed districts (Milange, Molumbo, Mopeia, and Morrumbala) plus one former IRS district (Maganja da Costa) and one control district (Lugela).

In Nampula Province, the Government of Mozambique, with support from the Global Fund, conducted IRS using Fludora Fusion only in Meconta, Murrupula, Erati, and Ribaue districts, and with Fludora Fusion and bendiocarb (Ficam) in Nampula, Angoche, Monapo, and Nacala Porto districts. VectorLink Mozambique performed monthly entomological monitoring using Prokopack aspirator, CDC light trap, and pit shelter collections in two intervention districts (Nampula and Erati) and one control district (Mogovolas). Cone wall bioassays were conducted in Nampula district and Erati. Annual insecticide susceptibility tests were carried out in the two sprayed districts of Erati and Nampula district, and the control district of Mogovolas.

Mosquito collections using the three methods described above demonstrated the presence of highly diverse species composition of anophelines, which included the two main malaria vectors *Anopheles funestus* s.l. and *An. gambiae* s.l., and other potential vectors and non-vectors such as *An. coustani*, *An. pretoriensis*, *An. tenebrosus*, *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, after IRS, *An. funestus* s.l. indoor resting densities were suppressed compared with pre-IRS densities in Zambezia Province (August to October). In Nampula Province, there was a decrease in the post-IRS indoor resting densities of *An. funestus* s.l. in Erati district but an increase in Nampula district. *An. gambiae* s.l. densities appeared to increase slightly, likely because of the rapid build-up of breeding habitats due to 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 using CDC light traps as a proxy for human landing catches. *An. funestus* s.l. tended to bite predominantly indoors at most sites. Biting activity seemed to follow human sleeping patterns, with peak indoor biting activity starting at around 10–11 pm and in the early morning hours, mainly at 2–3 am. This kind of vector behavior makes indoor vector control interventions (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 bendiocarb (Ficam) lasted at least three months. Actellic 300CS had low residual efficacy of less than two months in Milange, and in Molumbo it lasted for five months.

Results of insecticide susceptibility tests showed that local vectors are fully susceptible to pirimiphos-methyl, chlorfenapyr, clothianidin, and bendiocarb (except in Mopeia and Nampula districts, where resistance to bendiocarb was observed). Assays for pyrethroids (deltamethrin, permethrin, alpha-cypermethrin, and lambda-

cyhalothrin) 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 that depend on pyrethroid insecticides, and therefore the continued use of non-pyrethroid insecticides for IRS is important. Synergist assays with piperonyl butoxide (PBO) demonstrated full and partial restoration of vector susceptibility to pyrethroids (deltamethrin, lambda-cyhalothrin, alpha-cypermethrin, and permethrin) at most sites in Zambezia, indicating that PBO nets are a viable option for vector control to overcome the observed pyrethroid resistance in Zambezia. However, PBO only restored partial susceptibility of vectors' to pyrethroid insecticides in Nampula Province. The continued use of IRS with next generation insecticides is recommended for malaria vector control in the area.

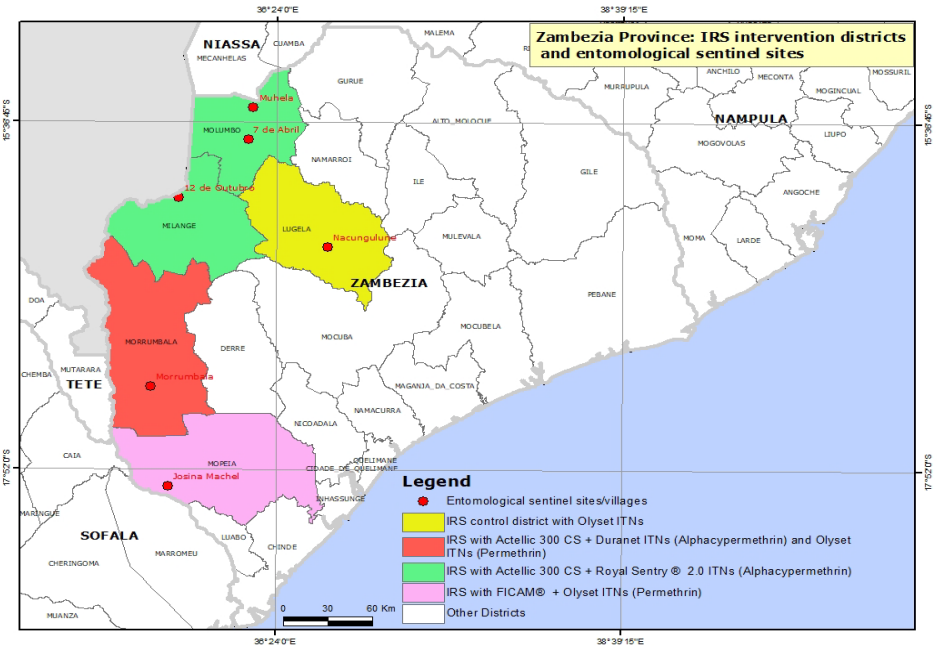
I. INTRODUCTION

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 2021, Zambezia implemented four spray campaigns under the PMI VectorLink Project. During the 2021 spray campaign, PMI VectorLink Mozambique conducted IRS in four target districts (Milange, Molumbo, Mopeia, and Morrumbala). VectorLink Mozambique also carried out entomological monitoring activities in Zambezia and supported the National Malaria Control Program's entomological activities in Nampula and five other provinces in the North and Central regions of the country to strengthen 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 covers the period from August 1, 2021, to June 30, 2022.

Entomological monitoring was conducted in three IRS intervention districts in six sentinel sites in Zambezia Province: Molumbo (7 de Abril and Muhela), Milange (3 de Fevereiro and 12 de Outubro), and Mopeia (Josina Machel and Eduardo Mondlane) and one non-intervention district, Lugela as a control district in two sentinel sites (Nhacungulune and Dabane) (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. were found mostly from June to September (dry season), while *An. gambiae* s.l. were found mostly from January to April (rainy season).

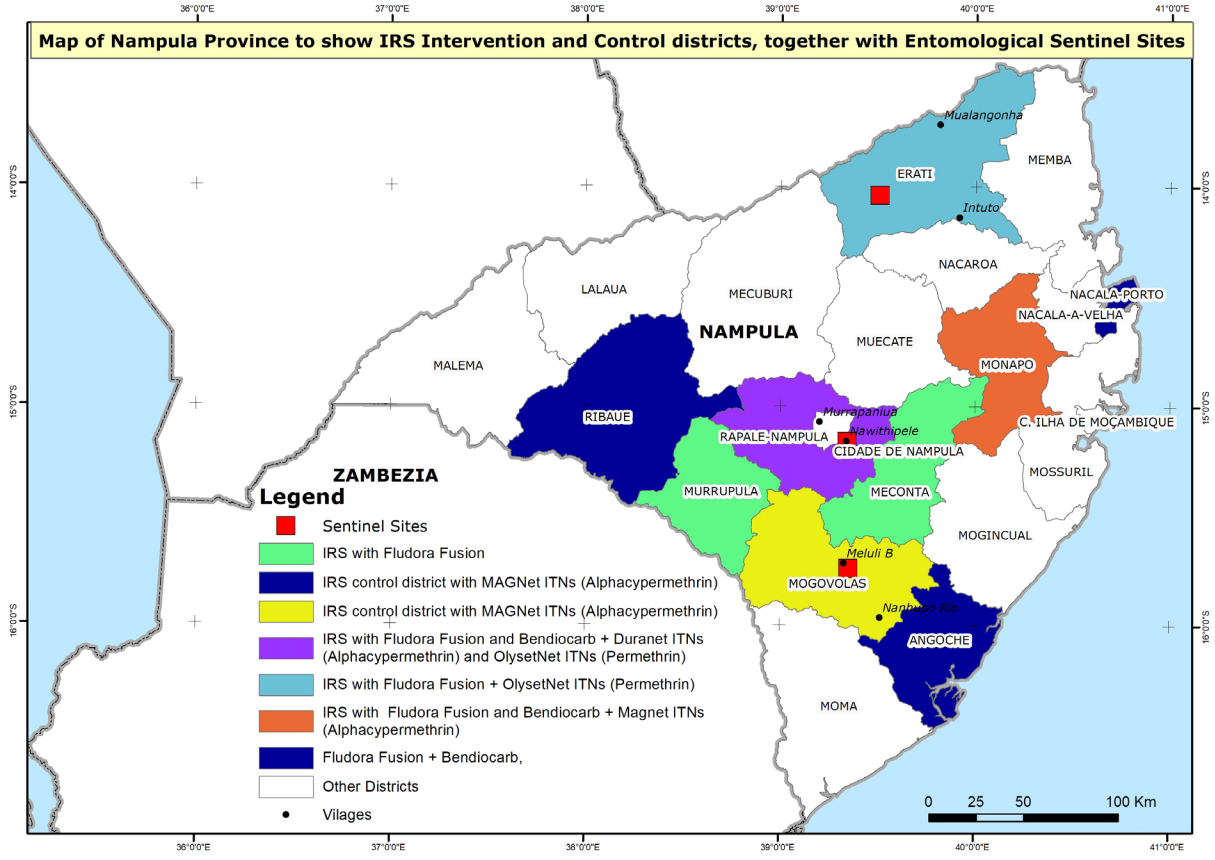
FIGURE 1A. ZAMBEZIA PROVINCE IRS INTERVENTION, CONTROL DISTRICTS, ENTOMOLOGICAL SENTINEL SITES, INSECTICIDE SPRAYED, AND INSECTICIDE-TREATED NETS DISTRIBUTED



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, Erati (Intuto and Mualangonha) and Nampula (Nawithipele and Murrapaniua), as well as in one control district, Mogovolas (Meluli B and Nanhupo Rio). The map of Nampula in Figure 1B shows the province's IRS intervention and control districts, together with the entomological sentinel sites.

As part of the 2020 universal coverage ITN campaign in Zambezia, Royal Sentry (alpha-cypermethrin based net), OlysetNet (permethrin-based net) and Duranet (alpha-cypermethrin based net) were distributed. In the 2022 campaign in Nampula, Permanent 3.0 (deltamethrin+PBO based net) were distributed. For pre-natal care, VEERALIN and Permanent 3.0 were distributed in Zambezia and Nampula Province respectively.

FIGURE 1B: NAMPULA PROVINCE IRS INTERVENTION CONTROL DISTRICTS, ENTOMOLOGICAL SENTINEL SITES, INSECTICIDES SPRAYED, AND INSECTICIDE-TREATED NETS DISTRIBUTED



2. METHODOLOGY

2.1 LONGITUDINAL MONITORING

Data were collected from August 2021 through June 2022, using Prokopack aspirators, Center for Disease Control and Prevention (CDC) light traps as proxy for human landing catches, and pit shelters.

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 three intervention districts, Milange (12 de Outubro and 3 de Fevereiro sentinel sites), Mopeia (Josina Machel and Eduardo Mondlane sentinel sites), and Molumbo (7 de Abril and Muhela sentinel sites), and in one control district, Lugela (Nhacungulune and Dabane sentinel sites).

In Nampula Province, Prokopack collections were conducted in two intervention districts, Erati (Intuto and Mualangonha sentinel sites) and Nampula (Nawithipele and Murrapaniua sentinel sites), and in one control district, Mogovolas (Meluli B and Nanhupo Rio sentinel sites).

Five houses in each of the two sentinel sites in each district were selected for Prokopack collections, totalling 10 houses per district. Prokopack collections were conducted from 6 am to 8 am over two consecutive days in each of the five houses in each sentinel site. The same houses were visited every two months for collections. The first collections were conducted two months prior to the 2021 IRS campaign, and collections continued after the campaign. In each house, one sleeping room was used for Prokopack collections. 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, then under beds and tables, and finishing with the roof or ceiling. Live mosquitoes in the cups were transferred first into small cages and then to paper cups. The mosquitoes were killed with chloroform, counted, and their abdominal stage was recorded on a data collection form. They were then placed in a petri dish for morphological identification and then 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 2021–June 2022 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 for human landing catches to collect primarily the host-seeking population to estimate human biting rates. With such traps, the person protected under a net is equivalent to a 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 sentinel sites listed for Prokopack collections in three intervention districts (Molumbo, Milange, and Mopeia), as well as in the control district (Lugela). Likewise, in Nampula Province, CDC light traps were installed in four houses in the two intervention districts of Erati (Intuto sentinel site) and Nampula district (Nawithipele sentinel site e) and the control 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/

of Mogovolas (Meluli B sentinel site). Every two months, the traps were set over three consecutive nights, from 6 pm to 6 am.

Traps were set both indoors and outdoors. Indoors, the traps were set about 1.5 m above the floor, in the bedroom at the foot of a bed with humans sleeping under treated bed nets.

CDC light traps were set up outdoors in a similar manner. The outdoor trap was placed 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 untreated 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 every two months for the CDC light trap collections in accordance with SOP 01/01.

2.1.3 PIT SHELTERS COLLECTIONS

Artificial pit shelters were used in each district. The pit shelters were located outside in the yard of each house in one of the sentinel sites of the district where Prokopack collections were also being done; each sentinel site had five pit shelters. In accordance with SOP13/01, sucking tubes were used to collect mosquitoes from 5 am to 9 am. In Zambézia Province, the pit shelters were located in Josina Machel sentinel site in Mopeia, 12 de Outubro sentinel site in Milange, Muhela sentinel site in Molumbo, and Nhacungulune sentinel site in Lugela (control district).

In Nampula Province, pit shelters collections were conducted in two intervention districts, Erati (Intuto sentinel site) and Nampula (Nawithipele sentinel site), and in one control district, Mogovolas (Meluli B sentinel site).

2.2 IRS QUALITY ASSAYS AND INSECTICIDE DECAY RATE MONITORING

In Zambezia Province, standard World Health Organization (WHO) cone bioassay tests were performed from November 2021 through June 2022 in Molumbo (7 de Abril sentinel site), through March 2022 in Milange (3 de Fevereiro sentinel site), and through April 2022 in Mopeia (Josina Machel sentinel site) to evaluate spray quality and residual efficacy of the insecticides used during the 2021 spray campaign. In Nampula Province, wall bioassays were conducted in Nampula district (Nawithipele) and Erati (Intuto) districts from September 2021 through July 2022. In both provinces, wall bioassays were conducted 24 hours after spraying and then monthly until the mortality rate was less than 80% for two consecutive months, SOP009/01[3].

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 the different cone 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 intervals for up to five days for both Fludora Fusion- and bendiocarb-exposed mosquitoes, and the percentage mortality was calculated in the replicates for each house and recorded according to the WHO protocol. When control mortality between 5 and 20% was observed, mortality was corrected using the Abbott formula (Abbott 1925).

[3] Complete SOPs are available at https://pmivectorlink.org/resources/tools-and-innovations/

2.3 VECTOR SUSCEPTIBILITY TESTING

From August to October 2021, unfed (not blood fed) adult *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, Morrumbala, and Lugela) and Nampula Province (Nampula district, Erati, and Mogovolas). Immature *An. gambiae* s.l. malaria vectors were also collected from different larval habitats in Zambezia (Maganja da Costa, Milange, Molumbo, Mopeia, Morrumbala, and Lugela districts) and Nampula (Nampula district, Erati, and Mogovolas) from January to April 2022, and reared to adults for susceptibility tests. Both species have different collection times because they are usually abundant at different seasons of the year. *An. gambiae* s.l., larvae are abundant during the raining season (January to April), but *An. funestus* s.l. larvae are difficult to find during this period. However, in the dry season (May to October), there is abundance of *An. funestus* s.l adults. 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 for 2021 IRS and to be used for 2022 IRS (pirimiphos-methyl and bendiocarb) and in nets (permethrin and alpha-cypermethrin) distributed were during the last mass distribution were prioritized for susceptibility tests in each of the 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 into holding tubes and paper cups. Mortality was recorded at 24 hours after exposure and followed for up to three days for chlorfenapyr and seven days for clothianidin. Where control mortality scored higher than 5% but below 20%, Abbott's formula 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 (2016) criteria 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 *An. gambiae* s.l. 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 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 1,983 mosquitoes from Zambezia Province and 638 from Nampula Province were morphologically identified as *An. funestus* s.l., *An. gambiae* s.l., *An. maculipalpis, An. coustani, An. tenebrosus,* and *An. rufipes* were sent to the INS laboratory to be analyzed by PCR for species identification, *kdr* and *Ace-1* resistant genes.

3. RESULTS: ZAMBEZIA

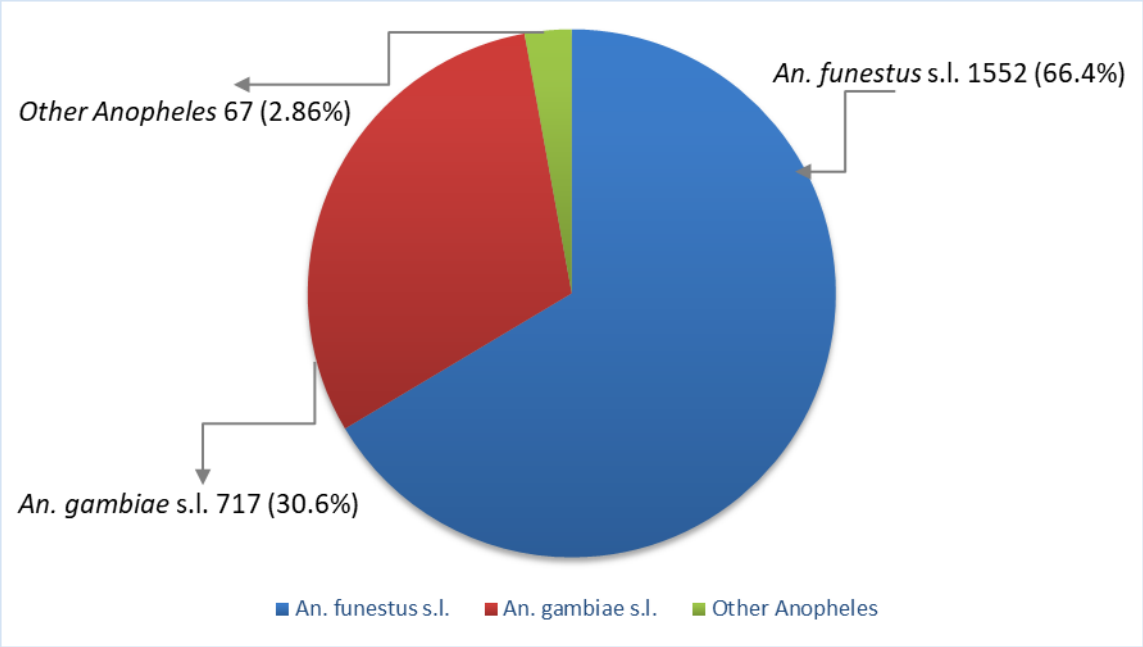
3.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT METHODS

Using the three collection methods (Prokopack, CDC light traps, and pit shelters), 2,336 anopheline mosquitoes belonging to seven different species and species complexes were collected in the three intervention districts and one control district of Zambezia Province. The anophelines included *An. funestus* s.l., *An. gambiae* s.l., *An. coustani*, *An. pretoriensis*, *An. tenebrosus*, *An. maculipalpis,* and *An. rufipes*. Table 1 and Figure 2 summarize the number of mosquitoes collected, by species and district. *An. funestus* s.l. was the most abundant anopheline collected, accounting for 66.4% of all collections, followed by *An. gambiae* s.l. at 30.6% and other anophelines at 2.9%.

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

Species collected	Milange	Molumbo	Mopeia	Lugela	Total per Species
An. funestus s.l.	476	54	695	327	1552
An. gambiae s.l.	262	162	134	159	717
An. coustani	0	3	2	2	7
An. maculipalpis	7	0	0	5	12
An. tenebrosus	16	1	21	0	38
An. rufipes	9		0	0	9
An. pretoriensis	0	0	1	0	1
Total	770	220	853	493	2336

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



3.1.1 PROKOPACK COLLECTIONS

Prokopack aspirators collected 312 *Anopheles* mosquitos (Table 2). Based on morphological identification, 257 of these belonged to *An. funestus* s.l. (82.37%) and 55 to *An. gambiae* s.l. (17.62%) (Table 2). No other species was collected using Prokopacks.

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

Mosquito Species /District	Milange	Molumbo	Mopeia	Lugela	Total
An. funestus s.l.	38	13	127	79	257
An. gambiae s.l.	9	8	8	30	55
Total	47	21	135	109	312

The indoor resting density of *An. funestus* s.l. estimated from Prokopack collections was relatively low in all intervention districts, especially one month after IRS. The highest density of *An. funestus* s.l. was observed before spray, in August 2021, at 5.3 mosquitoes per room per day in Mopeia district, the densities dropped immediately after IRS (from December to February), from 5.3 to 0 mosquitoes per room per day (Figure 3A). In Milange district, the densities of *An. funestus* s.l. were low before IRS and decreased to zero one month after IRS; they then increased to between 1.1 and 1.5 mosquitoes per room per day from February to April, two months after IRS (Figure 3A). The pattern of *An. gambiae* s.l. resting densities was different to that of *An. funestus* s.l., with the highest density observed three months after spray (in April 2022) in Lugela (control district), at 3.0 mosquito per room per day (Figure 3B). The density was 0 mosquitoes per room per day for all collection months up to February 2022.

In Lugela, the indoor resting density of *An. funestus* s.l. was about 2.4 mosquitoes per room per day before IRS. Although Lugela was not sprayed, after the IRS period (in November) indoor resting density there dropped over the months, for around seven months (from December up to June, it dropped continuously and slightly) to 0.6 mosquitos per room per day in June.

The *An. gambiae* s.l. indoor resting density pattern fluctuated but remained below 1.0 mosquito per room per day in the intervention districts throughout the monitoring season. In all intervention districts, the density of *An. gambiae* s.l. was close to zero from August to December 2021 and then increased slightly from February to April 2022 in Milange and Molumbo (Figure 3B). In Lugela, *An. gambiae* s.l. densities were zero from August to February and then increased to 3.0 mosquitoes per room per day in April.

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.

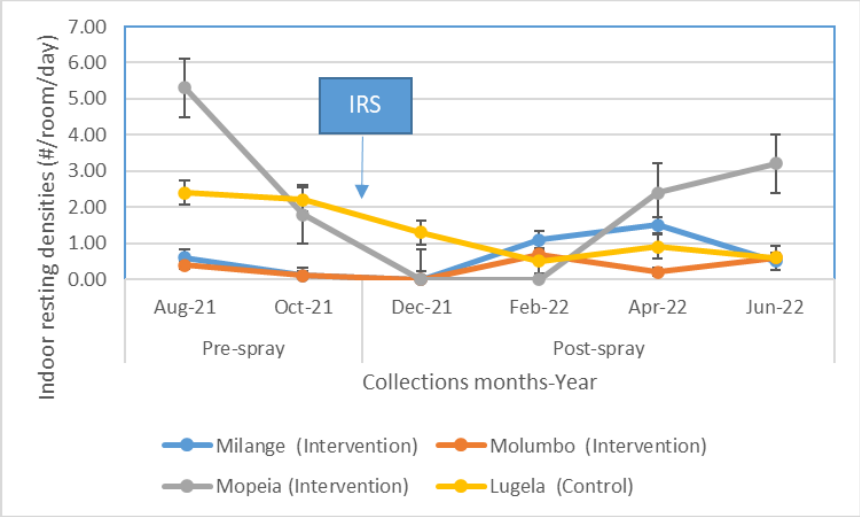
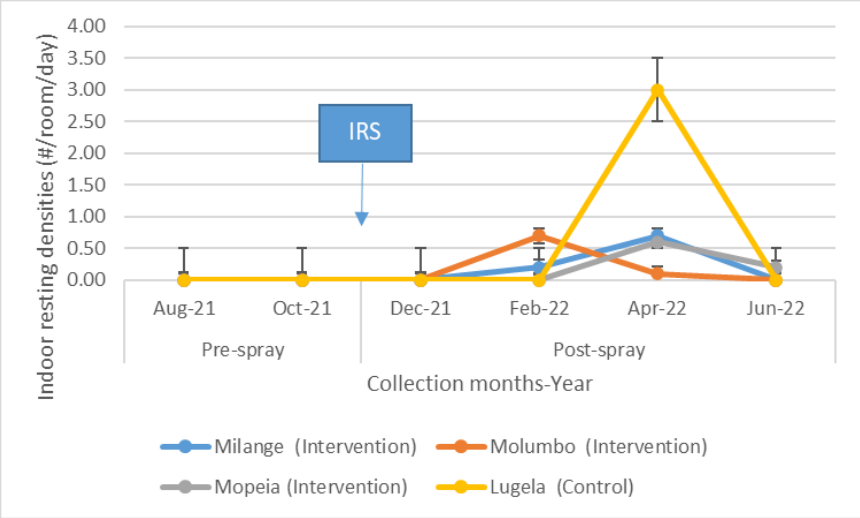


Figure 3B. *An. gambiae* s.l.



3.1.2 CDC LIGHT TRAP COLLECTIONS

The CDC light trap collections yielded a total of 1,947 *Anopheles* mosquitoes from the intervention districts (Milange, Molumbo, and Mopeia) and the control district (Lugela). Morphological identification showed that 1,228 (63.07%) were *An. funestus* s.l., 654 (33.59%) *An. gambiae* s.l., 7 (0.35%) *An. coustani*, 12 (0.61%) *An. maculipalpis*, 36 (1.84%) *An. tenebrosus,* 1 (0.05%) *An. pretoriensis*, and 9 (0.46%) *An. rufipes*. The highest proportion of the total collected *An. funestus* s.l. (the predominant species in most areas) was from Mopeia (42.92%), followed by Milange (34.77%), Lugela control site (19.29%), and Molumbo (3.01%) districts (Table 3). In previous years, the highest number of *An. funestus* s.l. have been collected in either Mopeia district or Lugela district. In 2020-2021 and 2019-2020 collection periods, the highest number of *An. funestus* s.l were collected in Lugela district. In the 2018-2019 and 2017-2018 collection periods, the highest number of *An. funestus* s.l were collected in Mopeia district. Table A1.2 in annex A has details on the proportion of the species by district and year.

TABLE 3. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN ZAMBEZIA PROVINCE USING CDC LIGHT TRAPS

Species collected	Milange	Molumbo	Mopeia	Lugela	Total Collected
An. funestus s.l.	427	37	527	237	1228(63.07)
An. gambiae s.l.	253	152	125	124	654(33.59)
An. coustani	0	3	2	2	7(0.35)
An. maculipalpis	7	0	0	5	12(0.61)
An. tenebrosus	16	1	19	0	36(1.84)
An. pretoriensis	0	0	1	0	1(0.05)
An. rufipes	9	0	0	0	9(0.46)
Total/district	712(36.56)	193(9.91)	674(34.61)	368(18.90)	1947

Note: Numbers in brackets are percentages.

Table A1.1 in the annex shows that, in terms of mean collections of mosquitoes per trap per night (m/t/n), *An. funestus* s.l., at 7.32 m/t/n over the six collection months, was most abundant in Mopeia, followed by 5.63 m/t/n in Lugela. This trend of high densities in the intervention districts rather than the control is not a surprise because historically, Mopeia has yielded more *An. funestus* s.l. than the other districts, sometimes including the control district.

Figure 4A shows that before IRS (in August 2021), *An. funestus* s.l. indoor trap densities in Lugela and Mopeia were around 4.08 m/t/n and 5.17 m/t/n, respectively, and less than 1.0 in Molumbo. The densities were less than 1.0 after IRS in Molumbo and Mopeia. In Milange, *An. funestus* s.l. densities were 4.08 in August and dropped to zero by October but started increasing in December: they went from 9.33 m/t/n in February to 13.83 m/t/n in April, which was the highest biting rate. This increase in biting rate of *An. funestus* s.l. was not expected in the rainy season because, in these areas and during this season, *An. gambiae* s.l. is usually in abundance. Collecting more *An. funestus* s.l. at this time was unexpected. This has not been the trend in the past and the reason for the increase in *An. funestus* s.l. is not clear at the moment.

Figure 4B shows that *An. gambiae* s.l. indoor density was low (less than 1 m/t/n) before IRS. This is expected due to the seasonality of the species where the densities are low during the dry season. One month after spray, in December, the densities remained low in all intervention districts, but they started increasing in February and peaked in April at 11.0, 5.75, 2.83, and 3.33 m/t/n in Milange, Molumbo, Mopeia, and Lugela, respectively.

In Milange the increases in the number of *An. gambiae* s.l., were relatively more significant. Milange is an inland district but heavy rains that occurred probably created favorable breeding habitats that caused a sharp increase in mosquito populations, mainly for *An. gambiae* s.l., as observed during the collection period after the heavy rains.

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

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

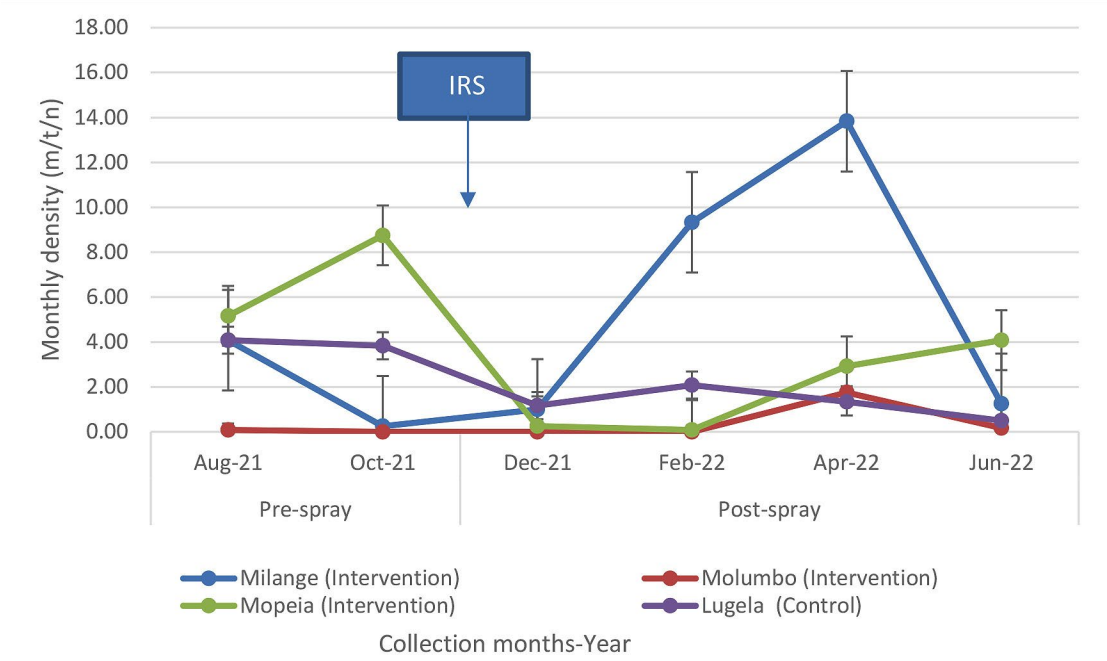
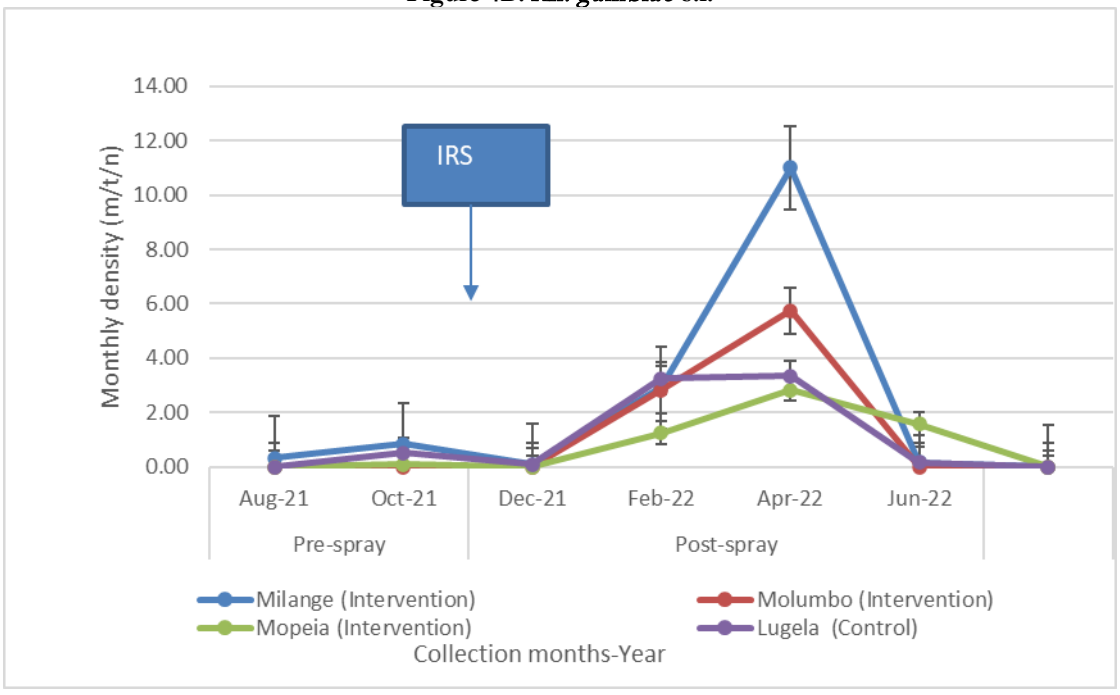


Figure 4B. *An. gambiae* s.l.



Outdoor CDC light trap density of *An. funestus* s.l. per trap per night showed varying densities in the four districts (Figure 5A). In Milange and Lugela, densities never exceeded 2.5 m/t/n; in Molumbo, densities were even lower, from 0.00 in August through February, and 0.08 m/t/n in April. Mopeia had the highest pre-spray density, 8.0 m/t/n, in October 2021, and its densities sharply decreased after IRS, but started to increase in April to a peak in June of 4.92 m/t/n. In February, outdoor densities of *An. funestus* s.l. was low in two intervention districts (Mopeia and Molumbo), while in Lugela (control), density remained higher at 1.25 m/t/n.

The outdoor densities of *An. gambiae* s.l. collected by CDC light trap, plotted in Figure 5B, show that in the pre-spray season the densities were low including in the control district. In December, after the spray campaign, the densities remained close to zero. From February to April, the outdoor density of *An. gambiae* s.l. peaked, reaching 1.92, 2.42, 2.75, and 3.25 m/t/n in Lugela, Molumbo, Mopeia, and Milange, respectively. The increase of *An. gambiae* s.l. in February and April could be attributed to an increase in breeding sites at the start of the rainy season, which lasted until June.

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

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

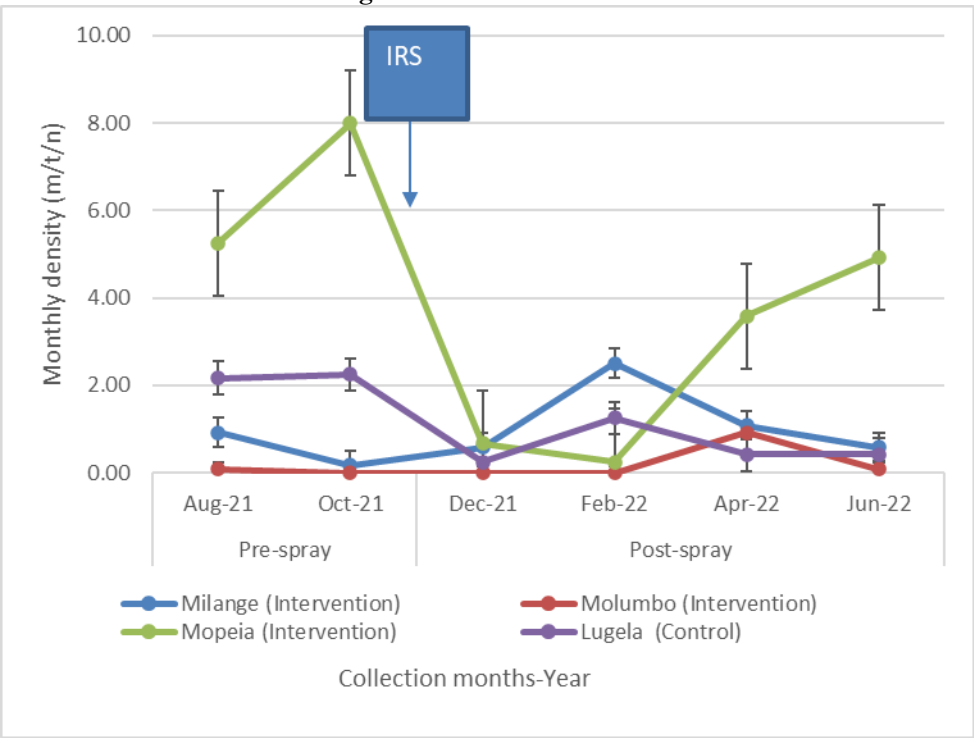
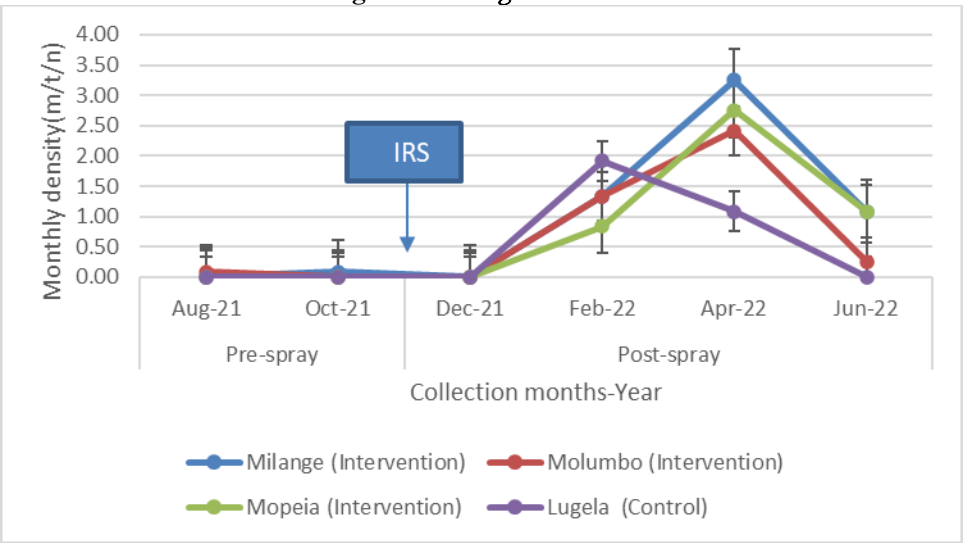


Figure 5B. *An. gambiae* s.l.



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 Mopeia, from 6.96 to 1.83 bites per person per night (b/p/n). In Milange, the density increased from 2.17 to 6.35 b/p/n. There also was a rise in Molumbo, albeit a more subtle one, from 0.04 to 0.48 b/p/n. As in Mopeia (intervention district), the mean indoor biting rate in Lugela (control) decreased, from 3.96 b/p/n before spray to 1.27 b/p/n after. Mean outdoor biting rates increased in Milange and Molumbo and decreased in Mopeia and Lugela (6.63 to 2.35 b/p/n and 2.21 to 0.58 b/p/n, respectively). For *An. gambiae* s.l., indoor and outdoor mean biting rates for all districts increased in the post-spray season relative to the pre-spray season. Again, this is because the post-spray period usually is followed by rain, which creates breeding sites for *An. gambiae* s.l. and consequently increases the population of *An. gambiae* s.l.

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
Milange	2.17	6.35	0.54	1.19	0.58	3.54	0.04	1.42
Molumbo	0.04	0.48	0.04	0.25	0.00	2.15	0.04	1.00
Mopeia	6.96	1.83	6.63	2.35	0.04	1.42	0.00	1.17
Lugela*	3.96	1.27	2.21	0.58	0.25	1.71	0.00	0.75

*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 and Molumbo. However, a significant difference was observed in Milange and Lugela ($p<0.05$), 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 Milange, Molumbo, and Lugela (control), showing an endophagic tendency.

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

District	*An. funestus* s.l.		X^2	*p-value*	*An. gambiae* s.l.		X^2	*p-value*
	# Collected indoors	# Collected outdoors			# Collected indoors	# Collected outdoors		
Milange	357	70	192.9	$7.4x10^{-44}$*	184	69	52.29	$4.83x10^{-13}$*
Molumbo	24	13	3.27	0.070	103	49	19.18	$1.19x10^{-5}$*
Mopeia	255	272	0.55	0.458	69	56	1.35	0.244
Lugela	156	81	23.73	$1.11x10^{-6}$*	88	36	21.81	$3.02x10^{-6}$*

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

The indoor and outdoor overnight biting rates of *An. funestus* s.l. are depicted in Figures 6A and 6B, respectively. Indoor and outdoor biting activity appeared to have different trends in each district. In Milange, indoor biting activity remained consistently above 0.58 bites per person per hour (b/p/h) for most of the night, starting at 6–7 pm and lasting until 5–6 am, with peak biting activity of 4.17 b/p/h observed early in the night, at 9–10 pm.

Mopeia had the highest outdoor biting activity in the early part of the night (from 6 pm up to 9 pm), and the highest peak biting rate, 3.50 b/p/h, was observed early in the night as well, around 10–11 pm, when most community members were going to bed.

In Lugela, the highest outdoor biting rate was 1.42 b/p/h, at 1–2 am. In Milange and Molumbo, outdoor biting followed a similar trend, with the significant biting activities occurring in early hours of the night, at 8–9 pm, and in the early morning at 2–3 am. Biting levels, however, were different: in Milange the peak was 1.0 b/p/n, while Molumbo had the lowest average biting rate and a peak of only 0.25 b/p/n at 8–9 pm, in the early night. The general observation here is that *An. funestus* s.l. appeared to feed actively at different times during the night, both indoors and outdoors. Comparing the biting rate observed in 2020- 2021 with the one from 2021-2022, in Milange, Mopeia and Lugela districts in general the biting rate was low in 2021-2022.

FIGURE 6. HOURLY BITING RATE OF *AN. FUNESTUS* S.L. CAUGHT BY CDC LIGHT TRAPS IN FOUR DISTRICTS OF ZAMBEZIA PROVINCE

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

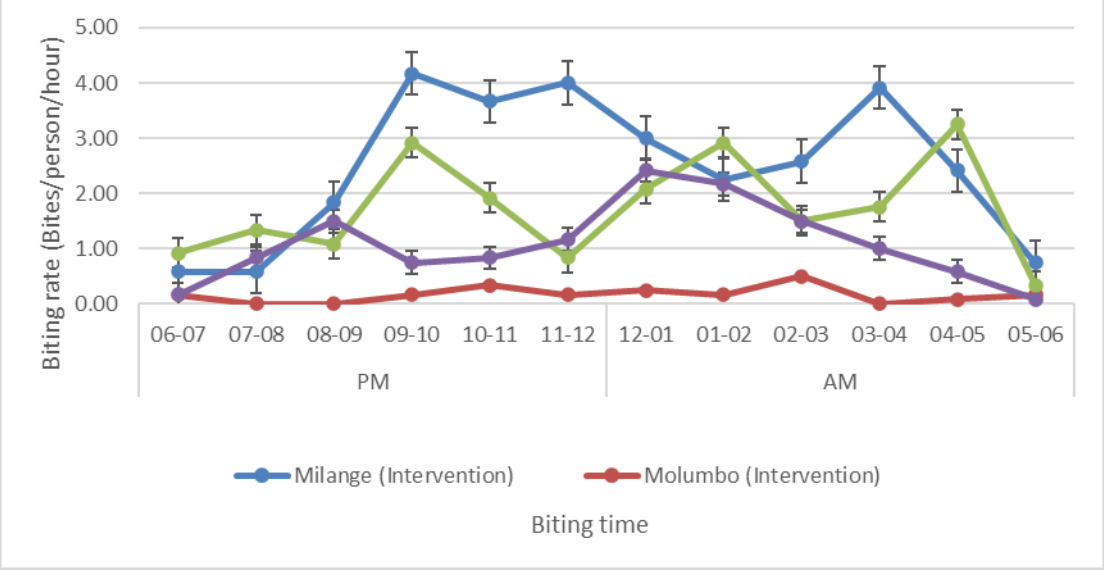
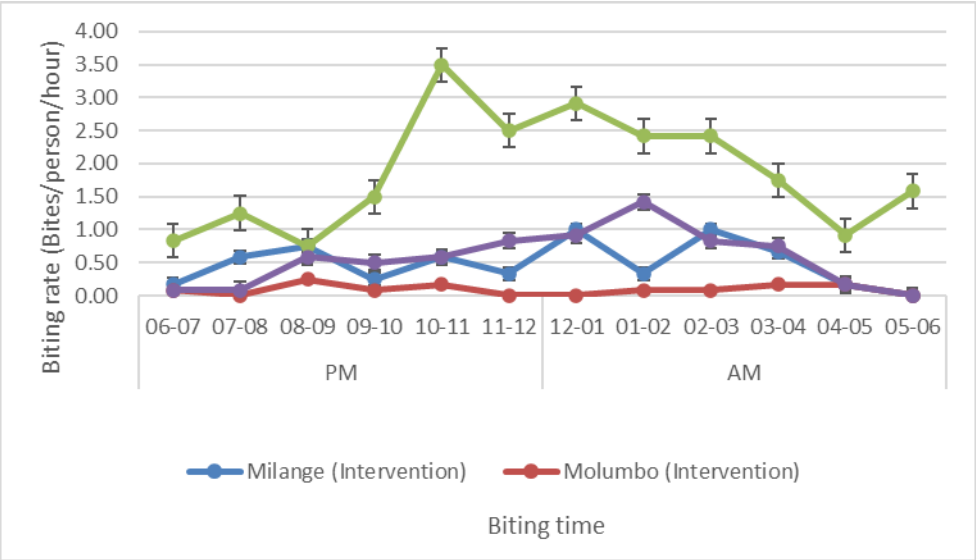


Figure 6B. *An. funestus* s.l. Outdoor



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 2.42 b/p/h from 6 pm to 6 am in all four districts. In Milange, the peak biting activity, 2.42 b/p/h, was observed at 10–11 pm indoors and at 1.58 b/p/h at 2–3 am outdoors. This indicates that *An. gambiae* s.l. bites while people are sleeping in the early night and early morning. In Lugela (control), the indoor biting rate was fairly uniform, at least from 8–9 pm to 3–4 am (varying from 0.5 to 1 bites/person/hour).

An. gambiae s.l. outdoor biting shows different trends from indoor biting in each district. Milange displayed the highest outdoor biting activity, followed by Molumbo, then Mopeia, and finally Lugela (control). In Lugela, the peaks were bimodal for outdoor biting, at 0.58 b/p/h at both 10–11 pm and 2–3 am. In Milange, Molumbo, and Mopeia, peak biting was 1.58 b/p/h at 2–3am, 0.67 b/p/h at 8–9pm and 2–3 am, and 0.58 b/p/h at 10–11pm and 3–4 am, respectively.

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

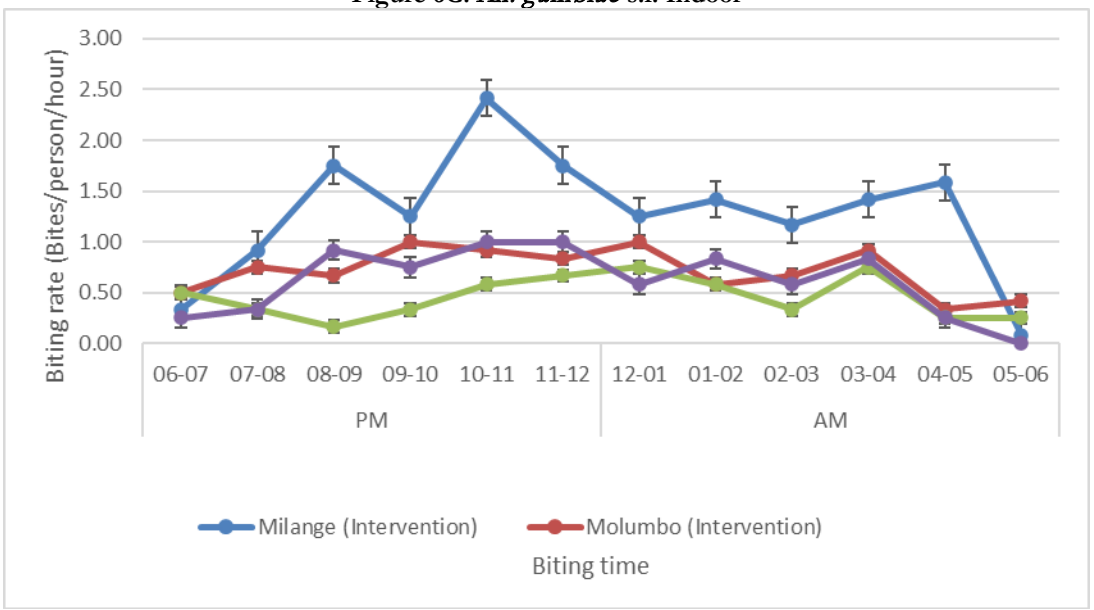
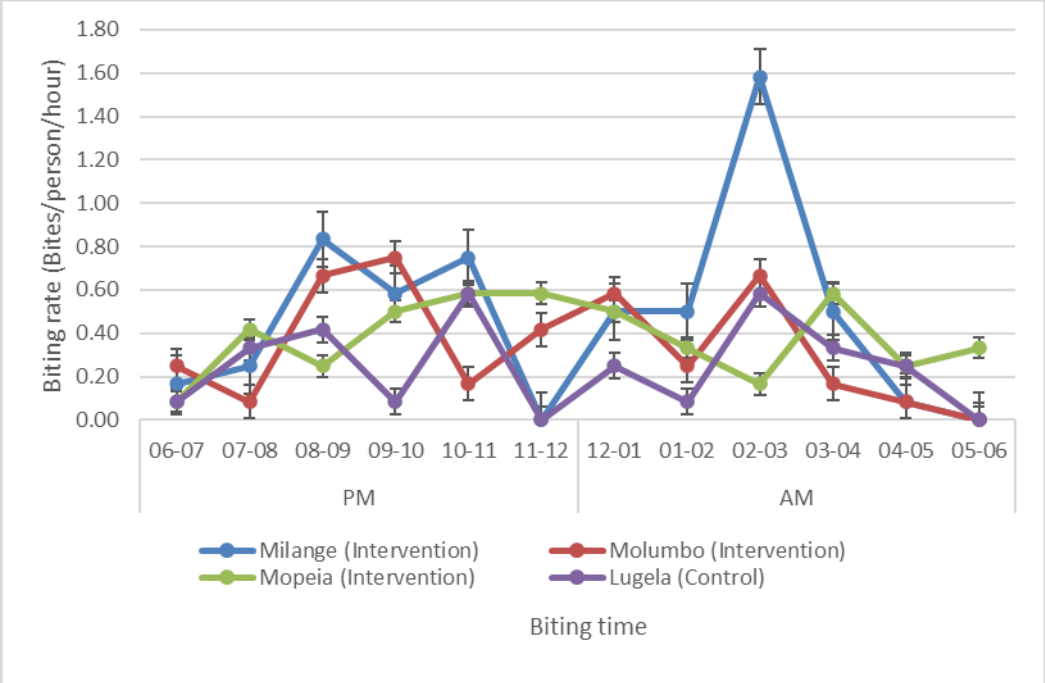


Figure 6D. *An. gambiae* s.l. Outdoor



3.1.4 PIT SHELTERS COLLECTION

Pit shelter collections yielded 77 mosquitoes over the collection period, a very low number. Of the 77 collected, 67 (87.01%) were *An. funestus* s.l., 8 (10.38%) *An. gambiae* s.l., and 2 (2.5%) were *An. tenebrosus* (Table 6).

TABLE 6. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN ZAMBEZIA PROVINCE USING PIT SHELTERS

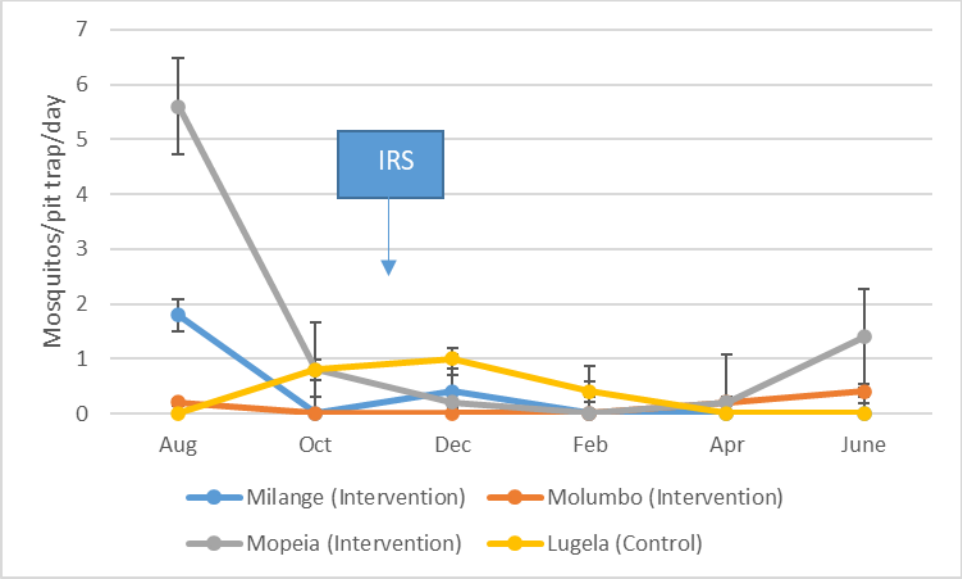
Mosquito Species /District	Milange	Molumbo	Mopeia	Lugela	Total
An. funestus s.l.	11	4	41	11	67 (87.01)*
An. gambiae s.l.	0	2	1	5	8 (0.38)
An. tenebrosus	0	0	2	0	2 (2.59)
Total	11	6	44	16	77

* Numbers in brackets are in percentage

Figures 7A and B present the outdoor densities of *An. funestus* s.l. and *An. gambiae* s.l. collected. Figure 7A shows that in Lugela and Molumbo, the outdoors resting densities of *An. funestus* s.l. were 0 and 0.2 mosquitos/pit trap/day, respectively, in August, before IRS. Densities were higher in Mopeia and Milange but all were below 2.0 mosquitoes/pit trap/day, except in Mopeia in August, when 5.6 mosquitoes/pit trap day were collected in August. In October 2021, the densities from all intervention sites decreased and continued to do so after IRS. In contrast, in Lugela, the densities increased from October to December, and then, from December to June, they decreased to zero. In Molumbo and Mopeia, the densities increased in June 2022.

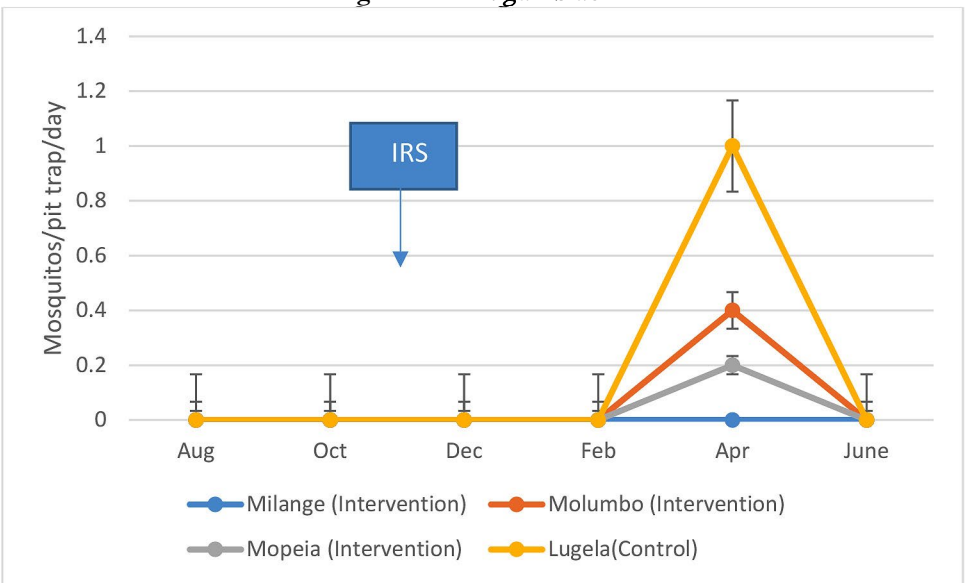
FIGURE 7. PIT SHELTER TRAP, DENSITY PER TRAP PER DAY IN MILANGE, MOLUMBO, MOPEIA, AND LUGELA DISTRICTS

Figure 7A. *An. funestus* s.l.



The *An. gambiae* s.l. outdoor densities presented in Figure 7B shows that this species was not collected in Molumbo, Mopeia, and Lugela until February 2022. The highest densities were obtained in April: in Lugela with 1.0 mosquito/pit trap/day, followed by Molumbo, with 0.4 mosquitoes/ pit trap/day and Mopeia with 0.2 mosquitoes/pit trap/day. In Milange, *An. gambiae* s.l. was not collected at all.

Figure 7B. *An. gambiae* s.l.



3.1.5 MOLECULAR ANALYSIS

In Zambezia a total of 1,983 mosquitos morphologically identified as *An. funestus* s.l., *An. gambiae* s.l., *An. maculipalpis, An. coustani, An. tenebrosus,* and *An. rufipes* were sent to the INS laboratory to be analyzed by PCR. Because of the earlier issues with the PCR reaction and using reagents from different sources that require

different protocols and standardization, the results were not received until July 2022 and, as a result, the samples could not be analyzed in time for the results to be included in this report. Results are expected when INS can start conducting genotyping, which was identified as the next step for sampling processing after technical assistance from CDC in August 2022. If the results are ready before this report is finalized, the report will be updated with the results; otherwise, an addendum will be submitted.

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 and decay rate monitoring of bendiocarb in Mopeia and Actellic 300 CS in Milange and Molumbo are shown in Figures 8 and 9.

3.2.1 QUALITY OF SPRAYING

For bendiocarb, mortality was scored at 100% in all houses tested with cone wall bioassays one day (24 hours) after spraying (T_0), (Figure 8).

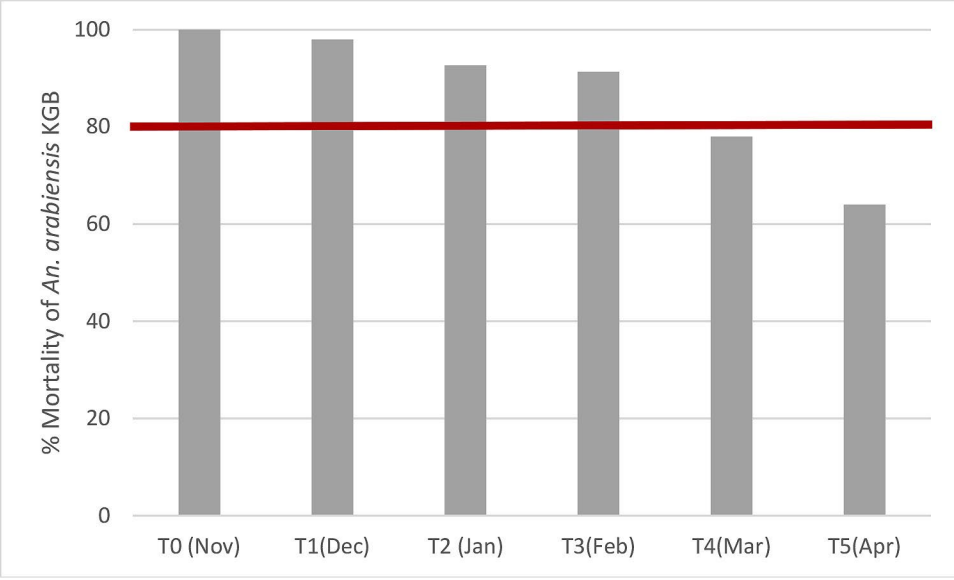
For Actellic 300 CS, mortality was scored at 99% one day after spraying in Milange and at 100% in Molumbo.

3.2.2 INSECTICIDE DECAY RATE

BENDIOCARB (FICAM) DECAY RATE

Wall bioassays for assessing bendiocarb (Ficam) spray quality and subsequent monitoring of the insecticide's decay rate was conducted in Josina Machel village in Mopeia. The first bioassay (T0) was conducted in November 2021 and elicited 100% mortality by day 1 (24hrs after spray) (Figure 8). Subsequent monthly cone bioassays resulted in 98% mortality. It was also noted that scores of 100% were observed only at T0 (24hrs after spray) and the residual bioefficacy of this product remained up to three months after IRS. Ficam demonstrated short bioefficacy in Mopeia, reaching the cut-off point in T4 (March) after spray with 78% mortality of *An. arabiensis* KGB susceptible strain. The monitoring of residual efficacy was stopped in T5 (April), at 64% mortality.

FIGURE 8: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF BENDIOCARB (FICAM) IN MOPEIA



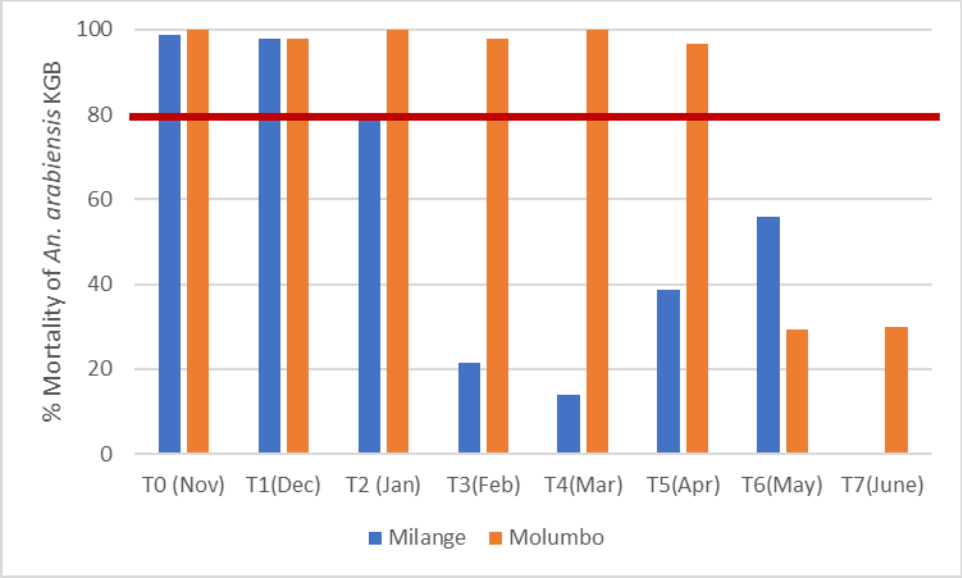
Red horizontal line indicates the 80% mortality cut-off point.

ACTELLIC 300 CS DECAY RATE

Cone wall bioassays for assessing Actellic 300 CS IRS quality assurance and subsequent monitoring of its decay rate were conducted in Milange and Molumbo (Figure 9).

Cone bioassays data showed low residual efficacy of Actellic 300 CS in Milange unlike in other years (2016/17 and 2017/18) when the product remained active for close to six months after spray. In Milange, the insecticide remained active for only one month after spray; mortality dropped to 98% in December. In February (T3), the efficacy of Actellic was below 80% (cut-off point) for two consecutive months. Because these results were so different from other years, follow-up tests were conducted until May (T6). Follow-up results were the same, with mortality of 56% observed at T6, confirming a low residual efficacy for Actellic 300 CS in Milange. In Molumbo, the residual efficacy lasted longer, up to April (T5); the cut-off point was observed in T6 (Figure 9).

FIGURE 9: SPRAY QUALITY ASSESSMENT AND RESIDUAL BIOEFFICACY OF ACTELLIC 300 CS IN MILANGE AND MOLUMBO



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

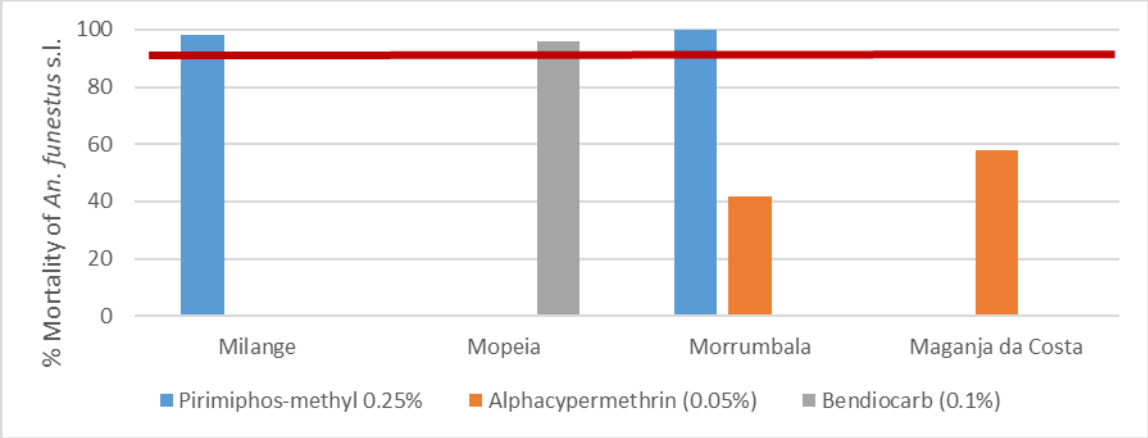
3.3 INSECTICIDE SUSCEPTIBILITY TESTS

Susceptibility tests against *An. funestus* s.l. and *An. gambiae* s.l. were conducted in Milange, Molumbo, Mopeia, Morrumbala and Lugela districts. *An. funestus* s.l. was exposed to pirimiphos-methyl (0.25%), alpha-cypermethrin (0.05%), and bendiocarb (0.1%) in Milange, Morrumbala, Maganja da Costa, and Mopeia districts. Also, *An. gambiae* s.l. was exposed to diagnostic dosages of permethrin (0.75%), pirimiphos-methyl (0.25%), clothianidin (2%), chlorfenapyr (100 µg/bottle), and bendiocarb (0.1%). In addition, 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 is planned to be used in the upcoming IRS campaign as well as the insecticide used on ITNs already distributed in each of the intervention and the control districts.

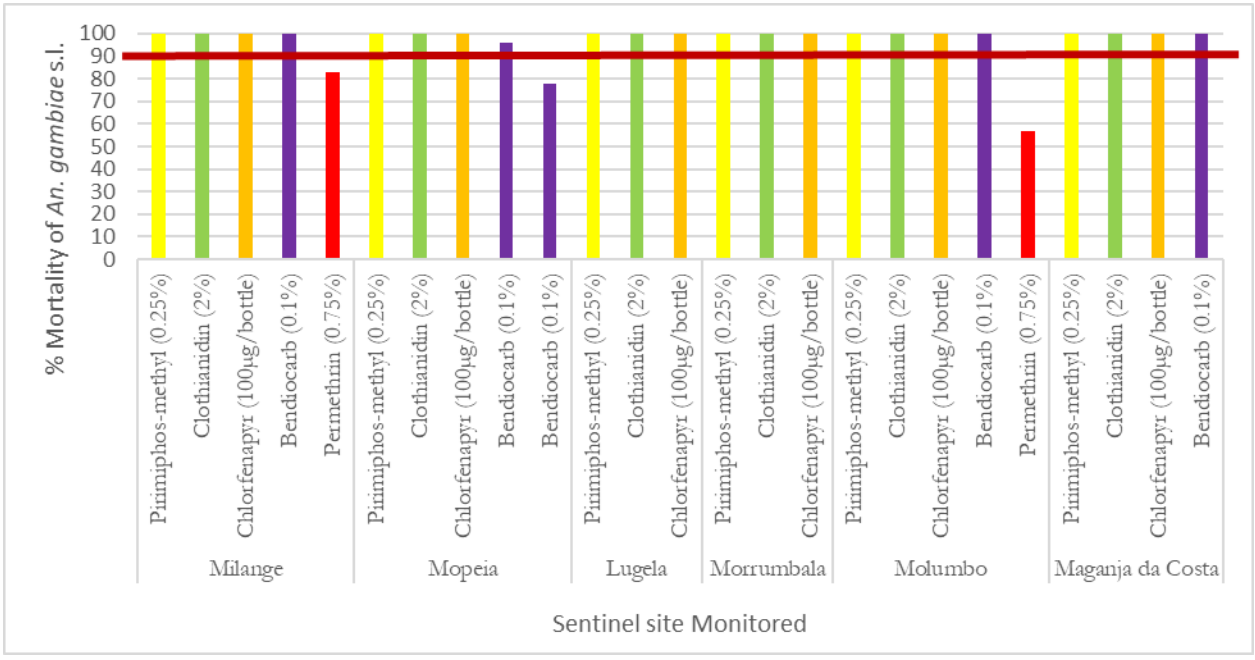
The mortality results presented in Figure 10 show that *An. funestus* s.l. was fully susceptible to pirimiphos-methyl 0.25% in Milange and Morrumbala, resistant to alpha-cypermethrin (0.05%) in Morrumbala and Maganja da Costa, and possible resistant to bendiocarb (0.1%) in Mopeia. Few *An. funestus* s.l. were collected and no other insecticide was tested on this population.

FIGURE 10. PERCENTAGE MORTALITY OF ADULT *AN. FUNESTUS* S.L. COLLECTED BY PROKOPACK FROM THE FIELD EXPOSED TO PIRIMIPHOS-METHYL, ALPHA-CYPERMETHRIN, AND BENDIOCARB



Susceptibility tests was conducted with *An. gambiae* s.l. samples collected from larvae and reared to adult. Results of the test presented in Figure 11 show that this species was fully susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr in all districts where the insecticides were tested. *An. gambiae* s.l. tested against bendiocarb in Milange, Molumbo, and Maganja da Costa also showed susceptibility. Resistance to permethrin (0.75%) was observed in Milange and Molumbo where the tests were conducted.[4] Resistance to bendiocarb (0.1%) was detected in Mopeia only. Clothianidin tests using Mero solvent was not tested in all districts because the insecticide and the Mero was received almost after the larval collection period. This protocol will be used in the upcoming testing season.

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.

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

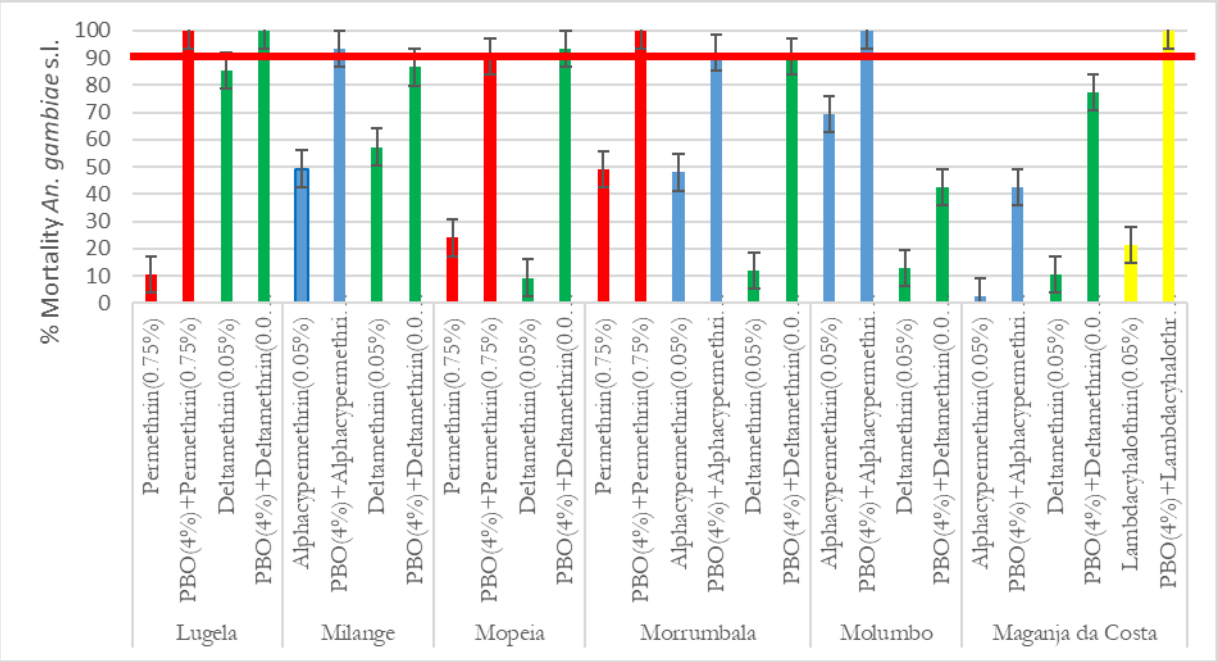
Note: 1) clothianidin and chlorfenapyr holding periods were up to three and seven days, respectively. The mortality for other insecticides was observed at a 24-hour holding period. 2) In Mopeia there was possible resistance to bendiocarb on the first test. This was repeated and confirmed resistance at the second round of test (second bar)

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 Lugela, Milange, Mopeia, Morrumbala, Molumbo, and Maganja da Costa. In Lugela, the synergist restored susceptibility to permethrin and deltamethrin to 100%. In Milange, susceptibility to alpha-cypermethrin and deltamethrin was partially restored to 93.3% and 86.6%, respectively. In Mopeia, susceptibility was partially restored to 90.6% for permethrin and to 93.3% for deltamethrin. In Morrumbala, susceptibility was fully restored (to 100%) for permethrin; it was partially restored to 92% for alpha-cypermethrin and 90.6% for deltamethrin. In Molumbo, susceptibility was fully restored (100%) to alpha-cypermethrin and partially restored (42.6%) to deltamethrin. In Maganja da Costa, PBO fully restored the susceptibility of lambda-cyhalothrin and partially restored it for alpha-cypermethrin (42.6%) and deltamethrin (77.3%).

In some districts, there was an indication of full involvement and partial involvement of monooxygenases as the mechanism of resistance on the range of the pyrethroids tested. 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 FIVE INTERVENTION DISTRICTS AND THE CONTROL LUGELA



4. RESULTS: NAMPULA PROVINCE

4.1 ANOPHELINE SPECIES COLLECTED BY THE DIFFERENT METHODS

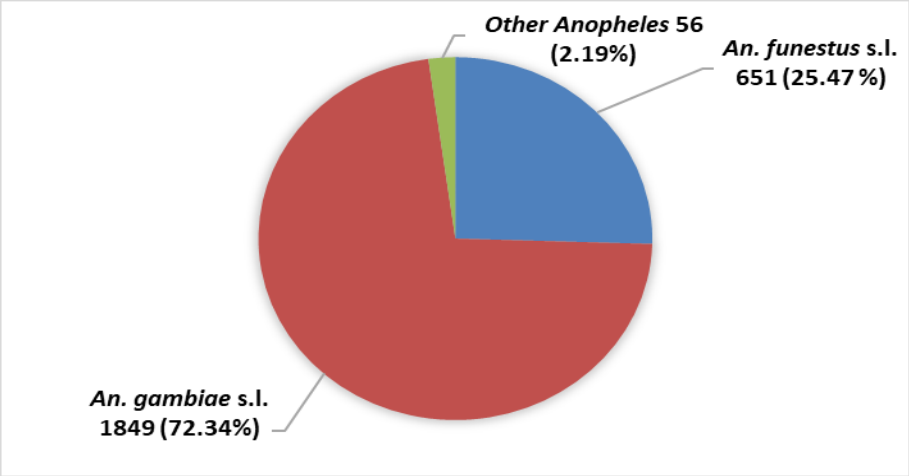
Using the three collection methods (Prokopack, CDC light traps indoors and outdoors, and pit shelters), 2,556 anopheline mosquitoes belonging to six different species and species complexes were collected: *An. funestus* s.l., *An. gambiae* s.l., *An. pretoriensis*, *An. rufipes*, *An. tenebrosus*, and *An. maculipalpis*. Table 7 and Figure 13 lays out the number of mosquitoes collected by species and by district. *An. gambiae* s.l. was the most abundant anopheline across all sites, accounting for 72.34% of all collections, followed by *An. funestus* s.l. at 25.47%, and the other anophelines at 2.19%. Compared with the previous years, the number of mosquitoes collected was high in 2020 – 2021 when collections were monthly and low in 2021 – 2022 when collections were bi-monthly.

Anopheles gambiae s.l. was the predominant species collected across all four years. In 2020-2021 and 2018-2019 collection periods, the highest number of *An. gambiae* s.l were collected in Erati district, and in the 2019-2020 collection period, the highest number of *An. gambiae* s.l was collected in Nampula district. Table A2.2 in annex A has details of the proportions of mosquito species by district and year.

TABLE 7. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN NAMPULA PROVINCE USING ALL COLLECTION METHODS

Species Collected	Erati	Nampula District	Mogovolas	Total per Species
An. funestus s.l.	187	318	146	**651**
An. gambiae s.l.	376	496	977	**1,849**
An. pretoriensis	4	1	6	**11**
An. rufipes	30	11	1	**42**
An. tenebrosus	0	0	2	**2**
An. maculipalpis	1	0	0	**1**
Total	**598**	**826**	**1,132**	**2,556**

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



A total of 1,849 *An. gambiae* s.l. mosquitoes were collected, 63 using Prokopack (3.41%), 16 using pit shelters (0.87%), and 1,770 using CDC light traps indoors and outdoors (95.72%). A total of 651 *An. funestus* s.l. were collected, including 59 from Prokopack aspirators (9.06%), 971 using CDC light traps indoors and outdoors (90.17%), and 5 using pit shelter (0.77%) collections.

4.2 PROKOPACK COLLECTIONS

Prokopack collections yielded 123 *Anopheles* mosquitoes (Table 8). Based on morphological identification, 63 (51.22%) of these belonged to *An. gambiae* s.l., 59 (47.97%) to *An. funestus* s.l., and 1 (0.81%) to *An. pretoriensis*.

TABLE 8. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN NAMPULA PROVINCE USING PROKOPACK ASPIRATORS

Mosquito Species/District	Erati	Nampula District	Mogovolas	Total
An. funestus s.l.	13	43	3	**59**
An. gambiae s.l.	31	11	21	**63**
An. rufipes	0	1	0	**1**
Total	**44**	**55**	**24**	**123**

Over the reporting period, the *An. funestus* s.l. resting densities were consistently low (<1 mosquito/ room/day) in all districts over most months except in June 2022 in Nampula district (Figure 14A). The highest mean indoor resting density for *An. gambiae* s.l. was observed in October 2021, estimated at 0.85 mosquitoes per room per day in Mogovolas and at 0.75 mosquitoes per room per day in Erati (Figure 14B).

In Nampula district, indoor resting density increased from 0.0 *An. funestus* s.l./ room/day in August 2021 to 0.25 *An. funestus* s.l./room/day in February 2022, and then to a peak of 1.75 *An. funestus* s.l./ room/ day in June 2022 (Figure 14A). There was a decline in the indoor resting density in Erati, from 0.25 *An. funestus* s.l./room/day in August 2021 to 0.0 *An. funestus* s.l./room/day from February to April 2022 and then an increase to 0.20 *An. funestus* s.l./room/day in June 2022. Resting density in Mogovolas increased from 0.0 *An. funestus* s.l./room/day in August 2021 to 0.10 *An. funestus* s.l./room/day in October 2021. It decreased to 0.0 *An. funestus* s.l./room/day from December 2021 to April 2022 and increased to 0.05 *An. funestus* s.l./room/day in June 2022. Indoor resting density of *An. gambiae* s.l. was less than 1.0 mosquitos/room/day in the intervention and control districts throughout the reporting period. The highest densities were observed in October 2021 in Mogovolas (0.85 *An. gambiae* s.l./room/day) and Erati (0.75 *An. gambiae* s.l./room/day), and in February 2022 in Nampula district (0.50 *An. gambiae* s.l./room/day) (Figure 14B). This was after IRS with Fludora Fusion in Erati and Nampula districts.

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

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

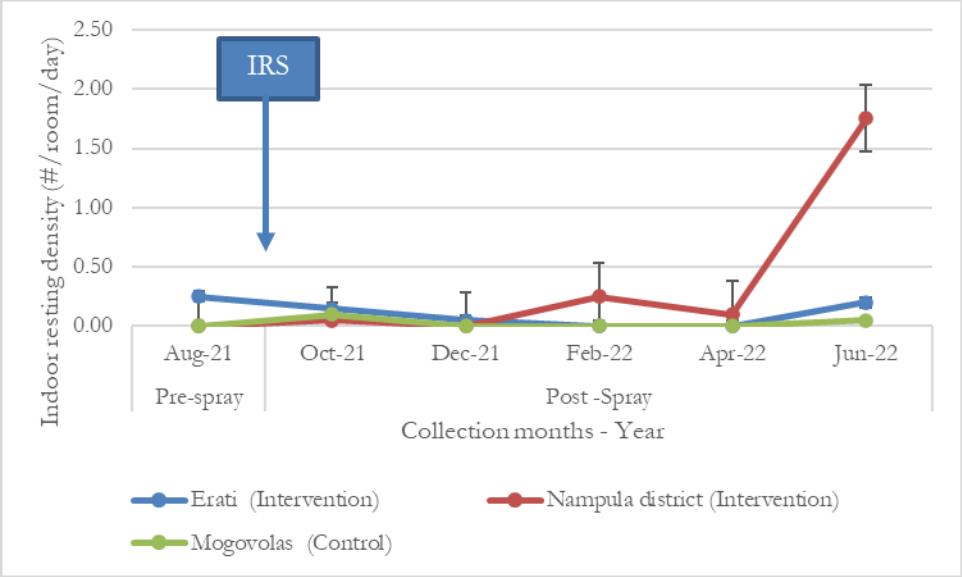
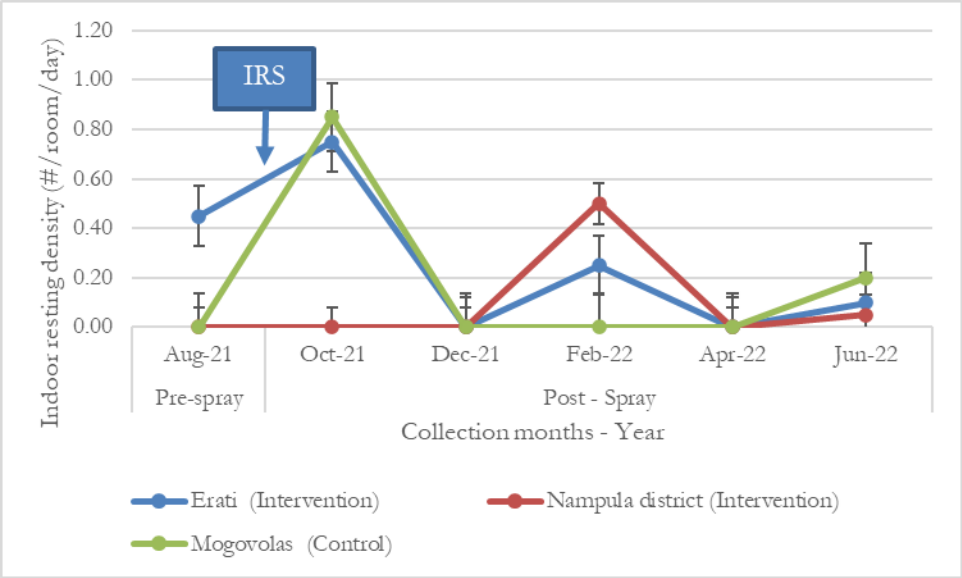


Figure 14B: *An. gambiae* s.l.



4.3 CDC LIGHT TRAP COLLECTIONS

The CDC light trap collections yielded a total of 2,395 *Anopheles* mosquitoes from two intervention districts (Nampula district and Erati) and the control district (Mogovolas). Morphological identification of the mosquitoes revealed that 587 (24.51%) were *An. funestus* s.l., 1,770 (73.90%) *An. gambiae* s.l., 25 (1.04%) *An. rufipes*, 10 (0.42%) *An. pretoriensis*, 2 (0.08%) *An. tenebrosus,* and 1 (0.04 %) *An. maculipalpis*. Mogovolas (control) district had the highest percentage of all *Anopheles* collected, 46.10%. The highest proportion of the total collected *An. gambiae* s.l. (the predominant species collected across all sites) was from Mogovolas (53.84%), followed by Nampula district (27.29%) and Erati (18.87%). Table 9 summarizes the total number of mosquitoes

collected per species per district and the respective percentages and number of mosquito species collected in each district using CDC light traps.

TABLE 9. 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
Erati	172	334	1	0	4	14	**525 (21.92)**
Nampula district	272	483	0	0	1	10	**766 (31.98)**
Mogovolas	143	953	0	2	5	1	**1,104 (46.10)**
Total collected	**587 (24.51)**	**1,770 (73.90)**	**1 (0.04)**	**2 (0.08)**	**10 (0.42)**	**25 (1.04)**	**2,395 (100)**

As shown in Annex A, Table A2.1, over the reporting period, *An. funestus* s.l. was most abundant in Nampula district (mean collection of 3.78 m/t/n), followed by Erati (mean collection of 2.39 m/t/n). *An. gambiae* s.l. was the most abundant species in Mogovolas (13.24 m/t/n) and Nampula district (6.71 m/t/n). Mogovolas had the lowest mean collection for *An. funestus* s.l. (1.99 m/t/n) and Erati for *An. gambiae* s.l. (4.64 m/t/n). The highest densities of *An. funestus* were recorded in June in Nampula district and of *An. gambiae* s.l. were recorded in October in Mogovolas district.

Figure 15A shows that before IRS, in August 2021, *An. funestus* s.l. indoor densities in Erati, Nampula district, and Mogovolas districts were 2.92 m/t./n, 1.67 m/t/n, and 2.75 m/t/n, respectively. In Erati, the densities dropped to zero in April and remained below 1.0 after IRS with a slight increase (to 2.50 m/t/n) observed in June 2022. In Mogovolas, the density dropped to zero from February to April followed by an increase to 0.95 m/t/n in June. Nampula district showed the highest densities relative to the other districts through the reporting period with densities remaining below 2.0 m/t/n until April and increased to 8.67 m/t/n in June. The highest density in Nampula district might be associated with the availability of more favorable breeding sites in the area to this species. The overall trend indicates that after IRS, densities of *An. funestus* s.l. tended to decrease until February, after about five months after IRS.

Figure 15B shows the *An. gambiae* s.l. indoor densities were 3.17 m/t/n in Mogovolas, 2.17 m/t/n in Erati, and 0.42 m/t/n in Nampula district just before IRS. Density decreased in December and increased in February in Erati and Nampula districts. In Mogovolas, it increased in April. The highest was observed in the rainy month of February in Nampula district, when it reached 17.33 m/t/n. The highest in Erati was 6.50 m/t/n in February, and in Mogovolas, it was 16.67 m/t/n in October. This increase observed in both the interventions and control districts might be attributable to the rainy season.

FIGURE 15. INDOOR LIGHT TRAP DENSITY OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN THETHREE DISTRICTS IN NAMPULA PROVINCE

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

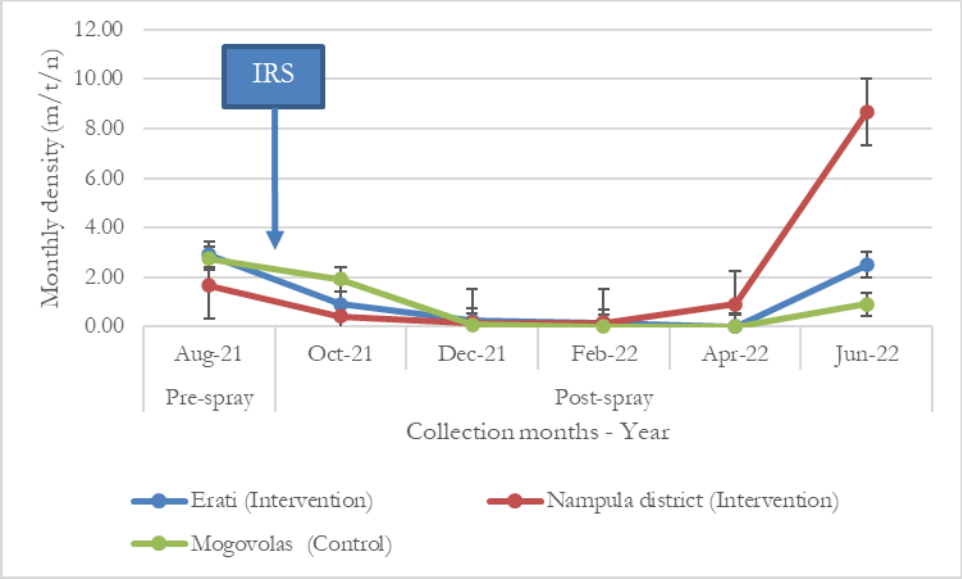
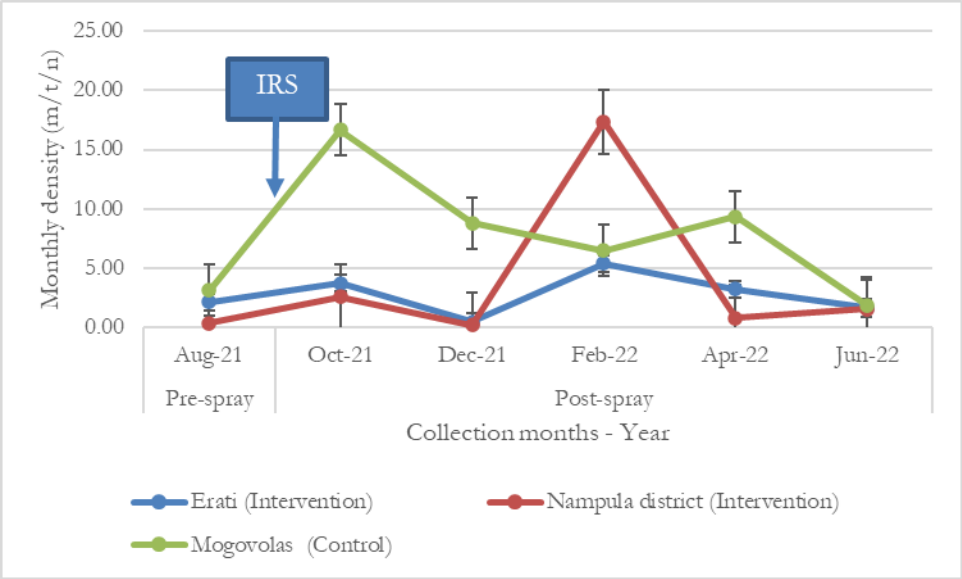


Figure 15B: *An. gambiae* s.l.



The outdoor densities of *An. funestus* s.l. in the pre-spray in Erati, Nampula district, and Mogovolas districts were 4.67 m/t./n, 1.58 m/t/n, and 2.17 m/t/n, respectively (Figure 16A). After IRS, from October 2021 to April 2022, the monthly densities of *An. funestus* s.l. were below 1.00 m/t/n in Erati and increased to 2.08 m/t/n in June 2022. In Mogovolas, the outdoor densities of *An. funestus* s.l. decreased to close to zero in December and to zero from February to April and then increased to 1.42 m/t/n in June. In Nampula district, the densities dropped to close to zero from October to December 2021 and increased to 7.08 m/t/n in June 2022. The outdoor densities of *An. gambiae* s.l. before IRS show that Erati had average densities of 1.67 m/t/n, and around zero in the other districts (Figure 16B). However, the densities of *An. gambiae* s.l. increased to 13.25 m/t/n in Mogovolas in October, and in February to 14.00 m/t/n in Nampula district and 4.58 m/t/n in Erati. The increase of *An. gambiae* s.l. in February is likely related to the rainy season.

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

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

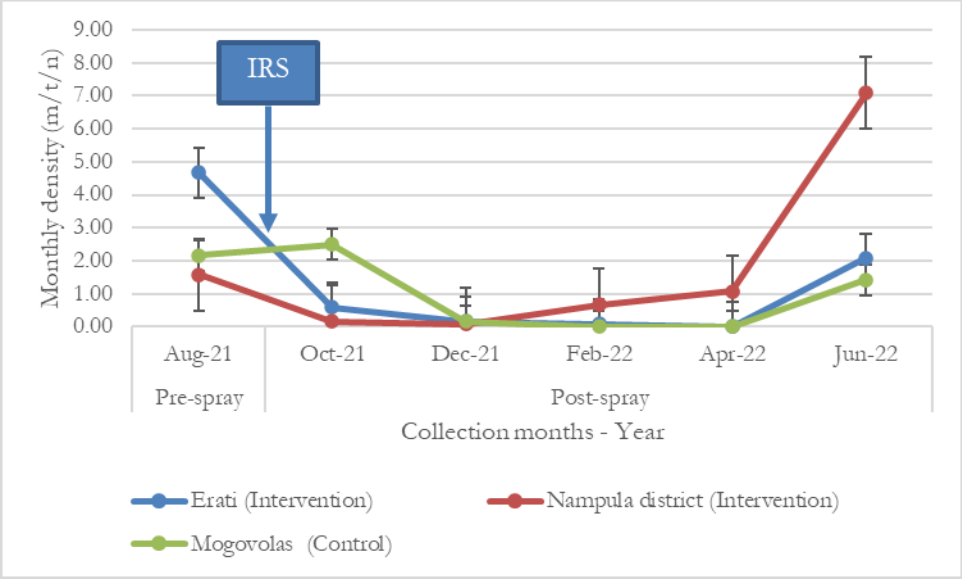
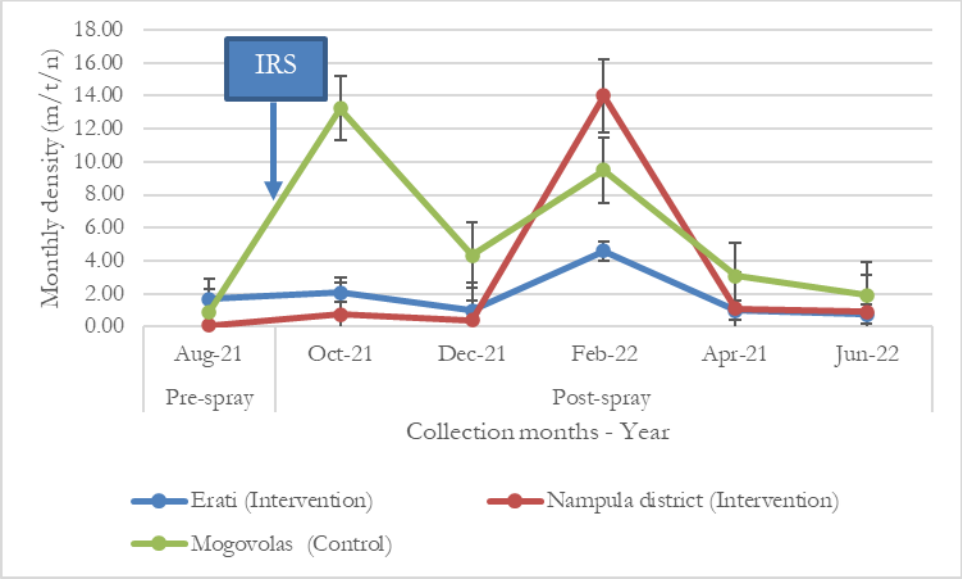


Figure 16B: *An. gambiae* s.l.



4.3.1 BITING TIME AND LOCATION BASED ON CDC LIGHT TRAP COLLECTIONS

Table 10 shows no significant difference between the total numbers of *An. funestus* s.l. samples collected indoors and outdoors ($p>0.05$) in all districts. In Erati (intervention) and Mogovolas (control) districts, where *An. funestus* s.l. showed more exophagic tendencies, the differences were not significant ($p>0.05$). In Nampula district (intervention), where *An. funestus* s.l. showed a more endophagic tendency, the difference was not significant ($p>0.05$).

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

TABLE 10. COMPARISON OF TOTAL NUMBER OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED BY CDC LIGHT TRAP INDOORS AND OUTDOORS IN THREE 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
Erati	81	91	0.581	0.446	201	133	13.844	0.000*
Nampula district	144	128	0.941	0.332	276	207	9.857	0.002*
Mogovolas	68	75	0.343	0.558	557	396	27.199	< 0.0001*

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

Table 11 summarizes the combined outdoor and indoor collections from the intervention and control districts with mean b/p/n for each species. The control district showed a slightly higher overall biting rate (1.310 b/p/n) than the intervention districts (0.824 b/p/n), but the difference was not significant ($p>0.05$). For *An. funestus* s.l. alone, a slightly higher biting rate (1.753 b/p/n) was observed in the intervention districts compared to the control district (1.014 b/p/n); however, the difference was not significant ($p>0.05$). For *An. gambiae* s.l. alone, the control district showed two fold higher biting rates (6.79 b/p/n) than the intervention districts (3.03 b/p/n) with a significant difference ($p<0.05$).

TABLE 11. MOSQUITO SPECIES COLLECTED BY CDC LIGHT TRAP AND THEIR COMBINED OUTDOOR AND INDOOR MEAN BITING RATES IN ERATI AND NAMPULA INTERVENTION DISTRICTS AND MOGOVOLAS CONTROL DISTRICT

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.	505	288	1.753	146	144	1.014
An. gambiae s.l.	872	288	3.028	977	144	6.785
An. pretoriensis	5	288	0.017	6	144	0.042
An. tenebrosus	0	288	0.000	2	144	0.014
An. maculipalpis	1	288	0.003	0	144	0.000
An. rufipes	41	288	0.142	1	144	0.007
Total	**1,424**	**1,728**	**0.824**	**1,132**	**864**	**1.310**

Table 12 shows that, after IRS, the *An. funestus* s.l. indoor and outdoor biting rates increased in Nampula district and decreased in Erati and Mogovolas (control). The *An. gambiae* s.l. indoor and outdoor biting rates showed a notable increase after spraying in all the IRS targeted districts. These observations were expected as they reflect the natural increase in vector populations after the rainy season. For *An. gambiae* s.l., the observations were the same in previous year. For *An. funestus* s.l., observations were different. In 2018/19, *An. funestus* s.l. decreased after IRS in all sites, both indoors and outdoors; in 2019/20, *An. funestus* s.l. decreased after IRS both indoors and outdoors only in Monapo; and in 2020/21 *An. funestus* s.l. again decreased after IRS both indoors and outdoors.

TABLE 12. 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
Erati	2.92	0.77	4.67	0.58	2.17	2.92	1.67	1.88
Nampula	1.67	2.07	1.58	1.82	0.42	4.52	0.08	3.43
Mogovolas*	2.75	0.58	2.17	0.82	3.17	8.65	0.92	6.42

*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. There was a steady increase in biting activity for several hours, both indoors and outdoors in Erati and Nampula districts. Most indoor bites took place between 6 pm and 3 am, peaking at 2.42 b/p/h in Nampula district at 12 am–1 am. In Erati, indoor bites peaked at 1.33 b/p/h at 10 pm–11 pm, and in Mogovolas at 0.92 b/p/h at 2 am–3 am.

Most outdoor bites took place between 6 pm and 3 am, peaking at 9 pm–10 pm for Nampula district (1.92 b/p/h), at 11 pm–12 pm (1.58 b/p/h) in Erati, and at 6 pm–7 pm and 2 am–3 am (1.08 b/p/h) in Mogovolas. The number of mosquitoes biting during these peak times was higher in the IRS district (Nampula district) than in the IRS (Erati) and unsprayed (Mogovolas) districts.

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 COLLECTIONS

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

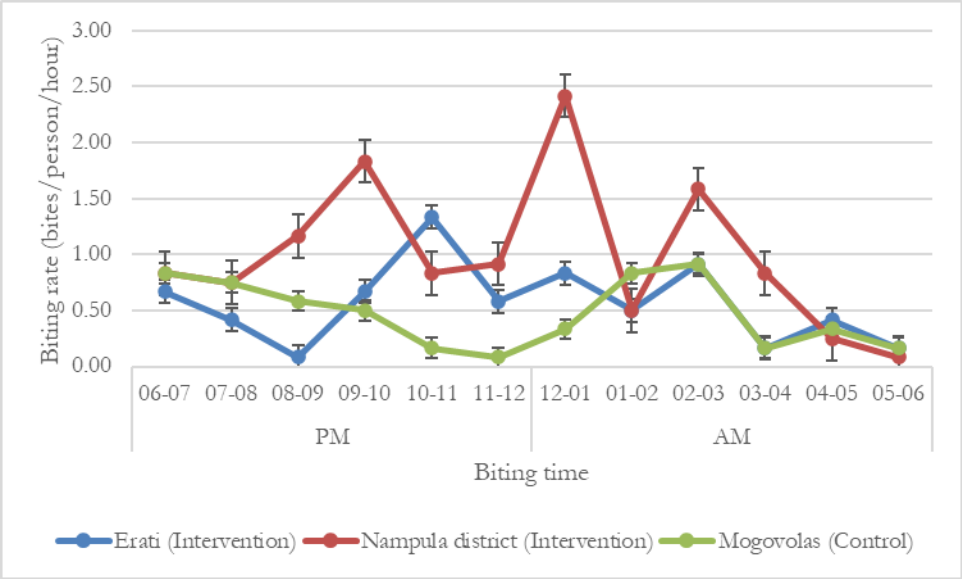
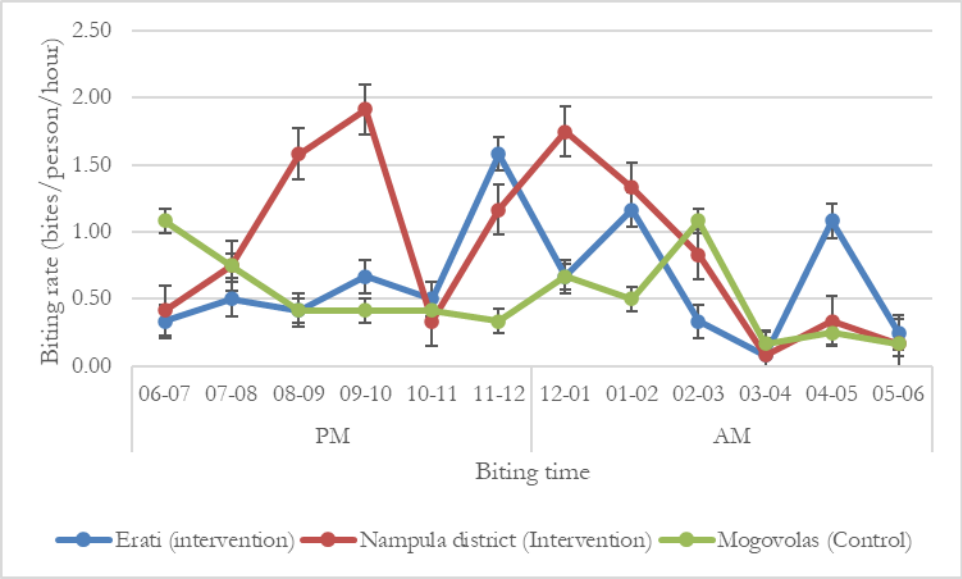


Figure 17B. *An. funestus* s.l. Outdoor



Figures 17C and 17D show the overnight biting pattern for *An. gambiae* s.l. indoors and outdoors. Both indoor and outdoor biting activities in Nampula district, Erati, and Mogovolas started at 6 pm. The *An. gambiae* s.l. bites took place between 6 pm and 6 am both indoors and outdoors in Nampula district, Erati, and Mogovolas. Indoor bites peaked at 12 am–1 am in Mogovolas (5.08 b/p/n), at 1 am–2 am in Nampula district (2.83 b/p/n), and at 10 pm–11 pm in Erati (2.50 b/p/n). Outdoor bites peaked at 7 pm–8 pm for Mogovolas (4.42 b/p/h), at 11 pm–12 pm for Nampula district (1.92 b/p/h), and at 12 pm–1 pm for Erati (1.42 b/p/h). The number of mosquitoes biting during these peak times were higher in the unsprayed district (Mogovolas) than in the IRS districts (Erati and Nampula district).

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

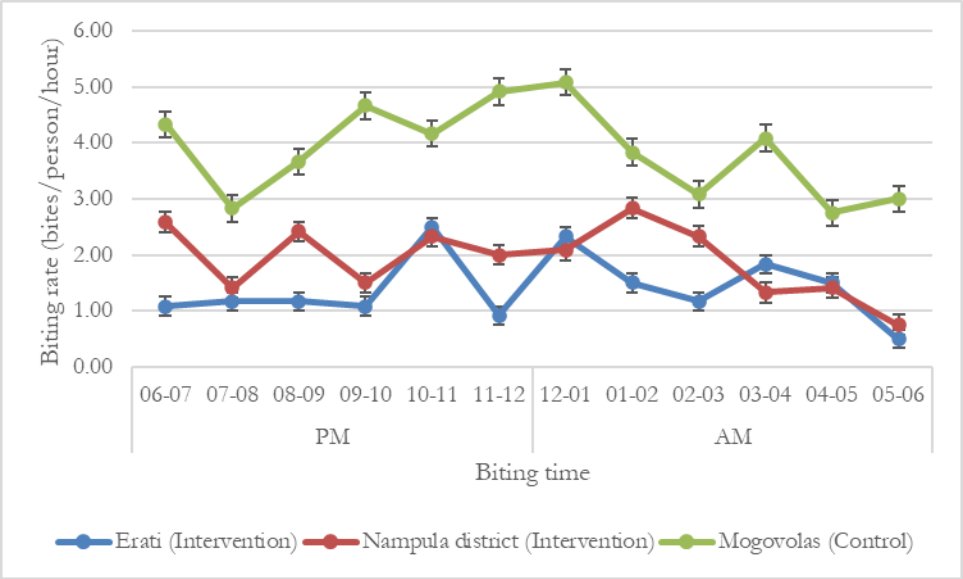
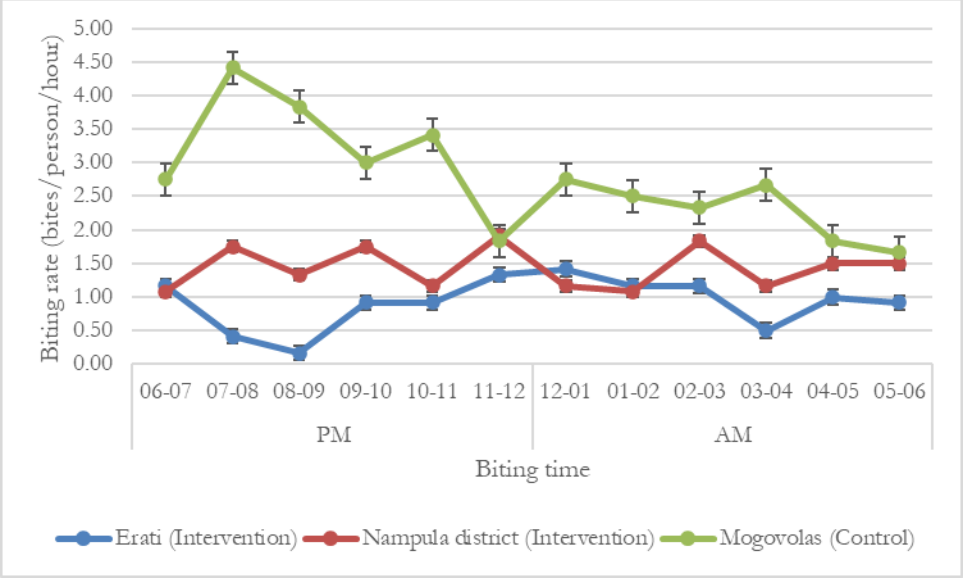


Figure 17D. *An. gambiae* s.l. Outdoor



4.4 PIT SHELTERS COLLECTION

Pit shelter collections yielded very few (38) mosquitoes over the collection period. Five mosquitoes (13.16%) were *An. funestus* s.l., 16 (42.11%) were *An. gambiae* s.l., 16 (42.11%) were *An. rufipes,* and 1 (2.63%) were *An. pretoriensis* (Table 13).

TABLE 13. NUMBER OF MOSQUITOES COLLECTED BY SPECIES AND BY DISTRICT IN NAMPULA PROVINCE USING PIT SHELTERS

Mosquito Species/District	Erati	Nampula	Mogovolas	Total
An. funestus s.l.	2	3	0	5 (13.16)*
An. gambiae s.l.	11	2	3	16 (42.11)
An. rufipes	16	0	0	16 (42.11)
An. pretoriensis	0	0	1	1 (2.63)
Total	29	5	4	38

* Numbers in brackets are in percentage

In Nampula district, the mean outdoor resting density of *An. funestus* s.l. was 0.60 mosquitoes/pit trap/day before IRS in August and dropped in October to zero (Figure 18a). In Erati, the mean outdoor density was 0.20 mosquitoes/pit trap/day before and after the October spray campaign; it dropped to 0.0 in December. In Mogovolas (control), the mean indoor densities were 0.0 for the reporting period.

The mean outdoor resting density of *An. gambiae* s.l. was 1.40 mosquitoes/pit trap/day in February 2022 in Erati (Figure 18B). In Nampula district, the mean outdoor density increased after IRS, while in Mogovolas, it dropped to zero after IRS in October 2021 and increased to 0.40 mosquitoes/pit trap/day in December. Overall, the outdoor densities were generally low for both species.

FIGURE 18. PIT SHELTER TRAP, DENSITY PER TRAP PER DAY IN ERATI, NAMPULA, AND MOGOVOLAS DISTRICTS

Figure 18A. *An. funestus* s.l.

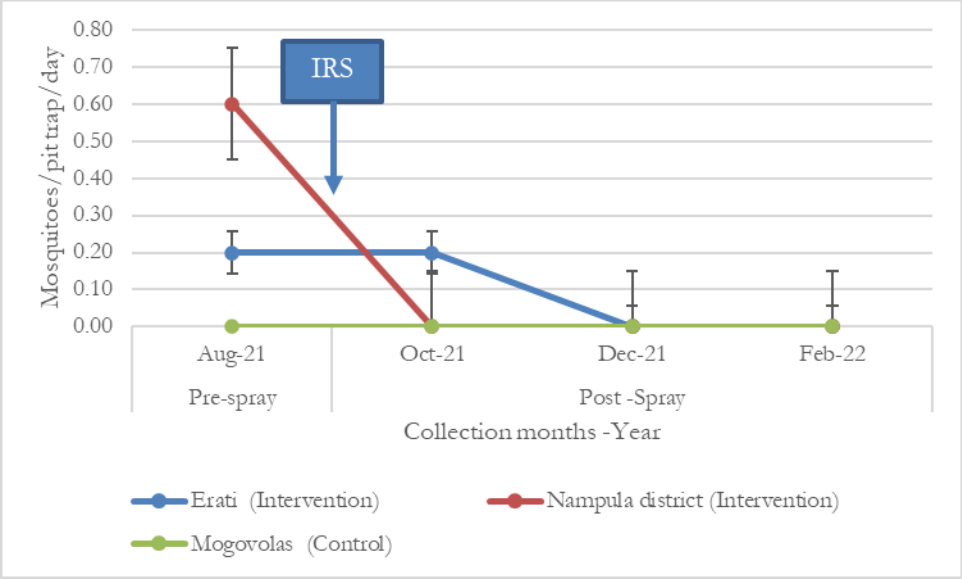
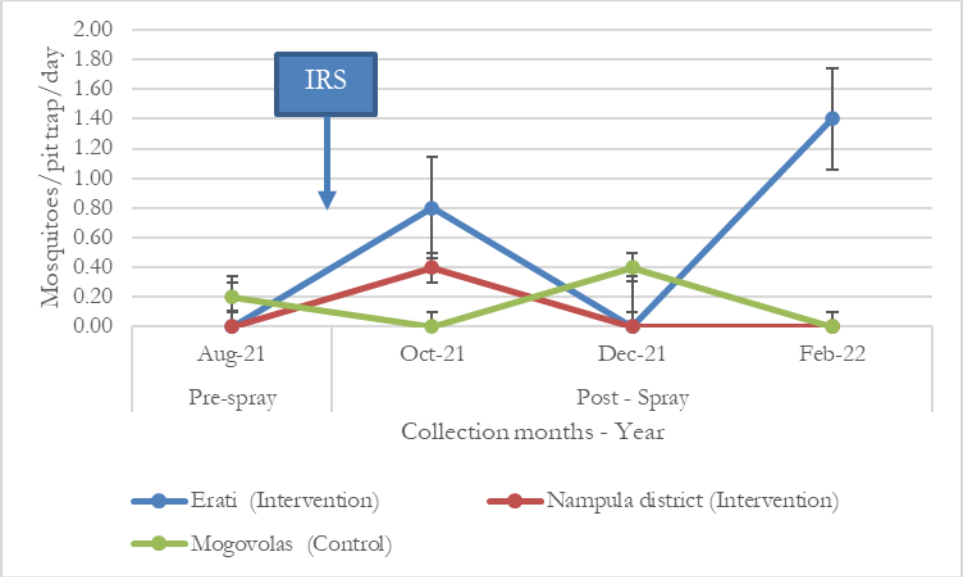


Figure 18B. ***An. gambiae*** **s.l.**



4.5 CONE WALL BIOASSAYS

During spray operations in September 2021, 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 (Intuto) and Nampula district (Nawithipele) and bendiocarb in Nampula district (Muriaze) are shown below (Figures 19 and 20, respectively).

4.5.1 QUALITY OF SPRAY

For Fludora Fusion (Erati and Nampula districts), mortality scored at T_0 was 100% in all houses tested with cone wall bioassays 48 hours after spraying. For bendiocarb (Nampula district only), mortality at T_0 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 strain upon exposure to all three types of sprayed surfaces (cob blocks, mud, and cement). As expected for bendiocarb (Ficam), high levels of knockdown were observed 30 minutes after exposure to almost all sprayed substrates, whereas Fludora Fusion (clothianidin and deltamethrin) elicited low knockdowns at 30 minutes after exposure. Fludora Fusion demonstrated its typical slow-acting characteristic where mosquitoes were observed to survive up to 48 hours after exposure, at which time 100% mortality was recorded. The results obtained from these wall assays strongly suggest that the spray teams were skilled in applying the insecticide uniformly, resulting in high 24-hour and 48-hour mortalities for bendiocarb and Fludora Fusion, respectively.

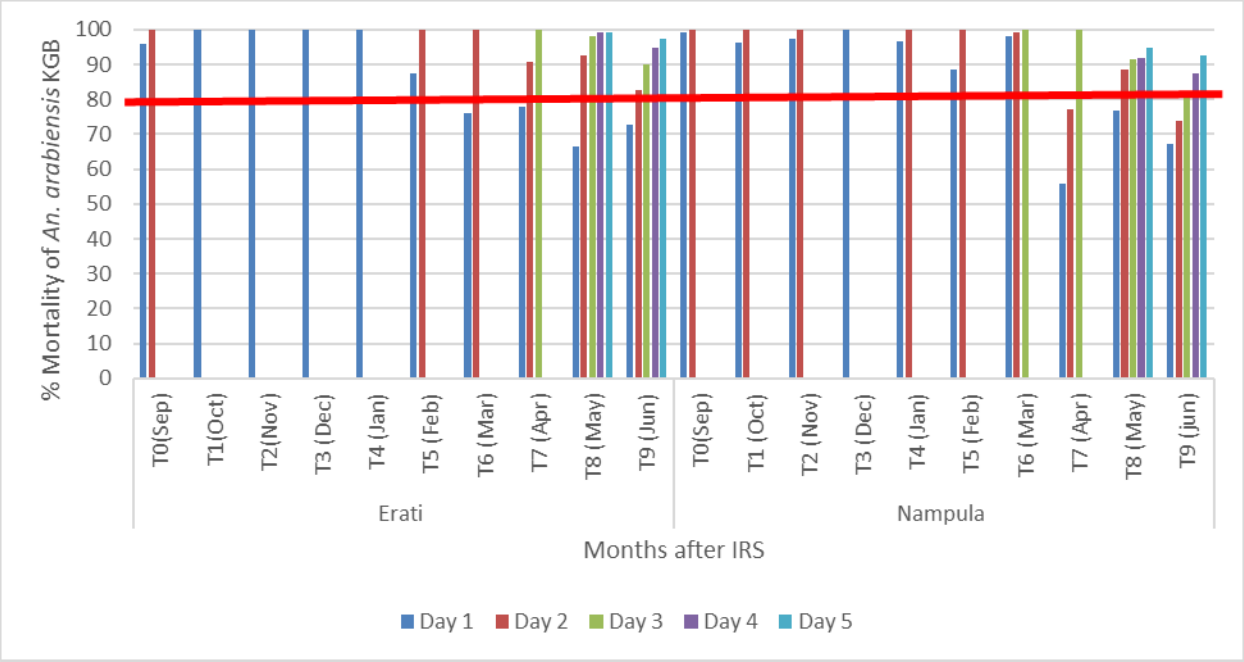
4.5.2 INSECTICIDE DECAY RATE

FLUDORA FUSION DECAY RATE

Cone wall bioassays for assessing quality of spraying with Fludora Fusion and subsequent monitoring of its decay rate were conducted in Erati district (Intuto) and Nampula district (Nawithipele). The first bioassay (T_0) was conducted in September 2022 and elicited a 100% mortality by day 2 after exposure (Figure 19). Subsequent monthly cone bioassays resulted in more than 80% mortality at day 5 up to 10 months in Erati and Nampula district districts. It was also noted that scores of 100% mortality was observed up to eight months after IRS in both Erati and Nampula district. There was a notable increase in the number of days when 100% mortality was achieved, from two days during the first five months to five days by the eighth month in Erati. In Nampula

district, there was a notable increase in the number of days when 100% mortality was achieved from two days during the first five months to three days by the sixth to seventh months after IRS. 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 19. RESULTS OF CONE WAL BIOASSAYS ON WALLS SPRAYED WITH FLUDORA FUSION IN ERATI AND NAMPULA DISTRICT

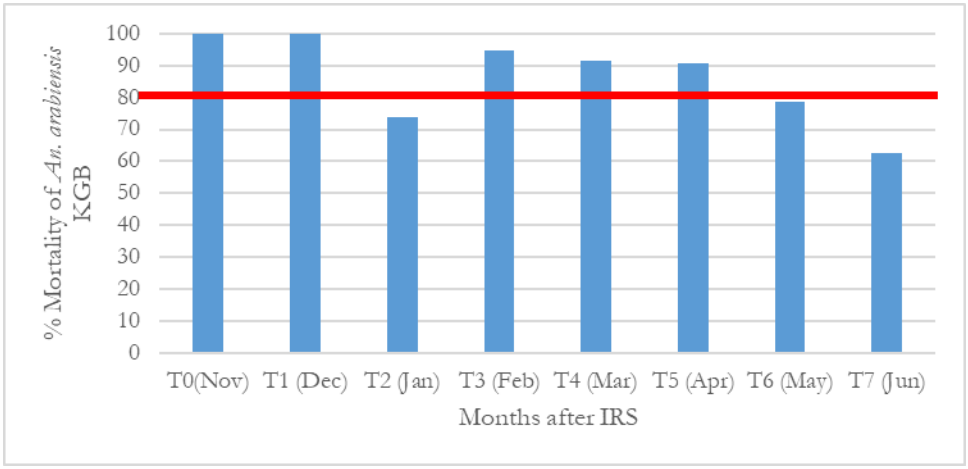


Red line indicates 80% mortality cut-off point.

4.5.3 BENDIOCARB DECAY RATE

Cone wall bioassays for assessing spray quality with bendiocarb and subsequent monitoring of its decay rate were conducted in Nampula district (Muriaze). The first bioassay (T_0) was conducted in November 2021, eliciting 100% mortality (Figure 19). Subsequent cone bioassays observed a first drop in mortality, to 74%, two months after spray (T_2), followed immediately by a recovery in month 3 (T_3) to 94.67% mortality. A subsequent drop below the cut-off point to 78.67% was observed six months (T_6) after spray, persisting during month 7 and therefore calling for termination of the monitoring. These result show that bendiocarb remained efficacious for up to five months.

FIGURE 20. RESULTS OF CONE WALL BIOASSAYS ON WALLS SPRAYED WITH BENDIOCARB IN NAMPULA DISTRICT



Red line indicates 80% mortality cut-off point.

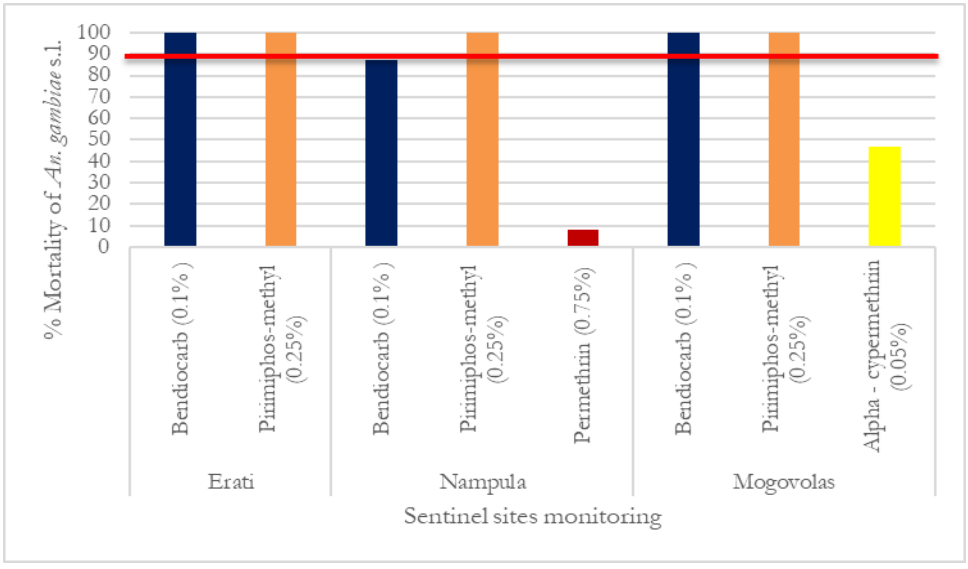
4.6 WHO SUSCEPTIBILITY TESTING

Susceptibility tests against *An. gambiae* s.l. were conducted from January through April 2022 in Erati, Nampula district, and Mogovolas districts, by exposing the *An. gambiae* s.l. to diagnostic dosages of bendiocarb (0.1%), pirimiphos-methyl (0.25%), permethrin (0.75%), and alpha-cypermethrin (0.05%). The project also conducted synergist assays with PBO for permethrin and alpha-cypermethrin.

WHO susceptibility tests indicated that *An. gambiae* s.l. mosquitoes from Mogovolas and Erati districts were fully susceptible to bendiocarb (with 100% mortality). For Nampula district, mosquitoes were resistant to it (87% mortality). *An. gambiae* s.l. were also found to be fully susceptible to pirimiphos-methyl (0.25%) at all sentinel sites with 100% mortality (Figure 21).

In Nampula district, *An. gambiae* s.l. was resistant to permethrin (0.75%), with 8% mortality. The vector was also observed to be resistant to alpha–cypermethrin (0.05%) in Mogovolas, with 46.77% mortality.

FIGURE 21. 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

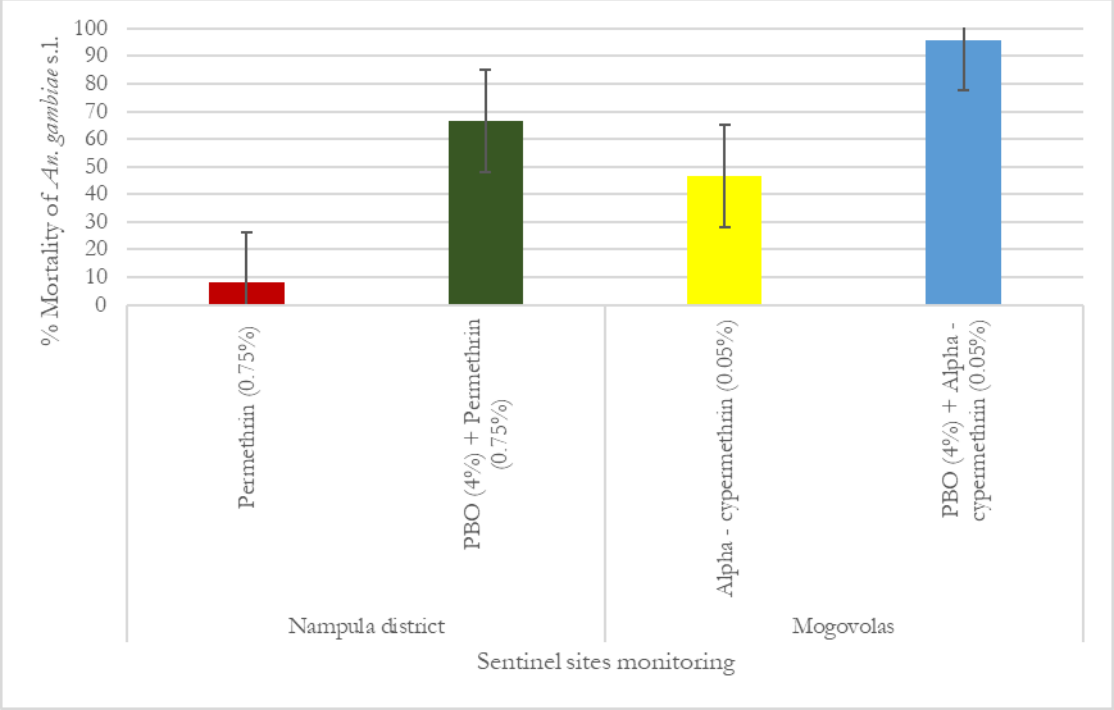


Red horizontal line indicates the 90% mortality cut-off point for susceptibility.

4.6.1 SYNERGIST ASSAYS USING WHO TUBE TESTS

Pre-exposure of *An. gambiae* s.l. to PBO seemed to increase susceptibility to permethrin and alpha-cypermethrin but did not restore full susceptibility (Figure 21). The mortality rates increased from 8.0% with permethrin alone to 66.7% with permethrin + PBO in Nampula district, and from 46.77% with alpha-cypermethrin alone to 96.0% with alpha-cypermethrin + PBO in Mogovolas district. However, for both insecticides (permethrin and alpha-cypermethrin), 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 22 shows that the pre-exposure to PBO resulted in only partial restoration of susceptibility to permethrin in Nampula district and alpha-cypermethrin in Mogovolas district.

FIGURE 22. MORTALITY OF *AN. GAMBIAE* S.L. EXPOSED TO ALPHA-CYPERMETHRIN, DELTAMETHRIN, AND PERMETHRIN WITH AND WITHOUT PBO IN NAMPULA DISTRICT (JANUARY–MARCH 2022)



5. Discussion and Lessons Learned

5.1 Zambezia Province

The entomological surveillance conducted in Zambezia employed three main collection methods: Prokopack aspirators, CDC light traps, and outdoor pit shelters. The CDC light traps were set next to humans sleeping under treated nets indoors and outdoors. Anophelines collected by these methods were identified using the morphological identification key, revealing the presence of seven anopheline species, with *An. funestus* s.l. being the most abundant (66.4%), followed by *An. gambiae* s.l. (30.6%) and other species, which accounted for 2.86%. Together, the two dominant vectors constituted 97% of the anopheline population collected. The greatest diversity of anopheline species was collected using CDC light traps, with seven species. Pit shelters collected three species and Prokopack two.

In all four districts *An. funestus* s.l. was more abundant than *An. gambiae* s.l. This finding is consistent with what was reported in the previous annual reports, although it counters the emerging dominance of *An. gambiae* s.l. reported in Milange last year. It seems that the species abundance might have been shifting between *An. funestus* s.l. to *An. gambiae* s.l. and vice versa, and there is a need to monitor the emerging dynamics of species composition in this area.

Low levels of indoor resting *An. gambiae* s.l. were recorded in both the intervention and control districts compared to *An. funestus* s.l., for which higher numbers were recorded in most districts.

Due to the gap in knowledge on outdoor resting behaviors, outdoor pit shelter collections were introduced to gather more data. Unfortunately, few mosquitoes were collected through this method and so it is difficult to draw conclusions. However, improving collection efforts to match the Prokopack collections can potentially improve the number of mosquitoes and determine the trends in outdoor resting behaviors in the future.

Monthly indoor resting patterns show *An. funestus* s.l. to be more abundant in Lugela (control) and Mopeia, mostly during the dry season (August–October) with a peak in August for Lugela district and April, June, and August for Mopeia district. This is an indication of more prolonged abundance of this species mainly in Mopeia district. *An. gambiae* s.l. was found relatively more frequently during the rainy season but the densities were very low, and the peak period could not be clearly established. Nevertheless, in Lugela, it seemed to have peaked in April as was noted in other districts.

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 one district. In Mopeia, a reduction in the biting rate was observed, which reduced threefold 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. *An funestus* s.l. collected indoor increased slightly from February to April in Milange, from April to June in Mopeia, from February to April in Molumbo and in Lugela it decreased from February to June. For the outdoor collections, *An. funestus* s.l. slight increase was observed from April to June in Mopeia, a similar trend with the indoor, while in Milange and Lugela, slight decrease was observed from February to June. In Milange the outdoor biting rate was the same with the indoor one; In Molumbo slight increase was observed from February to April, the same trend with the indoor. However, for the *An. gambiae* s.l., there was an increase in biting rates at all sentinel sites, even in low densities. 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. funestus* s.l. and *An. gambia*e s.l. show that most biting occurs in the early night when people are going to bed or are sleeping 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.

In Milange significant differences between indoor and outdoor biting rates was observed in both species and the higher biting occurred indoor for *An. funestus* s.l. ($p<0.05$; $=192.9$); for *An. gambiae* s.l. ($p<0.05$, $=52.29$).

In Molumbo no significant difference was observed between indoor and outdoor biting activities of *An. funestus* s.l. ($p>0.05$; $X^2=3.27$), while for *An. gambiae* s.l. the indoor biting activity was observed mostly indoor ($p<0.05$; $X^2=19.18$). In Mopeia no significant difference between indoor and outdoor biting rates was observed in both species, *An. funestus* s.l. ($p>0.05$; $X^2=0.55$) and *An. gambiae* s.l. ($p>0.05$; $X^2=1.35$); Lugela and Milange showed significant differences between indoor and outdoor and the indoor biting was higher for both species with $p < 0.05$; $X^2=23.73$ for *An. funestus* s.l. and $p < 0.05$; $X^2=1.35$ for *An. gambiae* s.l..

Molecular results are not yet available due to problems with standardization of the protocol associated with reception of the reagents from different sources. The results are expected after a technical assistance visit from CDC. The visit will support the INS to troubleshoot and validate the results obtained so far. The project will include the results in October if they are ready or an addendum report with the results will be submitted later.

The quality of IRS assessed by cone wall bioassays showed that spray teams did not underdose the spraying in all districts, demonstrating quality skills in consistent and uniform application of insecticides across districts. Subsequent monthly cone wall bioassays to monitor insecticide decay rates found that bendiocarb (Ficam) remained effective only three months after spray in Mopeia. Actellic 300 CS had different residual efficacy, lasting active only one month after spray in Milange and five months in Molumbo. The result for Actellic residual efficacy is different from previous results from Mozambique, where it has lasted up to six months.

In Milange, when it was observed that the residual efficacy results were not as expected, cone wall bioassay were conducted up to T6 to see if changes could happen, In addition, 5 more houses in the sentinel sites were added to the number of houses for cone wall bioassay. The results and the decay rate mortality remained below 80% as observed previously on the first 5 houses. In addition, the project conducted post-spray quality assurance on batches of the remaining Actellic 300CS from 2021 spray campaign. No issues were reported on the content of the active ingredient. Lastly, homeowners did not report modifying the walls of their home.

Insecticide susceptibility test results show that local vectors are fully susceptible to pirimiphos-methyl, chlorfenapyr, and clothianidin. The team identified *An. gambiae* s.l resistance to bendiocarb in Mopeia. Possible resistance to bendiocarb was also detected in Mopeia against *An. funestus* s.l. 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 2,556 anopheline mosquitoes were collected in Nampula Province using Prokopack, CDC light traps, and pit shelter traps. The anopheline mosquitoes were found to belong to six different species complexes: *An. funestus* s.l., *An. gambiae* s.l., *An. pretoriensis*, *An. rufipes*, *An. tenebrosus*, and *An. maculipalpis*: *An. gambiae* s.l. and *An. funestus* s.l. were the major vectors, making up 72.34% and 25.47%, respectively, of the mosquitoes that were collected. These entomological monitoring results indicate that *An. gambiae* s.l. remains the predominant *Anopheles* vector species in all sites. The greatest diversity of anopheline species was collected using CDC light traps, with six species out of the total six collected, followed by pit shelters with four species collected and Prokopack with three species collected.

Low levels of indoor resting mosquitoes were recorded in Erati and Mogovolas (control) districts for *An. funestus* s.l., compared to *An. gambiae* s.l., for which more were collected in three districts.

Due to the gap in knowledge on outdoor resting behaviors, outdoor pit shelter collection was introduced to bridge this gap in knowledge. Unfortunately, few mosquitoes were collected through this method, making it

difficult to draw conclusion. However, continuous collection with more collection efforts as described earlier can potentially determine outdoor resting behavior trends in the future.

Monthly indoor resting patterns show *An. funestus* s.l. is more abundant in Nampula district (intervention) and mostly during the dry season (May–October) with a peak in February and August in Nampula district. In Erati, the peak was observed in August. *An. gambiae* s.l. was found relatively more frequently during the rainy season but the indoor resting decreased after IRS in Erati and Mogovolas (control district) and increased in Nampula district.

CDC light trap collections show that indoor and outdoor *An. funestus* s.l. densities in Erati and Mogovolas decreased after IRS and were much lower than in Nampula district after IRS.

An. gambiae s.l. densities both indoors and outdoors was high in Mogovolas district. *An. gambiae* s.l. biting activity demonstrated an increase in both control and intervention (Erati and Nampula district) districts. These findings could potentially be an outcome of seasonal abundance of vector species during the rainy season.

Our findings show that IRS has contributed to the reduction of the indoor resting density in Erati. Insecticide-treated nets might have also contributed to a reduction in biting rates of *An. funestus* s.l. in Erati and Mogovolas. However, there was an increase in *An. gambiae* s.l. biting rates at all sentinel sites even with low densities. 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.

Although the peak indoor and outdoor biting time of *An. funestus* s.l. across all sentinel sites was between 6 pm and 3 am, biting continued indoors and outdoors in the morning hours of 5 am to 6 am, when residents were starting to awake and go outdoors. This might not be a favorable situation for any indoor-based malaria vector control intervention. The collection period in the evening was extended to 5 pm and 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 6 am both indoors and outdoors by *An. gambiae* s.l. in Erati, Nampula district, and Mogovolas (control) districts and continued until the morning hours of 5 am to 6 am. Based on these observations, collections are now being conducted from 5 pm to 7 am 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 district exhibited a residual efficacy for at least for 10 months after spraying, longer than the five months of residual efficacy of bendiocarb in Nampula district.

Based on the data collected so far, *An. gambiae* s.l. was resistant to bendiocarb and permethrin in Nampula district, and alpha-cypermethrin in Mogovolas. The National Malaria Control Program should not consider these insecticides for IRS in the future until the population is no longer resistant. *An. gambiae* s.l. remained susceptible to pirimiphos-methyl across all sentinel sites tested. However, the vector is resistant to bendiocarb in Nampula district. The synergist assays showed that PBO did not fully restore susceptibility to pyrethroid insecticides in the area, but mortality rates increased to 66.7% in Nampula district for permethrin and 96% in Mogovolas for alpha - cypermethrin. This might indicate that PBO nets with permethrin may not be a good 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. 1 CDC LIGHT TRAP DATA FROM MONTHLY COLLECTIONS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. IN FOUR DISTRICTS OF ZAMBEZIA PROVINCE: MILANGE, MOLUMBO, MOPEIA, AND LUGELA

Districts	Species	2021			2022			Total and annual average densities	
		Aug	Oct	Dec	Feb	Apr	June		
Milange	*An. funestus* s.l.	60	5	19	142	179	22	427	680
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	5.00	0.42	1.58	11.83	14.92	1.83	5.63	
	An. gambiae s.l.	4	11	1	51	171	15	253	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.33	0.92	0.08	4.25	14.25	1.25	3.51	
Molumbo	*An. funestus* s.l.	2	0	0	0	32	3	37	189
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.17	0.00	0.00	0.00	2.67	0.25	0.51	
	An. gambiae s.l.	1	0	0	50	98	3	152	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.08	0.00	0.00	4.17	8.17	0.25	2.11	
Mopeia	*An. funestus* s.l.	125	201	11	4	78	108	527	652
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	10.42	16.75	0.92	0.33	6.50	9.00	7.32	
	An. gambiae s.l.	0	1	0	25	67	32	125	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.08	0.00	2.08	5.58	2.67	1.74	
Lugela	*An. funestus* s.l.	75	73	17	40	21	11	237	361
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	6.25	6.08	1.42	3.33	1.75	0.92	3.29	
	An. gambiae s.l.	0	6	1	62	53	2	124	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.00	0.50	0.08	5.17	4.42	0.17	1.72	
Total		**267**	**297**	**49**	**374**	**699**	**196**	**1882**	

TABLE A1.2. PROPORTION OF *AN. FUNESTUS* S.L. COLLECTED DURING THE LAST FIVE CYCLES OF ENTOMOLOGICAL MONITORING IN ZAMBEZIA PROVINCE

Districts	Years of collection				
	2021-2022	2020-2021	2019-2020	2018-2019	2017-2018
Maganja da Costa	NA	18.77	13.31	6.55	24.01
Milange	34.77	16.57	5.50	7.62	22.08
Mopeia	42.92	28.89	33.26	50.97	53.90
Molumbo	3.01	NA	NA	NA	NA
Lugela	19.30	35.77	47.93	34.86	NA

TABLE A2.1. CDC LIGHT TRAP DATA FOR MONTHLY COLLECTIONS OF *AN. FUNESTUS* S.L. AND *AN. GAMBIAE* S.L. COLLECTED IN NAMPULA PROVINCE

Districts	Species	2021			2022			Total & Average Densities/Month/Night	
		Aug	Oct	Dec	Feb	Apr	June		
Erati	*An. funestus* s.l.	91	18	5	3	0	55	**172**	**506**
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	7.58	1.50	0.42	0.25	0.00	4.58	**2.39**	
	An. gambiae s.l.	46	70	18	120	51	29	**334**	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	3.83	5.83	1.50	10.00	4.25	2.42	4.64	
Nampula	*An. funestus* s.l.	39	7	3	10	24	189	**272**	**755**
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	3.25	0.58	0.25	0.83	2.00	15.75	**3.78**	
	An. gambiae s.l.	6	40	8	376	23	30	**483**	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	0.50	3.33	0.67	31.33	1.92	2.50	6.71	
Mogovolas	*An. funestus* s.l.	59	53	3	0	0	28	**143**	**1096**
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	4.92	4.42	0.25	0.00	0.00	2.33	**1.99**	
	An. gambiae s.l.	49	359	158	192	149	46	**953**	
	Trap nights	12	12	12	12	12	12		
	Mean # Mosq/trap/night	4.08	29.92	13.17	16.00	12.42	3.83	**13.24**	
Total		**290**	**547**	**195**	**701**	**247**	**377**	**2,357**	

TABLE A2.2. NUMBER OF MOSQUITOES COLLECTED BY SPECIES DURING THE LAST FOUR CYCLES OF ENTOMOLOGICAL MONITORING IN NAMPULA PROVINCE

Districts	Species	Years of collection			
		2021 - 2022	2020 -2021	2019 - 2020	2018 - 2019
Erati	*An. funestus* s.l.	187	167	306	341
	An. gambiae s.l.	376	1,744	887	988
	An. pretoriensis	4	43	1	0
	An. rufipes	30	21	5	0
	An. tenebrosus	0	1	1	0
	An. maculipalpis	1	3	1	0
	An. ziemanni	0	0	1	0

Districts	Species	Years of collection			
		2021 - 2022	2020 -2021	2019 - 2020	2018 - 2019
Monapo	*An. funestus* s.l.	NA	4	72	181
	An. gambiae s.l.		9	106	445
	An. pretoriensis		1	0	1
	An. rufipes		0	0	0
	An. tenebrosus		0	0	0
	An. maculipalpis		0	0	0
Nampula district	*An. funestus* s.l.	318	631	834	891
	An. gambiae s.l.	496	1,392	1,227	926
	An. pretoriensis	1	6	0	0
	An. rufipes	11	51	0	3
	An. tenebrosus	0	1	0	0
	An. maculipalpis	0	8	0	0
	An. coustani	0	0	1	2
Mogovolas	*An. funestus* s.l.	146	240	NA	NA
	An. gambiae s.l.	977	907		
	An. pretoriensis	6	4		
	An. rufipes	1	20		
	An. tenebrosus	2	0		
	An. maculipalpis	0	0		
Total		**2,556**	**5,253**	**3,442**	**3,778**