THE PMI VECTORLINK ANGOLA PROJECT

ANNUAL ENTOMOLOGY REPORT

MAY 2021–JUNE 2022

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Abt Associates | 6130 Executive Boulevard | Rockville, MD 20852
T. 301.347.5000

abtassociates.com

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ACRONYMS

CDC	(U.S.) Centers for Disease Control and Prevention
DHIS2	District Health Information Software 2
DPS	*Direcções Provincial de Saúde* (Provincial Health Directorate)
ELISA	Enzyme-Linked Immunosorbent Assay
ICCT	*Instituto de Controlo e Combate a Tripanossomíase* (Institute for the Control and Combat of Trypanosomiasis
INIS	*Instituto Nacional de Investigação em Saúde* (National Institute of Health Research)
ITN	Insecticide-treated Net
IRS	Indoor residual spraying
Kdr	Knock-down resistance
LT	Light trap
NMCP	National Malaria Control Program
PBO	Piperonyl butoxide
PCR	Polymerase chain reaction
PMI	(U.S.) President's Malaria Initiative
PSI	Population Services International
SOP	Standard operating procedures
STTA	Short-term technical assistance
WHO	World Health Organization

EXECUTIVE SUMMARY

In collaboration with the National Malaria Control Program (NMCP), the U.S. President's Malaria Initiative (PMI) VectorLink Project conducted entomological monitoring in Angola between May 2021 and June 2022 in seven provinces, including six PMI-focus provinces (Cuanza-Norte, Lunda-Sul, Lunda-Norte, Malanje, Uíge, and Zaire) and two additional non PMI-focus provinces (Huambo and Luanda) chosen based on existing insectary infrastructure and entomology capacity. Monitoring vector bionomics to determine species composition and malaria transmission intensity was conducted in three provinces (Huambo, Luanda, and Lunda-Norte) using indoor CDC light traps (LTs). A maximum of 60 LT collections were planned for each collection month at each site. Collected mosquitoes were morphologically identified to species and a proportion of the known malaria vectors *Anopheles funestus* s.l. and *An. gambiae* s.l. sent to the molecular laboratory for species identification and sporozoite infection assays. Insecticide susceptibility tests were conducted in six provinces (Cuanza-Norte, Huambo, Lunda-Sul, Malanje, Uíge, and Zaire) to inform insecticide-treated net (ITN) procurement decisions made by NMCP, PMI and other partners. World Health Organization (WHO) susceptibility bioassays using *An. gambiae* s.l. were conducted with the pyrethroids, deltamethrin, alpha-cypermethrin, and permethrin, with and without pre-exposure to the synergist piperonyl butoxide (PBO), while CDC bottle bioassays were done to determine the susceptibility to the pyrrole insecticide chlorfenapyr at the various sites. A proportion of pyrethroid resistant and susceptible samples were sent to the molecular laboratory for species PCR analysis and *kdr* analysis.

Anopheles funestus s.l. was the most abundant vector collected through LT from Huambo and Luanda provinces combined (96.7%) with a very low overall proportion of *An. gambiae* s.l. (2.2%). There was low *An. funestus* s.l. biting rate in Huambo between the months of June to September (<1 host seeking vector/trap/night) while rate of *An. funestus* s.l. biting was high throughout the collection period in Luanda from July 2021 to April 2022 (>10 vectors/trap/night). The entomological inoculation rate will be calculated in Year 4 following sporozoite ELISA testing of samples to determine *Plasmodium falciparum* infection rates.

There was widespread pyrethroid resistance in five provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire) with confirmed resistance to two or more of the pyrethroid insecticides tested (deltamethrin, alpha-cypermethrin, and permethrin). In Huambo Province, there was either full susceptibility or probable resistance to the pyrethroids tested. Pre-exposure to the synergist PBO either fully or partially restored susceptibility to pyrethroid resistant vectors. All vectors were fully susceptible to chlorfenapyr at all the sites tested. Due to the widespread distribution of pyrethroid resistance the NMCP and partners supporting ITN campaigns should not deploy pyrethroid only nets in these provinces, if possible. The recommendation is for distribution of ITNs containing PBO or dual active ingredient nets.

Local capacity was strengthened through a variety of measures, including training and working closely with at least three mosquito brigade staff in each of eight provinces, STTA (short term technical assistance) in March 2022 from a Senior CDC Entomologist on laboratory work flow and molecular sample processing protocol trainings, as well as attendance of two representatives from Angola at a project-wide VectorLink Regional VectorLink Collect DHIS2 training. VectorLink is playing a lead role in supporting the process of importation of WHO Entomology modules for Angola with expectations that the data will be used for evidence-based decision making.

Another objective of this reporting period was to work with the NMCP to establish of the country's first susceptible *An. gambiae* colony, which will help support the sustainability of entomological capacity in Angola. The team attempted to rear a cohort of susceptible *An. gambiae* s.s. Kisumu eggs received from CDC Atlanta in a container space at INIS laboratory in Luanda Province. Optimal rearing conditions (temperature and humidity) were ultimately achieved after troubleshooting; however the mosquito larvae did not survive past F1 generation due to an unintentional fumigation in proximity to the insectary. PMI and VectorLink, with support from the NMCP, are currently in the process of formalizing a collaboration with ICCT (*Instituto de Controlo e Combate a Tripanossomíase*) on a larger and more sustainable insectary space in Luanda, with plans to establish a susceptible mosquito colony in the project's 2022-23 work plan year.

Susceptibility tests in two provinces, Luanda and Lunda-Norte, were not conducted during the reporting period, due to limited numbers of malaria vectors collected to conduct the tests. In some provinces, it was difficult to collect sufficient quantities of *An. gambiae/An. funestus* larvae to test all insecticides. Thus, in most provinces only two of the three pyrethroids were tested and the tests extended beyond the planned time frame. VectorLink is actively locating and mapping new potential larval habitats to ensure completion of future susceptibility assays.

1. INTRODUCTION

Malaria remains a serious public health problem in Angola despite sustained malaria control strategies. Malaria is endemic throughout the country and is the principal cause of morbidity and mortality. Although the entire population is at risk, there is significant heterogeneity in transmission intensity across the country. Historically, the northeast provinces of Cabinda, Cuanza-Norte, Lunda-Norte, Lunda-Sul, Malanje, and Uíge are hyperendemic, the central and coastal provinces of Benguela, Bie, Cuanza-Sul, Huambo, Luanda, Moxico, and Zaire are mesoendemic with stable transmission; while the four southern provinces bordering Namibia have highly seasonal transmission and are prone to epidemics[1]. *Plasmodium falciparum* is the primary malaria parasite in Angola and accounts for an estimated 87% of malaria cases. The other three *Plasmodium* species known to cause human malaria—*P. vivax*, *P. malariae*, and *P. ovale*—are also present and cause about 7%, 3%, and 3% of reported malaria cases, respectively [2]. There are five *Anopheles* species responsible for transmission in the country: *Anopheles gambiae* s.s., *An. funestus*, *An. melas* (in coastal areas), *An. arabiensis*, and *An. pharoensis* (in southern, unstable mesoendemic areas). In addition, *An. rufipes* and *An. coustani* have been identified as secondary vectors in Huambo and Zaire provinces[1].

Angola's National Malaria Strategic Plan 2021-2025 outlines a four-pronged vector control strategy which aims to protect at least 80% of the population at risk by 2025 which includes insecticide-treated nets (ITNs), indoor residual spraying (IRS), prevention of malaria in pregnancy, and larviciding. A survey in 2016 showed that 31% of households in Angola owned at least one ITN [3]. By comparison, across all countries in sub-Saharan Africa in 2016, 80% of households owned at least one ITN [4]. PMI and the Global Fund have been key partners supporting procurement and distribution of ITNs. Angola took a major step in 2017 towards universal coverage of ITNs, conducting a three-phase nationwide mass campaign in 2017–2018, which resulted in the distribution of 6,693,503 ITNs to 2,379,943 registered household across 13 provinces. Following the campaign, a survey conducted in 2019 showed that 55.4% had access to a net in Cuanza-Sul Province (use: access ratio 0.87), 51% in Cunene Province (0.69 use: access ratio) and 33.3% in Uíge (0.77 use: access)[5]. The country is currently implementing a mass ITN campaign which was launched in Malanje and Cuanza-Norte in April 2022[6,7]. PMI is supporting distribution of standard ITNs to Cuanza-Norte, Lunda-Norte, Lunda-Sul, Malanje, Uíge, and Zaire. Global Fund is supporting distribution of PBO nets to Benguela and Cuanza-Sul. The NMCP supported the MOH with the procurement and will support ITN distribution to the remaining provinces not covered by PMI and Global Fund.

From 2012 to 2016, the PMI Africa Indoor Residual Spraying Program conducted longitudinal entomological surveillance activities in Angola, collecting data on key entomological indicators from three provinces (Cunene, Huambo, and Malanje), in collaboration with the *Direcções Provincial de Saúde* (DPS). In 2014, PMI constructed and established an insectary in Huambo, the first in Angola since the end of the civil war. PMI also supported the training of technicians on basic malaria entomology techniques as well as on-the-job training and mentorship to continuously improve their skills. PMI also supported training of provincial and municipal health authorities from nine provinces representing three malaria transmission zones (Benguela, Cunene, Huambo,

[1] U.S. President's Malaria Initiative Angola Malaria Operational Plan FY 2022. Retrieved from: www.pmi.gov.
[2] Malaria National Strategic Plan 2021-2025
[3] Instituto Nacional de Estatística - INE/Angola, Ministério da Saúde - MINSA/Angola, Ministério do Planeamento e do Desenvolvimento Territorial (MINPLAN) and ICF. 2017. Inquérito de Indicadores Múltiplos e de Saúde em Angola 2015-2016. Luanda, Angola e Rockville, Maryland, EUA: INE, MINSA, MINPLAN and ICF
[4] Koenker H, Arnold F, Ba F, Cisse M, Diouf L, Eckert E, et al. Assessing whether universal coverage with insecticide-treated nets has been achieved: is the right indicator being used? Malar J. 2018;17:355.
[5] VectorWorks 2019
[6] NMCP- Periodical Updates
[7] Population Services International- ITN Distribution Week 1 Magazine

Huila, Luanda, Malanje, Namibe, Uíge, and Zaire) where insecticide resistance was evaluated in 2015-2016. From 2016 to 2019, PMI did not support entomological monitoring activities in Angola.

PMI re-established support to entomological activities through the VectorLink project starting in 2019. Between January-March 2020, VectorLink conducted one month of entomological surveillance in two sites—Huambo and Lunda-Sul provinces—to determine species composition, vector behavior, and vector susceptibility to different insecticides. In Year 2, the project continued to build entomological capacity within the NMCP/National Department of Public Health, *Instituto Nacional de Investigação em Saúde* (INIS), and DPS mosquito brigades in each province. Between November 2020 and April 2021, the project conducted monthly community-based entomological surveillance in Huambo Province and insecticide resistance testing in six provinces (Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire).

In Year 3 (May 2021-April 2022), VectorLink continued monthly community-based entomological surveillance in Huambo Province and piloted this activity in Luanda in July 2021 and Lunda-Norte in December 2021. Insecticide resistance testing was conducted in seven provinces (Cuanza-Norte, Huambo, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire). The team also oversaw the set-up of an additional container at INIS in Luanda, designed to support a local susceptible *An. gambiae* colony. During the reporting period, VectorLink received three batches of eggs of susceptible *An. gambiae* mosquitoes and tried to rear the eggs to adults. The attempts were unsuccessful due to issues with temperature and humidity controls, as well as unintentional effect of fumigation around the facility where the insectary was located.

This report presents results from entomological monitoring conducted from May 2021 to April 2022 for community-based vector surveillance and December 2021 to June 2022 for insecticide resistance testing, as well as key project contributions to country-level capacity strengthening efforts.

2. METHODOLOGY

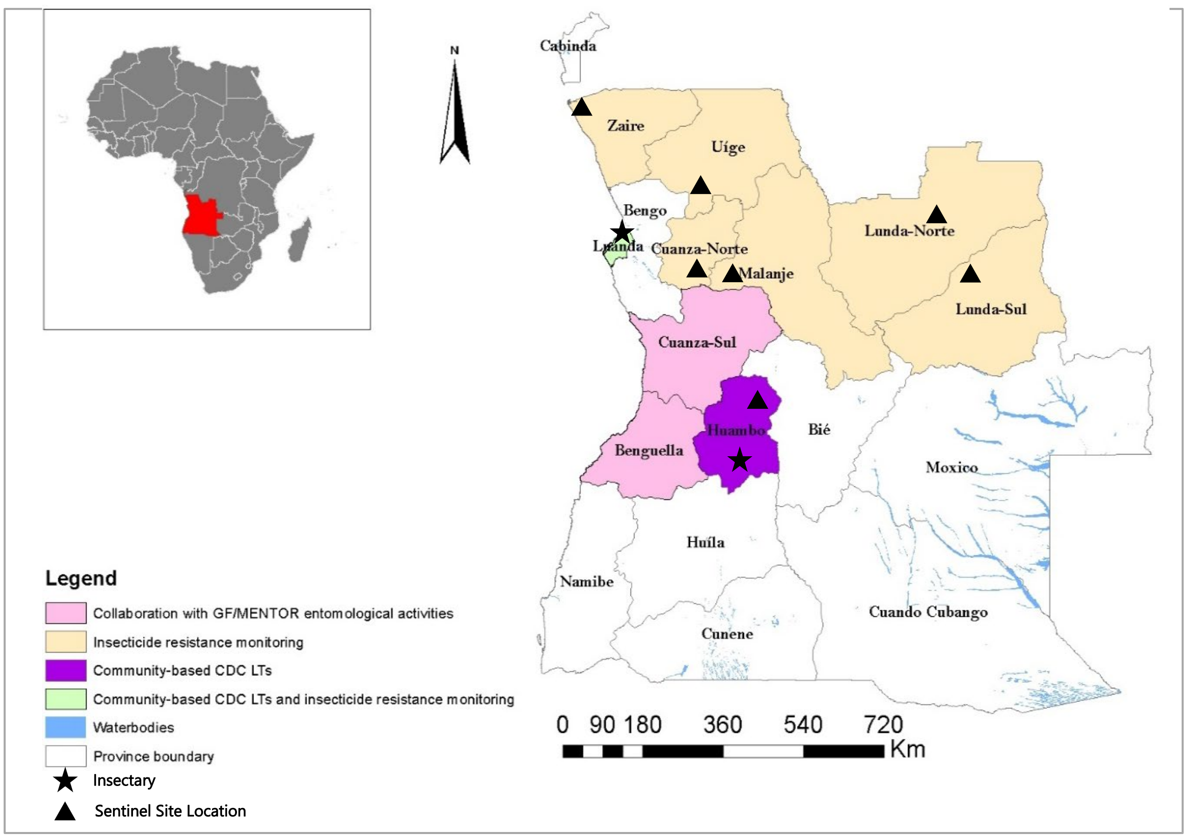
Details of VectorLink standard operating procedures used for WHO susceptibility tests, bottle bioassays, CDC light trap, and insectary rearing can be found online[8].

2.1 STUDY SITES

Between December 2021 and June 2022, insecticide resistance monitoring was conducted in sites across seven provinces—five PMI-focus provinces (Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire) and two provinces (Huambo and Luanda) chosen based on existing insectary infrastructure and entomology expertise. Insecticide susceptibility data was collected primarily to inform ITN procurement decisions made by the NMCP and partners. VectorLink Angola worked together with NMCP mosquito brigades in each province to collect insecticide susceptibility data during peak mosquito abundance months (December 2021 to June 2022) and conducted training of brigade staff as part of capacity development. Section 3 contains more training details.

Monthly vector bionomics monitoring was planned in three provinces (Figure 1 and Table 1). Monthly CDC LT collections continued in Huambo and started in Luanda in July 2021 using community-based surveillance, with regular support from VectorLink entomology technicians. In December 2021, the team piloted community-based surveillance in Lunda-Norte.

Figure 1: Map Showing Location of VectorLink Angola Sentinel Sites for Entomological Monitoring and Susceptibility Testing, and Partner Insectaries



[8] https://pmivectorlink.org/ resources/tools-and-innovations/.

The activities were conducted according to the PMI VectorLink Angola Year 3 work plan (Table 1) and the indicators covered are in line with the PMI technical guidance[9].

Table 1: Summary of PMI VectorLink Angola Entomological Activities (2021-2022)

Activity	Purpose	Sites	Timeline	Frequency	Status
Insecticide Susceptibility Testing	To determine vector susceptibility to three pyrethroid insecticides with and without the synergist PBO, and to chlorfenapyr.	Lunda-Sul, Cuanza-Norte, Luanda, Malanje, Huambo, Zaire and Uíge.	December 2021-June 2022	About two sites per month (during peak season).	Completed in six out of eight target provinces. No susceptibility data for Lunda-Norte. In Luanda, *An. azevedoi* was tested but this is not a targeted species or known malaria vector.
Community Ento Surveillance: Monthly species composition, vector density, human biting rate and EIR	To gather more detailed longitudinal information on malaria vector dynamics and behavior.	Huambo Province (Ngandarinha Village), Luanda Province (Mulundu Village), and Lunda-Norte Province (Camaquenzo I Village).	• May 2021-April 2022 in Huambo • July 2021-April 2022 in Luanda. • December 2021-April 2022 in Lunda-Norte	Trapping in a target of 60 houses per site per month	Completed in Huambo and Luanda provinces (Note that in Sept 2021 only 33/60 houses were completed in Luanda due to challenges with CDC LTs). Lunda-Norte piloted from Dec 2021-Apr 2022. All 60 houses were completed in only Feb and Mar 2022. See Section 3.4.
Molecular assays	To identify mosquito species of the *An. gambiae* s.l. and *An. funestus* s.l. species complex, and determine mechanisms of pyrethroid resistance (*kdr*), and *P. falciparum* sporozoite rates.	Cuanza-Norte, Huambo, Luanda, Lunda-Norte, Lunda-Sul, Malanje, Uíge, and Zaire.	July-September 2022	Throughout the year	Sample selection occurred for Year 2 samples and dissection of those samples have started and is ongoing. *A subsample of Y2 was tested at CDC

Note: *kdr*=knockdown resistance, PBO=piperonyl butoxide, PCR=polymerase chain reaction.

2.2 Indoor CDC LT (Community-Based Longitudinal Surveillance Pilot)

In November 2020, VectorLink Angola started a pilot of community-based entomological surveillance in Huambo Province. In 2021, in July and December, respectively, the team started to conduct similar pilots both in Luanda and Lunda-Norte provinces. For Huambo, Luanda and Lunda-Norte the project recruited a community member from Ngandarinha, Mulundu and Camaquenzo I Village, respectively, and after training, provided them with CDC LTs, batteries, solar panels for charging batteries, a magnifying glass, and other entomology supplies needed to preserve and store mosquito samples. The community collectors and local mosquito brigade staff received basic training on mosquito collection and basic morphological identification to genus level. VectorLink technicians collected GPS coordinates of 60 houses, labelled house doors, and subsequently the community collector surveyed three different houses per night located within walking distance of their own house using CDC LTs. The CDC LTs were hung indoors overnight, and collections were conducted for 20 nights per month for a maximum of 60 houses per month (same houses each month). Table 2 below shows the sampling frequency and timing of all indoor CDC LT collections.

VectorLink technicians provided close supervision and continuous training, traveling to the field regularly to reinforce the project standard operating procedures (SOPs) and ensure that data forms were fully completed, and mosquito samples were correctly stored and labelled (Figure 2). Community collectors separated mosquito specimens by genus and VectorLink technicians subsequently conducted mosquito species identification using the key of Coetzee 2020 [10]. Community collectors are being gradually trained so that they can carry out morphological species identification in the near future. When setting CDC LTs, the community collector also

[9] PMI Technical Guidance FY 2022

[10] Coetzee, M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malar J 19, 70 (2020)

gathered basic information through a questionnaire administered to the head of the household regarding the number of ITNs in the household, number of people that slept/did not sleep under a net the previous night, and physical inspection of ITN labels to record the brands of ITN present. Once collected and identified to species, mosquito samples were stored at -20°C in the freezer until molecular analyses, to take place at INIS in Luanda. Data was recorded on paper forms by the community collectors before being updated by VectorLink technicians after mosquito identification and sent to the data clerk for data entry in VectorLink Collect.

Table 2: Summary of Collection Methods

Collection method	Time	Frequency	Sample
Indoor CDC LT	6:00 p.m. to 6:00 a.m.	3 houses per day, 20 days a month	60 houses per month
Larval collections	9:00 a.m. to 2:00 p.m.	Daily	As many larvae as possible from several locations

Figure 2: Photograph of the Solar Panel Charging System for CDC LTs in Mulundu Village, Huambo Province (A), Community Collector Demonstrating Retrieval of Mosquitoes from a CDC LT as VectorLink Technician looks on (B), *Anopheles* Mosquitoes from CDC LT Stored Individually for Molecular Analysis (C)

2.3 LARVAL COLLECTIONS

From December 2021 to June 2022, VectorLink conducted susceptibility testing in six provinces (testing was not possible in Luanda and Lunda-Norte provinces; see Section 2.4). Larval collections were conducted daily by the provincial mosquito brigades in close coordination with a Senior VectorLink Entomology Technician in Cuanza-Norte, Luanda, Lunda-Sul, Malanje, Uíge, and Zaire (Figure 3). In Huambo, larval collections were conducted daily by three VectorLink Entomology Technicians. A sub-sample of *Anopheles* mosquitoes reared from larvae were identified morphologically (Coetzee, 2020) before testing to try and ensure the target vector species were used. After completion of insecticide testing, all mosquitoes were killed and morphologically identified to corroborate species.

2.4 Insecticide Susceptibility Testing, Synergist Assays, and CDC Bottle Bioassay Testing

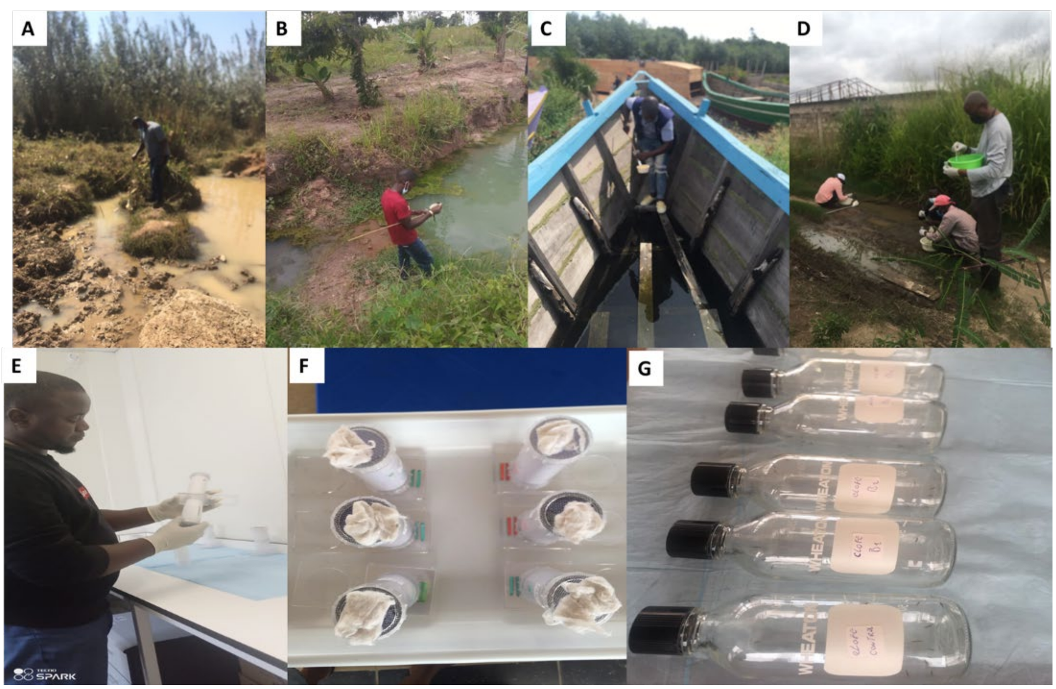
Insecticide susceptibility and synergist bioassay were conducted in the six study sites (Cuanza-Norte, Huambo, Lunda-Sul, Malanje, Uíge, and Zaire) by Senior VectorLink Entomology Technicians.

Insecticides tested in 2021-2022 (in order of priority):

- Deltamethrin 0.05% with and without pre-exposure to PBO 4%
- Alpha-cypermethrin 0.05% with and without pre-exposure to PBO 4%
- Chlorfenapyr 100 µg/bottle (tested in CDC bottle bioassays)
- Permethrin 0.75% with and without pre-exposure to PBO 4%

Mosquitoes were collected from the field as larvae and reared to adults before conducting susceptibility testing with adult *An. gambiae* s.l. following VectorLink SOPs that are based on WHO procedures. Figure 3 shows larval collection sites in some provinces and performance of susceptibility assays.

Figure 3: Photographs of *An. funestus* s.l. and *An. gambiae* s.l. Larval Collections (A, B, C, D), WHO Susceptibility Tests (E), Holding Tested Mosquitoes for 24 Hours at Controlled Temperature and Humidity (F), CDC Bottle Assays (G)



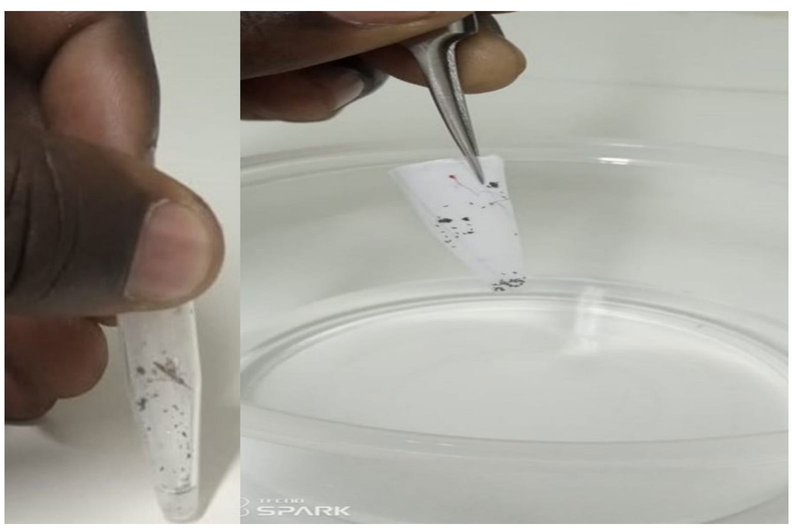
WHO susceptibility tests were conducted for deltamethrin, alpha-cypermethrin, and permethrin with and without pre-exposure to 4% PBO papers for 60 minutes followed by transfer to insecticide-treated papers for 60 minutes. All insecticide-treated filter papers were purchased at the diagnostic concentration from the WHO collaborating center, Universiti Sains Malaysia. Exposure tests were always accompanied by negative control tests in which mosquitoes were exposed to filter papers impregnated with silicone oil, with additional control tests of pre-exposure to 4% PBO papers followed by silicone oil papers for synergist tests. Mortality at 24 hours post-exposure was the primary outcome measure. Four replicates of 25 *An. gambiae* s.l. were exposed to each insecticide.

CDC bottle bioassays were completed in six sites (Cuanza-Norte, Huambo, Lunda-Sul, Malanje, Uíge, and Zaire) to determine the susceptibility status of *An. gambiae* s.l. to chlorfenapyr using a diagnostic dose of 100 μg/bottle. Four replicates of 20-25 female *An. gambiae* s.l. were exposed for 60 minutes to chlorfenapyr 100 μg/bottle. After exposure period, the mosquitoes were removed from the bottle, transferred to paper cups, and supplied with a sugar solution. Mortality was recorded every 24 hours for 72 hours total. After completion of mortality recording, mosquitoes were stored individually in Eppendorf tubes in a freezer for future molecular analysis. Each tube was labelled with key information such as date of test, site, mosquito species, insecticide tested and whether the mosquito was susceptible or resistant. Eppendorf tubes were placed in Ziploc bags and labelled according to the insecticide tested and site.

Despite the team's various efforts, insecticide resistance testing was not able to be completed in Luanda with the priority vector species in Year 3. One of the key challenges in this province was the difficulty in finding permanent *An. gambiae* s.l. breeding sites. Larvae found at breeding sites previously mapped as *An. gambiae* s.l. were found to be *An. azevedoi/listeri* (mosquito not known yet as malaria vector but further research to understand its role is needed).

In Luanda Province, where the major vector species is *An. funestus* s.l., Prokopack aspiration was done in April and May 2022 to collect adult mosquitoes for forced oviposition, followed by rearing of offspring for susceptibility tests. Prokopack aspirations were conducted early in the morning by sweeping the aspirator close to the wall, ceiling, and furniture for approximately 15 minutes per house (depending on size of the house) to collect resting mosquitoes. Blood-fed *An. funestus* adults were then taken back to the Luanda insectary and, after three days placed into individual Eppendorf tubes lined with a small piece of moist filter paper for forced oviposition (Figure 4). Eggs were subsequently washed into larval pans and larvae reared to adults. Larvae were fed with a combination of Tetramin tropical fish food and pond algae (based on feedback from University of the Witwatersrand). Although, some adults did emerge, they were not enough for testing.

Figure 4: Photograph of *An. funestus* s.l. Eggs Produced by Forced Oviposition on Filter Paper in an Eppendorf Tube



Despite great effort over several months, susceptibility testing was also not possible in Lunda-Norte Province. The team could not find enough *An. gambiae* s.l. larvae despite searches across a wide area and at different seasonal time points in the year (rainy season and dry season) to complete any tests.

2.5 LABORATORY ANALYSIS

Molecular analysis was conducted using samples collected in Year 1 from Huambo and Lunda-Sul provinces for training and optimization of approved molecular analysis protocols for malaria vectors through a STTA from a Senior CDC Entomologist who visited Angola in March 2022. Dissection of samples collected from the community-based surveillance in Huambo Province in Year 2 has started and molecular analysis is intended to begin in August 2022. In addition, samples collected from seven provinces for insecticide susceptibility testing will be included in molecular analysis scheduled to begin in August 2022. An amendment of this Annual Report with Year 2 results is expected in late 2022. A pre-determined number of mosquito samples from monthly collections and from susceptibility tests will be tested by PCR to determine species composition (see Table 3). The proportion of *An. gambiae* s.l. and *An. funestus* s.l. tested will vary by site depending on species composition.

Table 3: Minimum Number of Samples for Laboratory Analysis at INIS, Luanda

Sentinel Site	WHO Pyrethroid Susceptibility Test Species ID	WHO Pyrethroid Susceptibility *kdr* Frequency (L1014F/L)	CDC LT Species ID	CDC LT Sporozoite ELISA	TOTAL
			Year 1 Samples		
Huambo	n/a	n/a	144	218	362
Lunda-Sul	100	50	172	634	956
Subtotal Year 1	**100**	**50**	**316**	**852**	**1,318**
			Year 2 Samples		
Cuanza-Norte	100	50	n/a	n/a	150
Huambo	n/a	n/a	270 (All *An. gambiae* s.l. collected in Y2 + up to 45 *An. funestus* per month)	720	990
Luanda*	n/a	n/a	n/a	n/a	0
Lunda-Sul	100	50	n/a	n/a	150
Malanje	100	50	n/a	n/a	150
Uíge	100	50	n/a	n/a	150
Zaire	100	50	n/a	n/a	150
Subtotal Year 2	**500**	**250**	**270**	**720**	**1,740**
Total Year 1 + Year 2	**600**	**300**	**586**	**1,572**	**3,058**

*Samples collected in Luanda were identified as *An. azevedoi* and therefore will not undergo lab analysis.

2.6 DATA MANAGEMENT AND ANALYSIS

The DHIS2-based VectorLink Collect instance for entomological data management has been used in Angola since 2020. VectorLink Monitoring and Evaluation Specialists trained and supported VectorLink Angola entomologists, technicians, and data clerks remotely on updated data workflows—including field paper collections, technical reviews, data entry, data cleaning, and analytics—to support the generation and use of high-quality entomological data. All entomological data collected in Angola in this reporting period was managed within VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. In 2022/2023, the NMCP, INIS, and PMI will have ongoing access to these results dashboards to support timely decision-making.

The following formula was used to calculate entomological indicators:

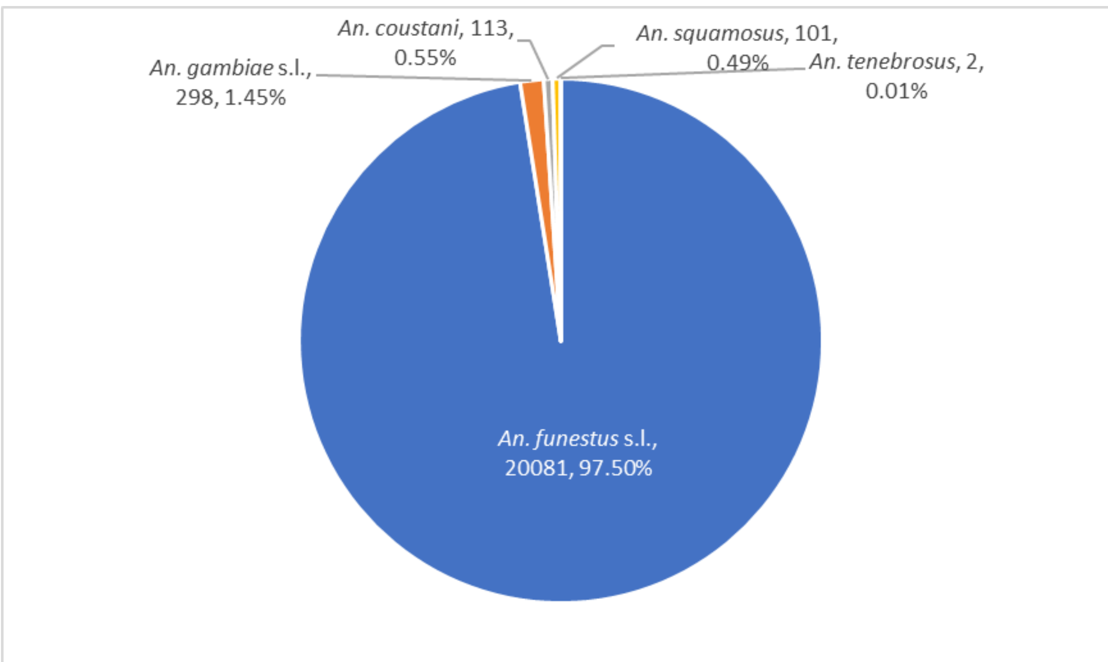
- Vector density (number/trap/night) = total number of *Anopheles* species collected by CDC LT during a specific period / total number of trap-nights.

3. Results

3.1 Malaria Vector Species Composition in Huambo and Luanda

From May 2021 to April 2022, a total of 20,595 *Anopheles* mosquitoes were collected using indoor CDC-LT in two sentinel sites, consisting of 96.7% *An. funestus* s.l., 2.2% *An. gambiae* s.l., 0.5% *An. coustani*, and 0.5% *An. squamosus* (Fig. 5).

Figure 5: Total Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Huambo and Luanda Sentinel Sites (May 2021–April 2022)



Anopheles funestus s.l. were more abundant than *An. gambiae* s.l. in both Huambo (89.0% versus 4.2%) and Luanda (99.0% versus 1.0%) (Figures 6 and 7). Other species identified in Huambo were *An. coustani* and *An. squamosus* and in Luanda *An. coustani* and *An. tenebrosus*.

Figure 6: Total Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Ngandarinha, Huambo Province (May 2021-April 2022)

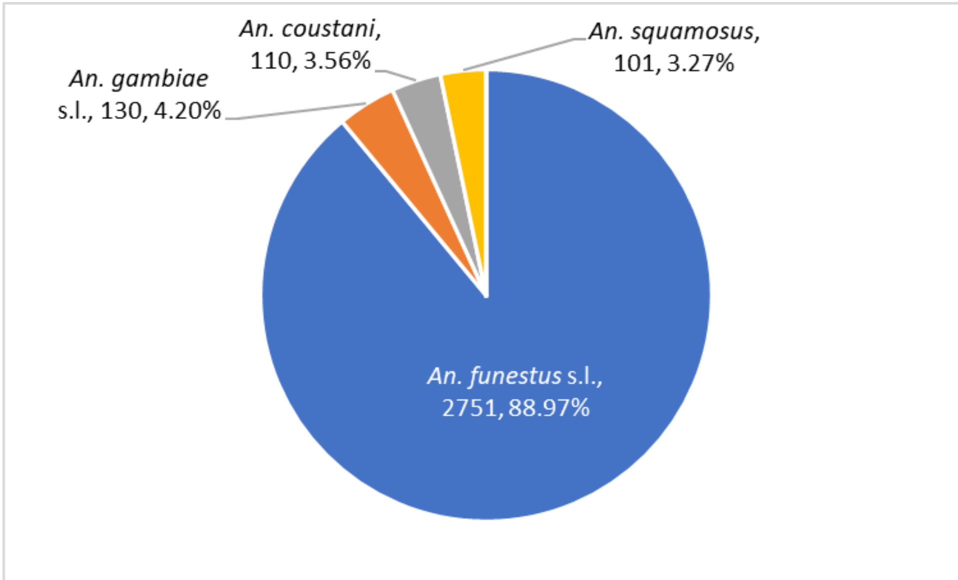
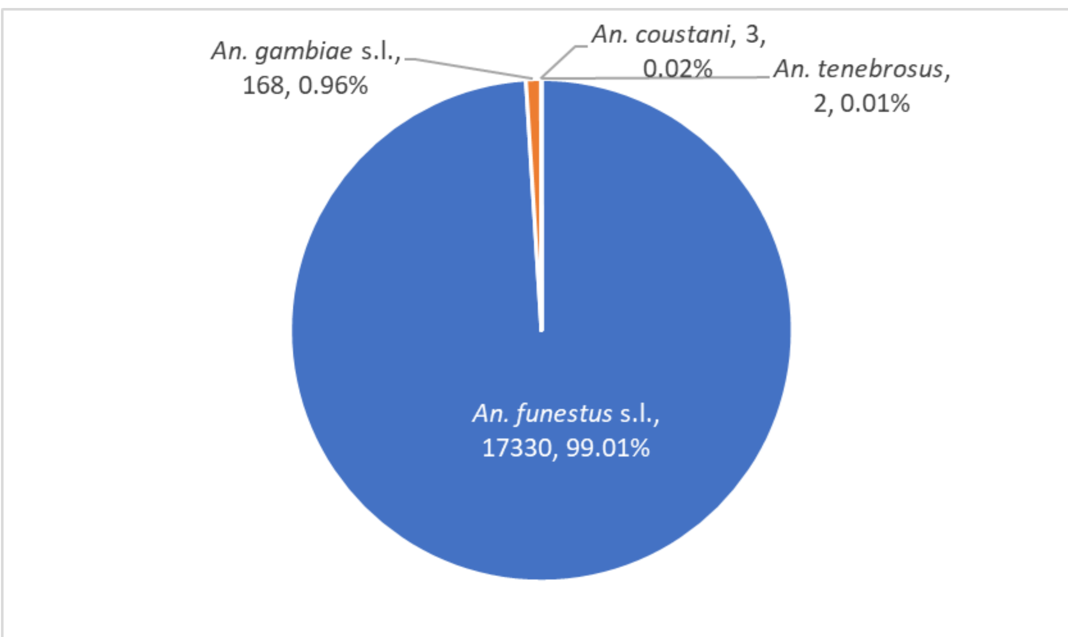


Figure 7: Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Mulundu Village, Luanda Province (July 2021-April 2022)



3.2 Malaria Vector Density (by Indoor CDC LT)

Maximum collection efforts were attained in Huambo, a total of 720, that is 60 trap collections per month for 12 months during the period May 2021 to April 2022. There were 573 out of a maximum 600 collections in Luanda which translates to 95.5% collection effort. Luanda did not attain 100% collection effort because of maintenance issues with the CDC LTs in September 2021, which were subsequently resolved. The total numbers of vectors collected during each monthly collection effort by site during the reporting period are provided in Annex A.

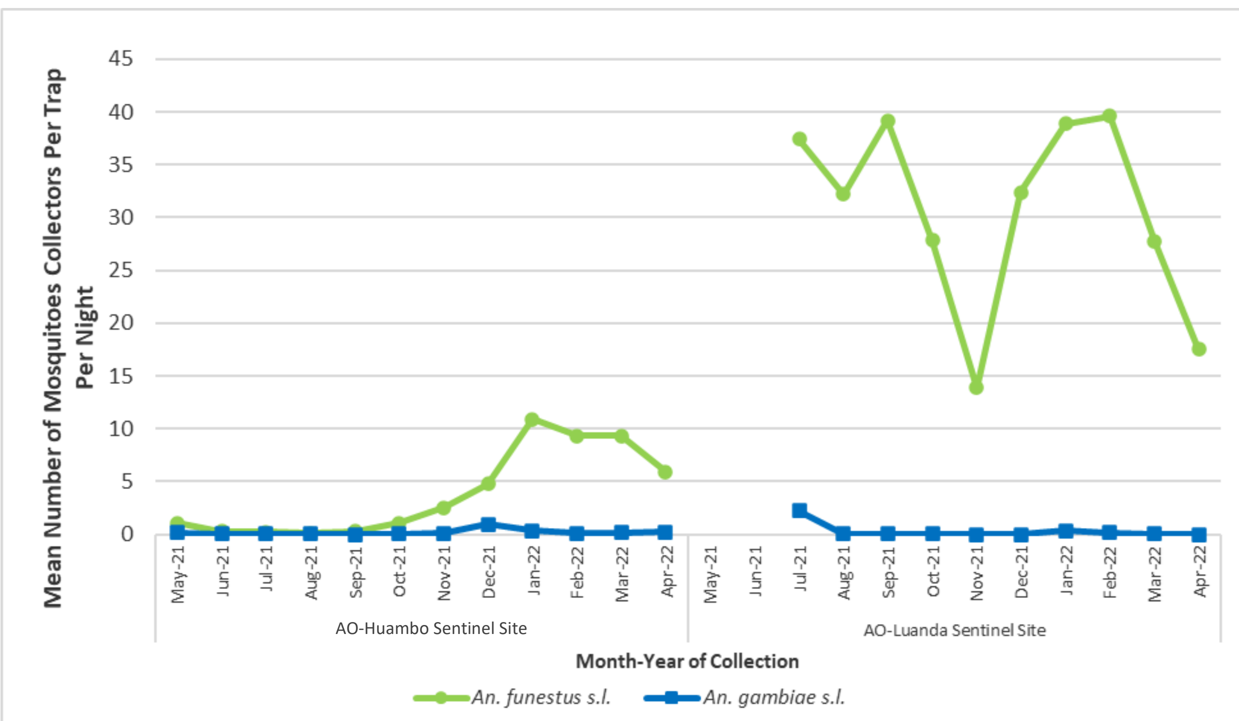
Figure 8 shows the density (mean number of primary malaria vector species *An. funestus* s.l. and *An. gambiae* s.l.) collected per trap per night in Ngandarinha Village, Huambo Province and Mulundu Village, Luanda Province between May 2021 to April 2022. Overall, *An. funestus* s.l. was the malaria vector species with the highest density at the site in Huambo (3.8 versus 0.2 vectors/trap/night) and Luanda (30.2 versus 0.3 vectors/trap/night). The total number of *An. funestus* s.l. collected in Luanda was about 6 times more than that collected in Huambo and the overall density (number/trap/night) in Luanda was about 8 times higher in density in Huambo.

In Huambo, monthly *An. funestus* s.l. densities were between 0-1 vector/trap/night from May to October 2021 with a rise in density between November 2021 and April 2022, reaching a peak of 10.9 vectors/trap/night in January 2022. This may be linked to high rainfall during December 2021 and January 2022. Monthly rainfall and the number of rainy days for Huambo and Luanda are provided in Figures 9 and 10 respectively.

In Luanda, monthly densities of *An. funestus* s.l. were above 10 vectors/trap/night throughout the collection period with a peak density of 39.6 vectors/trap/night in February 2022. This was followed by a decrease in mean numbers collected to 17.5 *An. funestus* s.l. per trap per night in April 2022. The lowest monthly density (13.9) was in November 2021.

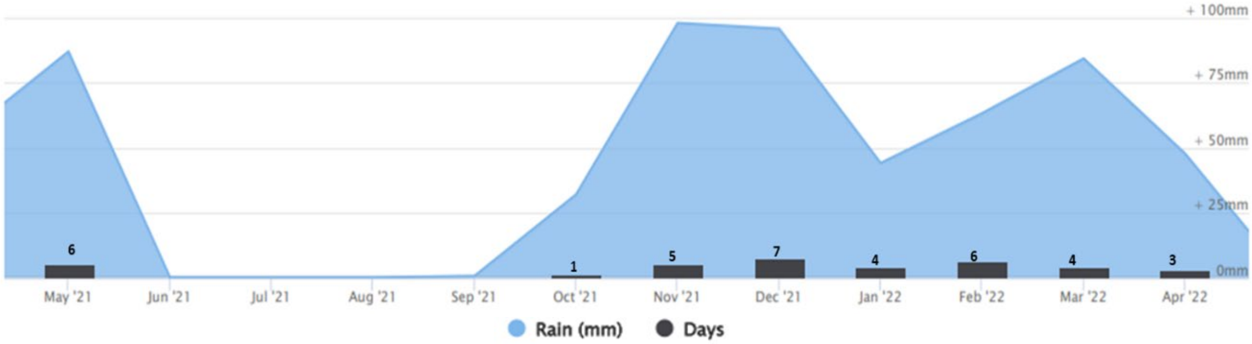
Anopheles gambiae s.l. monthly densities were generally low in both provinces (0.0 to 2.2 vectors/trap/night), with the highest density of 2.2 vectors occurring in July 2021 in Luanda Province. Peak monthly density in Huambo occurred in December 2021 (1.0 vectors/trap/night).

Figure 8: Mean Number of *An. funestus* s.l. and *An. gambiae* s.l. Collected per Night by Indoor CDC LT in Ngandarinha Village (Huambo Province) and Mulundu Village (Luanda Province) (May 2021-April 2022)



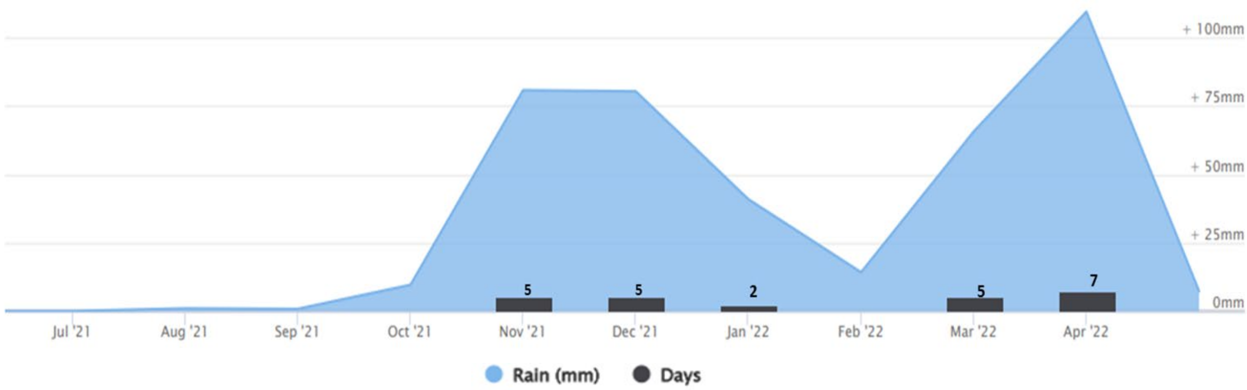
Note: Maximum of 60 CDC LT were used per month.

Figure 9: Rainfall (mm) and Number of Rain Days per Month in Huambo Province (May 2021-April 2022)



Source: https://www.worldweatheronline.com/huambo-weather-averages/huambo/ao.aspx

Figure 10: Rainfall (mm) and Number of Rain Days per Month in Luanda Province (July 2021-April 2022)



Source: https://www.worldweatheronline.com/luanda-weather/cuanza-norte/ao.aspx

3.3 Abdominal Stage of Collected Mosquitoes from May 2021-April 2022

Most vectors collected by indoor CDC LTs from May to December 2021 in Huambo and Luanda were unfed: 90.3% *An. funestus* s.l. and 95.7% *An. gambiae* s.l. A small percentage were either gravid or half gravid 1.3 % *An. funestus* s.l. and 0.7% *An. gambiae* s.l. (Figures 11 and 12). In October 2021, abdominal stage proportions for *An. gambiae* s.l. across sites was 50% fed and 50% unfed (one unfed from Luanda and one fed from Huambo). This was different from trends observed in previous collections and months where the majority of *An. gambiae* s.l. were unfed, indicating possible higher biting frequency in this month. However, generalizations cannot be made given the low sample size (n=2). The origin of blood meal will be identified by PCR once molecular capacity is established in country (see Section 3.2 below).

Figure 11: Proportion of *An. funestus* s.l. Mosquitoes by Abdominal Stage Collected by Indoor CDC LT In Huambo and Luanda Province (May 2021-April 2022)

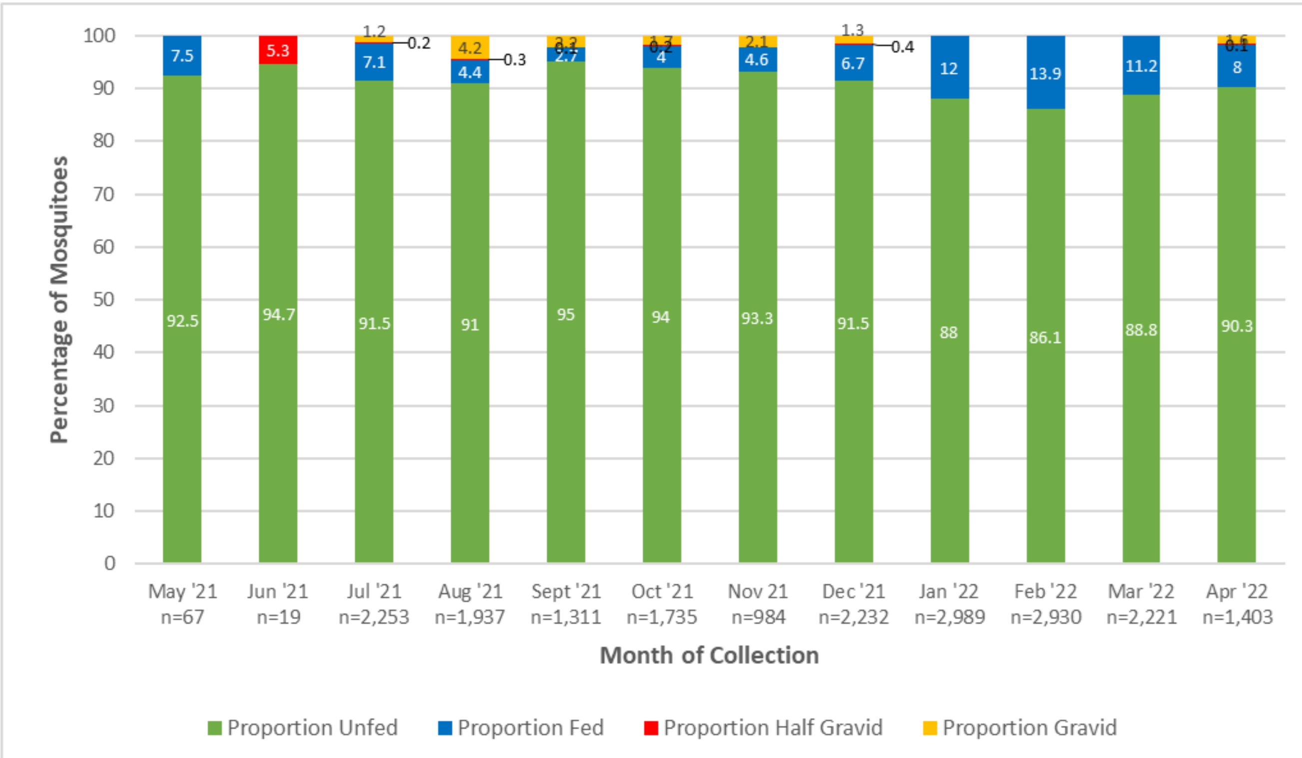
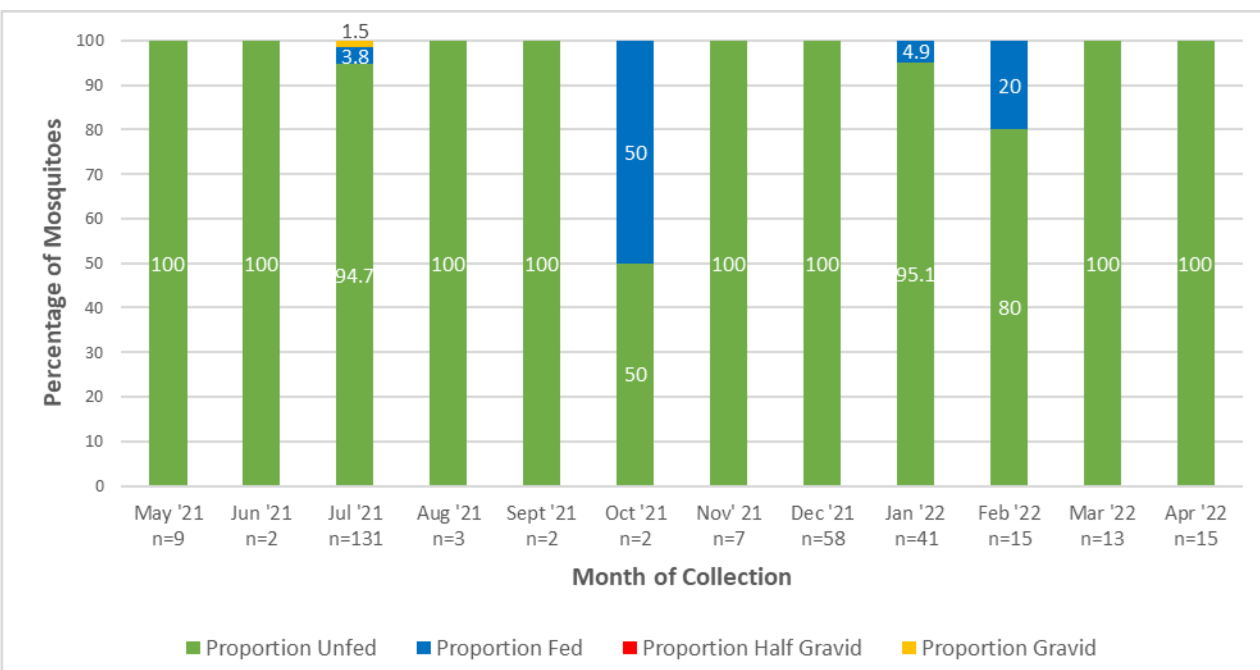


Figure 12: Proportion of *An. gambiae* s.l. Mosquitoes by Abdominal Stage Collected by Indoor CDC LT in Huambo and Luanda Provinces (May 2021-April 2022)

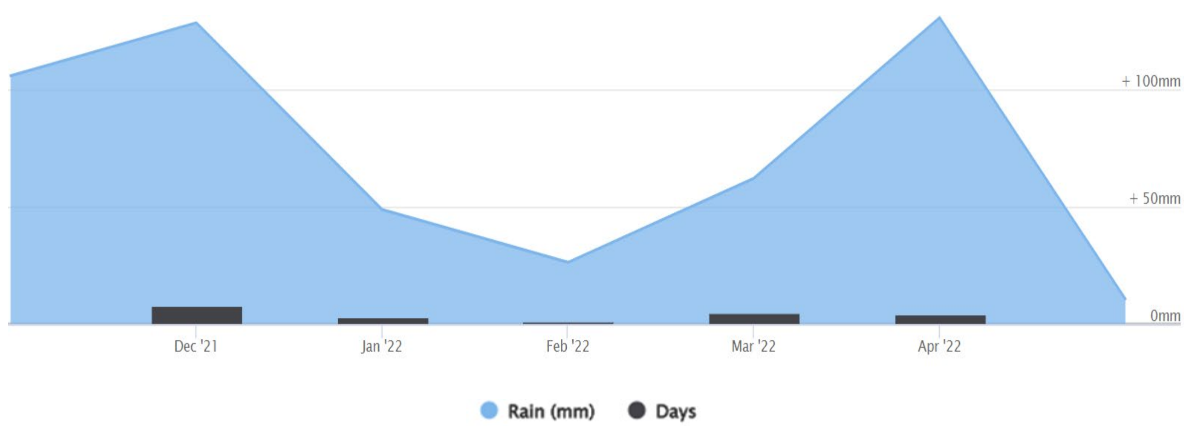


3.4 COMMUNITY-BASED ENTOMOLOGICAL MONITORING PILOT IN LUNDA NORTE

3.4.1 BACKGROUND

Lunda Norte Province is located in the northern part of the country and shares a large border with the Democratic Republic of the Congo. Figure 13 shows the amount of rainfall and number of rain days in this province. Based on the high malaria burden in this province (14.9%) and hyperendemicity of transmission, the NMCP requested entomological monitoring be conducted in this province. The PMI VectorLink team began piloting community-based surveillance there starting in December 2021, after identifying potential sites, community collector, and conducting a training. After concluding the training, the team encountered significant challenges, including cultural myths and reluctance to accept to new approaches for data collection from both community collector and the community where team was collecting data. The team also faced significant logistical challenges in conducting in-person supervision, given a pause in interprovincial flights due to COVID which meant the site was only accessible by a two-day trip from Luanda via car. The VectorLink Entomology Coordinator and Technician returned at the end of January 2022 to evaluate the issues, re-train the community collector, and provide additional sensitization to the community about the purpose of the activity and its importance for gathering data to inform for malaria control. The team also worked closely with the DPS, who were supportive in mitigating some of the challenges. Ultimately, the team was able to conduct two months of complete collections (60 houses per month) and three months of partial collections (see Annex A for the specific number) between December 2021 and April 2022.

Figure 13: Rainfall (mm) and Number of Rain Days per Month in Lunda Norte Province (December 2021-April 2022)

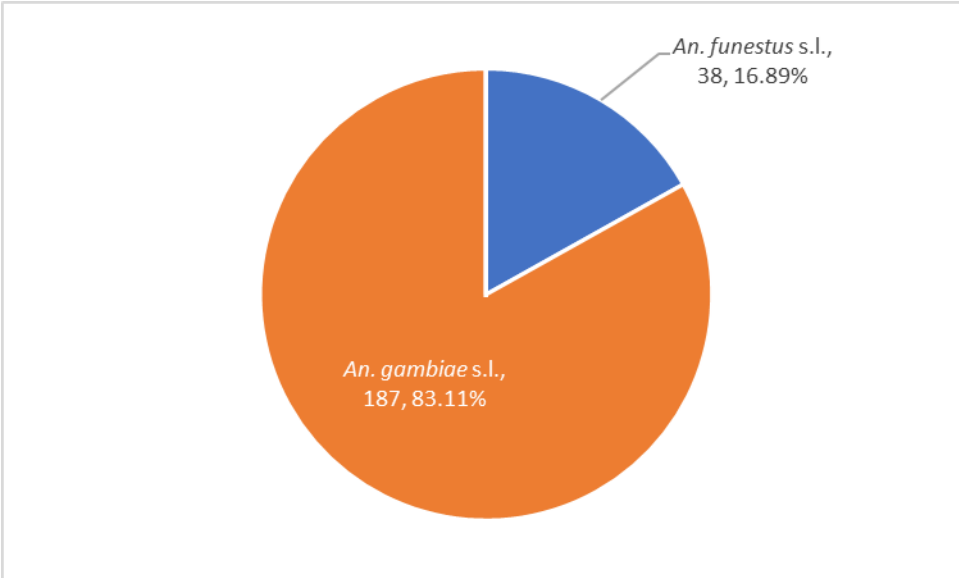


Source: https://www.worldweatheronline.com/lunda-weather/lunda-norte/ao.aspx

3.4.2 SPECIES COMPOSITION IN LUNDA NORTE

In Lunda Norte, only two *Anopheles* species were identified during the reporting period; *An. gambiae* s.l. which was the most abundant at 83.0% and *An. funestus* s.l. at 17.0% (Figure 14).

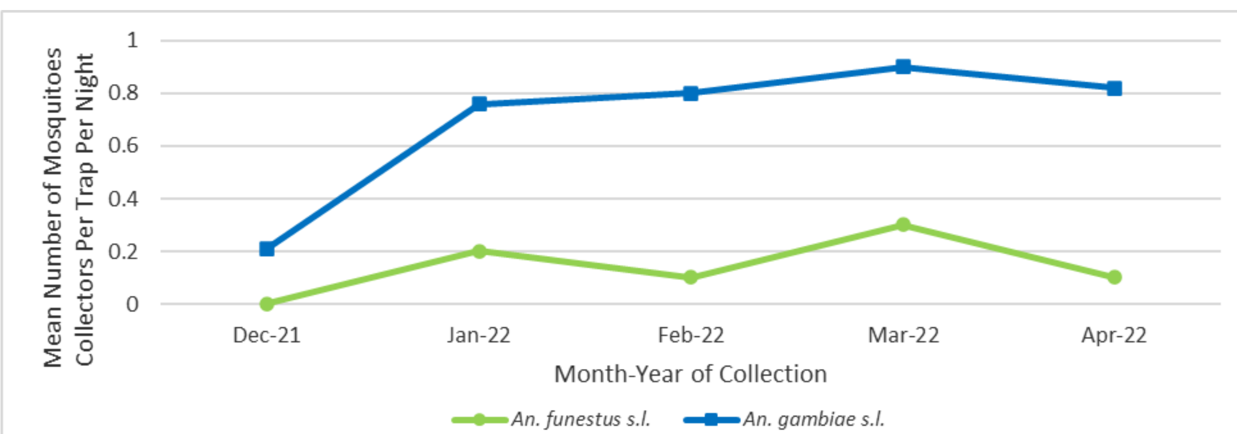
Figure 14: Species Composition of *Anopheles* Mosquitoes Collected by Indoor CDC LTs in Camaquenzo I Village, Lunda Norte Province (December 2021-April 2022)



3.4.3 Malaria Vector Density in Lunda Norte

A total of 225 *Anopheles* vectors were collected from Lunda Norte from 259 collections out of a target of 300 collections for the period December 2021 to April 2022 (86% collection effort) (see Annex A for details of number of collections and numbers of vectors collected per month). Vector density in Lunda Norte was low during the pilot community-based vector surveillance period (December 2021-April 2022) with monthly densities below 1 vector/trap/night throughout the period. *Anopheles gambiae* s.l. density was higher than *An. funestus* s.l. ranging between 0.2-0.9 vector/trap/night versus 0-0.3 vector/trap/night for *An. funestus* s.l. The highest vector density for each species was observed in March.

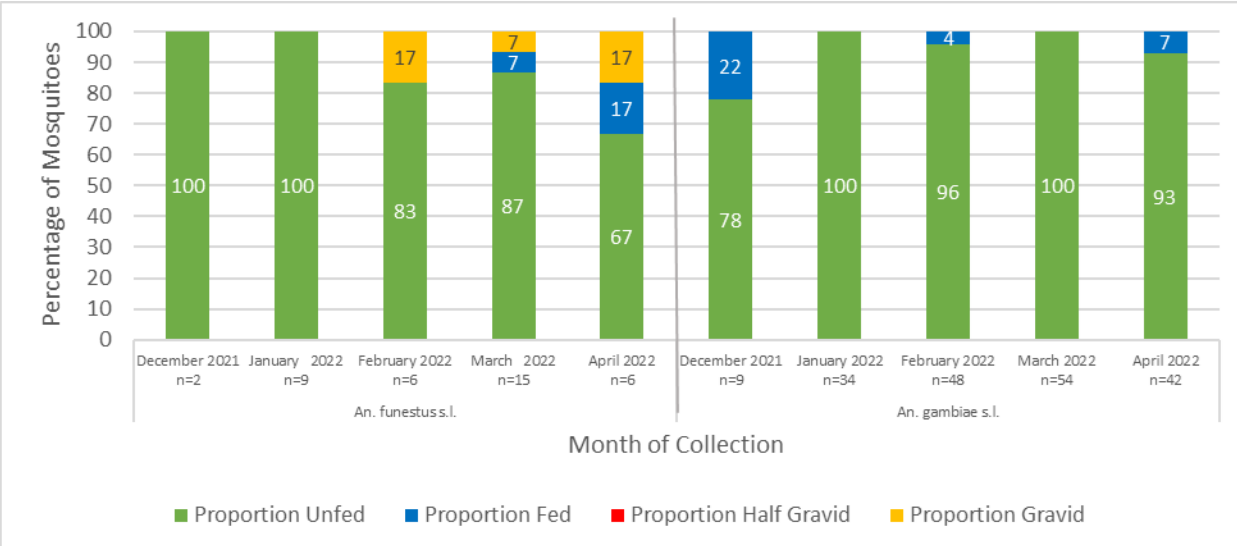
Figure 15: Mean Number of *An. funestus* s.l. and *An. gambiae* s.l. Collected per Night by Indoor CDC LT in Camaquenzo I Village, Lunda-Norte Province (December 2021-April 2022)



3.4.4 Abdominal Stage in Lunda Norte

Most vectors collected by indoor CDC LTs in Lunda Norte were unfed (86.8% *An. funestus* and 96.3% *An. gambiae* s.l.). A small percentage of *An. funestus* s.l. were either fed (5.3%) or gravid (7.9%), while for *An. gambiae* the remainder of the vectors were fed (3.7%). The gravid *An. funestus* s.l. vectors were collected between February 2022-April 2022.

Figure 1616: Proportion of *An. funestus* s.l. and *An. gambiae* s.l. Mosquitoes by Abdominal Stage Collected by Indoor CDC LT in Lunda-Norte Province (December 2021-April 2022)

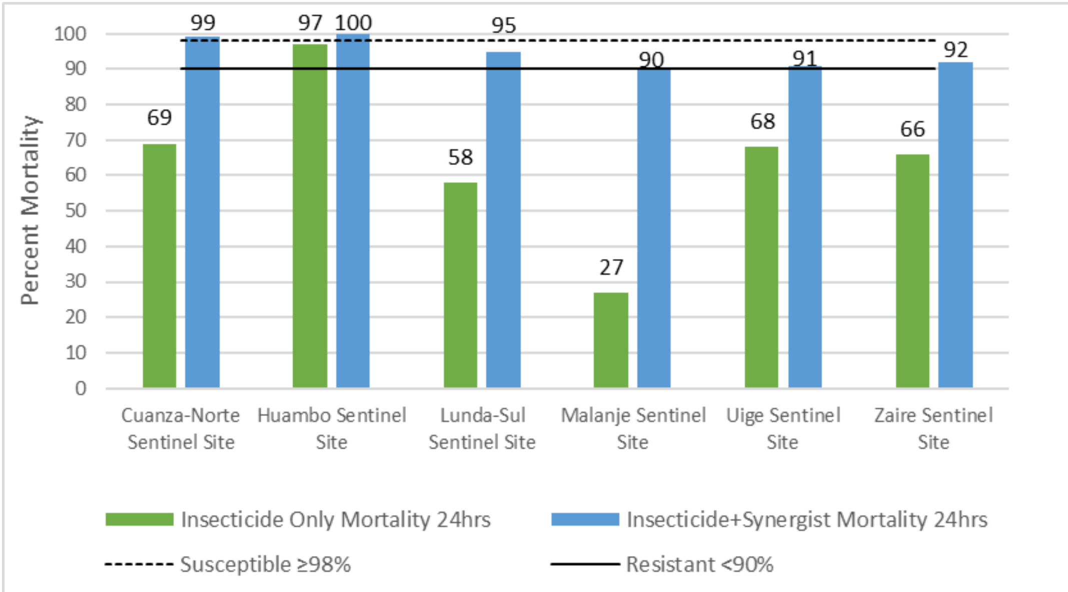


3.5 Insecticide Susceptibility, PBO Synergist, and CDC Bottle Assay Results

Insecticide susceptibility testing was conducted with *An. gambiae* s.l. that were collected as larvae in six sites Cuanza-Norte, Huambo, Lunda-Sul, Malanje, Uíge, and Zaire. VectorLink Angola Senior Entomology Technicians conducted insecticide susceptibility testing with at least two pyrethroid insecticides with and without PBO synergist pre-exposure in each site. Insecticide resistance status was based on the standard WHO criteria: <90% mortality (confirmed resistance), 90-97% mortality (probable resistance), and ≥98% mortality (susceptible).

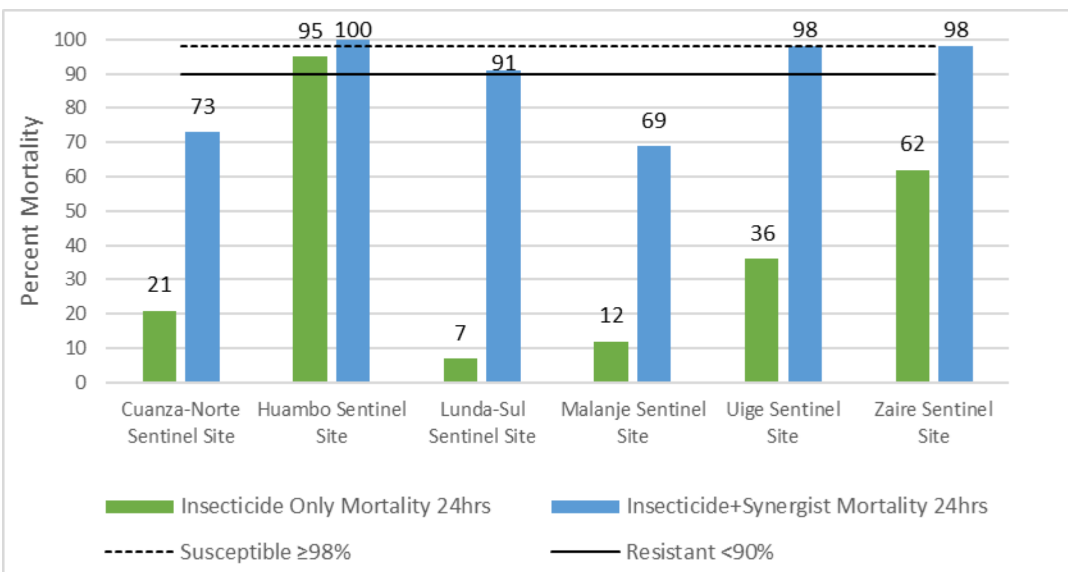
Deltamethrin (0.05%) was tested in all six sites and there was confirmed resistance in five out of six sites (Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire) with mortality ranging from 27% in Malanje to 69% in Cuanza-Norte (Figure 17). There was probable resistance in Huambo with mortality of 97%. Pre-exposure to PBO followed by deltamethrin exposure resulted in susceptibility being fully restored in Huambo and Cuanza-Norte provinces and in large increases in absolute mortality in the other provinces, though susceptibility wasn't fully restored. Alpha-cypermethrin was tested in all six sites and produced a similar trend, with confirmed resistance in five sites (Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire) mortality ranging from 7% in Lunda-Sul to 62% in Zaire). PBO pre-exposure resulted in full restoration of susceptibility in Huambo, Uíge and Zaire, partial restoration in Lunda-Sul , and absolute mortality increased, but susceptibility not restored in Cuanza-Norte and Malanje (Figure 18). Permethrin susceptibility tests were performed in three provinces (Huambo, Malanje and Uíge). Permethrin resistance was observed in Malanje (mortality of 4%) and Uíge (mortality of 20%) while there was full susceptibility in Huambo. PBO pre-exposure resulted in increases in absolute mortality, but not restoration of susceptibility in Malanje and Uige (Figure 19).

Figure 17: Percentage Mortality of *An. gambiae* s.l. in WHO Tube Tests with Deltamethrin (0.05%) and PBO (4%) + Deltamethrin (0.05%)



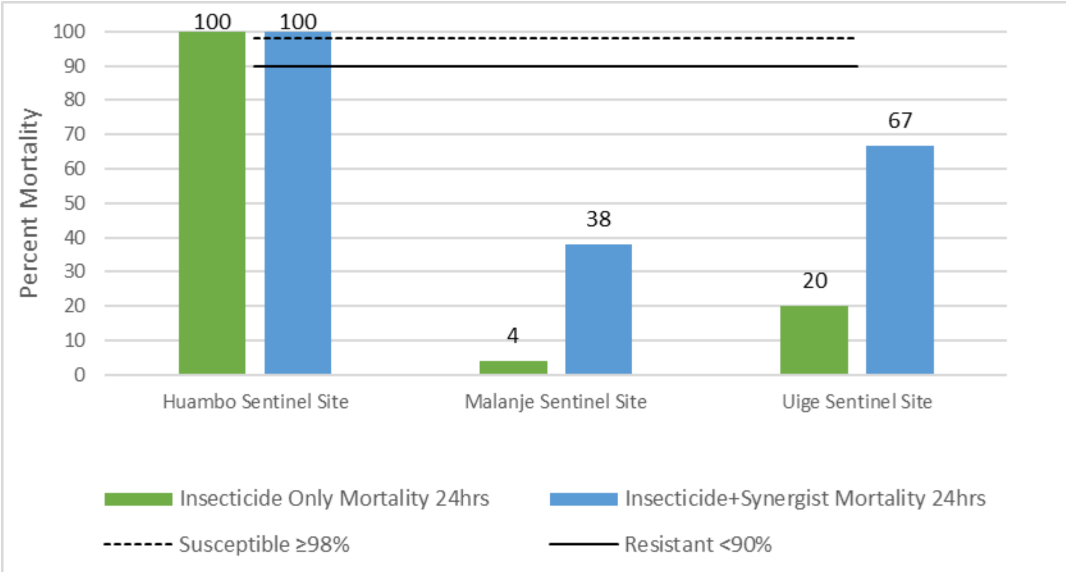
Note: Approximately 100 mosquitoes were tested per insecticide.

Figure 18: Percentage Mortality of *An. gambiae* s.l. in WHO Tube Tests with Alpha-cypermethrin (0.05%) and PBO (4%) + Alpha-cypermethrin (0.05%)



Note: Approximately 100 mosquitoes were tested per insecticide.

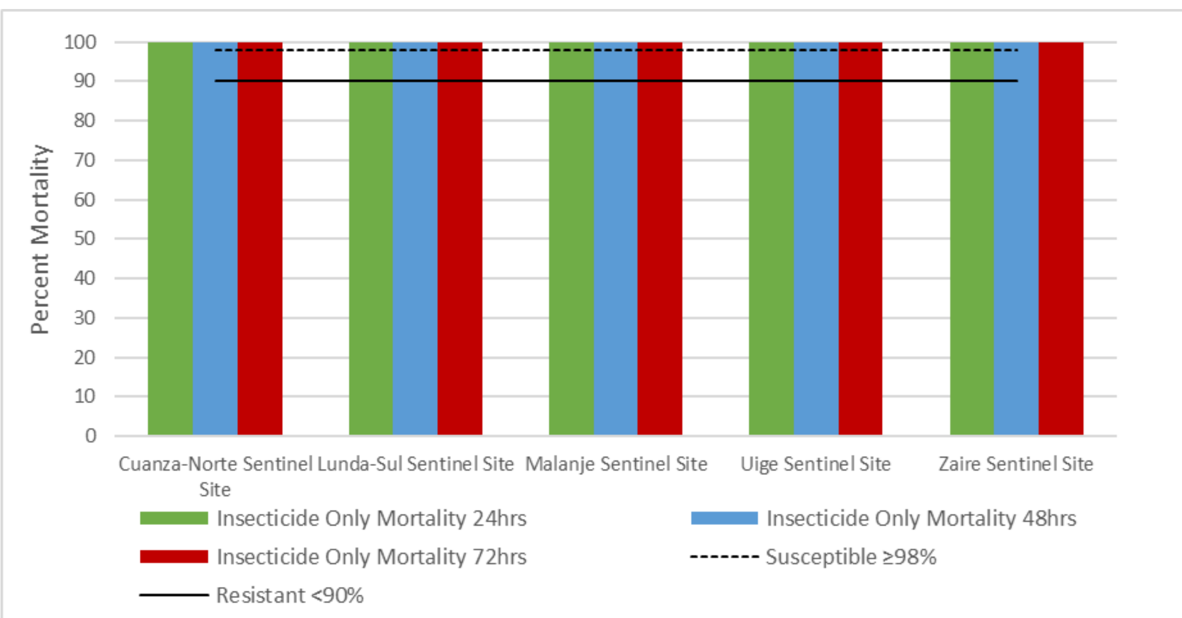
Figure 19: Percentage Mortality of *An. gambiae* s.l. in WHO Tube Tests with Permethrin (0.75%) and PBO (4%) + Permethrin (0.75%)



Note: Approximately 50-100 mosquitoes were tested per insecticide.

CDC bottle bioassays using the PMI VectorLink recommended dose of 100 µg/bottle for chlorfenapyr produced 100% mortality in the five sites tested (Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire) within 24 hours of exposure (Figure 20). This indicates that *An. gambiae* s.l. from Cuanza-Norte, Lunda-Sul, Malanje, Uíge, and Zaire are fully susceptible to chlorfenapyr.

Figure 20: Percentage Mortality of *An. gambiae* s.l. in Bottle Bioassays with Chlorfenapyr (100 µg/bottle) 24, 48, and 72 Hours after Exposure



Note: Approximately 100 mosquitoes were tested per insecticide.

Note that no susceptibility data are presented for Lunda-Norte and Luanda as it was not possible to collect sufficient mosquitoes for testing due to reasons stated in Section 2.4.

4. Capacity Building and Sustainability

The VectorLink Angola Entomology Coordinator and VectorLink Entomology Technicians continued to work closely with the NMCP, INSP/INIS, DPS (including mosquito brigades), and other in-country partners to strengthen entomological capacity. In Year 3, the project focused on building basic provincial capacity related to insecticide susceptibility testing and larval search and monitoring.

4.1 Provincial-Level Entomological Trainings

The VectorLink Senior Entomology Technicians conducted on-site, hands-on training for three mosquito brigade staff in each of six provinces prior to insecticide susceptibility testing (from December 2021-June 2022). NMCP staff participated and did supervision during field work in Cuanza-Norte, Huambo, and Uíge provinces. The training focused on methods for collection of mosquito larvae and adults, including collection of geolocation data for each site using GPS, basic morphological identification to genus level, insecticide susceptibility tests, and proper sample handling and storage. This helped to ensure the quality of work in the field through adherence to standard protocols and built provincial capacity for long-term sustainability. Mosquito brigade staff did not conduct susceptibility bioassays, but they observed susceptibility bioassays being conducted by VectorLink Senior Entomology Technicians. At the end of the training, all mosquito brigade attendees were able to collect *Anopheles* larvae, recognize key characteristics of *An. funestus* s.l. and *An. gambiae* s.l. adults, and were familiar with project SOPs for susceptibility testing. Since 2019, the project has trained over 30 mosquito brigade members, 28 of which were still engaged with project activities this year.

4.2 VectorLink Regional Entomology Training

VectorLink Angola was expected to support one participant from the NMCP or VectorLink Angola staff to attend a six-day regional entomology training in Dakar, Senegal. The emphasis for this training was to build capacity of NMCPs in entomology best practices. Due to COVID-19, this training was shifted to August 2022.

4.3 Short-Term Technical Assistance (STTA)

One STTA trip occurred during the reporting period to provide support to the in-country team. In March 2022, a CDC Senior Entomologist traveled to Angola to provide technical assistance in the form of a two-week training of VectorLink and INIS staff to establish laboratory work flow, laboratory inventory maintenance, molecular database management processes, and molecular protocol training activities.

4.4 VectorLink Regional Laboratory Training

VectorLink Angola planned to support one participant from INIS to participate in a regional advanced laboratory training to be organized by the VectorLink home office together with PMI and CRID (Center for Research in Infectious Diseases) in Cameroon. The training was expected to occur over 12 days and focus on PCR for species identification, resistance mechanisms, blood meal source, and ELISA/PCR for sporozoite infection detection. Due to COVID-19, this training was cancelled, and country-specific support has been provided by the VectorLink Molecular Entomologist.

4.5 VECTOR CONTROL WORKING GROUP MEETINGS

In collaboration with NMCP and other vector control partners, VectorLink Angola supported five vector control working group meetings (in August, October, and December 2021 and February and April 2022) to facilitate review of country-level entomological data and inform vector control decision-making. Meetings were held through a combination of in-person and remote participation by stakeholders including the NMCP, INIS, The MENTOR Initiative, the Clinton Health Access Initiative (CHAI) and USAID/PMI, and covered several important topics such as:

- Draft, revision, and discussion of National Malaria Strategic Plan 2021-2025
- Draft, revision, and discussion on definition of entomological indicators for integration in WHO Entomology Modules
- Updates and presentations from different stakeholders on the progress of entomological activities in country and challenges and next steps.

4.6 COLLABORATION WITH GLOBAL FUND/MENTOR

In Quarter 2 of 2021, Global Fund/MENTOR started entomological surveillance and insecticide resistance activities in Benguela and Cuanza-Sul in addition to entomology activities associated with the IRS activities in Cuando-Cubango and Cunene. VectorLink and Global Fund/MENTOR met weekly or bi-weekly to discuss progress of activities, results, challenges faced in the field as well as possible solutions and next steps to try to sort out challenges faced. Continued harmonization of entomology methods and procedures and data collection continued throughout the reporting period.

4.7 INSECTARIES

An insectary management and responsibilities document has been written and is being updated when necessary. Controllers and equipment for temperature and humidity control (essential for maintaining a susceptible colony) have been procured and some trials were done in the Luanda insectary located at INIS. Temperature and humidity were measured daily in the insectary and the optimal mosquito rearing conditions (temperature and humidity) were achieved. Similar temperature and humidity control process was instituted in the insectary in Huambo province with success. The project was also able to maintain healthy rabbits to be used in the feeding of the future susceptible colony. The rabbits are regularly checked and looked after by a veterinary doctor.

A cohort of *An. gambiae* s.s. Kisumu eggs received from CDC Atlanta were used to start the insectary in Luanda. The cohort survived to the F1 generation, but did not survive due to outdoor fumigation activities conducted around the facility. Lack of coordination with the NMCP on scheduled fumigation activities led to this unfortunate incident and highlighted the need for good communication and understanding of responsibilities well outlined for insectary success. A new possible collaboration with ICCT for Year 4 of the project is currently being discussed to provide a new insectary space in a better location and easier to protect from fumigation and other types of vector control interventions that may kill the susceptible colony when eggs arrive again in the last months of this year.

It has also been discussed with NMCP and ICCT to provide technicians that VectorLink will train and supervise to support with rearing and maintenance of the future susceptible colony.

5. Discussion

Anopheles funestus s.l. was the most abundant vector collected from CDC LTs inside houses in both Huambo and Lunda provinces combined (96.7%), being predominant in both provinces, Huambo (89.0%) and Luanda (99.0%). There was a very low overall proportion of *An. gambiae* s.l. in the two provinces. There was a relatively low biting rate from *An. funestus* s.l. vectors in Huambo during the non-peak period (between June 2021 to September 2021), (<1 host seeking vector/trap/night) peaking in high season between January 2022 to March 2022 (>9 host seeking vectors/trap/night). In Luanda Province, there was high biting rate from *An. funestus* s.l. throughout the reporting period (July 2021 to May 2022) with >10 host seeking female *An. funestus* s.l./trap/night each month. The entomological inoculation rate will be calculated in Year 4 following sporozoite ELISA testing of samples to determine *P. falciparum* infection rates.

Overall, most of the mosquitoes collected from both Huambo and Luanda using the indoor CDC LTs were host seeking vectors, 90.3% of *An. funestus* s.l. and 95.6% *An. gambiae* s.l. were unfed. Based on the monthly trends, the proportion of unfed *An. funestus* s.l. fell below 90% in the months of January to March 2022 and for *An. gambiae* s.l. in February 2022 (excluding the 50% observed in October 2021 with the small sample size of 2) only a small percent was fed or gravid. The origin of blood meal among the few fed mosquitoes will be identified by PCR later this year once molecular capacity is expanded in the country. Unlike Huambo and Luanda, *An. gambiae* s.l. was the most abundant vector among the collections done in Lunda Norte, and similar to the two provinces, most of the vectors from the CDC LTs in Lunda Norte were unfed.

Insecticide susceptibility tests showed that pyrethroid resistance is widespread in Angola. There was confirmed resistance to one or more of the pyrethroid insecticides tested (deltamethrin, alpha-cypermethrin and permethrin) in all the provinces except Huambo where there was either full susceptibility or probable resistance. Synergist bioassays with PBO pre-exposure increased mortality rates among deltamethrin, alpha-cypermethrin, and permethrin resistant vectors in all sites tested. Susceptibility was either fully or partially restored in most instances. The degree of absolute mortality increase, however, varied among sites and insecticide type. The highest increase was observed in Lunda-Sul for PBO/alphacypermethrin (from mortality of 7% to 97%). Susceptibility increased for PBO/deltamethrin exposures from 3-63%, PBO/alphacypermethrin 5-84% and PBO/permethrin 34-47%. In the future, it would be useful to determine the pyrethroid resistance intensity as WHO states that when resistance is confirmed at the 5× and especially at the 10× concentrations, operational failure is likely. There was full susceptibility to chlorfenapyr in all the five provinces tested: Cuanza-Norte, Lunda-Sul, Malanje, Uíge and Zaire.

The wide distribution of pyrethroid resistance feeds into the rationale for the NMCP, PMI, and other partners to procure non-pyrethroid ITNs for future net distribution campaigns. One alternative ITN option to mitigate pyrethroid resistance is deployment of PBO synergist nets with deltamethrin, alpha-cypermethrin or permethrin. Although susceptibility was not fully restored in several sites, the general increase in mortality when a PBO synergist was used indicates that ITNs containing PBO may provide greater control, particularly with deltamethrin or alpha-cypermethrin, than standard nets. Dual active ingredient (dual AI) nets such as Interceptor G2 and Royal Guard are also an option. Interceptor G2 should be considered in Angola, as susceptibility to chlorfenapyr was recorded in all five sites tested with 100% mortality. The increased cost for procuring PBO or dual AI nets is a big challenge for Angola, however the transition to these new types of nets is occurring with Global Fund distributing PBO nets to Benguela and Cuanza-Sul as part of the current mass campaign. It will be important to monitor the durability of PBO nets in Angola to help inform the NMCP and stakeholders regarding choice of ITNs for future mass campaigns.

Data on insecticide resistance in Lunda Norte remains a missing data element to inform the strategic deployment of vector control in this province. Several challenges were identified and lessons learned during the

Lunda Norte community entomological surveillance pilot are being used to improve entomological activities overall, including increased and more consistent engagement with the provincial and local stakeholders to educate and advocate for entomology activities led in partnership by NMCP, Vectorlink Angola, and local community.

After experiencing challenges with the establishment of a susceptible colony, VectorLink investigated several parameters such as temperature, humidity, and water on wild mosquitoes species to determine the optimal conditions to support the emergence of eggs and rearing of larvae to pupae stage. The project procured temperature and humidity loggers and captured data at several points throughout the day and experiments were later done to achieve the optimal temperature and humidity. The team monitored and recorded all conditions and procedures that were used to support the ultimate emergence of adult Kisumu mosquitoes. Close collaboration with the NMCP and ICCT in Year 4 on the new proposed insectary location will hopefully allow the establishment of the colony.

Local capacity was strengthened throughout the reporting period through a variety of measures. These included working closely with at least three mosquito brigade staff in each of eight provinces (the majority of which have worked with the project since its inception or for multiple years), an STTA from a Senior CDC Entomologist on molecular capacity building to VectorLink and INIS, as well as VectorLink Regional DHIS2 training. Challenges encountered included being unable to collect large numbers of larvae in any of the study provinces, limiting most provinces to only two of the three pyrethroids were tested. It also meant it was not possible to conduct any pyrethroid resistance intensity assays. This was partly due to the unpredictable rainfall patterns and partly due to limited local knowledge on the location of productive larval sites. VectorLink leads and supports the process of importation of WHO entomology modules for Angola with expectations to be used for decision making and policy changes.

Annex A: Number of Vectors Collected Per Site Per Month in Huambo, Luanda, and Lunda-Norte Provinces (May 2021-April 2022)

Site	Month Year	Collection Efforts	Species				
			An. funestus s.l.	*An. gambiae* s.l.	*An. coustani*	*An. squamosus*	*An. tenebrosus*
Huambo	May-21	60	67	9	2	2	0
	Jun-21	60	19	2	1	6	0
	Jul-21	60	12	2	1	15	0
	Aug-21	60	5	1	2	7	0
	Sep-21	60	16	0	0	1	0
	Oct-21	60	66	1	1	0	0
	Nov-21	60	150	7	1	2	0
	Dec-21	60	289	58	9	13	0
	Jan-22	60	656	20	16	16	0
	Feb-22	60	556	5	4	5	0
	Mar-22	60	560	10	11	24	0
	Apr-22	60	355	15	62	10	0
Luanda	Jul-21	60	2,241	129	1	0	0
	Aug-21	60	1,932	2	1	0	0
	Sep-21	33	1,295	2	0	0	0
	Oct-21	60	1,669	1	0	0	0
	Nov-21	60	834	0	0	0	0
	Dec-21	60	1,943	0	1	0	0
	Jan-22	60	2,333	21	0	0	1
	Feb-22	60	2,374	10	0	0	1
	Mar-22	60	1,661	3	0	0	0
	Apr-22	60	1,048	0	0	0	0
Lunda Norte	Dec-21	43	2	9	0	0	0
	Jan-22	45	9	34	0	0	0
	Feb-22	60	6	48	0	0	0
	Mar-22	60	15	54	0	0	0
	Apr-22	51	6	42	0	0	0
Total		**1,552**	**20,119**	**485**	**113**	**101**	**2**