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THE PMI VECTORLINK MADAGASCAR FINAL ENTOMOLOGICAL MONITORING REPORT

SEPTEMBER 2020 – JULY 2021

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ACRONYMS

CDC	Centers for Disease Control and Prevention
CS	Capsule Suspension
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-Linked Immunosorbent Assay
GFATM	Global Fund for AIDS Tuberculosis and Malaria
HLC	Human Landing Catch
HBR	Human Biting Rate
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Net
KD	Knockdown
NMCP	National Malaria Control Program
NMF	New Malaria Funding
ODC	Outdoor Collection
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
WG	Wettable Granulation
WHO	World Health Organization

EXECUTIVE SUMMARY

In Madagascar, malaria remains a major health problem and one of the leading causes of mortality., and severe malaria highly contributes to the overall reported deaths in the country. Malaria epidemiology varies considerably within the different regions of the country, though, the entire population is at risk. To better control the disease, the national malaria control programme (NMCP), through its National Strategic Plan, has stratified the country into malaria epidemiologic clusters based on malaria endemicity and included indoor residual spraying (IRS) and mass distribution of insecticide treated nets (ITNs) as the two main strategies to control malaria vectors in the country. Malaria control and/or elimination interventions are defined based on each epidemiological cluster. Since 2008, Madagascar is being supported by both the US President's Malaria Initiative (PMI) and the Global Fund for AIDS Tuberculosis and Malaria (GFATM) for the implementation of successful IRS campaigns through provision of funds, staff and technical guidance. From 2017 to date, PMI and other malaria stakeholders have sprayed approximately 48 districts across the country. During the 2020 IRS campaign, the PMI VectorLink Madagascar Project covered over 82 communes in three districts in the Atsimo Andrefana (Southwest) region with blanket IRS (Tulear II, Sakaraha, and Betioky districts) and in two districts in Ihorombe region (Ihosy and Iakora). Pirimiphos-methyl (Actellic® 300 CS), an organophosphate insecticide was sprayed in Betioky district. The districts of Tulear II and Sakaraha were sprayed with SumiShield®50 WG, a neonicotinoid insecticide. Fludora® Fusion, a mixture of deltamethrin and clothianidin, was used in the districts of Ihosy and Iakora. The spray campaign was conducted from November 2 to December 3, 2020, in all five districts.

PMI VectorLink Madagascar also conducted entomological monitoring activities including vector surveillance to assess malaria vector density, species composition, seasonal patterns, biting behavior, sporozoites rates, and parity of *Anopheles* mosquitoes in 12 sentinel sites. The entomological monitoring sites included: i) four IRS sites and two control (non-IRS) sites, ii) two sites in areas where IRS was withdrawn in 2019 (Antsikafoka, commune of Mahambo, Fenerive Est district and Ampasimpotsy, Manakara district) after four and three years of IRS blanket coverage respectively in each site, iii) two control sites in areas where IRS was withdrawn in 2019 (Vavatenina and Marofarihy), iv) one site in a district in an elimination setting (Anamakia, Antsiranana I district) (non-IRS) and v) one site in a district where the NMCP adopted only mass distribution of ITNs as unique malaria vector control strategy (Ankilivalo, Mahabo district). Data on vector species composition, density and behavior was collected using human landing catches (HLCs) and vector resting behavior using prokopack and mouth aspirators, indoors and outdoors in pit shelters and resting places. One month's data was collected prior to the spray campaign to serve as a baseline for both IRS and control sites, and subsequent monthly data was collected after spraying for nine months.

Additionally, wall bioassay tests were conducted to assess the quality of spray within one week of spray, and monthly thereafter to monitor the bio-efficacy of the sprayed insecticide until the residual efficacy of the insecticide was below 80% for two consecutive months. Insecticide susceptibility was also conducted in 13 sentinel sites, including the 12 sentinel sites where vector surveillance was conducted, and one site, located in an unsafe area (district of Sakaraha), where the team managed to perform only susceptibility tests and cone bioassays.

Ten *Anopheles* species (*An. gambiae* s.l., *An. funestus* s.l., *An. mascarensis, An. coustani, An. squamosus/cydippis, An. rufipes*, *An. pharoensis*, *An. pretoriensis, An. fuscicolor* and *An. pauliani)* were collected during the monitoring period and all methods included. A total of 20,255 adult mosquitoes were collected, including 5,792 (28.6%) female *Anopheles* and 14,463 (71.4%) culicine mosquitoes.

The most abundant *Anopheles* species was *An. gambiae* s.l., representing 78.0% (n= 4,519) of the total *Anopheles* mosquitoes collected. *An. funestus* s.l. and *An. mascarensis,* accounted for 4.6 % (n=268) and 0.4% (n=26) of the

collected species respectively and represented the secondary vectors of malaria in the country. *Anopheles coustani* (6.3%; n= 363), reported as a probable vector in Madagascar was present in nine out of the 12 sites surveyed. *An. squamosus/cyddipis* (5.5%; n= 319), *An. rufipes* (2.8%, n= 165), *An. pharoensis* (2.1%; n= 120), *An. pretoriensis* (0.03%; n= 2), *An. fuscicolor* (0.08%; n= 5) and *An. pauliani* (0.08%; n=5) represented the *Anopheles* collected.

A total of 4,843 *Anopheles* mosquitoes were collected using HLC, including 3,761 (77.7%) *An. gambiae* s.l. Also, 333 *An. gambiae* s.l., 22 *An. funestus* s.l., and eight *An. coustani* were collected indoors using the prokopack aspiration method. In addition, 342 *An. gambiae* s.l., 14 *An. funestus* s.l., seven *An. mascarensis,* and 15 *An. coustani* were collected outdoors using both mouth and prokopack aspirators, mostly from artificial pit shelters.

During the baseline collection conducted IRS, *An. gambiae* s.l. indoor human biting rates (HBRs) ranged from 1.7 bites per person per night (b/p/n) in Irina (Ihosy district), to 2.8 b/p/n in Ranotsara Nord, Iakora district. The outdoor HBRs ranged from 0.2 b/p/n in Irina to 6 b/p/n in Ranotsara Nord. In all IRS sentinel sites, the majority of *An. gambiae* s.l. exhibited exophagic tendencies before IRS, except in Tsaragiso. The low mean biting rates, observed in some sites, during the baseline as compared to post-spray could be explained by the limited availability of breeding sites before the rainy season when the baseline data was collected. *An. gambiae* s.l. was actively biting between 8pm and 11pm at all sites both indoors and outdoors.

The wall bioassays conducted during the first week of the IRS campaign to assess the quality of the spraying indicated that the spray was good in all sites with 100% mortality recorded for all the structures tested. Fumigant effect was recorded for Actellic® 300 CS (100%) within one week after spraying in Bezaha. One month after spray (T1), mortality was 50%, then dropped to 35% at T2 and 0% at T3. For SumiShield® 50 WG and Fludora Fusion, no airborne effect was observed in Tsaragiso, Kiliarivo, Irina and Ranotsara, where both insecticides were sprayed. Furthermore, Actellic® 300 CS lasted for at least four months for both tested walls (mud and wood) as the tests could not be completed for the fifth and sixth month post spraying due to the COVID-19 lock-down. However, the bioassays conducted at month seven and month eight post-spraying showed that the residual efficacy was below the efficacy threshold of 80%. SumiShield® 50 WG and Fludora Fusion lasted at least for seven and eight months respectively on both tested surfaces in the districts where they were sprayed.

An. gambiae s.l. was susceptible to pirimiphos-methyl, clothianidin, chlorfenapyr in 13 sites where the tests were conducted. Susceptibility to deltamethrin and/ or permethrin was observed in Tsaragiso, Ampasimpotsy, Marofarihy, Ranotsara Nord, Irina, Mahasoa, Kiliarivo, Ankilivalo, Bezaha, and Anamakia. However, *An. gambiae* s.l. was resistant to deltamethrin and/or permethrin in Vavatenina, Antsikafoka, Marofatika, Ankilivalo and Bezaha. The intensity of deltamethrin and permethrin resistance was still low in Antsikafoka, Ankilivalo, Marofatika and Bezaha with 100% mortality recorded at five time-diagnostic doses (5x) of deltamethrin and/or permethrin, and moderate resistance intensity in Vavatenina (100% mortality with 10x for deltamethrin and permethrin). PBO fully restored susceptibility to both pyrethroids tested in the areas of resistance.

The vector surveillance data confirms the site population density, biting, and behavior trends as observed in previous years, which is still appropriate for IRS timing in the targeted IRS sites. However, the early evening peak biting time observed at all sites and the exophagic tendency observed in ten out of the twelve vector surveillance sites, calls for appropriate communication for use of vector control tools. Furthermore, the vector densities are still low in Madagascar, and susceptibility remains in most locations to most of the insecticides used for public health vector control measures, though a few sites are now showing some resistance to deltamethrin and permethrin. The data will continue supporting the NMCP on vector control tools and strategy selection, knowing that the country is embarking in larviciding pilot activities in selected districts in the country.

1. INTRODUCTION

In Madagascar, malaria is endemic and about 90% of the population of the country is affected. However, the entire population is considered to be at risk for the disease. Under the U.S. President's Malaria Initiative (PMI) funded indoor residual spraying (IRS) projects, Abt has been implementing high-quality IRS campaigns since 2012, and gathered the most comprehensive vector control entomological data in several countries including Madagascar. As part of the 2019-2020 activities, the PMI VectorLink Madagascar project implemented IRS in two regions and five districts, and entomological vector surveillance in twelve sites and susceptibility tests in thirteen sites. The 2020 spray campaign was conducted from November 2 to December 3, 2020, during which 203,028 eligible structures were found and 197,787 structures sprayed with an overall coverage rate of 97.4% for all five districts while protecting 833,483 people from the burden of malaria. IRS was conducted using Actellic® 300CS (organophosphate) in Betioky district, SumiShield® 50WG (neonicotinoid) in Tulear II and Sakaraha districts in the Atsimo Andrefana region (Southwest), and Fludora Fusion (pyrethroid and neonicotinoid) in Ihosy and Iakora districts, Region of Ihorombe.

PMI VectorLink Madagascar conducted entomological monitoring, including baseline and post-spray data collection. Entomological monitoring was conducted in sprayed districts as well as in control sites, in former IRS/Exit Plan districts and their control, in a district in elimination setting (Anamakia) and in a non-sprayed district of the Menabe region, where only ITN is the vector strategy adopted (Ankilivalo). Monthly longitudinal vector surveillance was conducted using human landing catches (HLCs), indoor and outdoor resting collections using prokopack and mouth aspiration. Additionally, insecticide susceptibility tests were conducted once a year per site to assess the vector's insecticide resistance status in sprayed and non-sprayed sites. Spray quality and insecticide residual life was conducted for a week during the spray period and monthly thereafter respectively.

Entomological surveillance plays a critical role as it allows vector control programs to make informed decisions and evaluate interventions. The impact of IRS on vector density, resting and feeding behavior will help identify effective insecticides against local vectors to guide vector control programming in Madagascar.

2. METHODOLOGY

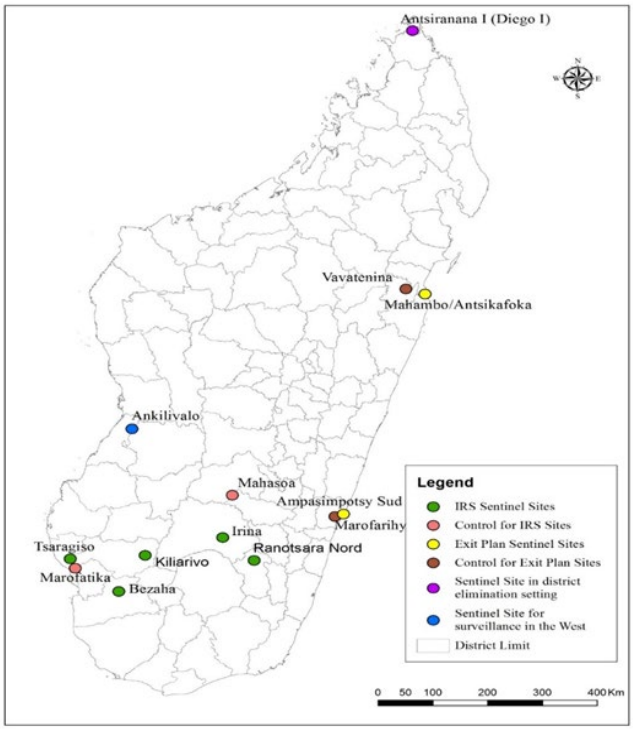
From September 2020 to July 2021, PMI VectorLink Madagascar conducted longitudinal entomological surveillance in 12 out of the 13 selected sites by the NMCP, including the 2020 IRS sites, as well as insecticide resistance monitoring in 13 sites (comprising of the 12 sites used for longitudinal entomological monitoring, plus one site in the district of Sakaraha, where only IRS insecticide decay rate and insecticide susceptibility tests were conducted). Longitudinal monitoring could not be performed in Sakaraha due to insecurity in the area.

2.1 Vector Bionomics Monitoring

2.1.1 Study Sites

Adult mosquito collections were conducted from September 2020 to July 2021 in sentinel sites and districts selected by the NMCP, including Vatovavy Fitovinany region (Manakara district), Analanjirofo region (Fenerive Est and Vavatenina districts) as IRS was withdrawn in 2019. In those regions, an exit plan was put in place to record entomological data during the period when IRS was previously conducted. The same activities were also conducted in Menabe region (Mahabo district: site of Ankilivalo), a non-IRS district and Antsiranana I (site of Anamakia), a district in elimination setting due to the absence of sentinel sites in the West of the country. In Atsimo Andrefana (Tulear II, Tulear I, Sakaraha, and Betioky districts), and in Ihorombe (Ihosy and Iakora districts), collections were conducted from October 2020 (a month before the 2020 IRS campaign) to July 2021 (Figure 1).

Figure 1: Entomological Monitoring Site Locations and District Boundaries



All sentinel sites and status, where entomological surveillance was performed during the 2020 PMI VectorLink Madagascar work plan period of performance, are listed in Table 1 below.

Table 1: List of Sentinel Sites and Status

Region	District	Sentinel Site Location	Status	Years as Sentinel Site
Analanjirofo (East Coast)	Fenerive Est	Mahambo/ Antsikafoka	No IRS in 2019*/ITNs	2014 - 2021
Analanjirofo (East Coast)	Vavatenina	Vavatenina	Control for East*/ITNs	2014 - 2021
Vatovavy Fito Vinany	Manakara	Ampasimpotsy	No IRS in 2019*/ITNs	2017 - 2021
Vatovavy Fito Vinany	Manakara	Marofarihy	Control for Vatovavy Fitovinany*/ITNs	2017 - 2021
Atsimo Andrefana	Tulear II	Tsaragiso	IRS/ITNs	2018 - 2021
Atsimo Andrefana	Sakaraha	Kiliarivo	IRS/ITNs	2018 - 2021
Atsimo Andrefana	Betioky	Bezaha	IRS/ITNs	2018 - 2021
Atsimo Andrefana	Tulear II	Marofatika	Control for Atsimo Andrefana (Southwest)/ITNs	2018 - 2021
Ihorombe	Ihosy	Irina	IRS/ITNs	2019 - 2021
Ihorombe	Iakora	Ranotsara Nord	IRS/ITNs	2019 - 2021
Haute Matsiatra	Ambalavao	Mahasoa	Control for Ihosy and Iakora districts/ITNs	2019 - 2021
Menabe	Mahabo	Ankilivalo	Entomological monitoring in malaria control area. No IRS ITNs	2019 - 2021
Diana	Antsiranana I (Diego I)	Anamakia	Entomological monitoring in malaria elimination settings. No IRS/No ITN	2019 - 2021

*Sites maintained as part of the Exit Plan Strategy (post IRS).

2.1.2 Adult Mosquito Collections

Baseline entomological data was collected in the targeted areas one month before the IRS campaign, then followed by post-spray monthly longitudinal monitoring. Data on species composition, vector densities, and vector behavior were collected using HLCs. The HLCs were conducted during two consecutive nights in three houses per site per month from 6:00 pm to 6:00 am. HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human acting as host attractant (mosquito collectors) following SOP 02/01. With legs exposed to attract host-seeking mosquitoes, one human bait was seated indoors and another one outdoors in each house for two consecutive nights per month for a total of six person-nights indoors and six person-nights outdoors per site per month. The collectors switched between indoors and outdoors on an hourly basis to control for potential differences in attractiveness. The collectors used flashlights and hemolysis tubes to collect mosquitoes that landed on their legs before they could be bitten. The tubes were covered with cotton after individual collection of mosquitoes. The teams transferred the mosquitoes hourly to custom-made labelled bags over a 12-hour period.

Indoor and outdoor resting collections were performed using prokopack aspirator (SOP 11/01) and mouth aspiration (SOP 13/01) (Table 2). The prokopack aspiration method was carried out between 6:00 a.m. and 8:00 a.m. to collect indoor resting mosquitoes in ten houses per site, monthly following the SOP 11/01, while both prokopack and mouth aspiration methods were conducted outdoors during one day per month and per site in resting places such as pit shelters constructed by VectorLink, tree holes, zebu pens, etc.

CDC light trap was specifically used in Anamakia, located in a district in malaria elimination setting, where vector density is very low. All mosquitoes collected through each method were morphologically identified to genus. *Anopheles* mosquitoes were identified to species or species complex by microscope, using simultaneously the identification keys of Grejbine 1966, Gillies and Coetzee 1987. After morphological identification, *An. gambiae* s.l. from each site was dissected to estimate the indoor and outdoor parity rate. The abdominal status of all female *Anopheles* collected by aspiration methods, was determined and sorted into four categories: unfed, blood-fed, half-gravid, and gravid. All mosquitoes were preserved on silica gel in Eppendorf tubes for further laboratory processing to identify sibling species, resistance mechanisms, infection status, and source of blood meal, using polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA). The collection times and sampling methods are shown in Table 2.

Table 2: Longitudinal Monitoring Adult Mosquito Collection Methods

Collection method	Time	Frequency	Sample
HLC	6:00 pm to 6:00 am	Two nights per site per month	Three houses per site (indoor/ outdoor)
Indoor resting (Prokopack)	6:00 am to 8:00 am	One day per month	Ten houses per site
Outdoor resting collection (ODC)	6:00 am to 8:00 am	One day per month by Prokopack and mouth aspirator in outdoor resting places and/or pit shelter, up to a 10-meter distance from the houses.	Ten outdoor resting places and/or shelters per site
CDC Light Trap	6:00 pm to 6:00 am	Two nights per month in one site	Two houses (indoor/ outdoor)

2.2 Insecticide Resistance Monitoring

From October 2020 through January 2021, VectorLink Madagascar completed insecticide resistance monitoring in 13 sites across the country during the rainy season, including the twelve longitudinal monitoring sites. Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats within the district, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day-old adult females using World Health Organization (WHO) tube tests (SOP 06/01) and CDC bottle assays (SOP 04/01). For each tube test, about 80–100 female *An. gambiae* s.l. were tested against the insecticide (in four batches of 20–25) and an additional number of 40–50 female *An. gambiae* s.l. was tested in two control tubes (20–25 each) in parallel. The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), lambda-cyhalothrin (0.05%) and pirimiphos-methyl (0.25%) were tested in all sites. When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five and 10 times the diagnostic concentration of permethrin, deltamethrin, alpha-cypermethrin. Clothianidin 2% papers were treated locally using a protocol designed by PMI VectorLink (SOP 17/01). The susceptibility testing was conducted as described above, and the mortality was recorded up to seven days post-exposure. Synergist assays with piperonyl butoxide (PBO) were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol to determine the involvement of cytochrome P450s in pyrethroid resistance. A high percent mortality and/or reversal of susceptibility when pre-exposed to PBO indicates probable involvement of enzymes such as P450s in the resistance mechanism. CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle with one-hour exposure, and mortality was recorded every 24 hours up to three days (72 hours).

Furthermore, the susceptibility status of *An. funestus* s.l., *An. mascarensis* and *An. coustani* were tested against selected insecticides in areas of high density of each species. Adult-collected mosquitoes using mouth aspiration were used to perform the tests.
For all tests, when the mortality of the control was between 5% and 20%, corrected mortality was determined using Abbot's formula, and resistance and intensity were defined following the WHO criteria (WHO 2016):
Resistance status at diagnostic doses:

- 98% or greater mortality indicates susceptibility.
- Between 90% and 97% mortality indicates possible resistance.
- Less than 90% mortality indicates confirmed resistance.

Resistance intensity with mortality at 5x and or mortality at 10x:

- 98–100% at 5x: Low resistance
- <98% at 5x and 98–100% at 10x: moderate resistance
- <98% at 10x: high resistance

2.3 Quality Assurance of IRS and Insecticide Decay Rate

WHO cone bioassays were used to determine the spray quality and residual efficacy of each insecticide on sprayed surfaces (SOP 09/01). The tests were conducted using wild-caught larvae reared into adults at each sentinel site. The susceptibility status of the local vector *An. gambiae* s.l, was confirmed against the insecticide sprayed in the area, using WHO tube tests (SOP 06/01), before mosquitoes from the same population were used for the cone bioassay. Cone bioassays were conducted within one week after the IRS spray campaign started, to evaluate the quality of the spray. The residual bio-efficacy of the insecticides was then monitored monthly using the same protocol. Two common types of surfaces were selected from each of the different sites: mud and wood. The cones were placed on the treated walls at 0.5m, 1m, and 1.5m above the ground. Ten female *An. gambiae* s.l. were introduced per cone and exposed for 30 minutes. After the 30 minutes, the exposed mosquitoes were retrieved back in the corresponding disposable cups, and the cups were then placed in a rack covered with a damp towel to create favourable humidity for the mosquitoes in the laboratory where they were held for up to five days due to the slow-acting nature of the insecticides. The number of mosquitoes knocked down after 30 minutes and 60 minutes and the number dead after every 24 hours of holding were recorded up to five days for SumiShield and Fludora Fusion, while Actellic mortality was determined after 24 hours.

Additionally, the fumigant effect of the sprayed insecticides was conducted in the same houses used for cone bioassays. Ten female *An. gambiae* s.l. were introduced in a small cage (15cmx10cm), placed on a chair approximately 10 cm from a sprayed wall and about one meter above the floor. The surface was covered with clean paper to ensure there is no contamination of the cage with the insecticide sprayed on the wall. The mosquitoes were exposed for 30 minutes and then transferred to paper cups and fed with 10% glucose soaked in cotton. The knockdown effect was recorded 30 minutes post-exposure. Mortality was recorded after a 24-hour holding period for Actellic and up to five days for the clothianidin-based insecticides. A control cage was set outside under a tree in the shade. Fumigant tests were conducted monthly until mortality was <50% during two consecutive months. For both tests, when the mortality of the control was between 5% and 20%, corrected mortality was determined using Abbot's formula.

2.4 Molecular Analysis

Samples of malaria vectors will be tested at the NMCP laboratory for molecular analysis and the results will be reported once available. Insecticide resistance in mosquitoes may be related to target site mutations. Among them, resistance to pyrethroids and DDT is described as a substitution of amino acid leucine to either phenylalanine (L1014F, referred as *kdr* -West) or serine (L1014S, referred as *kdr*-East) at the position 1,014 in the sodium channel gate. For organophosphate and carbamate insecticide, target site mechanism, known as *ace-1* is a substitution of an amino acid glycine to serine at position 119. Samples of *An. gambiae* s.l. will be randomly selected per site within the WHO susceptibility tested mosquitoes and will be analyzed to determine species

identification and assess molecular markers of insecticide resistance. The DNA of each individual mosquito will be extracted using the protocol designed by Collins et al, 1987. The presence of *kdr*-West and East mutations will be characterized using the protocol described by Martinez-Torres et al. (1998) and Huynh et al. (2007) for *kdr*-West and *kdr*-East respectively, while the *ace*-1 mutation will be characterized following the protocol of Weill et al. (2004).

Adult *An. gambiae* s.l. and *An. funestus* s.l. from the 12 sites surveyed and collected using HLCs will be molecularly identified to sub-species as *An. gambiae* s.s., *An. coluzzii*, *An. merus* or *An. arabiensis* or members of *An. funestus* group for both complex of species by the NMCP laboratory. The sporozoite infection rate of subsamples of mosquitoes collected from each site by HLC will be also determined using the ELISA protocol for identification of *Plasmodium falciparum* circumsporozoite infection.

2.5 Data Presentation and Statistical Analysis

The District Health Information Software (DHIS2)-based VectorLink Collect database was used for entomological data management in Madagascar for the first time in 2020. The PMI VectorLink home office staff trained VectorLink Madagascar entomologists and database managers on updated data workflows, including field paper collections, technical reviews, data entry, data cleaning, and analytics, to generate and support the use of high-quality entomological data. All entomological data collected in Madagascar in 2020 were analyzed in VectorLink Collect. The platform includes comprehensive dashboards to synthesize vector bionomics and insecticide resistance summary results. By the end of 2021, key stakeholders, including NMCP, and PMI, will all have ongoing access to these results dashboards to support timely decision making.

3. RESULTS

3.1 Vector Bionomics Monitoring

Vector bionomics activities were conducted in the twelve sites from September 2020 to July 2021. However, activities were suspended in April and May 2021 due to country lockdown and in compliance with the PMI VectorLink Project's Mitigation Measures and Modifications for Vector Control Monitoring Activities in the Context of COVID-19.

3.1.1 All Method Species Composition

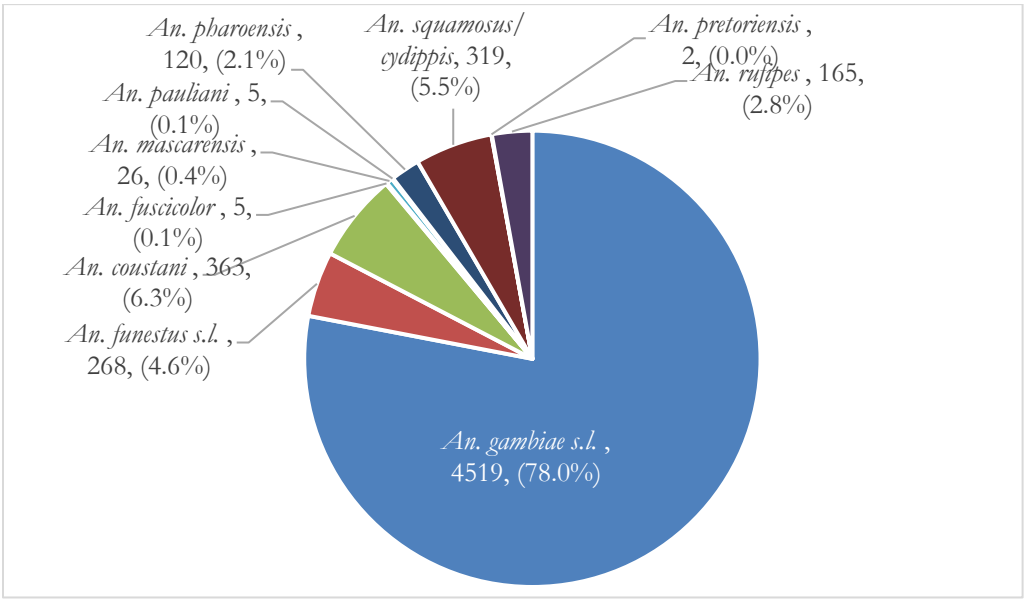
A total of 20,255 mosquitoes were collected from all the 12 sentinel sites from September 2020 to July 2021 in Vatovavy Fitovinany region (Ampasimpotsy and Marofarihy), in Analanjirofo region (Antsikafoka and Vavatenina), in Diana region (Anamakia) and Menabe region (Ankilivalo), and from October 2020 to February 2021 in Atsimo Andrefana region (Betioky, Tulear II, Sakaraha) and Ihorombe region (Irina, Ranotsara Nord, Mahasoa (control)), using HLC, indoor and outdoor resting collection with prokopack and mouth aspirators, and CDC light trap in one site (Annex A Table A1).

Using HLCs, 16,277 (80.4%) mosquitoes were collected, including 4,843 *Anopheles* mosquitoes, 2,235 (11.0%) including 419 *Anopheles* mosquitoes using indoor resting collection with prokopack, 1,537 (7.6%) including 432 *Anopheles* mosquitoes collected outdoors using prokopack and mouth aspirators. The site of Anamakia where CDC light traps were only used, yielded 206 (1.0%) mosquitoes including 98 *Anopheles* mosquitoes.

A total of 5,792 (29.3%) of all the mosquitoes collected were *Anopheles*; and 5,176 (89.4%) of those were confirmed malaria vectors or potential vectors in Madagascar: *Anopheles gambiae* s.l. (n= 4,519), *An. funestus* s.l. (n= 268), *An. mascarensis* (n= 26), and *An. coustani* (n= 363) (Figure 2, Annex A Table A1). The other *Anopheles* included *An. pharoensis* (n=120), *An. pretorensis* (n=2), *An. rufipes* (n=165), *An. squamosus/cydippis* (n=319), *An. pauliani* (n=5) and *An. fuscicolor* (n=5).

Anopheles gambiae s.l. was collected at all sentinel sites and was the most common primary vector collected in the IRS areas. *Anopheles funestus* s.l. was collected in ten sites: Antsikafoka, Vavatenina, Ampasimpotsy, Marofarihy, Tsaragiso, Marofatika, Bezaha, Kiliarivo, Ankilivalo and Anamakia. During this investigation period, *Anopheles mascarensis* was collected in four sites (Vavatenina, Ampasimpotsy, Anamakia and Marofarihy). *An. coustani* was collected in nine sites (Vavatenina, Anamakia, Marofarihy, Mahasoa, Anamakia, Ampasimpotsy, Ankilivalo, Ranotsara Nord and Bezaha) (Figure 2, Annex A Table A1).

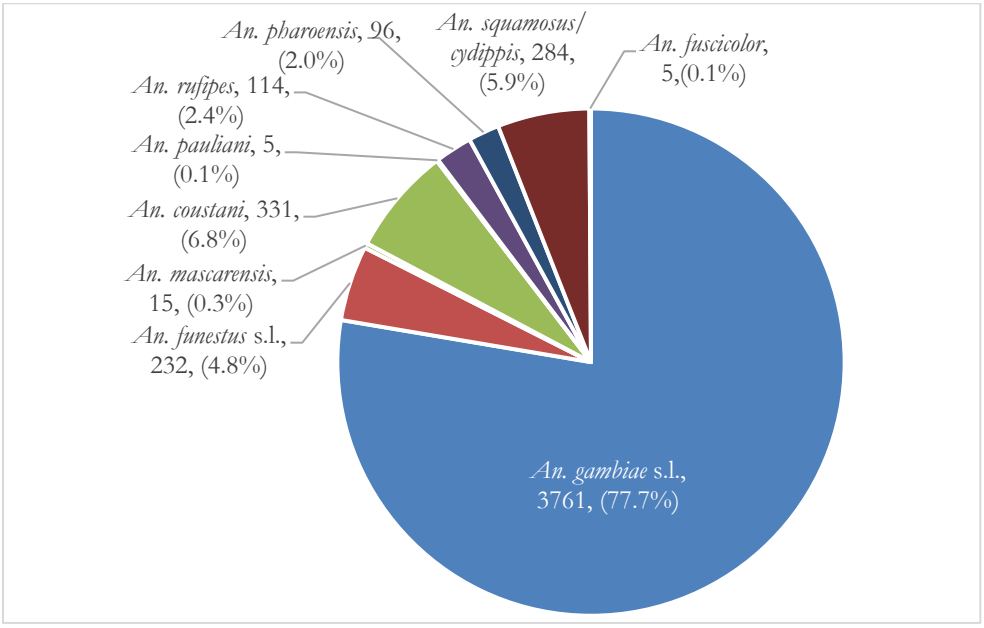
Figure 2: Species Composition of *Anopheles* Collected at all Sites, all Methods Included



3.1.2 Species Composition of HLC Collected Mosquitoes

The diversity of *Anopheles* species was recorded through HLC collections (nine of the ten species collected). *Anopheles gambiae* s.l. remained the major malaria vectors in Madagascar representing about 78% (n= 3,761) of the total *Anopheles* collected by HLCs (n= 4,843). *An. funestus* s.l. (4.8%, n= 232) and *An. mascarensis* (0.3%, n= 15) remained as secondary vector in addition to *An. coustani* (6.8%, n= 331) which was reported as a potential malaria vector in the country. *An. squamosus/cydippis* (5.9%, n= 284) and *An. rufipes* (2.4%, n= 114) were the other common *Anopheles* species that were collected (Figure 3, Annex A Table A2).

Figure 3: Species Composition of Malaria Vectors Collected by HLCs



3.1.3 Indoor Resting Density

The overall mean indoor vector density of *Anopheles gambiae* s.l. in all sites was low (0 to 1.4 vector per room per day) during the collection period. For the other vectors, the indoor resting density was almost null for *An. funestus* s.l., *An. mascarensis* and *An. coustani*. Due to the very low number of mosquitoes collected indoors over the collection period both in IRS and control sites, the team could not conduct an analysis of IRS implementation and the impact of IRS on indoor resting density (Annex A Table A3).

3.1.4 Outdoor Collections (ODC)

A total of 1,387 mosquitoes were collected using aspirators and /or prokopack in the twelve sites, resting outdoors in natural and pit shelters of which 552 (39.8%) were collected using mouth aspiration and 835 (60.2%) using prokopack aspiration (Table 3). The collection sites were composed of animal fences, ground holes, pit shelters, tree holes, vegetation, and other type of outdoor abandoned houses. Using mouth aspiration, the highest mosquito densities were collected in ground holes (22.5%, n= 124) and other shelters (23.7%, n= 131). With prokopack aspiration, the highest mosquito densities were recorded in pit shelters (42.3%, n= 353) and ground holes (28.4%, n= 237). Three hundred and seventy-one (371) malaria vectors were collected over both methods, including 342 (92.2%) *An. gambiae* s.l. from all sites, 14 (3.81%) *An. funestus* s.l. collected from seven sites (Ampasimpotsy, Anamakia, Antsikafoka, Bezaha, Mahasoa, Marofarihy and Vavatenina), 15 (4.0%) of *An. coustani* from two sites (Ankilivalo and Antsikafoka) (Annex A Tables A4 & A5).

Table 3: Outdoor Mosquitoes Collected Using Mouth and Prokopack Aspiration

Mouth aspiration

Type of outdoor shelter	Culicine	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	*An. pharoensis*	*An. mascarensis*	*An. rufipes*	*An. squamosus/ cydippis*	*Total*
Fence	45	10	3	0	0	0	0	0	**58**
Ground hole	92	24	0	4	0	0	0	4	**124**
Other type of outdoor shelter	80	45	1	2	0	0	2	1	**131**
Pit shelter	37	21	0	0	0	0	0	0	**58**
Tree	80	11	2	3	1	0	0	2	**99**
Vegetation	58	21	0	2	0	0	0	1	**82**
Total	**392**	**132**	**6**	**11**	**1**	**0**	**2**	**8**	**552**
Prokopack									
Type of outdoor shelter	Culicine	*An. gambiae* s.l.	*An. funestus* s.l.	*An. coustani*	*An. pharoensis*	*An. mascarensis*	*An. rufipes*	*An. squamosus/ cydippis*	*Total*
Fence	18	1	1	0	0	0	0	0	**20**
Ground hole	164	39	4	1	7	0	17	5	**237**
Other type of outdoor shelter	53	47	0	0	0	7	0	0	**107**
Pit shelter	270	69	3	2	3	0	0	6	**353**
Tree	11	9	0	0	0	0	3	0	**23**
Vegetation	44	44	0	0	0	0	2	0	**90**
	3	1	0	1	0	0	0	0	**5**
Total	**563**	**210**	**8**	**4**	**10**	**7**	**22**	**11**	**835**

Total both methods	**955**	**342**	**14**	**15**	**11**	**7**	**24**	**19**	**1,387**

3.1.5 Vector Density and Behavior per Site Status

An. gambiae s.l. and *An. coustani* showed an exophagic tendency in IRS, Exit Plan and control for Exit Plan sites (p<0.0001); but *An. gambiae* s.l. was endophagic in IRS control sites. In the only-ITN sites, non-IRS (Ankilivalo) and in that of elimination setting (Anamakia), *An. gambiae* s.l. bites similarly indoors and outdoors. *Anopheles coustani* was also exophagic in Ankilivalo, but there was no significant difference between both biting locations in Anamakia. Both the IRS control sites (Marofatika and Irina) and Exit Plan control site's *An. gambiae* s.l. and other vectors showed endophagic tendencies (Table 4).

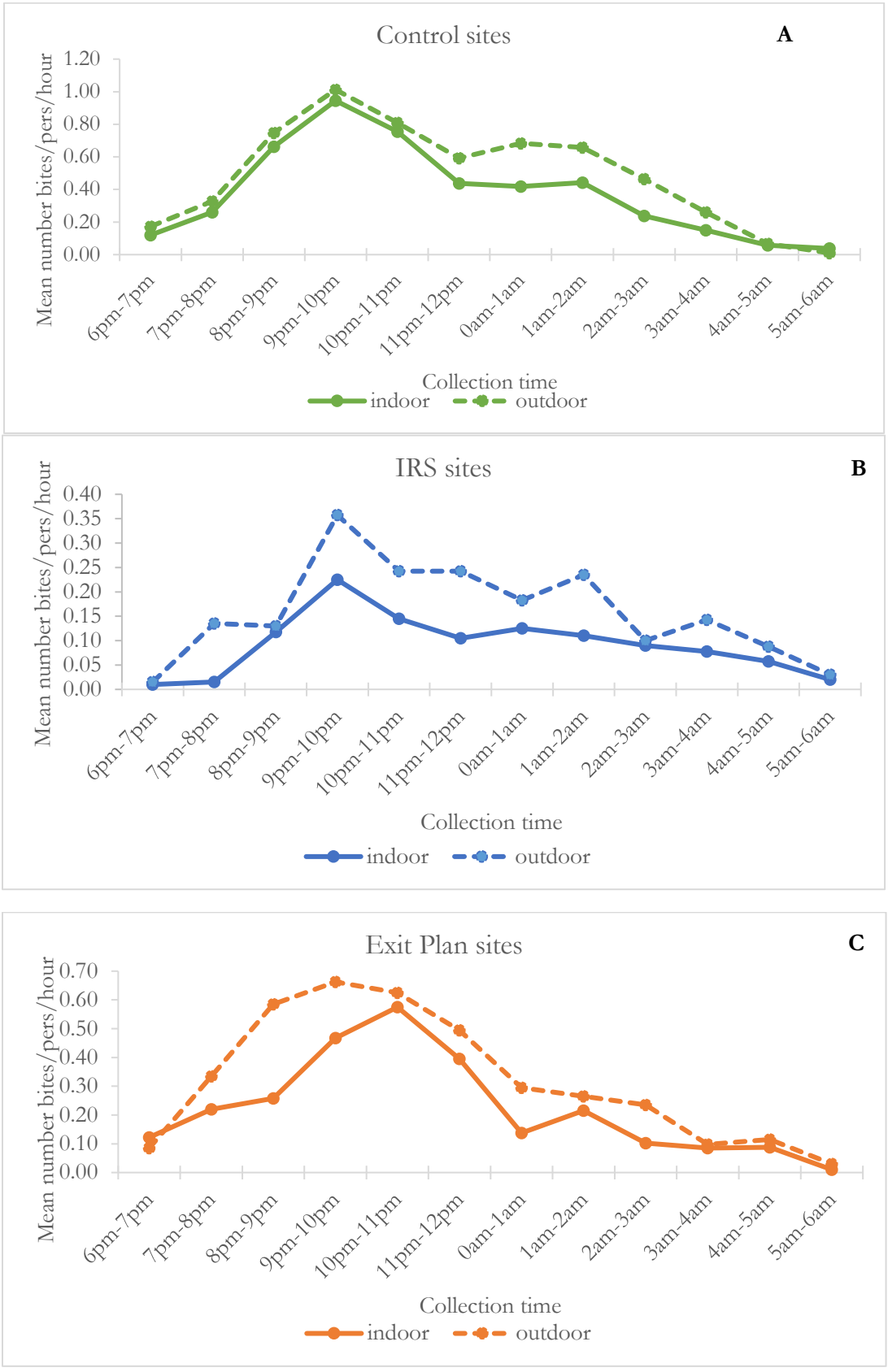
Table 4: Indoor Vs. Outdoor Biting of *Anopheles* Vectors Collected Using HLC

	Vector	# Indoor	# Outdoor	Exophagic index	P-value
IRS sites	*An. gambiae* s.l.	211	365	63.4%	<0.0001
	An. funestus s.l.	11	6	NA	NA
	An. coustani	9	20	69.0&	<0.0001
Control for IRS sites	*An. gambiae* s.l.	244	61	43.2%	<0.0001
	An. funestus s.l.	1	0	NA	NA
Exit Plan sites	*An. gambiae* s.l.	315	497	61.2%	<0.0001
	An. funestus s.l.	51	62	54.9%	= 0.3007
	An. mascarensis	5	4	NA	NA
	An. coustani	16	49	75.4%	<0.0001
Control for Exit Plan sites	*An. gambiae* s.l.	625	971	60.8%	<0.0001
	An. funestus s.l.	41	56	57.7%	=0.12775
	An. mascarensis	3	3	NA	NA
	An. coustani	21	60	74.1%	<0.0001
ITN sites (non IRS) Ankilivalo	*An. gambiae* s.l.	120	143	54.4%	=0.15612
	An. funestus s.l.	1	1	NA	NA
	An. coustani	42	65	60.7%	<0.0001
Elimination setting sites (non-IRS, non ITN) Anamakia	*An. gambiae* s.l.	60	71		=0.33651
	An. coustani	16	20		=0.50499

3.1.6 *An. gambiae* s.l. Biting Time

At all sites, *An. gambiae* s.l. bites mostly as early as 8pm through 10pm, both indoors and outdoors. All sites' peak biting time was recorded during this period with a mean of one bite per person per hour (b/p/h) in the control sites indoors and outdoors, 0.2 b/p/h and 0.4 b/p/h indoors and outdoors respectively in IRS sites and 0.6 b/p/h and 0.7 b/p/h indoors and outdoors respectively in the exit plan sites. The biting rates decreased at all sites after 11 pm (Figure 4; Annex A Tables A6 & A7).

Figure 4: Indoor and Outdoor Biting Time of *An. gambiae* s.l. per Site Status



3.1.7 Human Biting Rates

The mean human biting rate (HBR) of *An. gambiae* s.l. in the sprayed sites was higher a month before spraying during the baseline collection, in October 2020 and dropped after IRS. The mean outdoor HBR dropped from 2.7 b/p/n in October to 0.6 b/p/n in June 2021 after a slight increase in February (2.7b/p/n) and March (3.6 b/p/n). The mean indoor HBR was 2.3 b/p/n in October 2020 and dropped to 0.5 b/p/n in June 2021 after increasing during February (1.5 b/p/n) and March (2.3 b/p/n) collections, similarly to the outdoor collection trends (Figure 5).

In the control sites, the mean outdoor HBR was 3.8 b/p/n in September 2020, increased to its highest peak in February (17.9 b/p/n) before dropping to 11 b/p/n in March and 0.9 b/p/n in June. The indoor HBR was 1.6 b/p/n in September 2020, increased to the peak of 14 b/p/n in February 2021 before decreasing to 0.8 b/p/n in June (Figure 5).

In the remaining sites of Analanjirofo and Vatovavy Fitovinany, where IRS was withdrawn in 2019, and other unsprayed sites (Ankilivalo, Anamakia), the mean outdoor HBR was 4.5 b/p/n in September 2020 and dropped to 0.9 b/p/n in December 2020. The highest peak was observed in March 2021 with 7.5 b/p/n. The mean indoor HBR was 1.9 b/p/n in September 2020 and the peak was observed in March 2021 (4.6 b/p/n) (Figure 6).

Figure 5: Mean Indoor and Outdoor Human Biting Rates of *An. gambiae* s.l. in IRS and Control Sites

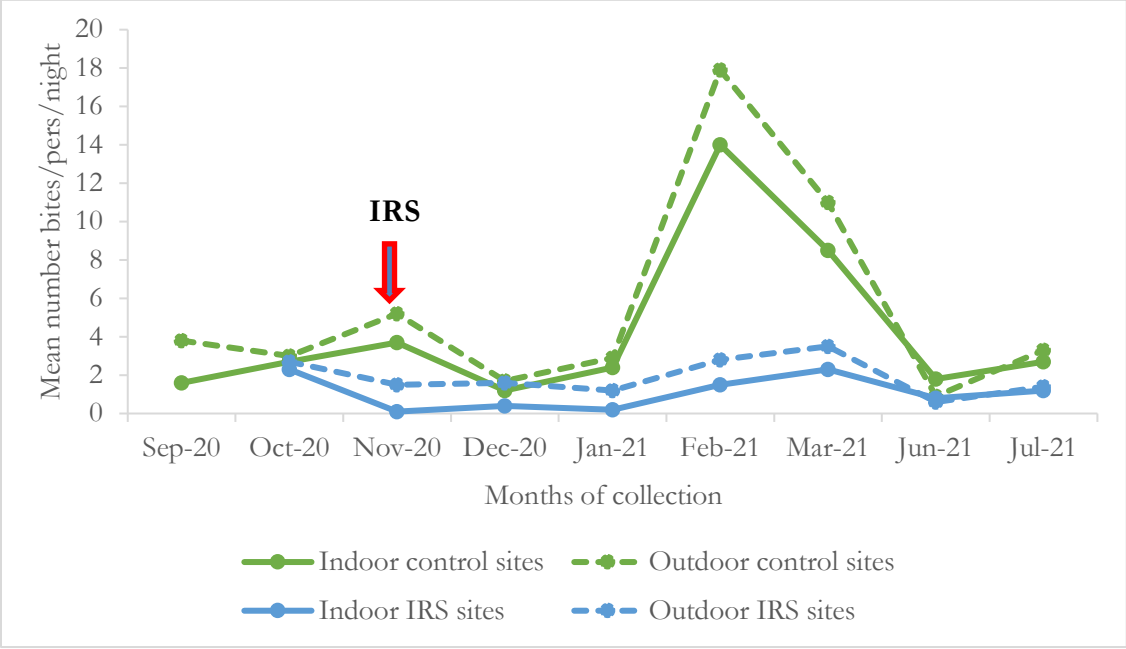
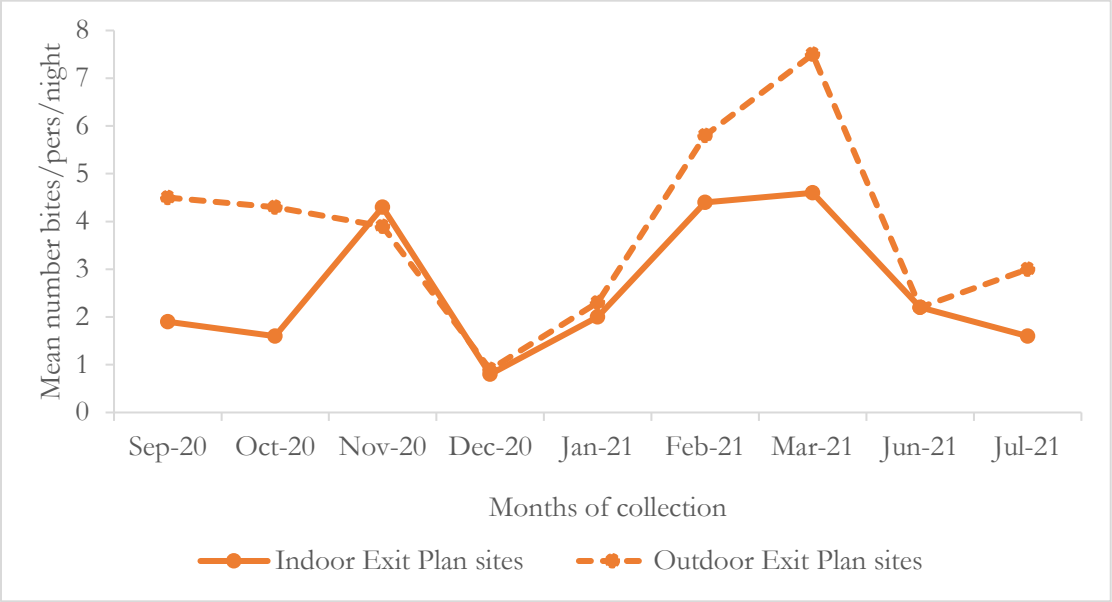


Figure 6: Mean Indoor and Outdoor Human Biting Rates of *An. gambiae* s.l. at the Sentinel Sites in Non-Sprayed Sites with Exit Plan (Previously Sprayed but Dropped) and Other Unsprayed Sites



3.1.8 Parity Rates

A total of 1,562 *An. gambiae* s.l. collected by HLC indoors from all sites were ovary-dissected for parity reading, including 864 from the control sites, 205 from the IRS sites and 493 for the other exit plan and unsprayed sites. Out of the 864 mosquitoes dissected in the control sites, 491(56.8%) were parous, 94/205 (45.8%) parous from the IRS sites and 183/493 (37.1%) from the exit plan sites. For outdoors, 2,610 *An. gambiae* s.l. were dissected including 1,106 from the control sites, 439 from IRS sites and 1,065 from the exit plan sites. Out of the total, 1,235 were parous including 608/1,106 (55.0%) parous from the control sites, 181/439 (41.2%) parous from the IRS sites and 446/1,065 (41.9%) parous from the exit plan sites.

However, the proportion of parous mosquitoes varied per month, per site and also per number of mosquitoes dissected per each month (Figure 7, Annex A Table A8).

Figure 7: Mean Indoor and Outdoor Parity of *An. gambiae* s.l. per Site

3.2 Insecticide Resistance Monitoring

3.2.1 *An. gambiae* s.l.

Figures 8 and 9 show the resistance status to the different insecticides tested against *An. gambiae* s.l. collected from the 13 different sites. Susceptibility of *An. gambiae* s.l. to deltamethrin and/ or permethrin was observed in Tsaragiso, Ampasimpotsy, Marofarihy, Ranotsara, Irina, Mahasoa, Kiliarivo, Ankilivalo, Bezaha, and Anamakia. The results also showed that *An. gambiae* s.l. was resistant to deltamethrin and/or permethrin in Vavatenina, Antsikafoka, Marofatika, Ankilivalo and Bezaha, while the resistance was suspected to permethrin in Kiliarivo (Figures 8 and Annex B Table B1). Also, *An. gambiae* s.l. of all sites showed full susceptibility to pirimiphos-methyl (Figure 9).

Pre-exposure of mosquitoes to PBO before exposure to deltamethrin and/or permethrin restored full susceptibility to both pyrethroids in areas of confirmed resistance such as in Vavatenina, Antsikafoka, Marofatika, Bezaha and Ankilivalo for deltamethrin and permethrin (Figure 10, Annex B Table B2).
The intensity assay performed with five times the diagnostic dose (5x) of deltamethrin and/or permethrin yielded 100% mortality in Antsikafoka, Ankilivalo, Marofatika and Bezaha, showing a low resistance intensity. Deltamethrin 5x and Permethrin 5x yielded 97% and 96% mortality, respectively, in Vavatenina, but 100% mortality was recorded with 10x giving a moderate resistance intensity (Figure 11, Annex B Table B3).

Figure 8: Susceptibility of *An. gambiae* s.l. to Pyrethroids (Deltamethrin, Permethrin and Alpha-Cypermethrin) in the Thirteen Sites Surveyed using WHO Tube Test.

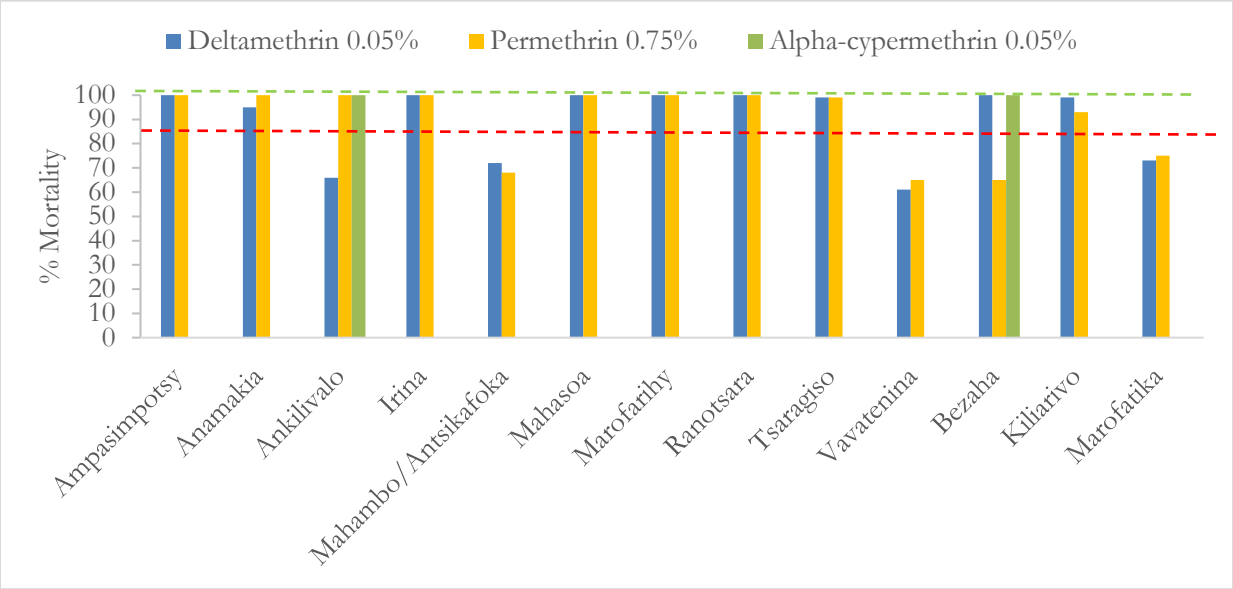


Figure 9: Susceptibility of *An. gambiae* s.l. to Pirimiphos-Methyl 0.25% in the Thirteen Sites Using WHO Tube Test

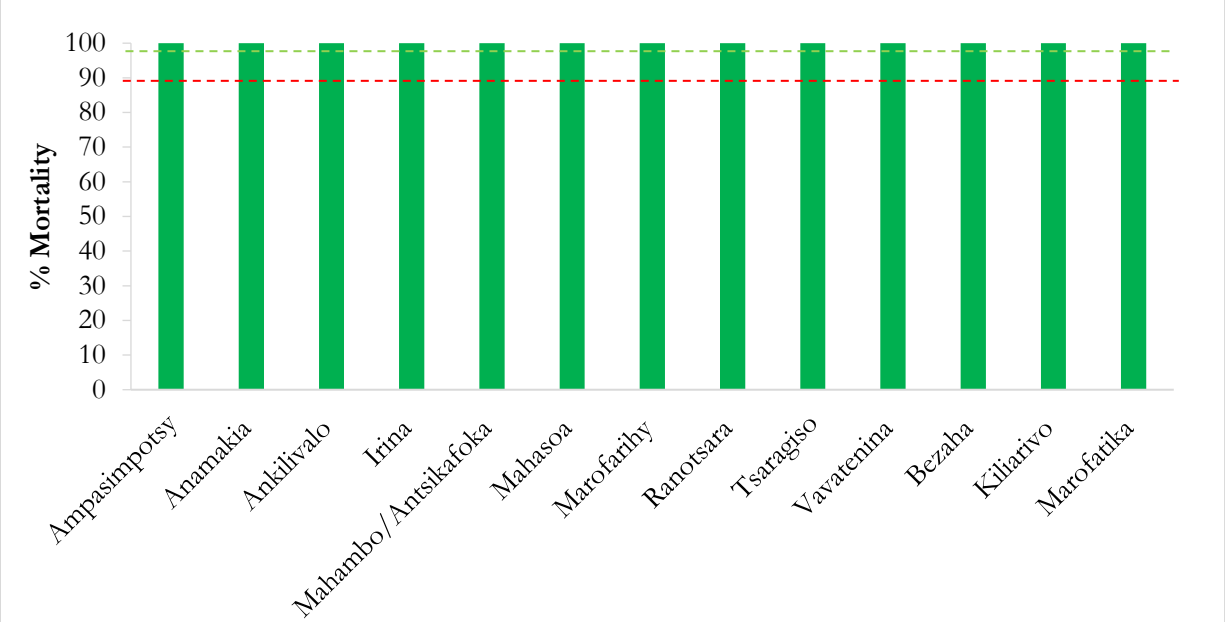


Figure 10: Piperonyl Butoxide Synergist Assay of *An. gambiae* s.l. Using WHO Tube Test in Sites where Resistance Was Recorded

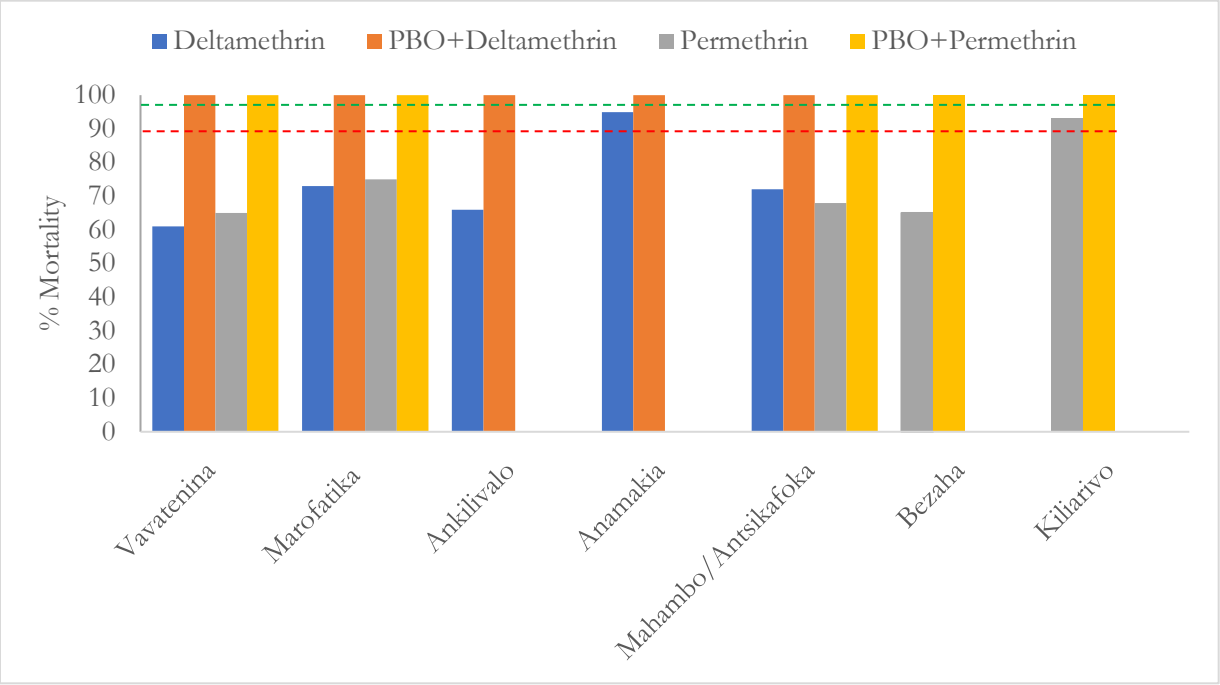
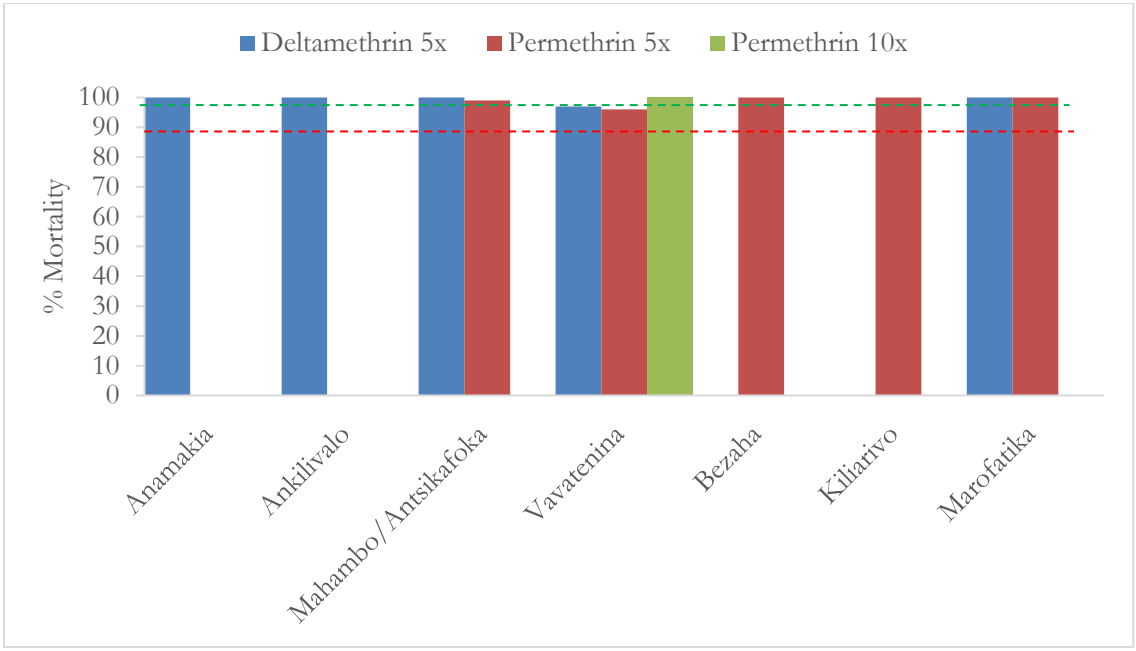


Figure 11: Pyrethroid Resistance Intensity of *An. gambiae* s.l. using WHO Tube Test in Sites where Resistance Was Recorded



For the slow acting insecticides, susceptibility of *An. gambiae* s.l. to clothianidin 2% was recorded at all sites after three days of mortality while susceptibility to chlorfenapyr 100 µg/bottle was recorded after 48 hours at all sites (Figures 12 and 13, Annex B4).

Figure 12: Susceptibility of *An. gambiae* s.l. to Clothianidin 2% in the Thirteen Sites Using the WHO Tube Test

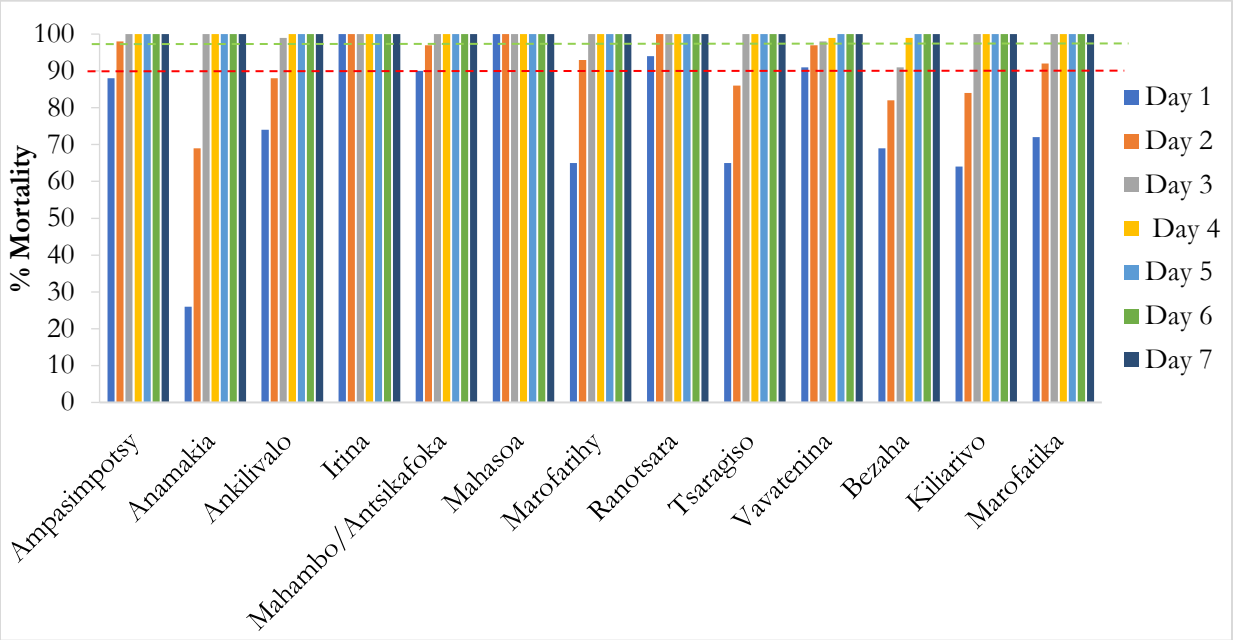
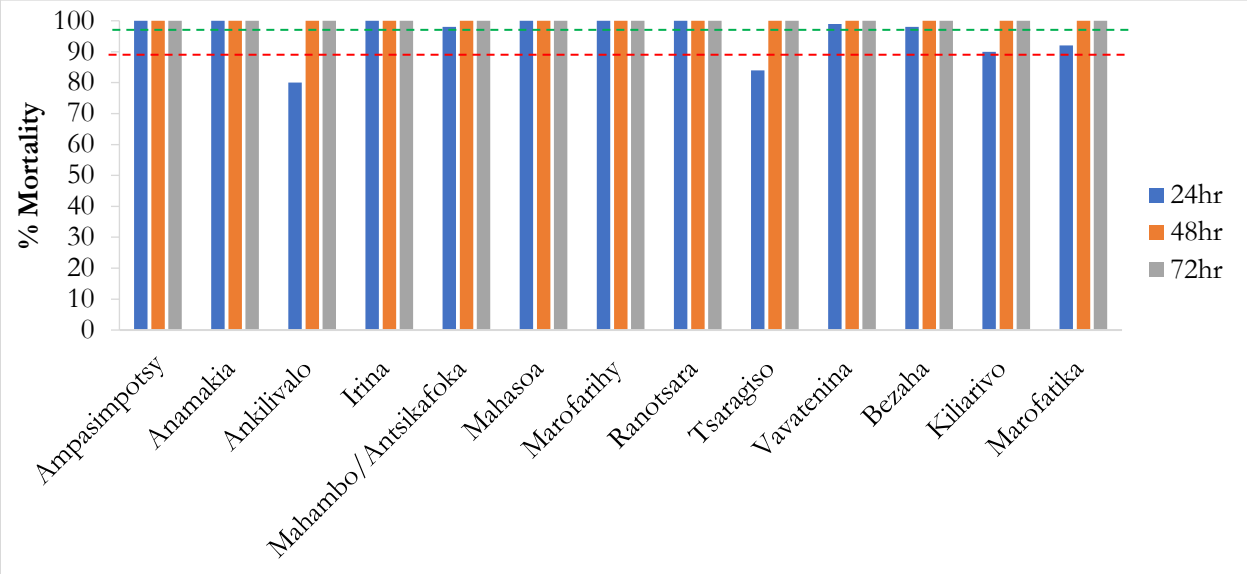


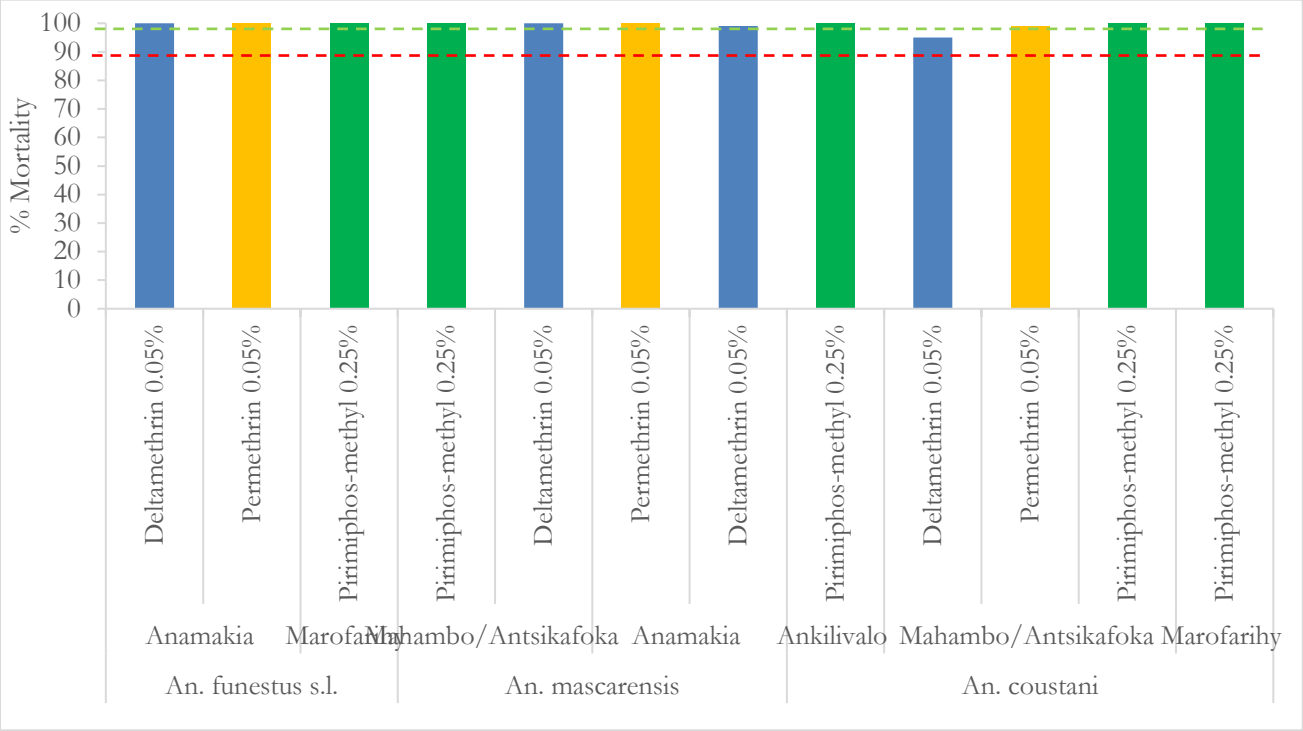
Figure 13: Susceptibility of *An. gambiae* s.l. to Chlorfenapyr 100 µg/Bottle against Using CDC Bottle Assays



3.2.2 Other *Anopheles*

The other vectors, *An. funestus*, *An. mascarensis* and *An. coustani* were fully susceptible to the insecticides tested (pirimiphos-methyl, deltamethrin and permethrin), except in Mahambo/Antsikafoka, where possible resistance was observed for deltamethrin against *An. coustani* (Figure 14).

Figure 14: Susceptibility of *An. funestus* s.l., *An. mascarensis*, *An. coustani* to Deltamethrin, Permethrin and Pirimiphos-Methyl in Selected Sites Using WHO Tube Test (Blue = Deltamethrin, Yellow = Permethrin and Green = Pirimiphos-methyl)



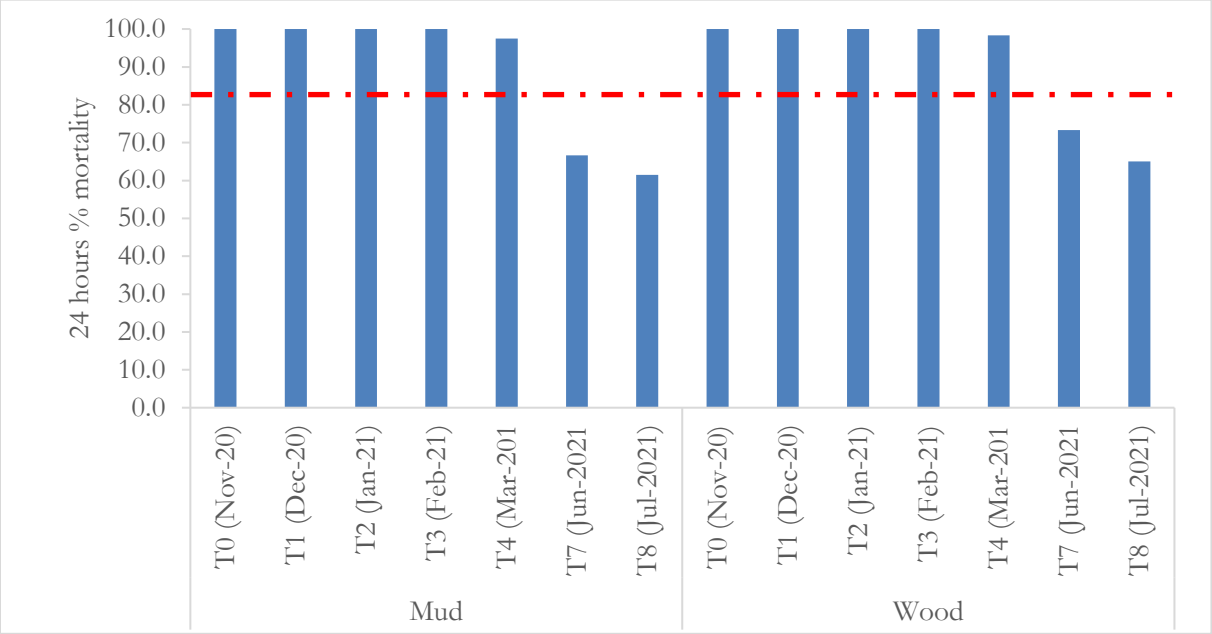
3.3 Cone Bioassay Results

In the five sprayed districts of the Atsimo Andrefana (Southwest) and Ihorombe regions, most houses were made of mud or wooden walls. These types of walls were selected for the bioassay. Furthermore, the initial cone bioassay tests conducted during the first week of the spray campaign, which served as quality control showed 100% mortality for all three insecticides sprayed on both types of wall. The results indicated good spray quality in all the five sprayed districts. Thereafter, the monthly insecticide residual life was conducted until the mortality of each surface tested fell under 80% for two consecutive months.

3.3.1 Actellic 300 CS Sprayed Surfaces

The residual life of Actellic 300 CS sprayed in Bezaha was for at least four months before the lockdown of the country, and impossibility to test the fifth, sixth, and sixth months. About 67% and 73% mortality was recorded on mud and wood surfaces respectively at T7 (seven months after spraying) and, 62% and 65% mortality at T8 (Figure 15, Annex C Table C1).

Figure 15: Residual Efficacy Observed for Pirimiphos-Methyl (Actellic® 300 CS) in Bezaha

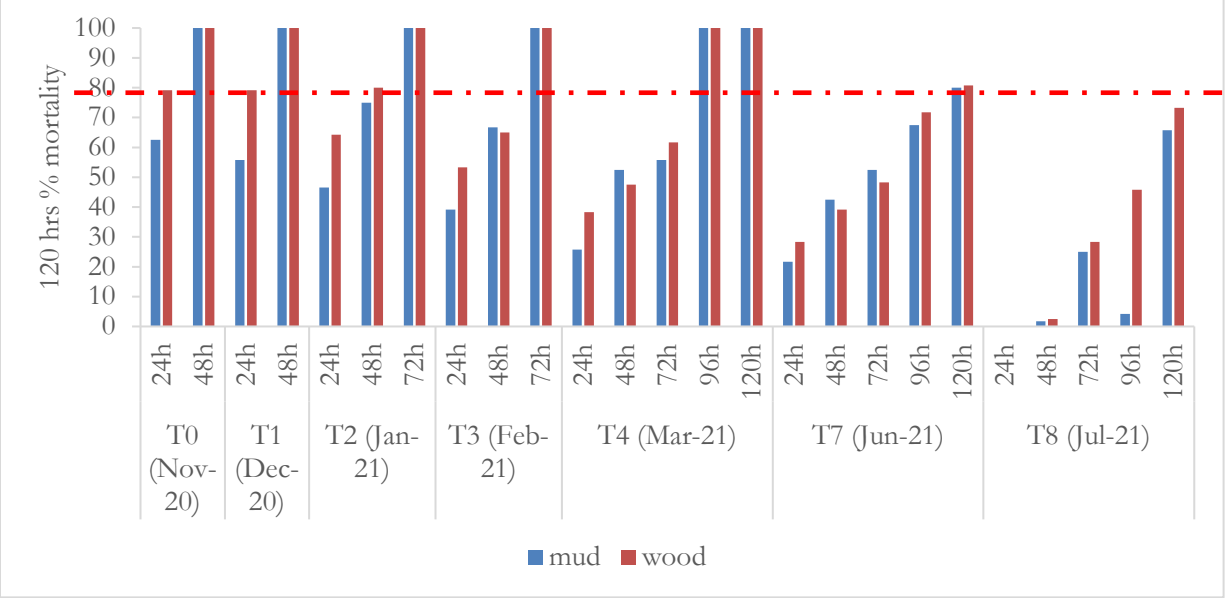


Red line represents the 80% efficacy threshold

3.3.2 SumiShield® 50 WG Sprayed Surfaces

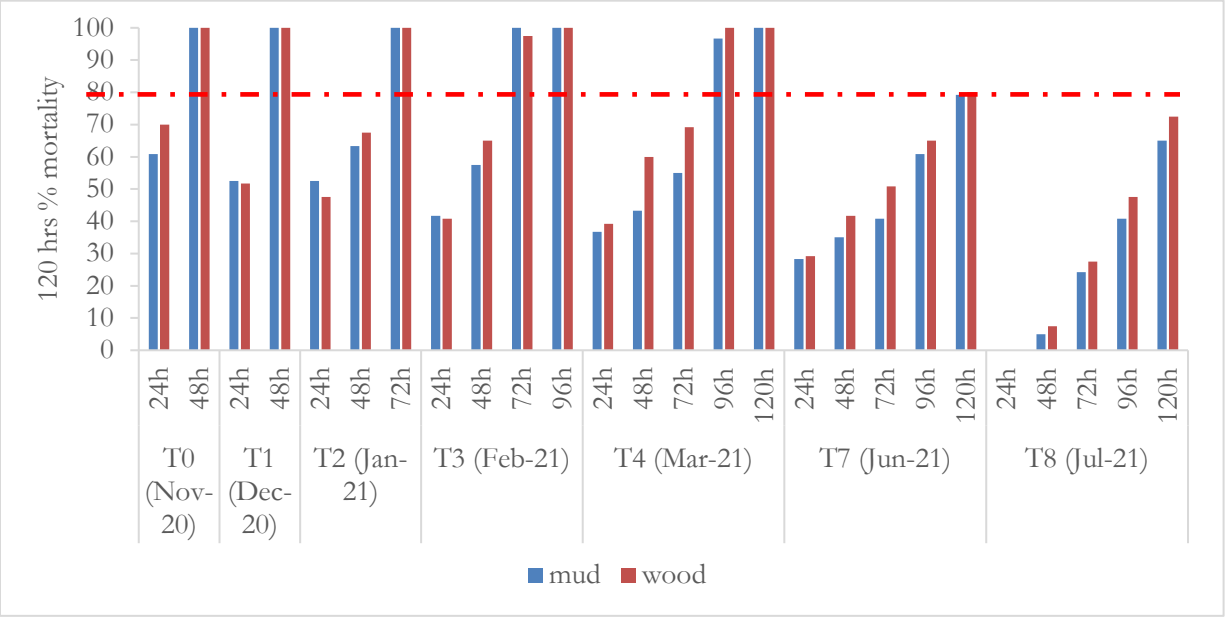
SumiShield was sprayed in two districts in Kiliarivo (Sakaraha district) and Tsaragiso (Tulear II district). The residual life of the insecticide lasted for about seven months in both districts and on both mud and wood surfaces even though there was no testing at T5 and T6. The insecticide efficacy dropped below the 80% threshold at eight months post spraying (Figures 16 & 17, Annex C Tables C2 & C3).

Figure 16: Residual Efficacy Observed for Clothianidin (SumiShield® 50 WG) in Kiliarivo Sakaraha District



Red line represents the 80% efficacy threshold

Figure 17: Residual Efficacy Observed for Clothianidin (SumiShield® 50 WG) in Tsaragiso, Tulear II District

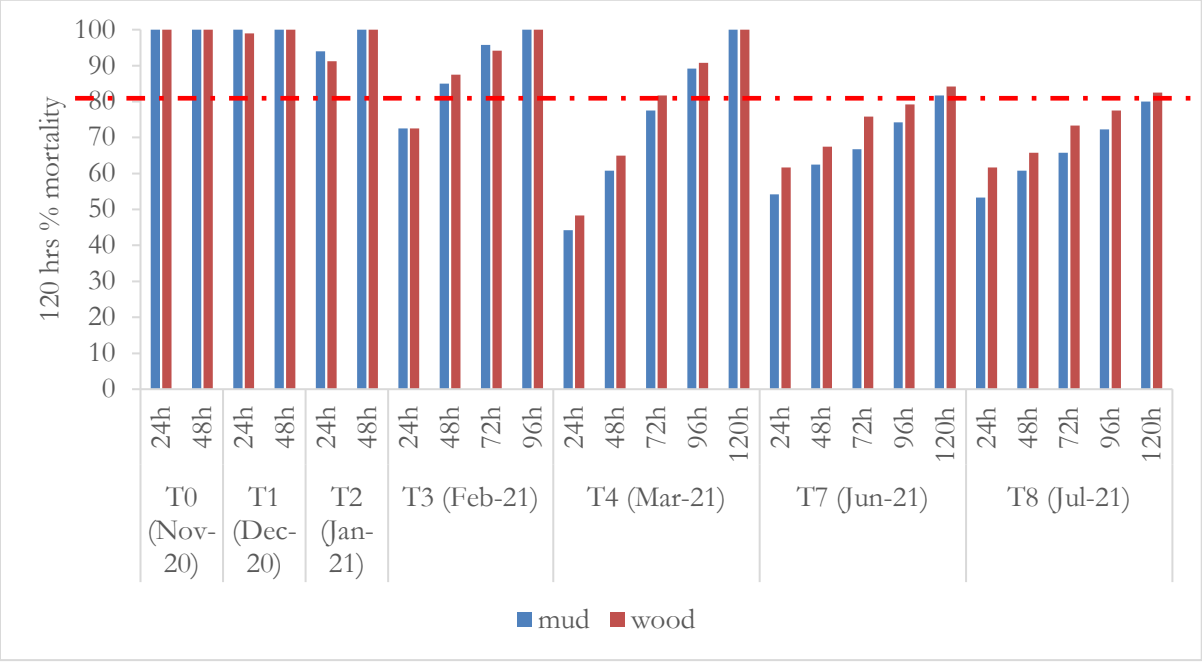


Red line represents the 80% efficacy threshold

3.3.3 Fludora Fusion® WP-SB Sprayed Surfaces

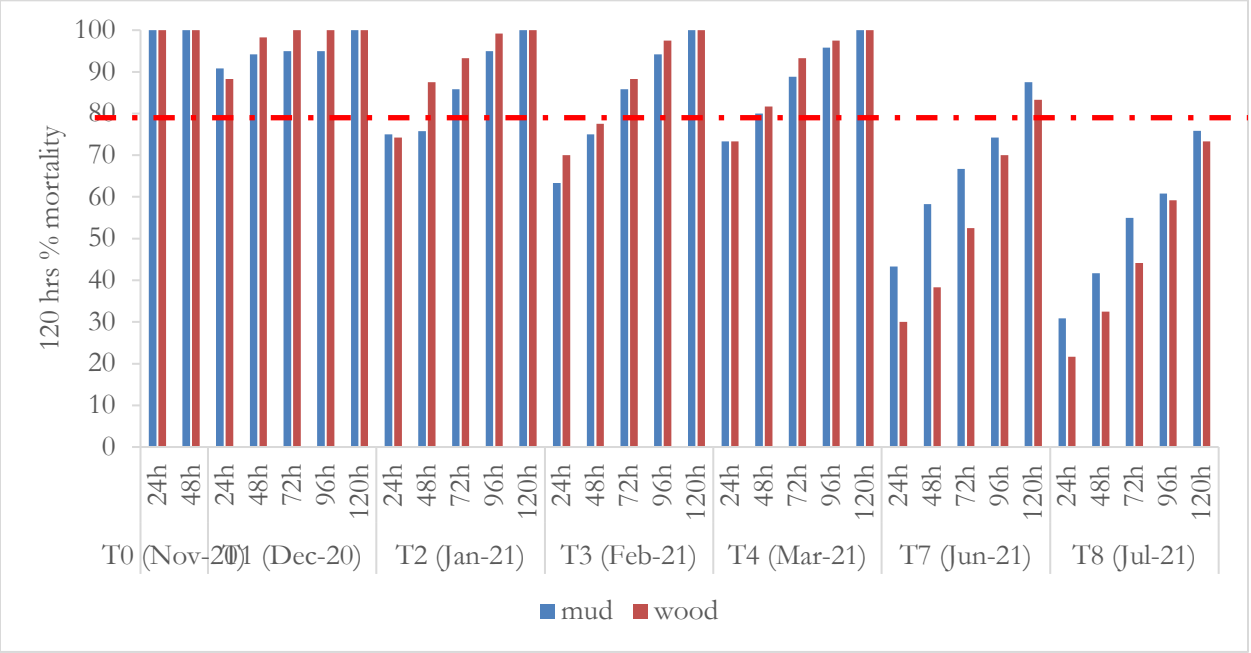
Fludora Fusion was sprayed in two districts, including Irina and Ranotsara. The residual efficacy dropped after eight months in Irina (Ihosy district) and Ranotsara (Iakora district). Tests conducted on both mud and wood surfaces showed that the insecticide lasted for eight months in Irina and seven months in Ranotsara before falling under the 80% threshold (Figures 18 and 19).

Figure 18: Residual Efficacy Observed for Fludora Fusion® in Irina, Ihosy District



Red line represents the 80% efficacy threshold

Figure 19: Residual Efficacy Observed for Fludora Fusion® in Ranotsara, Iakora District



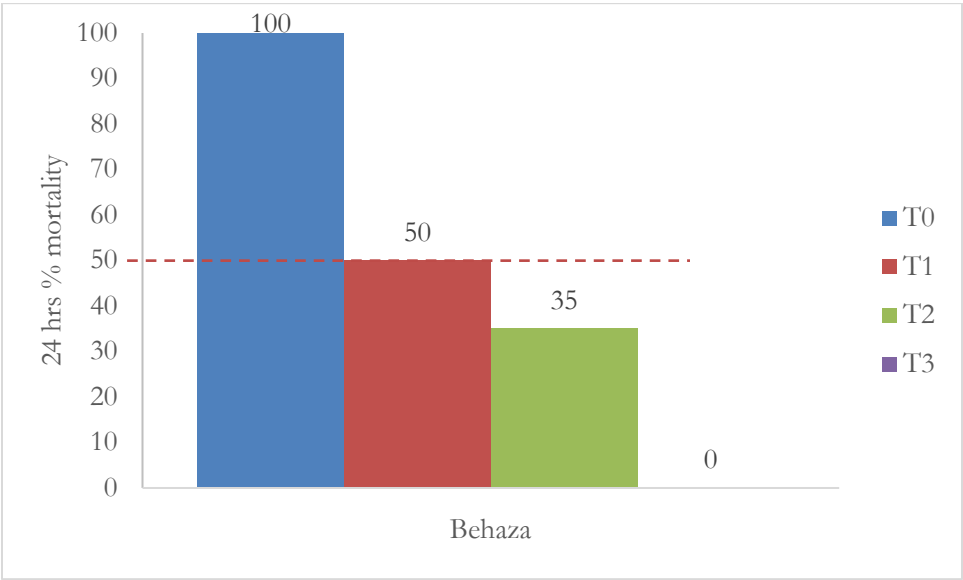
Red line represents the 80% efficacy threshold

3.4 Fumigant Effect of the Sprayed Insecticides

An airborne effect was observed with Actellic® 300 CS, which yielded 100% mortality during the test conducted within one week after spraying (T0) in Bezaha. The effect was observed for an additional month post spraying with about 50% mortality of the mosquitoes tested before dropping to 35% at T2 and 0% at T3 (Figure 20).

No post-spray airborne effect was recorded for Sumishield® 50 WG and Fludora Fusion.

Figure 20: Fumigant Effect of Actellic® 300 CS Sprayed in Behaza



Red line represents the 50% cut-off of the airborne effect

3.5 Molecular Analysis

All the mosquito samples sent to the NMCP molecular laboratory for analysis since 2020 are still not fully completed due to COVID-19 and the fact that the country was placed on lock-down several times since the pandemic started. An addendum will be prepared when data is available. Similarly, the 2021 samples have still not yet been submitted to a laboratory, and the country team is working on an alternative solution to have the samples analyzed.

4. CONCLUSIONS

The vector surveillance data showed a diversity of *Anopheles* mosquitoes across the country, of which *An. gambiae* s.l., *An. funestus* s.l., and *An. mascarensis* vector species were known as malaria vectors. However, *An. coustani*, present at different proportions in various sentinel sites was described as a potential vector after being reported as sporozoite carrier in the country. This diversity of *Anopheles* may need to be investigated for circumsporozoite detection similar to *An. coustani*. *Anopheles funestus* s.l. was found in humid/tropical and equatorial setting (Antsikafoka, Vavatenina and Ampasimpotsy) as well as in subdesert zone (Tsaragiso, Marofatika and Bezaha). Similarly, *An. coustani*, was collected in humid/tropical setting (Vavatenina, Ampasimpotsy and Anamakia), in subdesert zone (Bezaha) and in the fringe of the Central High Lands with tropical high-altitude climate (Irina and Mahasoa). *Anopheles gambiae* s.l. was the dominant vector collected in all sites using all collection methods for both outdoor and indoor collections.

Overall, the density of the vectors collected was low before and after IRS in the sprayed sites, similarly to the control sites even though higher peaks of more than 14 bites per person per night were recorded indoor in March and outdoors in April 2021 in the control sites. The March-April period coincided with the highest biting densities in all the sites. Also, the indoor resting density observed was so low that the number of vectors collected resting both indoors and outdoors did not allow the team to draw conclusions about any changes in resting behavior of the vectors or to assess the impact of IRS on indoor resting density. It was also recorded that the vectors rest outdoors as noted in previous annual reports. Furthermore, *An. gambiae* s.l. biting rates were highest during the first part of the night, as early as 8pm through 11pm, both indoors and outdoors unrelated to the site and interventions. This should be a concern for disease prevention strategies, such as ITNs or IRS, knowing that the population may not be in bed at that time of the night. The trends need to be monitored closely to advise the NMCP on appropriate measures to be undertaken, as well as population awareness.

Anopheles gambiae s.l. was susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr in all sites, including IRS areas, while few sites showed resistance to pyrethroid insecticides. The trends observed with pyrethroids need to be closely followed to avoid fixing of the mutation even though the resistance is still moderate. The same trend was observed in the previous year monitoring data and almost at the same sites. This requires allele frequency monitoring to estimate the level of the mutation in the vector populations. Furthermore, the fact that PBO was able to increase the pyrethroid susceptibility status, that could contribute to decision making in terms of ITN selection and distribution. As the country is still spraying all three available insecticides for IRS, an appropriate rotation plan will help preserve the susceptibility against the vectors in the country.

Cone bioassay tests conducted during the first week of the IRS campaign indicated good quality of spray with 100% mortality of *Anopheles gambiae* s.l. recorded after 24-hour post exposure for all structures tested and sprayed with Actellic 300 CS and Fludora Fusion and after two days for those sprayed with SumiShield 50 WG. The monthly monitoring of the insecticide decay rate for all three insecticides used (Actellic 300 CS, SumiShield and Fludora Fusion) showed that all the insecticides remained 100% effective, four months after IRS was conducted. The monitoring was stopped at the fifth and sixth months after spraying due to the COVID 19 pandemic lock-down; however, it resumed at T7 (seven months after spray) and the mortality dropped under 80% in Bezaha (sprayed with Actellic 300CS), while Sumishield 50 WG which was sprayed in Tsaragiso and Kiliarivo was effective for seven months. In Irina and Ranotsara (sprayed with Fludora Fusion), the observed mortality during the tests remained through the eighth month, before dropping a month later; thus, showing a residual life of about eight months. The trends are similar to those observed in previous reports where the efficacy of all insecticides lasted over six months.

The fumigant effect was only recorded for Actellic 300 CS in Madagascar while no airborne effect was observed for SumiShield 50 WG and Fludora Fusion sprayed structures.

5. ANNEX

All reported data could be visualized through the VectorLink Collect database (VLC)

5.1 Annex A: Longitudinal Monitoring

Table A1: Number of Mosquitoes Collected at Each Sentinel Site between September 2020 and July 2021

	Ampasi-mpotsy	Anamakia	Ankilivalo	Irina	Antsikafo-ka	Mahasoa (control IRS)	Marofari-hy (control Exit Plan)	Ranotsara	Tsaragiso	Vavatenina (control Exit Plan)	Bezaha	Marofatika (control IRS)	TOTAL
An. gambiae s.l.	721	273	352	161	171	203	541	245	239	1180	107	326	**4,519**
An. funestus s.l.	77	2	2	0	52	1	50	0	7	58	16	3	**268**
An. coustani	68	46	116	0	4	16	21	8	0	62	22	0	**363**
An. mascarensis	9	11	0	0	0	0	1	0	0	5	0	0	**26**
An. pharoensis	0	1	16	0	0	0	0	0	0	0	103	0	**120**
An. pretorensis	0	2	0	0	0	0	0	0	0	0	0	0	**2**
An. rufipes	1	10	0	6	0	148	0	0	0	0	0	0	**165**
An. squamosus	17	3	40	3	0	2	1	8	64	3	178	0	**319**
An. pauliani	0	0	0	0	0	0	0	0	0	5	0	0	**5**
An. fuscicolor	0	0	0	0	0	0	0	5	0	0	0	0	**5**
Culicine	1007	468	4126	254	580	764	2750	657	891	553	1312	1101	**14,463**
Total	**1,900**	**816**	**4,652**	**424**	**807**	**1,134**	**3,364**	**923**	**1,201**	**1,866**	**1,738**	**1,430**	**20,255**

Table A2: Number of Mosquitoes Collected by HLC and Human Biting Rates (Bites/Person/Night = b/p/n) during the Collection Period.

Sites	Period	*Anopheles gambiae* s.l.				*Anopheles funestus* s.l.				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**
Mahasoa (control IRS)	Sep-20	9	1.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	7	1.2	6	1.0	7	1.2	8	1.3
	Oct-20	8	1.3	12	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	9	1.5
	Dec-20	3	0.5	10	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	1.0	10	1.7
	Jan-21	3	0.5	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	8	1.3	8	1.3
	Feb-21	13	2.2	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	11	1.8	7	1.2
	Mar-21	11	1.8	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	13	2.2	12	2.0
	Jun-21	5	0.8	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jul-21	6	1.0	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Marofarihy (control Exit Plan)	Sep-20	11	1.8	37	6.2	1	0.2	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Oct-20	14	2.3	34	5.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Nov-20	26	4.3	46	7.7	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	2	0.3	0	0.0	0	0.0
	Jan-21	13	2.2	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	2	0.3	0	0.0	0	0.0
	Feb-21	36	6.0	65	10.8	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Mar-21	45	7.5	47	7.8	14	2.3	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	Jun-21	29	4.8	12	2.0	7	1.2	9	1.5	1	0.2	0	0.0	2	0.3	12	2.0	0	0.0	0	0.0
	Jul-21	2	0.3	13	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		0.0	0	0.0	0	0.0
Vavatenina (control Exit Plan)	Sep-20	8	1.3	25	4.2	3	0.5	3	0.5	0	0.0	1	0.2	3	0.5	9	1.5	0	0.0	0	0.0
	Nov-20	5	0.8	30	5.0	1	0.2	3	0.5	0	0.0	0	0.0	1	0.2	4	0.7	0	0.0	0	0.0
	Dec-20	0	0.0	13	2.2	0	0.0	4	0.7	0	0.0	0	0.0	0	0.0	8	1.3	0	0.0	1	0.2
	Jan-21	26	4.3	39	6.5	3	0.5	2	0.3	1	0.2	0	0.0	0	0.0	3	0.5	0	0.0	1	0.2
	Feb-21	261	43.5	346	57.7	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Mar-21	115	19.2	192	32.0	0	0.0		0.0	0	0.0	0	0.0	1	0.2	0	0.0	3	0.5	1	0.2
	Jun-21	0	0.0	3	0.5	5	0.8	9	1.5	1	0.2	1	0.2	1	0.2	3	0.5	0	0.0	0	0.0
	Jul-21	34	5.7	53	8.8	7	1.2	15	2.5	1	0.2	0	0.0	10	1.7	17	2.8	0	0.0	1	0.2
Marofatika (control IRS)	Oct-20	27	4.5	8	1.3	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Nov-20	36	6.0	18	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Dec-20	18	3.0	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Sites	Period	*Anopheles gambiae* s.l.				*Anopheles funestus* s.l.				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**
	Jan-21	15	2.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Feb-21	27	4.5	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Mar-21	32	5.3	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jun-21	9	1.5	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jul-21	23	3.8	10	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Irina	Oct-20	10	1.7	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	Nov-20	0	0.0	13	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3
	Dec-20	0	0.0	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2
	Jan-21	0	0.0	10	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Feb-21	8	1.3	17	2.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	1	0.2
	Mar-21	5	0.8	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jun-21	0	0.0	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jul-21	1	0.2	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Ranotsara	Oct-20	17	2.8	36	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
	Nov-20	0	0.0	10	1.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7
	Dec-20	0	0.0	12	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	Jan-21	1	0.2	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	2	0.3
	Feb-21	20	3.3	23	3.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5
	Mar-21	34	5.7	36	6.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5
	Jun-21	2	0.3	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	3	0.5	0	0.0	0	0.0
	Jul-21	4	0.7	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Tsaragiso	Oct-20	16	2.7	9	1.5	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	9	1.5	33	5.5
	Nov-20	3	0.5	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	5	0.8
	Dec-20	7	1.2	11	1.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	11	1.8
	Jan-21	4	0.7	10	1.7	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	2	0.3
	Feb-21	8	1.3	24	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Mar-21	13	2.2	27	4.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jun-21	4	0.7	3	0.5	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jul-21	15	2.5	12	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Sites	Period	*Anopheles gambiae* s.l.				*Anopheles funestus* s.l.				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**
Bezaha	Oct-20	11	1.8	19	3.2	7	1.2	2	0.3	0	0.0	0	0.0	0	0.0	5	0.8	6	1.0	17	2.8
	Nov-20	0	0.0	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2	9	1.5
	Dec-20	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	2	0.3	6	1.0
	Jan-21		0.0		0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	17	2.8
	Feb-21	0	0.0	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	21	3.5	37	6.2
	Mar-21	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2	19	3.2	52	8.7
	Jun-21	13	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	2	0.3	7	1.2	2	0.3
	Jul-21	9	1.5	12	2.0	0	0.0	2	0.3	0	0.0	0	0.0	3	0.5	4	0.7	21	3.5	19	3.2
Ampasimpotsy	Sep-20	22	3.7	48	8.0	1	0.2	5	0.8	1	0.2	2	0.3	4	0.7	9	1.5	0	0.0	0	0.0
	Oct-20	18	3.0	47	7.8	2	0.3	6	1.0	0	0.0	0	0.0	5	0.8	10	1.7	0	0.0	0	0.0
	Nov-20	75	12.5	58	9.7	1	0.2	0	0.0	0	0.0	0	0.0	5	0.8	15	2.5	1	0.2	5	0.8
	Jan-21	18	3.0	19	3.2	0	0.0		0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0
	Feb-21	63	10.5	92	15.3	1	0.2	3	0.5	2	0.3	2	0.3	0	0.0	2	0.3	0	0.0	7	1.2
	Mar-21	52	8.7	65	10.8	0	0.0	5	0.8	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7
	Jun-21	30	5.0	19	3.2	12	2.0	4	0.7	0	0.0	0	0.0	0	0.0	10	1.7	0	0.0	1	0.2
	Jul-21	5	0.8	15	2.5	16	2.7	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Anamakia	Dec-20	7	1.2	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	Jan-21	11	1.8	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Feb-21	20	3.3	26	4.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7	0	0.0	2	0.3
	Mar-21	22	3.7	25	4.2	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	3	0.5	0	0.0	0	0.0
	Jun-21	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	13	2.2	13	2.2	0	0.0	4	0.7
	Jul-21	0	0.0	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	2	0.3
Ankilivalo	Sep-20	12	2.0	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	0.8
	Oct-20	1	0.2	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	7	1.2
	Nov-20	2	0.3	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jan-21	9	1.5	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	3	0.5
	Feb-21	20	3.3	12	2.0	0	0.0	0	0.0	0	0.0	0	0.0	6	1.0	3	0.5	3	0.5	2	0.3
	Mar-21	27	4.5	39	6.5	1	0.2	1	0.2	0	0.0	0	0.0	34	5.7	62	10.3	2	0.3	7	1.2
	Jun-21	21	3.5	25	4.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0
	Jul-21	28	4.7	36	6.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	7	1.2	2	0.3

Sites	Period	*Anopheles gambiae* s.l.				*Anopheles funestus* s.l.				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**	**Indoor**	**Indoor Biting Rate**	**Out-door**	**Out-door Biting Rate**
Antsikafoka	Sep-20	1	0.2	25	4.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Nov-20	1	0.2	9	1.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Dec-20	3	0.5	2	0.3	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jan-21	10	1.7	13	2.2	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Feb-21	2	0.3	8	1.3	0	0.0	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Mar-21	9	1.5	50	8.3	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Jun-21	1	0.2	8	1.3	11	1.8	22	3.7	0	0.0	0	0.0	0	0.0	2	0.3	0	0.0	0	0.0
	Jul-21	5	0.8	19	3.2	6	1.0	6	1.0	0	0.0	0	0.0	1	0.2	1	0.2	0	0.0	0	0.0

Table A3: Total Number of Mosquitoes Collected by Prokopack Aspirator and Indoor Resting Density during the Collection Period

Species	Month	Tsaragiso		Bezaha		Irina		Ranotsara Nord		Marofatika		Mahasoa		Antsikafoka		Vavatenina		Ampasipotsy		Marofarihy		Ankilivalo		Anamakia	
		#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density
An. gambiae s.l.	Sep-20													0	0	0	0	6	0.6	13	1.3	6	0.6		
	Oct-20	7	0.7	3	0.3	5	0.5	2	0.2	14	1.4	7	0.7					5	0.5	4	0.4	4	0.4		
	Nov-20	5	0.5	0	0	8	0.8	0	0	9	0.9	3	0.3	1	0.1	2	0.2	9	0.9	6	0.6	2	0.2		
	Dec-20	6	0.6	1	0	4	0.4	0	0	6	0.6	4	0.4	0	0	2	0.2	0				0	0	5	0.5
	Jan-21	5	0.5	0	0	9	0.9	0	0	4	0.4	11	1.1	1	0.1	0	0	5	0.5	9	0.9	8	0.8	10	1
	Feb-21	18	1.8	0	0	7	0.7	0	0	6	0.6	15	1.5	0	0	4	0.4	8	0.8	11	1.1	12	1.2	13	1.3
	Mar-21	11	1.1	1	0.1	10	1	0	0	11	1.1	15	1.5	0	0	1	0.1	14		23	2.3	12	1.2	25	2.5
	Jun-21	3	0.3	6	0.6	5	0.5	1	0.1	3	0.3	5	0.5	0	0	0	0	1		11	1.1	9	0.9	1	0.1
	Jul-21	7	0.7	6	0.6	8	0.8	0	0	9	0.9	10	1	0	0	2	0.2	2		4	0.4	17	1.7	5	0.5
An. funestus	Sep-20													0	0	0	0	0	0	0	0	0	0		
	Oct-20	1	0.1	1	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.1	0	0		
	Nov-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0		
	Dec-20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0		0	0	0	0	0
	Jan-21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Feb-21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0.4	0	0	0	0
	Mar-21	0	0	0	0	0	0	0	0	0	0	1	0.1	0	0	0	0	0	0	1	0.1	0	0	0	0
	Jun-21	0	0	0	0	0	0	0	0	0	0	0	0	2	0.2	0	0	5	0.5	3	0.3	0	0	2	0.2
	Jul-21	0	0	4	0.4	0	0	0	0	0	0	0	0	0	0	0	0	3	0.3	0	0	0	0	0	0

Table A4: Total Number of Mosquitoes Collected Outdoor Using Mouth Aspiration (ODC) Method during the Collection Period.

	Ampasimpotsy	Ankilivalo	Irina	Antsikafoka	Mahasoa (control IRS)	Marofarihy (control Exit Plan)	Ranotsara	Vavatenina (control Exit Plan)	Bezaha	Total
An. coustani	7	2	0	0	0	0	0	2	0	11
An. funestus s.I.	5	0	0	0	0	0	0	1	0	6
An. gambiae s.l.	25	19	8	3	14	14	26	19	4	132
An. pharoensis	0	1	0	0	0	0	0	0	0	1
An. rufipes	0	0	0	0	2	0	0	0	0	2
An. squamosus	0	5	0	0	0	0	0	1	2	8
Culicine	58	130	0	32	8	112	9	39	4	392
Total	**95**	**157**	**8**	**35**	**24**	**126**	**35**	**62**	**10**	**552**

Table A5: Total Number of Mosquitoes Collected Outdoor Using Prokopack Aspiration (ODC) Method during the Collection Period.

	Anamakia	Ankilivalo	Irina	Mahasoa	Marofarihy	Tsaragiso	Bezaha	Marofatika	Total
An. coustani	1	2	0	0	0	0	1	0	4
An. funestus s.I.	0	0	0	0	3	1	4	0	8
An. gambiae s.l.	44	12	48	28	14	39	8	17	210
An. mascarensis	7	0	0	0	0	0	0	0	7
An. pharoensis	0	0	0	0	0	0	10	0	10
An. rufipes	4	0	1	17	0	0	0	0	22
An. squamosus	0	0	0	0	0	0	11	0	11
Culicine	56	57	37	95	89	97	67	65	563
Total	**112**	**71**	**86**	**140**	**106**	**137**	**101**	**82**	**835**

Table A6: Indoor Hourly Human Biting Rates of *An. gambiae* s.l.

Site status	Sentinel sites	18-19	19-20	20-21	21-22	22-23	23-24	24-1	1-2	2-3	3-4	4-5	5-6
Control Sentinel Sites	Mahasoa (control)	0.0	0.0	0.08	0.4	0.08	0.04	0.17	0.31	0.08	0.04	0.0	0.0
	Marofarihy	0.0	0.13	0.38	0.88	1.0	0.69	0.31	0.19	00	0.06	0.0	0.0
	Marofatika	0.46	0.6	1.5	1.3	1.4	0.67	0.9	0.98	0.77	0.44	0.21	0.13
	Vavatenina	0.02	0.31	0.69	1.2	0.54	0.35	0.29	0.29	0.1	0.06	0.02	0.02
	Mean	0.12	0.26	0.66	0.95	0.75	0.44	0.42	0.44	0.24	0.15	0.06	0.04
Exit Plan (Non-Sprayed)	Ampasimpotsy	0.19	0.44	0.58	1.2	1.6	1.3	0.29	0.25	0.02	0.04	0.0	0.0
	Anamakia	0.22	0.11	0.14	0.36	0.28	0.14	0.08	0.22	0.08	0.03	0.0	0.0
	Ankilivalo	0.06	0.31	0.27	0.21	0.29	0.08	0.1	0.29	0.25	0.23	0.35	0.04
	Mahambo/Antsikafoka	0.02	0.02	0.04	0.1	0.13	0.06	0.08	0.1	0.06	0.04	0.0	0.0
	Mean	0.12	0.22	0.26	0.47	0.58	0.39	0.14	0.22	0.10	0.09	0.09	0.01
IRS Intervention Sentinel Sites	Bezaha	0.0	0.0	0.02	0.13	0.02	0.08	0.08	0.13	0.0	0.02	0.0	0.02
	Irina	0.0	0.02	0.06	0.21	0.29	0.15	0.25	0.13	0.19	0.17	0.15	0.02
	Ranotsara	0.02	0.04	0.33	0.52	0.08	0.13	0.13	0.08	0.02	0.06	0.04	0.0
	Tsaragiso	0.02	0.0	0.06	0.04	0.19	0.06	0.04	0.1	0.15	0.06	0.04	0.04
	Mean	0.01	0.015	0.12	0.225	0.145	0.105	0.125	0.11	0.09	0.08	0.06	0.02

Table A7: Outdoor Hourly Human Biting Rates of *An. gambiae* s.l.

Site status	Sentinel sites	18-19	19-20	20-21	21-22	22-23	23-24	24-1	1-2	2-3	3-4	4-5	5-6
Control Sentinel Sites	Mahasoa (control)	0.00	0.02	0.10	0.25	0.21	0.00	0.27	0.33	0.04	0.02	0.02	0.00
	Marofarihy	0.00	0.40	1.00	0.96	0.96	0.88	0.56	0.35	0.27	0.17	0.04	0.00
	Marofatika	0.00	0.04	0.19	0.54	0.17	0.19	0.10	0.15	0.15	0.08	0.00	0.00
	Vavatenina	0.69	0.85	1.70	2.30	1.90	1.30	1.80	1.80	1.40	0.77	0.21	0.04
	Mean	0.17	0.33	0.75	1.01	0.81	0.59	0.68	0.66	0.47	0.26	0.07	0.01
Exit Plan (Non-Sprayed)	Ampasimpotsy	0.13	0.65	1.30	1.50	1.50	1.10	0.75	0.31	0.31	0.00	0.00	0.00
	Anamakia	0.17	0.25	0.31	0.39	0.25	0.19	0.08	0.14	0.08	0.08	0.03	0.00
	Ankilivalo	0.04	0.38	0.33	0.38	0.40	0.29	0.25	0.15	0.13	0.23	0.33	0.08
	Mahambo/Antsikafoka	0.00	0.06	0.40	0.38	0.35	0.40	0.10	0.46	0.42	0.08	0.10	0.04
	Mean	0.09	0.34	0.59	0.66	0.63	0.50	0.30	0.27	0.24	0.10	0.12	0.03
IRS Intervention	Bezaha	0.02	0.04	0.08	0.08	0.10	0.15	0.13	0.06	0.04	0.15	0.08	0.04
	Irina	0.00	0.02	0.02	0.35	0.33	0.19	0.06	0.40	0.06	0.04	0.02	0.02

Site status	Sentinel sites	18-19	19-20	20-21	21-22	22-23	23-24	24-1	1-2	2-3	3-4	4-5	5-6
Sentinel Sites	Ranotsara	0.00	0.13	0.13	0.33	0.35	0.48	0.44	0.33	0.17	0.25	0.21	0.06
	Tsaragiso	0.04	0.35	0.29	0.67	0.19	0.15	0.10	0.15	0.13	0.13	0.04	0.00
	Mean	0.02	0.14	0.13	0.36	0.24	0.24	0.18	0.24	0.10	0.14	0.09	0.03

Table A8: Indoor and Outdoor Parity Rates of *An. gambiae* s.l.

Sites	Month	Total indoor	Total parous	% Indoor Parous	Total outdoor	Total parous	% Outdoor Parous
Control sites	Sep-20	26	15	57.7	68	28	41.2
	Oct-20	49	33	67.3	53	30	56.6
	Nov-20	64	45	70.3	103	49	47.6
	Dec-20	21	15	71.4	29	15	51.7
	Jan-21	57	30	52.6	68	31	45.6
	Feb-21	336	140	41.7	433	169	39.0
	Mar-21	203	151	74.4	256	214	83.6
	Jun-21	43	21	48.8	20	12	60.0
	Jul-21	65	41	63.1	76	60	78.9
IRS sites	Oct-20	53	30	56.6	65	21	32.3
	Nov-20	3	0	0.0	36	22	61.1
	Dec-20	10	6	60.0	38	18	47.4
	Jan-21	5	3	60.0	28	10	35.7
	Feb-21	39	10	25.6	66	26	39.4
	Mar-21	65	24	36.9	83	37	44.6
	Jun-21	14	8	57.1	15	5	33.3
	Jul-21	16	13	81.3	27	16	59.3
Exit Plan sites	Sep-20	35	11	31.4	81	26	32.1
	Oct-20	19	6	31.6	116	33	28.4
	Nov-20	78	17	21.8	105	43	41.0
	Dec-20	10	7	70.0	68	24	35.3
	Jan-21	47	16	34.0	156	46	29.5
	Feb-21	105	30	28.6	176	62	35.2
	Mar-21	109	46	42.2	216	108	50.0
	Jun-21	52	18	34.6	62	44	71.0
	Jul-21	38	32	84.2	85	60	70.6

5.2 Annex B: Insecticide Susceptibility Tests

Table B1: Results of *An. gambiae* s.l. Susceptibility Tests

Insecticide Tested	Vavatenina			Antsikafoka			Tsaragiso			Marofatika			Kiliarivo		
	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status
Deltamethrin	100	61	R	100	72	R	100	99	S	NC	73	R	100	99	S
Permethrin	100	65	R	100	68	R	100	99	S	100	75	R	100	93	P
Pirimiphos-Methyl	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Clothianidin	100	100	S	100	100	S	100	100	S	100	100	S	NC	100	S
Chlorfenapyr	100	100	S	100	100	S	100	100	S	100	100	S	NC	100	S

Insecticide Tested	Ampasimpotsy			Marofarihy			Ranotsara Nord			Ankilivalo			Bezaha		
	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status
Deltamethrin	100	100	S	100	100	S	100	100	S	100	66	R	100	100	100
Permethrin	100	100	S	100	100	S	100	100	S	100	100	S	100	72	R
Pirimiphos-Methyl	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Clothianidin	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Chlorfenapyr	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S

Insecticide Tested	Irina			Mahasoa			Anamakia		
	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status	N# Tested	24h % * Mortality	Resistance Status
Deltamethrin	100	100	S	100	100	S	100	98	S
Permethrin	100	100	S	100	100	S	100	100	S
Pirimiphos-Methyl	100	100	S	100	100	S	100	100	S

Table B2. Results of Synergist Assay of *An. gambiae* s.l. (WHO Tube Test)

Site	insecticide	PBO Only Control: Total Tested	PBO Only Control Mortality 24hrs	Insecticide+PBO Total Tested	Insecticide+PBO Mortality 24hrs	Insecticide Only: Total Tested	Insecticide Only Mortality 24hrs	Solvent Only Control: Total Tested	Solvent Only Control Mortality 24hrs
Vavatenina (control)	Deltamethrin	25	5	25	100	100	61	50	0
Vavatenina (control)	Permethrin	25	0	25	100	100	65	50	0
Marofatika (control)	Deltamethrin	25	0	100	100	100	73	50	0
Marofatika (control)	Permethrin	25	0	100	100	100	75	50	0
Ankilivalo	Deltamethrin	25	0	25	100	100	66	50	0
Anamakia	Deltamethrin	25	0	25	100	100	95	50	0
Mahambo/Antsikafoka	Deltamethrin	25	0	25	100	100	72	50	0
Mahambo/Antsikafoka	Permethrin	25	0	25	100	100	68	50	0
Bezaha	Permethrin	25	0	25	100	100	65	50	0
Kiliarivo	Permethrin	25	0	100	100	100	93	50	0

Table B3: Results of Resistance Intensity Assay

Sites	Insecticide	Concentration	Mortality
Anamakia	Deltamethrin	0.25%	100
Ankilivalo	Deltamethrin	0.25%	100
Antsikafoka	Deltamethrin	0.25%	100

Sites	Insecticide	Concentration	Mortality
Antsikafoka	Permethrin	3.75%	99
Vavatenina (control Exit Plan)	Deltamethrin	0.25%	97
Vavatenina (control Exit Plan)	Permethrin	3.75%	96
Vavatenina (control Exit Plan)	Permethrin	7.50%	100
Bezaha	Permethrin	3.75%	100
Kiliarivo	Permethrin	3.75%	100
Marofatika (control IRS)	Deltamethrin	0.25%	100
Marofatika (control IRS)	Permethrin	3.75%	100

Table B4. Results of *An. gambiae* s.l. Susceptibility Tests to Clothianidin (Mortality Observed from Day1 to Day7)

Sites	KD 60min	24hrs	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Ampasimpotsy	46	88	98	100	100	100	100	100
Anamakia	7	26	69	100	100	100	100	100
Ankilivalo	25	74	88	99	100	100	100	100
Irina	100	100	100	100	100	100	100	100
Antsikafoka	39	90	97	100	100	100	100	100
Mahasoa (control IRS)	100	100	100	100	100	100	100	100
Marofarihy (control Exit Plan)	43	65	93	100	100	100	100	100
Ranotsara	87	94	100	100	100	100	100	100
Tsaragiso	5	65	86	100	100	100	100	100
Vavatenina (control Exit Plan)	28	91	97	98	99	100	100	100
Bezaha	35	69	82	91	99	100	100	100
Kiliarivo	8	64	84	100	100	100	100	100
Marofatika (control IRS)	7	72	92	100	100	100	100	100

Table B5: Results of *An. gambiae* s.l. Susceptibility Tests to Chlorfenapyr (Mortality Observed from Day1 to Day3)

Sites	KD 60min	24h	48h	72h
Ampasimpotsy	71	100	100	100
Anamakia	0	100	100	100
Ankilivalo	23	80	100	100
Irina	100	100	100	100
Mahambo/Antsikafoka	6	98	100	100
Mahasoa (control IRS)	100	100	100	100
Marofarihy (control Exit Plan)	83	100	100	100
Ranotsara	84	100	100	100
Tsaragiso	39	84	100	100
Vavatenina (control Exit Plan)	6	99	100	100
Bezaha	71	98	100	100
Kiliarivo	50	90	100	100
Marofatika (control IRS)	38	92	100	100

Table B6: Results of Other Vector Species Susceptibility Tests

Species	Sites	Insecticide Tested	Value (%)	Number tested	Resistance status
An. funestus s.l.	Anamakia	Deltamethrin	100	100	S
		Permethrin	100	100	S
	Marofarihy (control Exit Plan)	Pirimiphos-methyl	100	50	S
An. mascarensis	Antsikafoka	Pirimiphos-methyl	100	100	S
		Deltamethrin	100	100	S
	Anamakia	Permethrin	100	100	S
		Deltamethrin	99	100	S
An. coustani	Ankilivalo	Pirimiphos-methyl	100	100	S
	Antsikafoka	Deltamethrin	95	100	P
		Permethrin	99	99	100
		Pirimiphos-methyl	100	100	100
	Marofarihy (control Exit Plan)	Pirimiphos-methyl	100	100	100

5.3 Annex C: Sprayed Insecticide Residual Life

Table C1: Results of Cone Bioassay on Actellic 300 CS® (Mortality Observed 24h)

Sites	Period	Mud	Wood
Bezaha	(T0) November 2020	100	100
Bezaha	(T1) December 2020	100	100
Bezaha	(T2) January 2021	100	100
Bezaha	(T3) February 2021	100	100
Bezaha	(T4) March 2021	97.5	98.3
Bezaha	(T7) June 2021	66.7	73.3
Bezaha	(T8) July 2021	61.7	65

Table C2: Results of Cone Bioassay on Fludora fusion® and SumiShield® 50 WG (Mortality Observed from Day1 to Day5)

	Site	Period	Mortality	Mud	Wood
Fludora Fusion	Irina	Nov-20	24hrs	100	92.5
			Day 2	100	97.5
			Day 3	100	100
		Dec-20	24hrs	100	99.2
			Day 2	100	100
		Jan-21	24hrs	92.5	91.7
			Day 2	100	100
		Feb-21	24hrs	72.5	72.5
			Day 2	85	87.5
			Day 3	93.5	95.8
			Day 4	100	100
		Mar-21	24hrs	44.2	48.3
			Day 2	62.5	63.3
			Day 3	76.7	80.8
			Day 4	90.6	91

Site	Period	Mortality	Mud	Wood
		Day 5	100	100
	Jun-21	24hrs	54.2	61.7
		Day 2	62.5	67.5
		Day 3	66.7	75.8
		Day 4	74.2	78.8
		Day 5	80.8	83.1
	Jul-21	24hrs	53.3	60.8
		Day 2	60.8	66.7
		Day 3	65.8	73.3
		Day 4	72.6	77.5
		Day 5	79.4	82.5
Ranotsara	Nov-20	24hrs	78.3	77.5
		Day 2	91.7	94.2
		Day 3	100	100
	Dec-20	24hrs	100	86.7
		Day 2	100	100
	Jan-21	24hrs	66.7	75
		Day 2	77.5	89.2
		Day 3	91.7	92.5
		Day 4	95.8	99.2
		Day 5	100	100
	Feb-21	24hrs	63.3	69.2
		Day 2	75	76.7
		Day 3	85.8	87.5
		Day 4	94.2	97.5
		Day 5	100	100
	Mar-21	24hrs	70	77.5
		Day 2	80	84.2
		Day 3	88.3	93.3
		Day 4	95.8	97.5
		Day 5	100	100
	Juin 2021	24hrs	43.3	30
		Day 2	58.3	38.3

	Site	Period	Mortality	Mud	Wood
			Day 3	65	52.5
			Day 4	74.2	70
			Day 5	87.5	83.3
		Jul-21	24hrs	30.8	21.7
			Day 2	41.7	31.7
			Day 3	51.7	44.2
			Day 4	59.2	59.2
			Day 5	75.8	73.3
SumiShield 50WG	Tsaragiso	Nov-20	24hrs	60.8	70
			Day 2	100	100
		Dec-20	24hrs	52.5	51.7
			Day 2	100	100
		Jan-21	24hrs	52.5	47.5
			Day 2	63.3	67.5
			Day 3	100	100
		Feb-21	24hrs	41.7	40.8
			Day 2	57.5	66.7
			Day 3	100	100
		Mar-21	24hrs	35.8	39.2
			Day 2	45	60
			Day 3	55.8	70
			Day 4	100	100
		Jun-21	24hrs	27.5	29.2
			Day 2	36.7	41.7
			Day 3	44.2	50.8
			Day 4	60.8	65
			Day 5	79.2	82.5
		Jul-21	24hrs	0	0
			Day 2	5	7.5
			Day 3	24.2	27.5
			Day 4	40.8	47.5
			Day 5	65	72.5
	Kiliarivo	Nov-20	24hrs	62.5	79.2

Site	Period	Mortality	Mud	Wood
		Day 2	100	100
	Dec-20	24hrs	55.8	79.2
		Day 2	99.2	100
		Day 3	100	100
	Jan-21	24hrs	46.7	64.2
		Day 2	75	80
		Day 3	100	100
	Feb-21	24hrs	39.2	53.3
		Day 2	66.7	65
		Day 3	100	100
	Mar-21	24hrs	25.8	37.5
		Day 2	52.5	48.3
		Day 3	61.7	61.7
		Day 4	100	100
	Jun-21	24hrs	16.3	28.3
		Day 2	38.9	39.2
		Day 3	48.9	48.3
		Day 4	68.9	70
		Day 5	80	80.8
	Jul-21	24hrs	0	0
		Day 2	1.7	2.5
		Day 3	25	26.7
		Day 4	41.7	47.5
		Day 5	65.8	73.3