U.S. PRESIDENT'S MALARIA INITIATIVE

THE PMI VECTORLINK MADAGASCAR FINAL ENTOMOLOGICAL MONITORING REPORT

JULY 2019 – AUGUST 2020

SUBMITTED SEPTEMBER 18, 2020

Recommended Citation: The PMI VectorLink Project. Madagascar Entomological Monitoring Final Report: July 2019 – August 2020. Rockville, Maryland. The PMI VectorLink Project, Abt Associates Inc.

Contract: AID-OAA-I-17-00008

Task Order Number: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Approved on:

BOLD THINKERS DRIVING REAL-WORLD IMPACT

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ACRONYMS

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

EXECUTIVE SUMMARY

Background

Malaria remains a major health problem in Madagascar, and severe malaria is among the top five causes of reported overall deaths. Malaria epidemiology varies considerably in different regions of the country; however, the entire population is considered to be at risk for the disease. Among the five *Plasmodium* species that can infect humans, four are present in Madagascar (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*), with a predominance of *Plasmodium falciparum* at more than 90%. *Anopheles gambiae* s.l. represents the main malaria vector across the country and the predominant *Anopheles* species. However, additional species such as *An. funestus* s.l., *An. mascarensis* and *An. coustani* have been reported as malaria vectors in country.

The malaria National Strategic Plan has stratified the country into malaria epidemiologic clusters based on malaria endemicity. Malaria control and elimination interventions are defined based on each epidemiological cluster. Since 2008, Madagascar has worked with PMI to implement malaria vector control activities. Indoor residual spraying (IRS) and mass distribution of ITNs represent the two main malaria vector control strategies undertaken in the country.

In Madagascar, indoor residual spraying (IRS) is an important component of the malaria control strategy, as included in the country's National Strategic Plan. Madagascar is supported by both the US President's Malaria Initiative (PMI) and Global Fund (GF) for the implementation of successful IRS campaigns through provision of funds, staff and technical guidance. From 2017 to date, the country has sprayed approximately 48 districts across the country with the support of PMI and Global Fund for AIDS Tuberculosis and Malaria/New Malaria Funding (GFATM/NMF).

During the 2019 IRS campaign, the PMI VectorLink Madagascar Project has covered over 93 communes in four districts in the South West region with blanket IRS (Tulear II, Sakaraha, Betioky, Ampanihy districts) and in one district in Ihorombe region (Ihosy) The project team sprayed pirimiphos-methyl (Actellic® 300 CS), an organophosphate insecticide, in Betioky and Ampanihy districts. 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 district of Ihosy. The spray campaign was conducted from November 4 to November 30, 2020 in these five districts.

PMI VectorLink Madagascar conducted entomological monitoring activities including comprehensive vector monitoring activities on vector density, species composition, seasonal patterns, biting behavior, sporozoites rates, and parity of *Anopheles* mosquitoes from 12 sentinel sites. The entomological monitoring sites included four IRS sites and two control (non-IRS) sites, two sites in areas where IRS was withdrawn in 2019 (Mahambo (Fenerive Est) and Manakara, after four and three years of IRS blanket coverage respectively in each site). The monitored sites also included two control sites in areas where IRS was withdrawn in 2019 and were compared with one site in a district in an elimination setting (non-IRS) and one site in a district where the National Malaria Control Program (NMCP) adopted only mass distribution of insecticide treated nets (ITNs) as unique malaria vector control strategy. Data on vector species composition, density and behavior was collected using human landing catches (HLCs) and adult collections using Prokopack and mouth aspirators, indoors and outdoors in pit shelters. 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 about 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 for a maximum of eight months after which the residual insecticide was below 80% for two consecutive months. Insecticide susceptibility was also conducted in 13 sentinel sites: the 12 sentinel sites where comprehensive entomological monitoring was conducted, and one site, located in an unsafe area (district of Sakaraha), where only susceptibility tests and cone bio assay could be performed.

Results

The results of this report exclude data from two sites (Anamakia in the district of Diego I and Ankilivalo in the district of Mahabo), where activities began in December 2019 but were stopped in February 2020,due to the COVID-19 pandemic.

Vector density and seasonality: A total of 6,072 (33.9 %) female *Anopheles* and 11,846 (66.1 percent) culicine mosquitoes were collected during the monitoring period. The most abundant *Anopheles* species was *An. gambiae* s.l., representing 39.9% (n= 2,420) of the total *Anopheles* mosquitoes collected. The other two *Anopheles* species, *An. funestus* s.l. and *An. mascarensis,* which are also vectors of malaria in Madagascar, accounted for 9.2 % (n=556) and 3.7% (n=226) of the collected species respectively. *Anopheles coustani,* reported as a probable vector in one area of Madagascar (Nepomichene et al., 2015), was present in eight sites. A total of 612 (10.1 % of the total *Anopheles* mosquitoes collected) female *An. coustani* were collected during the monitoring period. The other *Anopheles*, composed of *An. fuscicolor*, *An. flavicosta*, *An. maculipalpis*, *An. pauliani*, *An. rufipes*, *An. ranci* and *An. squamosus/cyddipis* represented 37.2%(n= 2,258) of the total *Anopheles* collected.

A total of 77 *An. gambiae* s.l., 29 *An. funestus* s.l., and 7 *An. mascarensis* were collected indoors using the Prokopack aspiration method. In addition, 232 *An. gambiae* s.l., 29 *An. funestus* s.l. and 38 *An. mascarensis,* were collected outdoors with mouth aspirators and Prokopack, mostly from artificial pit shelters. The team collected 16,140 (90.1%) mosquitoes (all genera included) through human landing catches: 5,642 (35.0 %) indoors and 10,498 (65.0 %) outdoors; 3,391 (21.0 %) are known or possible malaria vectors.

Feeding time and location: At the baseline before indoor residual spraying (IRS), *An. gambiae* s.l. indoor human biting rates (HBR) ranged from 0.2 bites per person per night (b/p/n) in Manakaravay (Ampanihy district), to 4.2 bites per person per night in Tsaragiso (Tulear II district) and Bezaha (Betioky district). The outdoor human biting rates ranged from 0.2 (b/p/n) in Manakaravay to 6.2 (b/p/n) in Bezaha. In all IRS sentinel sites, the majority of *An. gambiae* s.l. exhibited exophagic tendencies before IRS, except in Tsaragiso. It has been noted that the vectors bite more outdoors than indoors in the IRS sites but no significant difference was observed in control sites. The low mean biting rates observed 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 throughout the night with variable peaks between sites.

Quality of spray and residual life: The results of wall bioassays indicated good spray quality in all sites with 100 % mortality recorded for all the structures tested at T0 (24 hours after spraying) and T1 (one month after spray). The fumigant test for Actellic® 300 CS shows no airborne effect of the insecticide, one month after spraying. The mosquito mortality rate with pirimiphos-methyl (Actellic® 300 CS), Sumishield® 50WG and Fludora Fusion® exceeded 80% over six months post-spray in the sprayed districts following the 2019 IRS campaign.

Susceptibility tests: The results of the vector susceptibility tests indicated susceptibility of *An. gambiae* s.l. to pirimiphos-methyl, clothianidin, bendiocarb and chlorfenapyr in all areas where the tests were conducted. However, *An. gambiae* s.l. is resistant to permethrin and deltamethrin in Vavatenina and Mahambo/Antsikafoka, to alpha-cypermethrin in Vavatenina and Tsaragiso, and to lambda-cyhalothrin in Vavatenina and Betaindambo. Suspected resistance to deltamethrin in Besakoa Bezaha and Betaindambo, to Dichlorodiphenyltrichloroethane (DDT) in Vavatenina and to permethrin in Betaindambo, was noted.

Molecular analysis: Mosquito samples will be sent to the NMCP laboratory for molecular analysis and Enzyme-Linked Immunosorbent Assay (ELISA) circumsporozoite tests. This report will be updated or amended as soon as the results of these tests are available.

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 implemented IRS since 2012, delivered high-quality IRS campaigns 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 monitoring activities in twelve sites.

Entomological monitoring was conducted in districts where Actellic® 300 CS, SumiShield® 50 WG and Fludora Fusion® were sprayed, as well as in control sites. Monthly indoor resting collections using Prokopack aspirators, human landing catches, outdoor collection of adult mosquitoes from pit shelters using Prokopack and mouth aspirators, cone bioassays, as well as insecticide susceptibility testing were conducted in selected sites.

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.

The objectives of the 2019-2020 entomological surveillance were:

- To identify the vector species composition and density
- To assess vector biting and resting behavior
- To assess the IRS quality and insecticide decay rates post-spray operations
- To assess vector susceptibility to different insecticides used for IRS and ITNs including pyrethroid (alpha-cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin), organophosphate (pirimiphos-methyl), carbamate (bendiocarb), organochlorine (DDT), pyrrole (chlorfenapyr) and neonicotinoid (clothianidin).

2. METHODOLOGY

From July 2019 to August 2020, PMI VectorLink Madagascar conducted longitudinal entomological surveillance in 10 of 12 selected sites by the NMCP, including the 2019 IRS sites and insecticide resistance monitoring in 11 of the 13 selected sites. The twelve sites selected include two sites added later (Diego I and Ankilivalo), where activities started in December 2019 but stopped in February 2020 due to the COVID-19 pandemic.

The number of Insecticide resistance monitoring sites was 11, including the 10 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 due to insecurity in the area.

2.1. Vector Bionomics Monitoring

2.1.1. Study Sites

The 2019 Madagascar IRS campaign was the first round of spraying with pirimiphos-methyl (Actellic® 300 CS) in Betioky and Ampanihy districts, within the South West region, the first round with SumiShield® 50 WG in Tulear II and Sakaraha districts in the South West region and the first round with Fludora Fusion® in Ihosy, Ihorombe region.

Table 1 below describes all the 2019/2020 entomological monitoring sentinel sites.

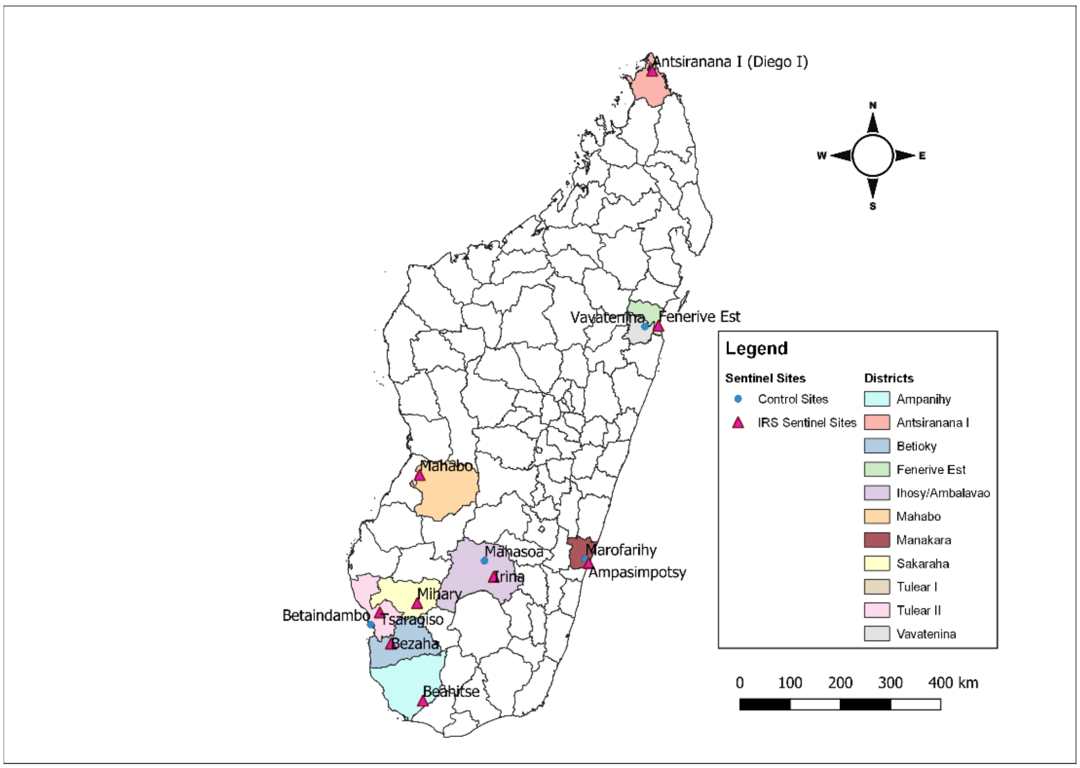
Table 1: List of Sentinel Sites

Region	District	Sentinel Site Location	Status	Years as Sentinel Site
Analanjirofo (East Coast)	Fenerive Est	Mahambo/ Antsikafoka*	ITNs mass distribution; No IRS since 2019, after five years of blanket IRS with Actellic 300 CS	2014–2020
Analanjirofo (East Coast)	Vavatenina	Vavatenina*	ITNs mass distribution; Control for East	2014–2020
Vatovavy Fito Vinany	Manakara	Ampasimpotsy*	ITNs mass distribution; No IRS since 2019 after two years of blanket IRS with Actellic 300 CS	2017–2020
Vatovavy Fito Vinany	Manakara	Marofarihy*	ITNs from mass distribution; Control for Vatovavy Fitovinany	2017–2020
Atsimo Andrefana	Tulear II	Tsaragiso	IRS with Actellic 300 CS in 2018 and Sumishield in 2019/ITNs mass distribution	2018–2020
Atsimo Andrefana	Sakaraha	Andasy	IRS with Actellic 300 CS in 2018 and Sumishield in 2019/ITNs mass distribution	2018–2020
Atsimo Andrefana	Ampanihy	Manakaravavy	IRS with Actellic 300 CS /ITNs mass distribution	2019
Atsimo Andrefana	Betioky	Besakoa/Bezaha	IRS with Actellic 300 CS /ITNs	2019–2020
Atsimo Andrefana	Tulear I	Betaindambo	ITNs mass distribution; Control for Atsimo Andrefana (South West)	2019–2020

Region	District	Sentinel Site Location	Status	Years as Sentinel Site
Ihorombe	Ihosy	Irina	IRS with Fludora Fusion/ITNs mass distribution	2019–2020
Haute Matsiatra	Ambalavao	Mahasoa	ITNs routine distribution; Control for Ihosy (Ihorombe)	2019–2020
Menabe	Mahabo	Ankilivalo**	ITNs mass distribution; Entomological monitoring in malaria control area; no IRS	2019–2020
Diana	Antsiranana I (Diego I)	Anamakia**	Entomological monitoring in malaria elimination setting; No IRS/ITNs routine distribution.	2019–20

*Sites maintained as part of the Exit Plan Strategy (post IRS).
** Sites where activities started in December 2019, but stopped after two months, due to Covid-19 pandemic.

Figure 1: Entomological Monitoring Site Locations and District Boundaries



2.1.2. Adult Mosquito Collections

Baseline entomological data was collected in the targeted areas, one month before the IRS campaign. Data on species composition, vector densities, and vector behavior were collected using human landing catches (HLCs) and following the VectorLink Standard Operating Procedure (SOP) 02/01, indoor resting collections by Prokopack aspiration (SOP 11/01), and outdoor resting collections using Prokopack and mouth aspirators (SOP 13/01) (Table 2). The team collected adult mosquitoes from July 2019 to August 2020 in Vatovavy Fitovinany region (Manakara district), and from August 2019 to May 2020 in Analanjirofo (Fenerive Est and Vavatenina districts) as IRS was withdrawn in those regions and an exit plan was put in place to record entomological data during the same period that IRS was conducted. However, the team discontinued activities in Analanjirofo in June 2020 due to restrictions put in place in the region due to the COVID-19 pandemic. In Atsimo Andrefana region (Tulear II, Tulear I, Sakaraha, Betioky and Ampanihy districts), and in Ihorombe region (Ihosy district), collections were conducted from October 2019 (one month before the 2019 IRS campaign) to August 2020.

HLCs were performed indoors and outdoors to collect adult mosquitoes landing on human baits using the VectorLink SOP 02/01.

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. The mosquitoes were sorted by species using simultaneously the identification keys of Grejbine 1966 / Gillies and Coetzee 1987. The abdominal status of all female *Anopheles* was determined and sorted into four categories: unfed, blood-fed, half-gravid, and gravid.

The Prokopack and mouth aspiration methods were also conducted outdoors during one day per month and per site in outdoor resting places such as pit shelters constructed by VectorLink, tree holes, zebu pens, etc. (Figure 2).

Figure 2: Outdoor Collection Places (A&B: Pit Shelters, C&D: Tree Holes, E&F: Flowers & Animal Fences)

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. *Anopheles gambiae* s.l. from each site was dissected to estimate parity rate. 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: one room per house, ten houses per site	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	Ten outdoor resting places and/or shelters per site

2.2. Insecticide Resistance Monitoring

From November 2019 through January 2020, VectorLink Madagascar completed insecticide resistance monitoring in 13 sites across the country including all IRS and control sites (Figure 1). Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats, 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 and Center for Disease Control and Prevention (CDC) bottle assays. For each tube test, about 100 female *An. gambiae* s.l. were tested against the insecticide (in four batches of 25) and an additional 40–50 were tested in two control tubes (20–25 each) in parallel (SOP 06/01).

The diagnostic concentrations of permethrin (0.75 percent), deltamethrin (0.05 percent), alpha-cypermethrin (0.05%), lambda-cyhalothrin (0.05%), bendiocarb (0.1%), pirimiphos-methyl (0.25%) and DDT (4%) were tested at the different sites. Resistance was defined following the WHO criteria (WHO, 2016):

- 98 % or greater mortality indicates susceptibility
- between 90 and 98 % mortality indicates possible resistance
- less than 90 % mortality indicates confirmed resistance

When insecticide resistance was confirmed, resistance intensity (high, moderate, and low) was also tested at five and 10 times the diagnostic concentration of pyrethroids.

Synergist assays with PBO were conducted for deltamethrin, permethrin, alpha-cypermethrin and lambda-cyhalothrin 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.

Clothianidin (2%) papers were treated locally following a protocol designed by VectorLink using the formulated product of SumiShield™ 50WG dissolved in distilled water. The susceptibility testing was conducted as described above, and the mortality was recorded up to seven days post-exposure.

CDC bottle assays were conducted using chlorfenapyr at the doses of 100µg/bottle. Testing was done following the VectorLink SOP 04/01 with one-hour exposure time and mortality recorded every 24 hours up to three days (72 hours).

2.3. Spray Quality and Residual Efficacy

WHO cone bioassays were used to determine the spray quality and the monthly residual efficacy of each insecticide on the various sprayed surfaces. The tests were conducted following the VectorLink SOP 09/01 and using adult mosquitoes emerged from wild-caught larvae. Prior to the test, the susceptibility of the local vector to the insecticide sprayed in the area was determined using WHO tube tests, 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 mosquitoes were exposed to the sprayed surfaces for 30 minutes and the "knock-down" rate was recorded at 30 minutes and 60 minutes post-exposure. The mortality was observed after 24 hours for Actellic sprayed sites, and five days for both Sumishield and Fludora Fusion sprayed sites.

To determine the fumigant effect of Actellic sprayed in the houses, 10 female *Anopheles gambiae* s.l. reared from wild caught larvae 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 that 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 (KD) effect was recorded 30 minutes post-exposure. Mortality was recorded after a 24-hour holding period. A control cage was set outside under a tree in the shade. Fumigant tests were conducted monthly until mortality was <20 percent during two consecutive months.

2.4. Molecular Characterization

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 analysed 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).

Adults *An. gambiae* s.l. and *An. funestus* s.l. from the 10 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 status 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.

3. RESULTS

3.1. Vector Bionomics Monitoring

3.1.1. Vector Species Composition

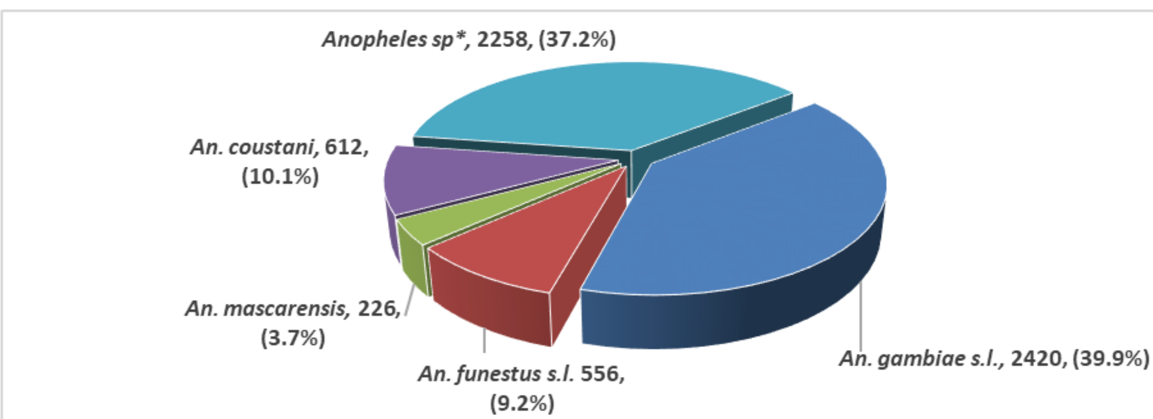
A total of 17,918 mosquitoes were collected from all the sentinel sites during the collection period: from July 2019 to August 2020 in Vatovavy Fitovinany region, from August 2019 to May 2020 in Analanjirofo, and from October 2019 to August 2020, in the South West and Ihorombe regions, using HLC, indoor Prokopack aspiration and outdoor resting collection with aspirators. In two sites of Analanjirofo region, activities stopped in May 2020 due to the COVID-19 outbreak. In the eleven other sites, activities were also interrupted in April, but started back up in June 2020, except in Diego I, where the activities could not be resumed. Activities stopped again in July and re-started in August 2020. Listed below are the number and proportion of total mosquitoes collected per sampling method:

- HLC: 16,140
- Indoor Collection with Prokopack: 945
- ODC: 833

A total of 6,072 (33.9%) of the mosquitoes collected were *Anopheles* and 3,814 (62.8%) of the total *Anopheles* represented known or potential malaria vectors in Madagascar: *Anopheles gambiae* s.l., *Anopheles funestus* group, *Anopheles mascarensis*, and *Anopheles coustani* (Figure 3).

Anopheles gambiae s.l. was collected at all sentinel sites and was the common primary vector collected in the IRS areas. *Anopheles funestus* s.l. was collected in eight sites Mahambo/Antsikafoka, Vavatenina, Ampasimpotsy, Marofarihy, Manakaravavy, Tsaragiso, Besakoa/Bezaha and Mahasoa. *Anopheles mascarensis* was collected in four sites: Mahambo/Antsikafoka, Vavatenina, Ampasimpotsy and Marofarihy. While *An. coustani* was found in eight sites: Mahambo/Antsikafoka, Vavatenina, Marofarihy, Ampasimpotsy, Irina, Mahasoa, Tsaragiso and Besakoa/Bezaha (Annex 1).

Figure 3: Species Composition of *Anopheles* Collected at all Sites, all Methods Included



**Anopheles* sp.: *An. squamosus/cydippis, An. pharoensis, An. pauliani, An. fuscicolor, An. brunipes*

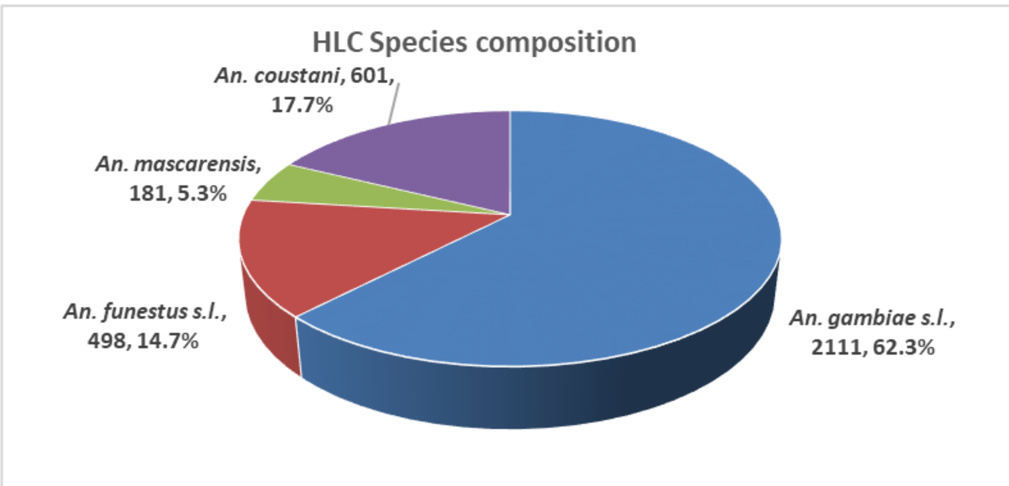
3.1.2. Human Landing Catches (HLC)

3.1.2.1. All Site Results

3.1.2.1.1 Species Composition

Anopheles gambiae s.l. and *An. funestus* s.l. remain the major malaria vectors in Madagascar. However, *An. mascarensis* and *An. coustani* were detected with sporozoite infection recently in the country (Nepomichene et al., 2015) and have therefore been considered as potential vectors of malaria in the country. The number of malaria vectors and potential vectors collected from ten sentinel sites out of 12 using HLCs (Annex II) are described in the Figure 4 below:

Figure 4: Species Composition of *Anopheles* Collected by HLCs



3.1.2.2. Indoor and Outdoor Vector Density

The vectors showed an exophagic tendency in all sites. The overall proportion of vectors and potential vectors collected outdoors was significantly higher than indoors (p<0.0001) (Table 3).

Table 3: Indoor Vs. Outdoor *Anopheles* Vectors Collected at All Sites Using HLC

Vector	# Indoor	# Outdoor	Exophagic index	P-value
An. gambiae s.l.	624	1487	70.4%	<0.0001
An. funestus s.l.	193	305	61.2%	<0.0001
An. mascarensis	39	142	78.5%	<0.0001
An. coustani	128	473	78.7%	<0.0001

3.1.2.3. IRS Sites and Control

3.1.2.3.1. Indoor and Outdoor Vector Density

The general trend observed was similar in the IRS sites with a mean of 68.6% of exophagic index for *An. gambiae* s.l., while lower index was recorded in the IRS control sites, though the number of vectors collected was very low. Table 5, in Annex, shows the monthly species composition, vector density and HBR in the different sites. Table 4 below shows the trend of endophagic rates in IRS sites and control sites.

Table 4. Indoor Vs Outdoor Density of Mosquitoes Collected by HLC in IRS and Control Sites

	Sites	*Anopheles gambiae* s.l. Indoor	*Anopheles gambiae* s.l. Outdoor	Exophagic rate (%)	*Anopheles funestus* s.l Indoor	*Anopheles funestus* s.l Outdoor	Exophagic rate (%)
IRS sites	Tsaragiso	73	103	58.5%	3	3	50.0%
	Bezaha	44	65	59.6%	0	0	0.0%
	Irina	33	123	78.8%	1	1	50.0%
	Manakaravavy	21	82	79.6%	0	1	100.0%
Total		**171**	**373**	**68.6%**	4	**4**	**50.0%**
Control sites	Betaindambo	17	27	61.4%	0	0	0.0%
	Mahasoa	114	91	44.4%	47	37	44.0%
Total		**131**	**118**	**47.4%**	**47**	**37**	**44.0%**

3.1.2.3.2. Human Biting Rates

The mean outdoor and indoor human biting rate (HBR) was higher a month before spraying during the baseline collection, in October 2019, in both IRS and control sites compared to post-IRS (10 b/p/n for sprayed sites and 2.5 b/p/n in control sites). The HBR dropped to 0.8 b/p/n, one month after spraying in the IRS sites, but remained the same in the control site (Figure 5, Annex II). However, the outdoor biting in the IRS sites was consistent and higher than the indoor biting and also higher than the control site biting throughout the collection months. Though the mean number of bites is low in the control sites, the trend observed could not allow a comparative analysis with the IRS sites. The vector population exhibited varying behaviors during each collection period in the control sites, whereby the mosquitoes either bite more indoors or outdoors depending on the month. The low biting rate observed in the control sites could also be associated with the fact that only two sites were selected and the average was compared to the four IRS site results; in addition, the control sites were situated in a different ecosystem and climate compared to the IRS sites.

In addition, the HBR was lower in Analanjirofo and Vatovavy Fitovinany, where IRS was withdrawn in 2019, compared to the control sites, despite the fact that all those sites were not sprayed. The team observed higher outdoor biting compared to indoor biting in both sets of sites and all collection months, except in March 2020 in the exit plan sites. (Figure 6, Annex II). This showed that both exit plan sites and selected control sites had similar trends in terms of vector behavior.

Figure 5: Monthly Distribution of Indoor and Outdoor Mean Human Biting Rates (bites/person/night: b/p/n) for An. gambiae s.l. at the Sentinel Sites in Sprayed Sites and Control Sites

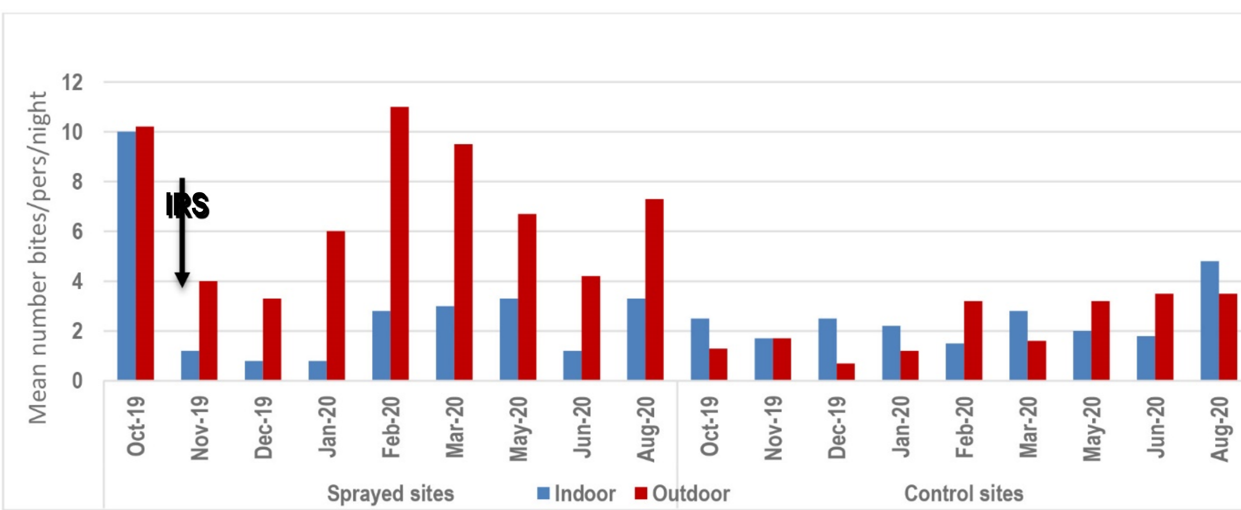
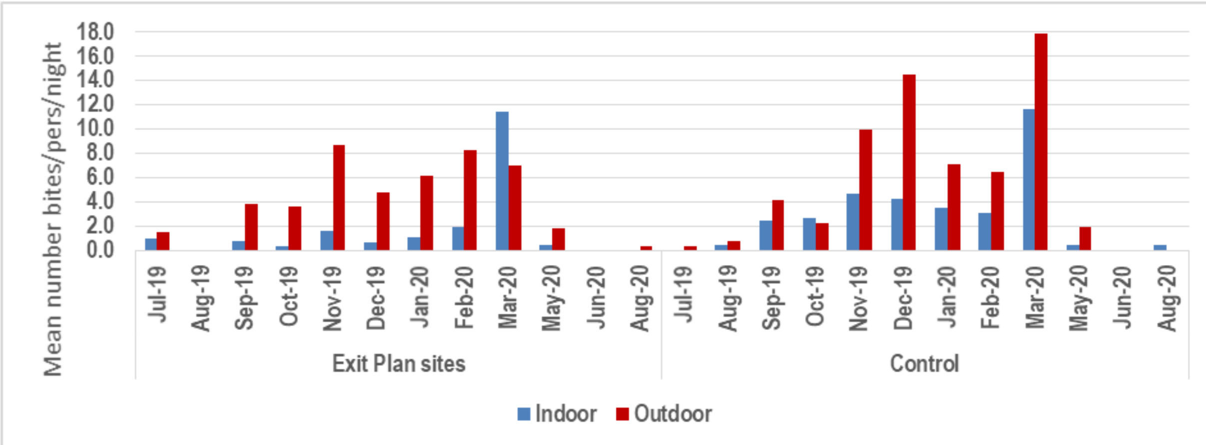


Figure 6: Monthly Distribution of Indoor and Outdoor Mean Human Biting Rates (bites/person/night: b/p/n) for An. gambiae s.l. at the Sentinel Sites in Non-Sprayed Sites with Exit Plan (Previously Sprayed but Dropped) and Control Sites



3.1.2.3.3. Biting Time

In the sprayed areas, the highest biting activities of *An. gambiae* s.l, were observed between 9.00 pm and 12.00 am without a distinct peak, both indoor and outdoor. In the control sites, the peak was observed between 12.00 am and 01.00 am. In the Exit Plan districts, the peak biting time was noted between 8.00 pm and 12.00 am, and between 9.00 pm and 11.00 pm in the control (Figures 7 and 8).

Figure 7: ***Anopheles gambiae*** **s.l. Biting Times at Sprayed Districts**

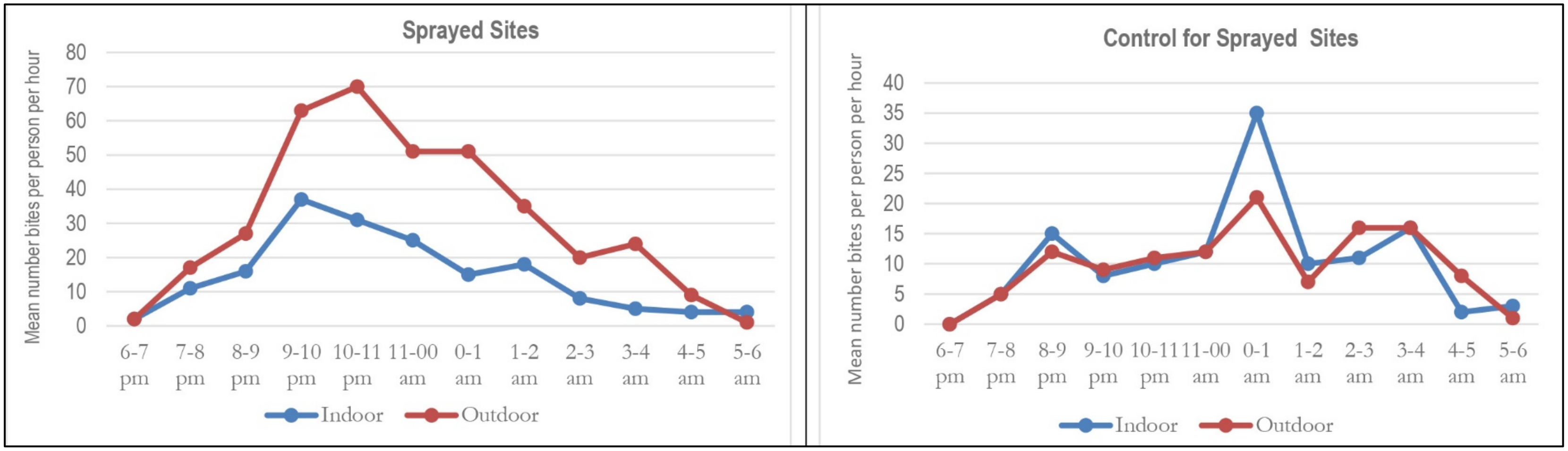
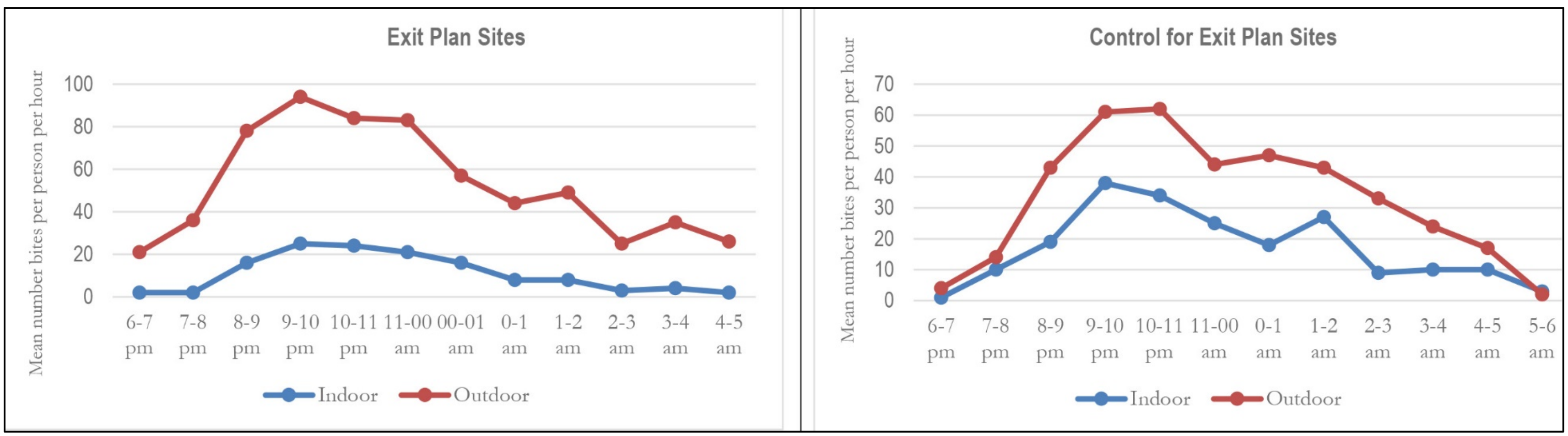


Figure 8: ***Anopheles gambiae*** **s.l. Biting Hours in Non-Sprayed Sites (Previously Sprayed but Dropped: Districts in Exit Plan)**



3.1.3. Indoor Resting Density

The indoor resting density of vectors at sprayed sites was low and varied from 0.1 to 0.6 *An. gambiae* s.l per room (f/r) collected at the sprayed sites and from 0 to 0.4 at the control sites during the baseline data collection in October 2019. During the post-spray period, 0 to 0.4 *An. gambiae* s.l. per room were collected at both the sprayed and control sites. For the other vectors, the indoor resting density was almost nil for both. *An. funestus* s.l., and *An. mascarensis*. The resting habit and impact of IRS on indoor resting density could not be confirmed using this indicator due to the low number of mosquitoes collected indoors over the collection period (Table 6 in Annex).

3.1.4. Outdoor Collections (ODC)

A total of 833 mosquitoes were collected outdoors using aspirators in ten sites in natural resting areas (tree holes) and pit shelters in Vatovavy Fitovinany, Analanjirofo, South West and Ihorombe regions. The vectors collected were composed of 232 (27.9%) *An. gambiae* s.l. from all sites, 29 (3.5%) *An. funestus* s.l. collected from six sites (Mahambo, Vavatenina, Marofarihy, Ampasimpotsy, Mahasoa and Tsaragiso), 38 (4.6%) of *An. mascarensis* from four sites (Mahambo/ Antsikafoka, Vavatenina, Marofarihy and Ampasimpotsy) (Table 7).

3.2. Parity Rate

At baseline, (one month prior to the start of the spray campaign), the average parity rate of *An. gambiae* s.l. was high (74.3%; n=148) in the sites to be sprayed. In the control sites, the parity rate was 80% (n=25)

During the seven months post IRS, which represents the effective period of the insecticide used in the area, there was a global reduction of the average parity rates, though the decrease of parous mosquitoes was different within sites and months.

Figure 9 below shows the decrease of the parity rate in Irina, right after spraying. It remains low (under 40%) until seven months post spraying. The insecticide used was Fludora Fusion.

Figure 9: Monthly Parity of *An. gambiae* s.l. in Sprayed Sites during the Collection Period

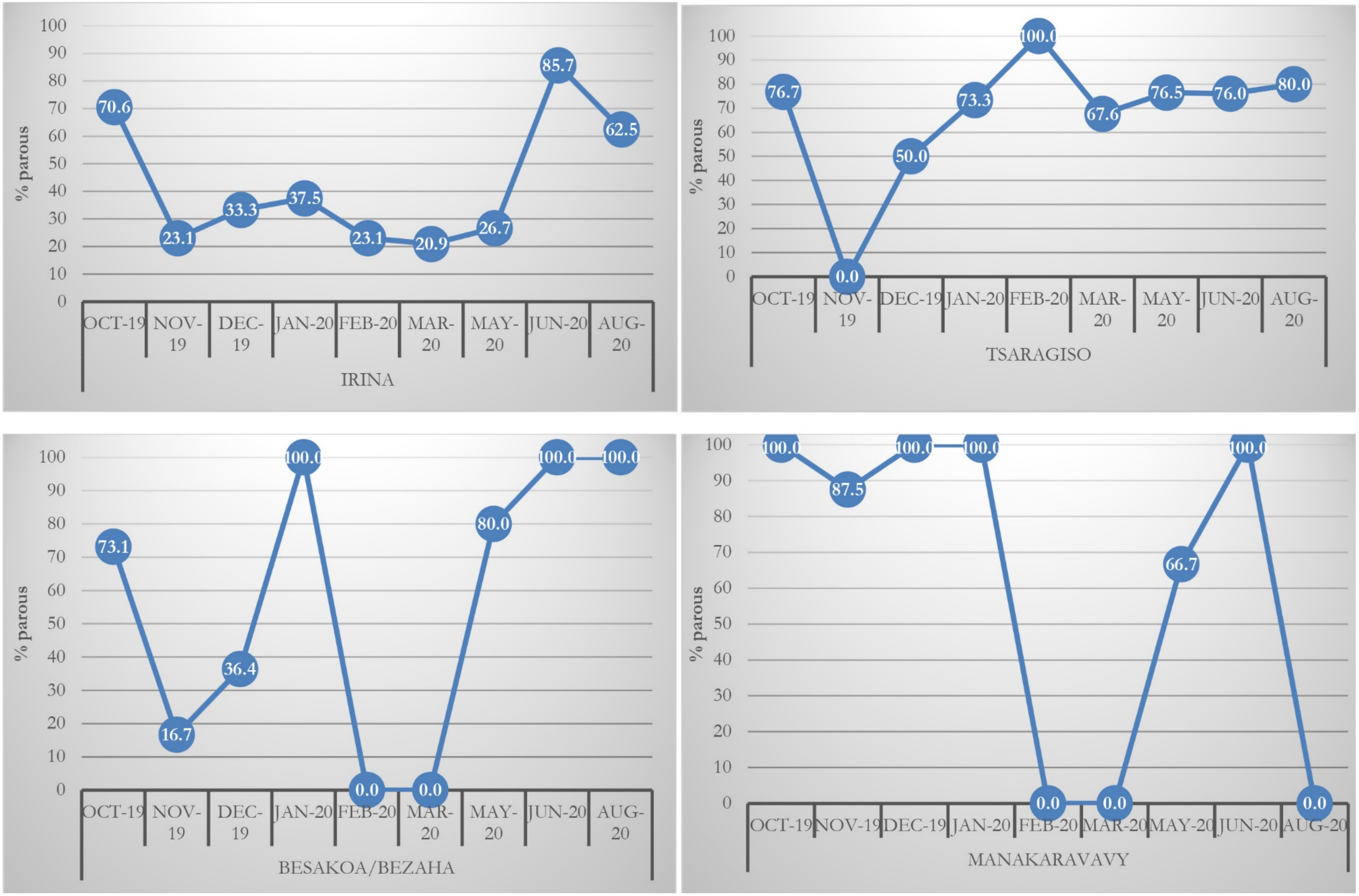
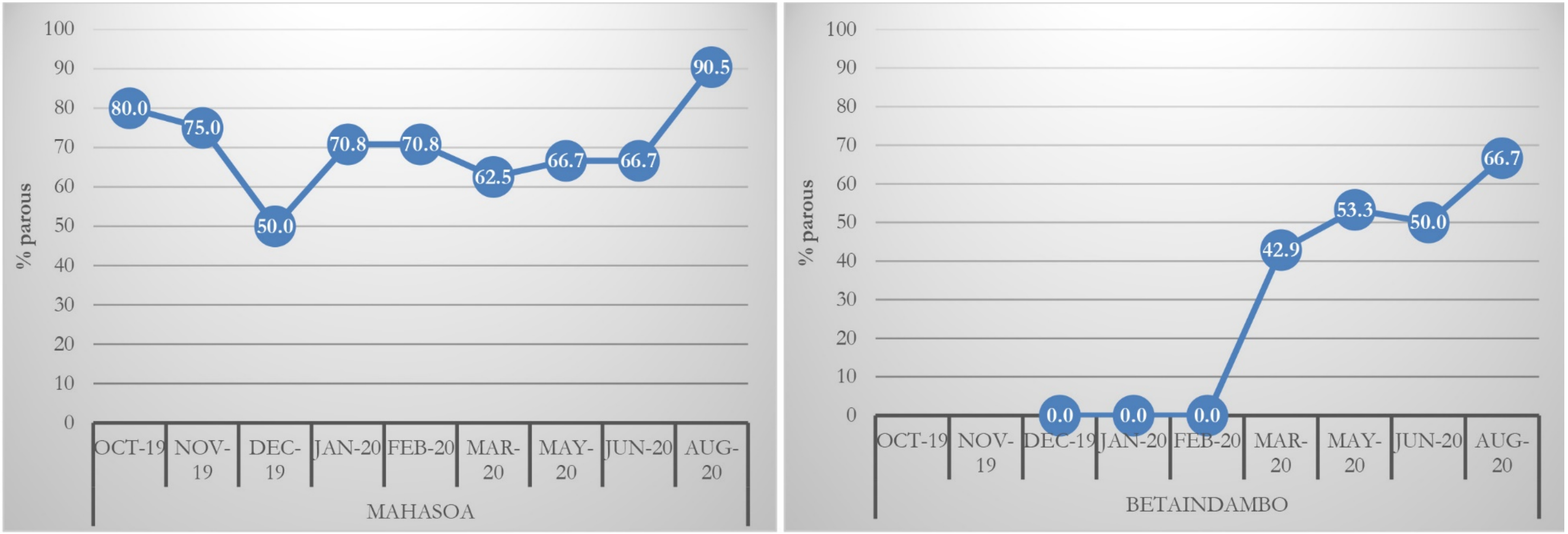


Figure 10: Monthly Parity of *An. gambiae* s.l. in Control Sites during the Collection Period



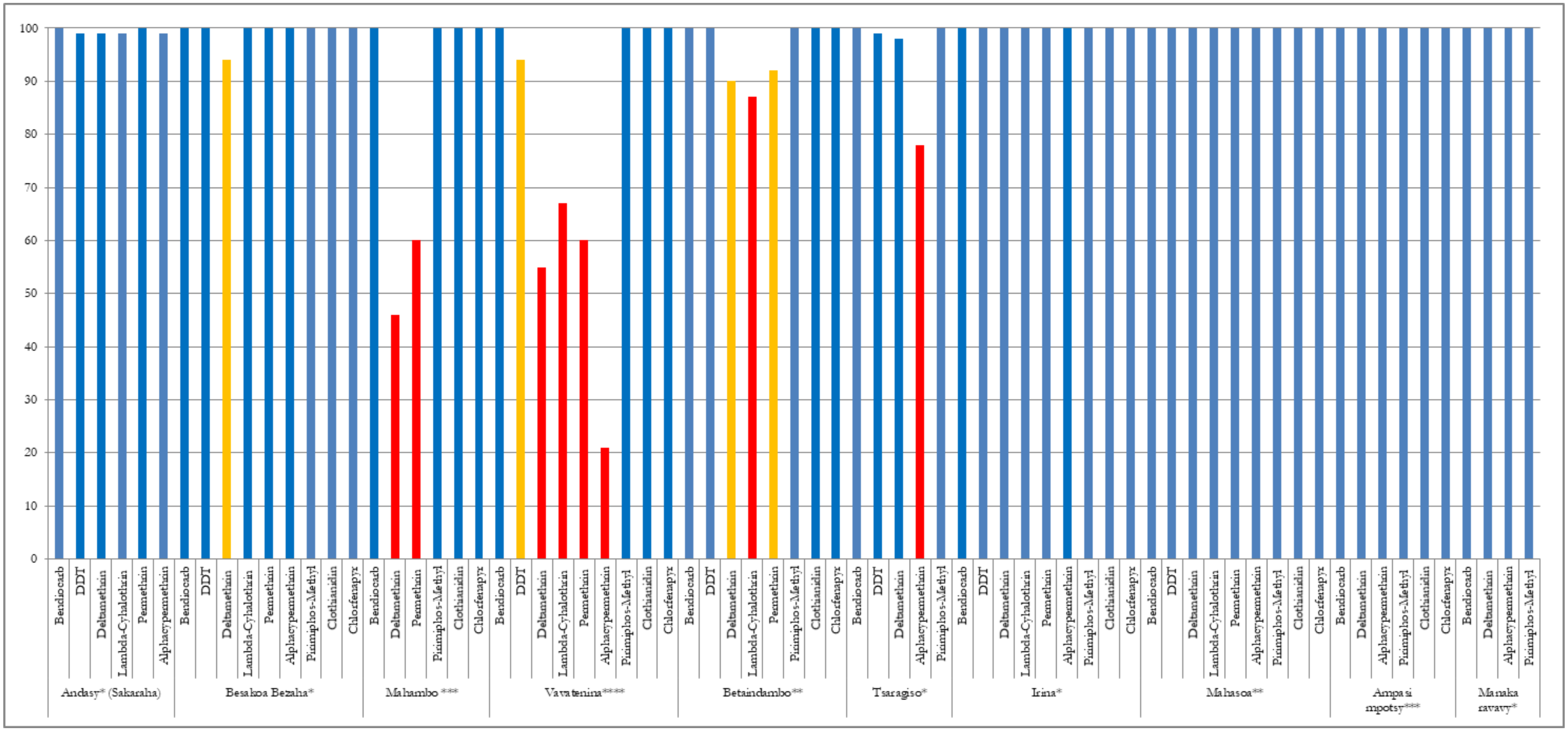
Error bars represent five percent percentage error bars.

3.3. Insecticide Susceptibility Test

3.3.1. WHO Susceptibility Test

The results of the vector susceptibility tests indicated susceptibility of *An. gambiae* s.l. to pirimiphos-methyl, clothianidin, bendiocarb and chlorfenapyr in all areas where the tests were conducted (Figure 11 and Annex V). However, *An. gambiae* s.l.'s resistance to permethrin and deltamethrin in Vavatenina and Mahambo/Antsikafoka, to alpha-cypermethrin in Vavatenina and Tsaragiso, and to lambda-cyhalothrin in Vavatenina and Betaindambo was detected. Suspected resistance to deltamethrin was noted in Besakoa Bezaha and Betaindambo, and to permethrin in Betaindambo.

Figure 11: Insecticide Susceptibility Status of *An. gambiae s*.l. using the Diagnostic Concentration of each Insecticide



Susceptible   Possible resistance   Confirmed resistance

*IRS sites; ** Control for IRS sites, *** Exit Plan sites,   **** Control for Exit Plan sites

3.3.2. Resistance Intensity Assays

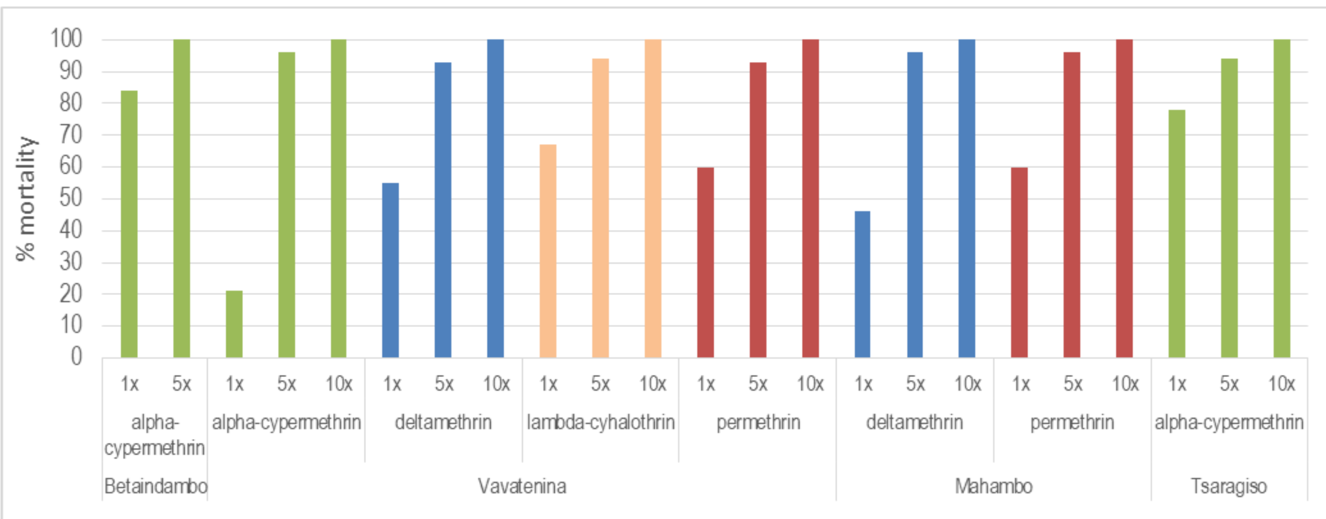
The intensity assay performed with five times the diagnostic dose (5x) of lambda-cyhalothrin yielded 100% mortality in Betaindambo (Tulear I), showing a low resistance intensity. With 5x alpha-cypermethrin dose, 96% and 94% mortality were recorded in Vavatenina and Tsaragiso, respectively. The subsequent concentration of ten times the diagnostic dose (10x) of alphacypermethrin tested in those two sites recorded 100% mortality, giving a moderate resistance intensity in Vavatenina and Tsaragiso (Figure 12).

Deltamethrin 5x yielded 96% and 93 % mortality in Mahambo and Vavatenina, respectively but 100% mortality was recorded with 10x in those two sites, giving a moderate resistance intensity.

Permethrin 5x showed 96% and 93% mortality in Mahambo and Vavatenina, respectively but 100% mortality was recorded with 10x in those two sites, giving a moderate resistance intensity.

Lambda-cyhalothrin yielded 94% and 100%mortality with 5x and 10x concentration, respectively, in Vavatenina, giving a moderate resistance intensity.

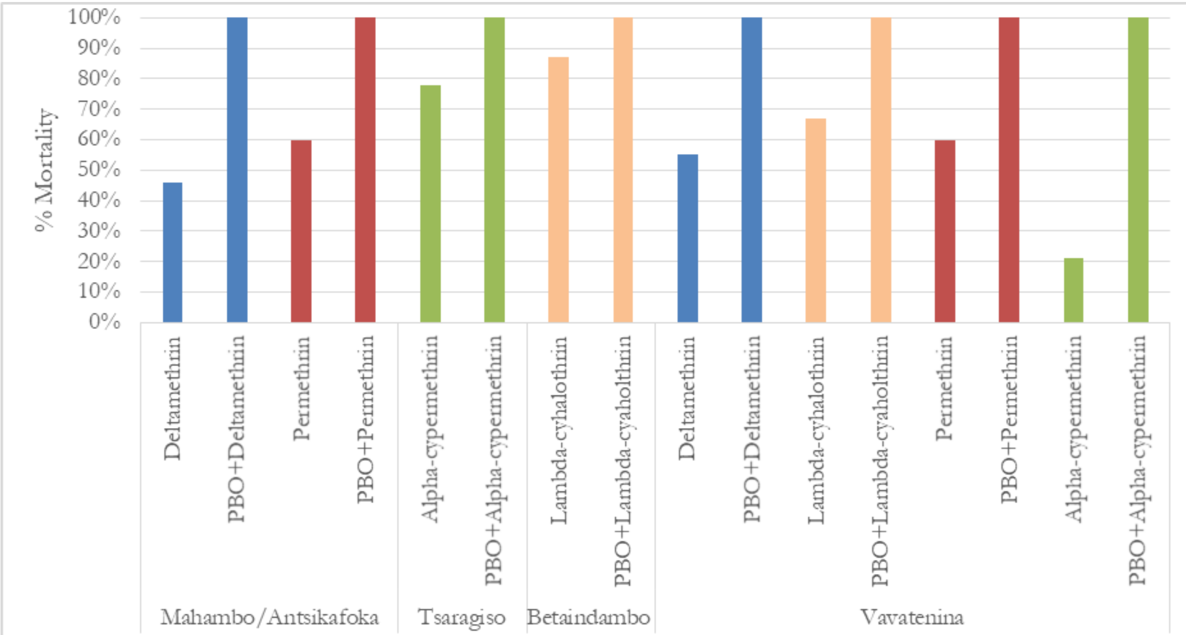
Figure 12. Resistance Intensity Observed for Alpha-Cypermethrin, Deltamethrin, Lambda-Cyhalothrin and Permethrin in Betaindambo, Vavatenina, Mahambo and Tsaragiso



3.3.3. Synergist Assays

PBO + deltamethrin and PBO + permethrin were tested in Vavatenina and Mahambo/ Antsikafoka, as well as PBO + lambda-cyhalothrin, in Vavatenina and Betaindambo, and PBO + alpha-cypermethrin in Vavatenina and Tsaragiso. For all tests, PBO restored full susceptibility (Figure 12).

Figure 13: Synergist Assay using PBO in Pyrethroid Resistant Sites



3.4. IRS Spray Quality and Insecticide Decay Monitoring

3.4.1. Cone Bioassay on Sprayed Surfaces

Cone bioassays completed after the spray campaign indicated good spray quality in all the five sprayed districts with 100% mortality recorded for all the structures tested at T0 and T1 and loss of airborne effect starting from T1 (Figure 9). In the five sprayed districts of the South West and Ihorombe regions, most houses are made of mud or wood.

The efficacy of all insecticides sprayed remained mostly for six months on all sprayed surfaces. Seven months after spray (T7), the residual efficacy decreased (under the threshold of 80%) in all the sites: for Actellic 300 CS, the 24h mortality rate is 72.5% on mud and 76.7 % on wood in Besakoa/Bezaha, 72.5% on mud and 79.1% on wood in Manakaravavy. For SumiShield 50 WG, the mortality rate after five days is 75% on mud and 77.5% on wood in Tsaragiso, and 76.7 % on mud and 77.5 % on wood in Andasy. The mortality rate with Fludora Fusion is 78.3 % on mud and 80.8 % on wood in Irina. Eight months after spray, it is confirmed that all the insecticides used for IRS (Actellic® 300 CS, Sumishield® 50WG and Fludora Fusion®) lost their efficacy with mortality under 80% in all the sites, on all surfaces during the two consecutive months of test (Figures 14-16).

Figure 14: Residual Efficacy Observed for Pirimiphos-methyl (Actellic® 300 CS) in Besakoa/Bezaha and Manakaravavy, in the South West Region

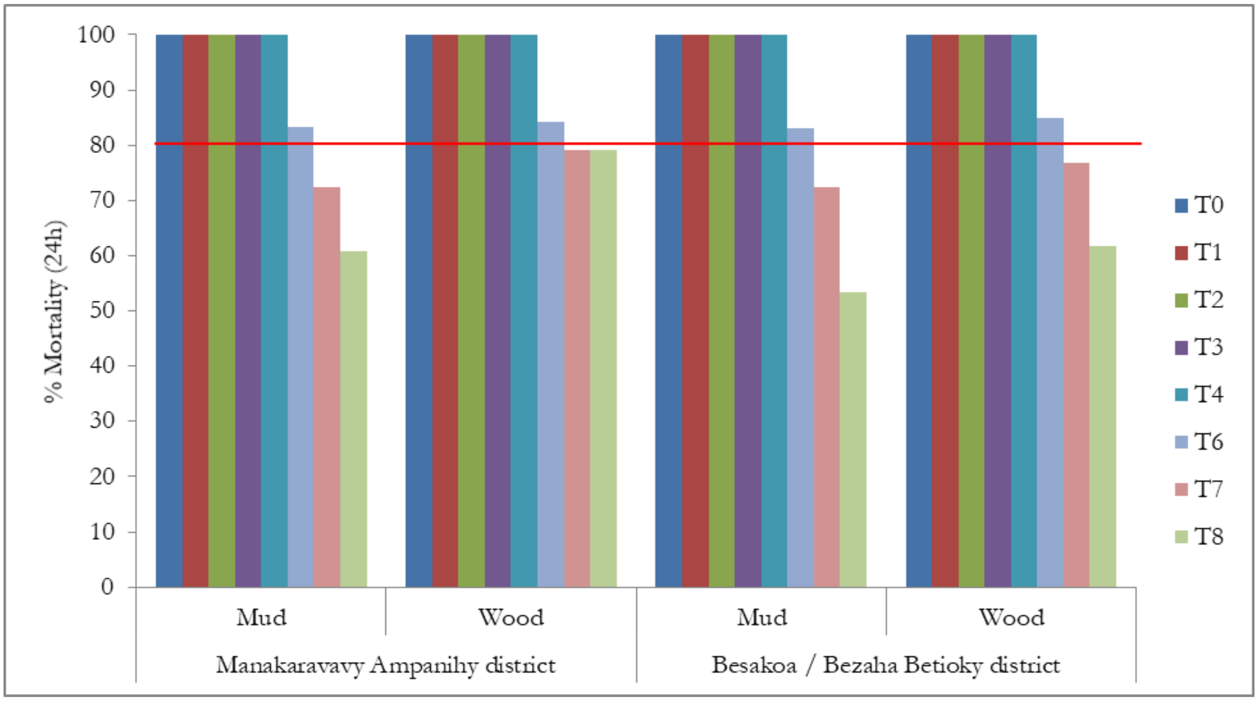


Figure 15: Residual Efficacy Observed for Clothianidin (SumiShield® 50 WG) in Andasy, Sakaraha District

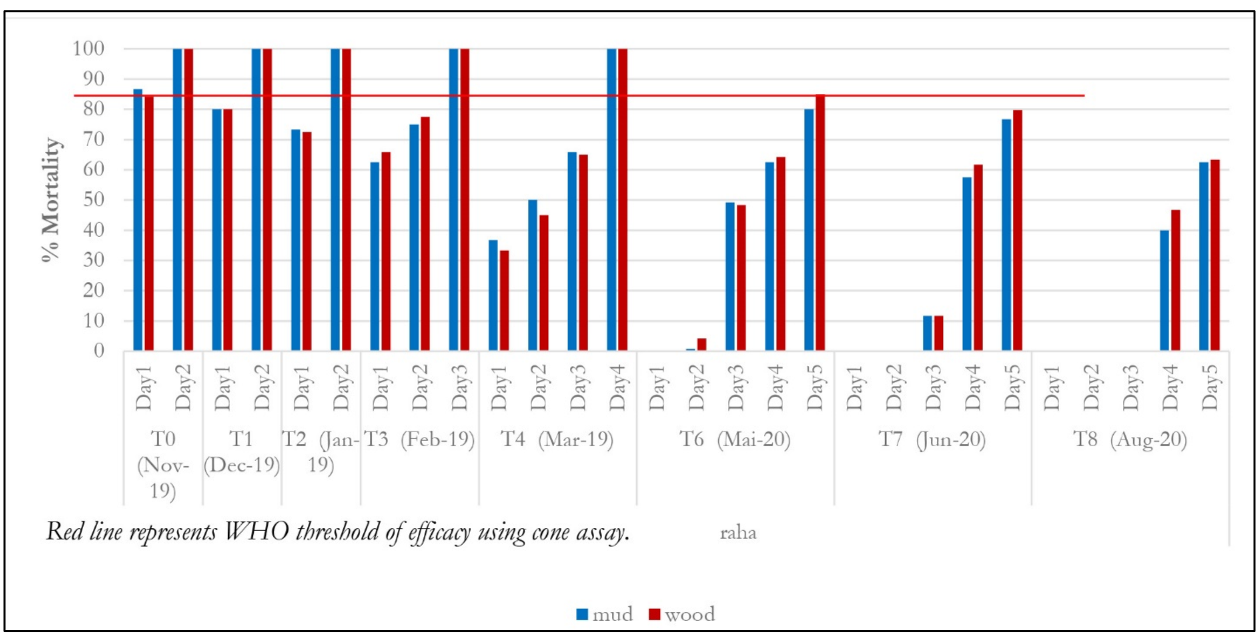


Figure 16: Residual Efficacy Observed for Clothianidin (SumiShield® 50 WG) in Tsaragiso, Tulear II District

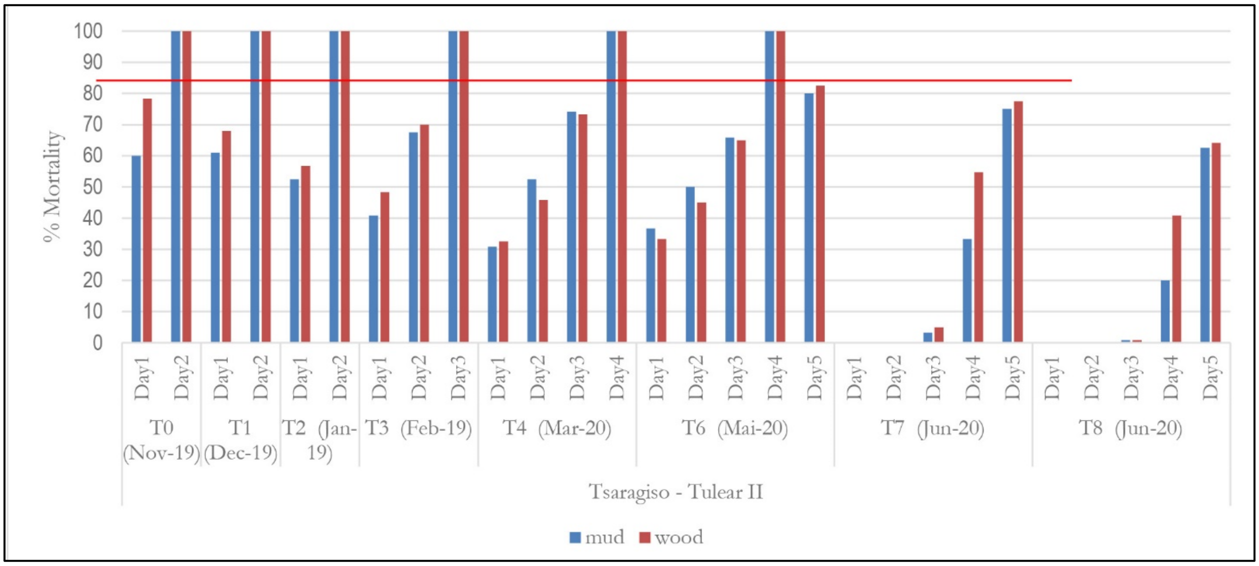
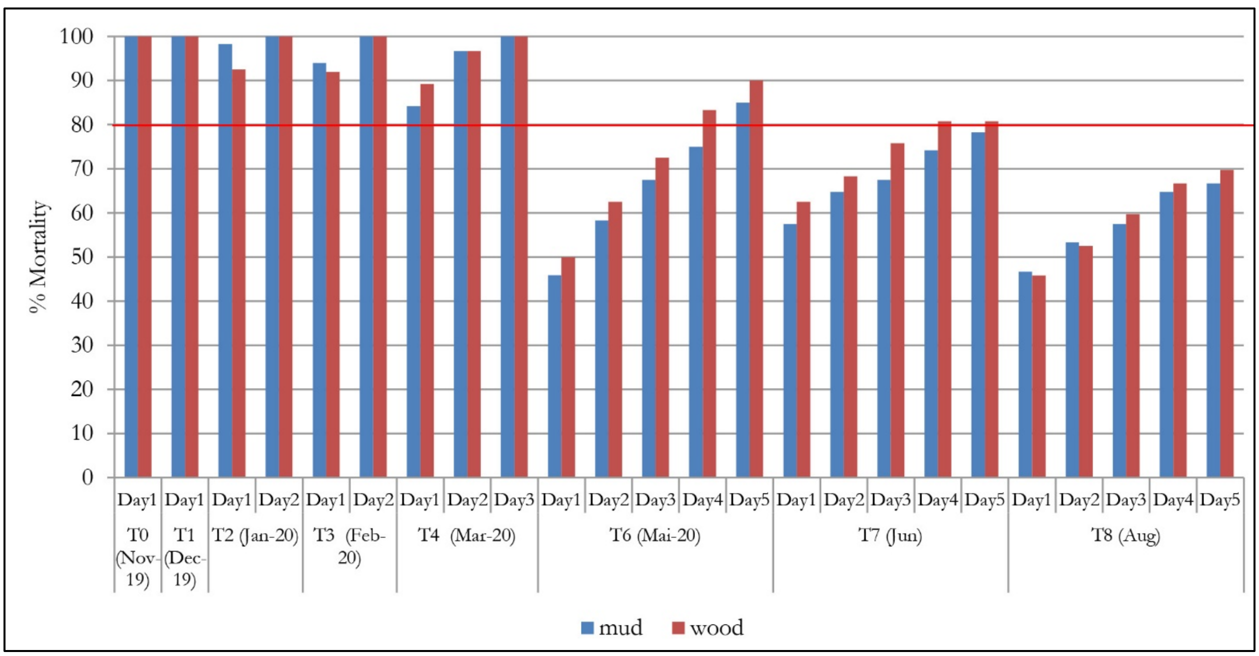


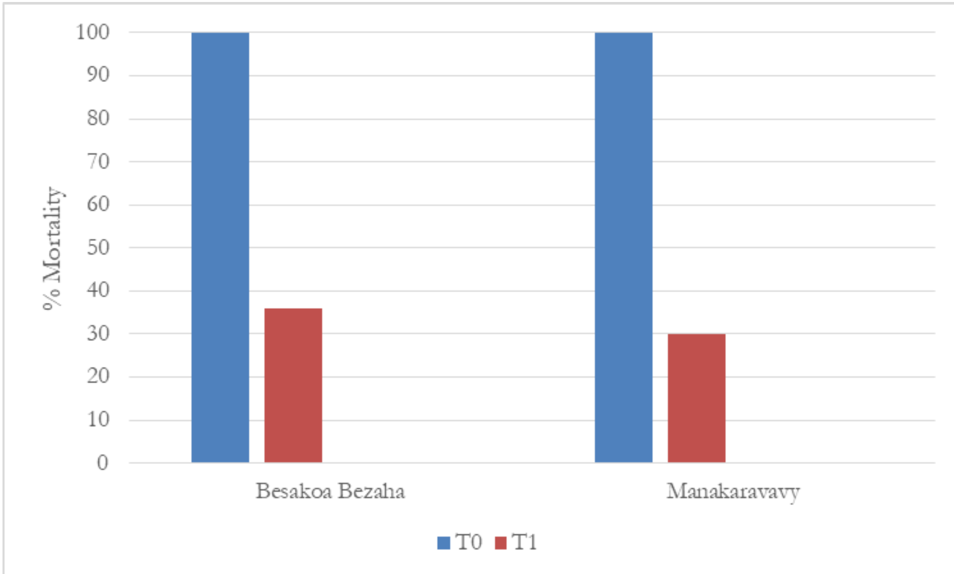
Figure 17: Residual Efficacy Observed for Fludora Fusion® in Irina, Ihosy District



3.4.2. Fumigant Effect of Pirimiphos-methyl (Actellic® 300 CS)

The results from the fumigant effect of pirimiphos-methyl (Actellic® 300 CS) showed that mortality was high (100 %) within one week after spraying (T0) in all the IRS sites. One month after spray (T1), it decreased to 36 percent in Besakoa/Bezaha and 30 percent in Manakaravavy. At T2 and T3, it dropped to 0% in Besakoa/Bezaha and Manakaravavy (Figure 18).

Figure 18: Fumigant Effect of Pirimiphos-Methyl



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4. CONCLUSIONS

The data collected indicate that *An. gambiae* s.l., *An. funestus* group, and *An. mascarensis* vector species and one implicated vector, *An. coustani*, are present at different proportions in various sentinel sites. *Anopheles coustani* was particularly present in eight of the sites while *An. gambiae* s.l. was the dominant vector collected in all sites. Furthermore, the number of anopheles mosquitoes collected this year seems to be lower than previous year reports. The trend could be attributed to the ecology and climate change in the collection sites.

Overall, the density of the vectors collected was low before and after IRS in the sprayed sites similarly to the control sites. Particularly, 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 habits of the vectors or to assess the impact of IRS on indoor resting density. Nonetheless, the longevity of the vector population observed after the first month post-IRS showed that the spray might have an impact on the mosquito populations and that was particularly observed in the site of Irina where Fludora Fusion was sprayed.

The outdoor biting and resting activity of mosquitoes in the spray areas was important and needs to be taken into consideration for effective vector control strategies in Madagascar. However, the selection of IRS control sites needs to be considered to enable comparison of results from sprayed and unsprayed areas for entomological impact evidence.

Larviciding could be a complementary vector control strategy in addition to the main interventions, IRS and/or ITN mass distribution in the area where the strategy could be implemented, especially where breading sites are few, fixed and identifiable.

Anopheles gambiae s.l. was susceptible to pirimiphos-methyl, clothianidin, bendiocarb and chlorfenapyr in all sites, including IRS areas. However, few sites have started developing resistance to pyrethroid insecticides, which are used for the impregnation of ITNs. The data gathered over years could help the NMCP on ITN campaign decision making.

Per the low or moderate resistance observed against the pyrethroid insecticides, in addition to the synergist assay results, Madagascar could start considering the new generation of PBO ITNs in the areas where resistance is observed for all upcoming ITN campaigns to brake the increase in insecticide resistance of the vectors.

Cone bioassay tests conducted during the first week of the IRS campaign indicated good quality of spray. The team recorded 100 percent mortality of *Anopheles gambiae* s.l. after 24-hour post exposure for all sprayed structures tested and all insecticides sprayed. This was confirmed through a repeat test one month after the structures were sprayed, where 100 percent mortality was still recorded for all structures even when the airborne effect of the insecticide was decreased significantly. The results for fumigant effect of pirimiphos-methyl 300 CS showed 100 percent mortality of the mosquitoes tested in Besakoa/Bezaha and Manakaravavy, the two sites sprayed with Actellic 300 CS, at T0. At T1, mortality was 36 percent in Besakoa/Bezaha and 30 percent in Manakaravavy. At T2 and T3, no fumigant effect was observed in both sites.

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 still remained effective over six months after IRS was conducted.

Per the results of the entomological surveys, it could be suggested that a rotation of Actellic CS, SumiShield WG and Fludora Fusion for IRS campaigns in Madagascar be done to enable insecticide resistance management. It was shown that these insecticides lasted long enough to cover the peak of malaria transmission season.

Limitations

The entomological surveillance data gathered could not be interpreted for comparison between sites (either sprayed or unsprayed or exit plan and controls) due to the fact that the sites were selected from different ecosystems and climates across the country. The non-selection of nearby control sites made it difficult to compare the data collected in the IRS sites to show evidence based entomological impact of the IRS activity undertaken because each site showed different entomological parameter trends.

The site of Sakaraha could not be investigated correctly due to security conditions. Sakaraha is located in a sprayed district and the longitudinal vector monitoring could not be conducted because of the needed night activities. Only cone bioassay and susceptibility tests were completed.

5. ANNEX

Table 5. Number of Mosquitoes Collected at Each Sentinel between July 2019 and August 2020 in Vatovavy Fitovinany, August 2019 and May 2020 in Analanjirofo, October 2019 and August 2020 in the South West and Ihorombe Regions.

	Mahambo (Fenerive Est)	Vavatenina	Betaindambo (Toliara I)	Tsaragiso (Toliara II)	Ampasipotsy	Manakaravavy	Marofarihy	Irina (Ihosy)	Mahasoa (Ambalavao)	Bezaha (Betioky)	Total
Anopheles gambiae s.l.	652	494	56	238	105	113	150	237	241	134	2420
Anopheles funestus	132	126	0	7	87	1	111	2	90	0	556
An. mascarensis	78	110	0	0	15	0	23	0	0	0	226
An. coustani	85	211	0	40	116	0	119	2	23	16	612
Anopheles sp.	218	99	0	71	247	20	301	40	226	1036	2258
Other Genus	604	1512	1979	1074	1721	1222	2064	344	338	988	11846
Total	1769	2552	2035	1430	2291	1356	2768	625	918	2174	17918

Table 6. Number of Mosquitoes Collected by HLC and Human Biting Rates (bites/person/night = b/p/n) between July 2019 and August 2020 in Vatovavy Fitovinany, August 2019 and May 2020 in Analanjirofo, October 2019 and August 2020 in the South West and Ihorombe Regions.

Sites	Month	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Out door	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)
Mahambo	September	0	0.0	9	1.5	1	0.2	15	2.5	0	0.0	39	6.5	1	0.2	47	7.8	0	0.0	138	23.0
	October	1	0.2	6	1.0	1	0.2	10	1.7	0	0.0	10	1.7	0	0.0	34	5.7	2	0.3	77	12.8
	November	6	1.0	46	7.7	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2	0	0.0	0	0.0
	December	1	0.2	26	4.3	0	0.0	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	January	5	0.8	36	6.0	1	0.2	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	February	12	2.0	50	8.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	March	66	11.0	361	60.2	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	May	2	0.3	11	1.8	46	7.7	45	7.5	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	0	0.0
Betaindambo	October	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	0.0	0	0.0
	November	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	0.0	0	0.0
	December	1	0.2	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	0.0
	January	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	February	0	0.0	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	March	5	0.8	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	May	2	0.3	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
	June	3	0.5	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	August	6	1.00	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Sites	Month	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Out door**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**
Tsaragiso	October	25	4.2	5	0.8	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	3	0.5
	November	0	0.0	4	0.7	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	29	4.8
	December	2	0.3	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	7	1.2
	January	4	0.7	19	3.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	9	1.5
	February	6	1.0	11	1.8	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2
	March	8	1.3	19	3.2	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	May	11	1.8	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
	June	4	0.7	12	2.0	0	0.0	1	0.2	0	0.0	0	0.0	14	2.3	26	4.3	9	1.5	8	1.3
	August	13	2.2	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Vavatenina	September	7	1.2	11	1.8	3	0.5	4	0.7	3	0.5	8	1.3	4	0.7	18	3.0	0	0.0	6	1.0
	October	5	0.8	7	1.2	5	0.8	1	0.2	12	2.0	25	4.2	4	0.7	22	3.7	2	0.3	13	2.2
	November	22	3.7	47	7.8	2	0.3	5	0.8	2	0.3	4	0.7	5	0.8	5	0.8	2	0.3	7	1.2
	December	24	4.0	74	12.3	5	0.8	17	2.8	3	0.5	6	1.0	23	3.8	54	9.0	6	1.0	12	2.0
	January	19	3.2	41	6.8	0	0.0	0	0.0	0	0.0	0	0.0	6	1.0	8	1.3	2	0.3	1	0.2
	February	16	2.7	33	5.5	3	0.5	4	0.7	0	0.0	3	0.5	1	0.2	1	0.2	1	0.2	4	0.7
	March	66	11.0	102	17.0	7	1.2	20	3.3	8	1.3	15	2.5	11	1.8	18	3.0	13	2.2	26	4.3
	May	3	0.5	9	1.5	12	2.0	35	5.8	7	1.2	11	1.8	9	1.5	20	3.3	0	0.0	4	0.7
Irina	October	9	1.5	17	2.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	5	0.8
	November	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	3	0.5
	December	0	0.0	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	January	1	0.2	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	February	11	1.8	40	6.7	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	5	0.8
	March	7	1.2	30	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	6	1.0
	May	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	6	1.0
	June	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
	August	1	0.2	4	0.7	0	0.0	1	0.2	0	0.0	0	0.0	1	0.2	0	0.0	1	0.2	0	0.0

Sites	Month	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Out door	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)	Indoor	Indoor (b/p/n)	Outdoor	Outdoor (b/p/n)
Mahasoa	October	15	2.5	8	1.3	6	1.0	3	0.5	0	0.0	0	0.0	1	0.2	1	0.2	29	4.8	24	4.0
	November	10	1.7	10	1.7	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	14	2.3	19	3.2
	December	14	2.3	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	12	2.0	14	2.3
	January	13	2.2	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	14	2.3	12	2.0
	February	9	1.5	15	2.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	13	2.2	12	2.0
	March	12	2.0	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	3	0.5	7	1.2
	May	10	1.7	9	1.5	6	1.0	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	2	0.3
	June	8	1.3	13	2.2	22	3.7	19	3.2	0	0.0	0	0.0	3	0.5	4	0.7	2	0.3	12	2.0
	August	23	3.8	19	3.2	13	2.2	11	1.8	0	0.0	0	0.0	0	0.0	6	1.0	1	0.2	4	0.7
Marofarihy	July	0	0.0	2	0.3	5	0.8	4	0.7	0	0.0	0	0.0	3	0.5	11	1.8	2	0.3	11	1.8
	August	3	0.5	5	0.8	3	0.5	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	September	8	1.3	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	3	0.5	0	0.0	0	0.0
	October	11	1.8	7	1.2	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	November	6	1.0	13	2.2	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7	7	1.2	41	6.8	82	13.7
	December	2	0.3	13	2.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	5	0.8
	January	2	0.3	2	0.3	2	0.3	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	5	0.8
	February	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	2	0.3
	March	4	0.7	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	3	0.5	3	0.5	3	0.5
	May	0	0.0	3	0.5	9	1.5	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0	16	2.7	15	2.5
	June	0	0.0	0	0.0	11	1.8	19	3.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	August	3	0.5	0	0.0	4	0.7	0	0.0	3	0.5	14	2.3	11	1.8	71	11.8	10	1.7	89	14.8
Manakaravavy Ampanihy	October	1	0.2	2	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7
	November	1	0.2	7	1.2	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	December	0	0.0	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	January	0	0.0	8	1.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	February	0	0.0	15	2.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Sites	Month	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Indoor	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Out door**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**	**Indoor**	**Indoor (b/p/n)**	**Outdoor**	**Outdoor (b/p/n)**
	March	1	0.2	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
	May	1	0.2	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	June	1	0.2	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	August	16	2.7	30	5.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0		
Ampasimpotsy	July	6	1.0	9	1.5	2	0.3	22	3.7	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0
	August	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	1	0.2
	September	5	0.8	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	22	3.7	30	5.0
	October	1	0.2	16	2.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	November	4	0.7	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	12	2.0
	December	3	0.5	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	4	0.7
	January	2	0.3	1	0.2	1	0.2	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	8	1.3
	February	0	0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	March	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	2	0.3	1	0.2	1	0.2	4	0.7
	May	1	0.2	0	0.0	7	1.2	14	2.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	26	4.3
	June	0	0.0	0	0.0	7	1.2	12	2.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	August	0	0.0	2	0.3	1	0.2	1	0.2	1	0.2	6	1.0	13	2.2	100	16.7	11	1.8	106	17.7
Bezaha	October	25	4.2	37	6.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	0	0.0	13	2.2	37	6.2
	November	6	1.0	7	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	27	4.5	41	6.8
	December	3	0.5	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	61	10.2	68	11.3
	January	0	0.0	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	104	17.3	144	24.0
	February	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	74	12.3	105	17.5
	March	2	0.3	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.2	33	5.5	21	3.5
	May	4	0.7	4	0.7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	27	4.5	42	7.0
	June	2	0.3	3	0.5	0	0.0	0	0.0	0	0.0	0	0.0	7	1.2	6	1.0	30	5.0	38	6.3
	August	2	0.3	6	1.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	18	3.0	51	8.5

Table 7: Total Number of Mosquitoes Collected by Prokopack Aspirator and Indoor Resting Density between July 2019 and August 2019 in Vatovavy Fitovinany, August 2019 and May 2020 in Analanjirofo, and 2019 and August 2020 in the South West and Ihorombe Regions.

Species	Month	Mahambo		Vavatenina		Irina		Mahasoa		Manakaravav y		Besakoa Bezaha		Tsaragiso		Betaindambo		Marofarihy		Ampasipotsy	
		#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density
An.gambiae s.l.	July																	0	0.0	0	0.0
	August																	0	0.0	0	0.0
	September	0	0.0	1	0.1													1	0.1	0	0.0
	October	0	0.0	1	0.1	5	0.5	4	0.4	1	0.1	6	0.6	4	0.4	0	0.0	4	0.4	1	0.1
	November	1	0.1	0	0.0	0	0.0	4	0.4	0	0.0	0	0.0	0	0.0	0	0.0	2	0.2	0	0.00
	December	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.00
	January	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	1	0.0	0	0.0	0	0.0	0	0.00
	February	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	0	0.0	0	0.0	3	0.3	0	0.0
	March	0	0.0	0	0.0	2	0.2	3	0.3	0	0.0	1	0.1	2	0.2	0	0.0	2	0.2	1	0.1
	May	0	0.0	0	0.0	1	0.1	0	0.0	0	0.0	2	0.2	4	0.4	0	0.0	0	0.0	1	0.1
	June					0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	3	0.3	0	0.0	0	0.0
	August					0	0.0	0	0.0	2	0.2	2	0.2	2	0.2	3	0.0	0	0.0	0	0.0
An. funestus	July																	0	0.0	0	0.0
	August																	0	0.0	0	0.0
	September	0	0.0	1	0.1													0	0.0	0	0.0
	October	0	0.0	0	0.0	0	0.0	2	0.2	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	0	0.0
	November	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	0.0	0	0.0
	December	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	0.0	0	0.0
	January	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	0.0	0	0.0
	February	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	0.0	0	0.0
	March	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	0.0	0	0.0
	May	3	0.3	0	0.0	0	0.0	2	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.3	3	0.3
	June					0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	8	0.8	4	0.4
	August					0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.1	1	0.1

Species	Month	Mahambo		Vavatenina		Irina		Mahasoa		Manakaravavy		Besakoa Bezaha		Tsaragiso		Betaindambo		Marofarihy		Ampasipotsy	
		#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density	#	Vector Density
	July																	0	0.0	0	0.0
	August																	0	0.0	0	0.0
	September	2	0.2	0	0.0													0	0.0	0	0.0
	October	0	0.0	1	0.1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	November	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	0.0	0	0.0
An. mascarensis	December	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	0.0	0	0.0
	January	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	0.0	0	0.0
	February	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	0.0	0	0.0
	March	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	0.0	0	0.0
	May	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	0.0	0	0.0
	June					0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	August					0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	3	0.3	1	0.1

Table 8: Total Number of Mosquitoes Collected by Outdoor Collection with Aspirator (ODC) Method between July and December 2019 in Vatovavy Fitovinany, August and December 2019 in Analanjirofo, October and December 2019 in the South West and Ihorombe Regions.

	Mahambo (Fenerive Est)	Vavatenina	Marofarihy	Ampasipotsy	Irina (Ihosy)	Mahasoa (Ambalavao)	Betaindambo (Toliara I)	Behatse	Tsaragiso (Toliara II)	Bezaha (Betioky)	Total
An. gambiae s.l.	13	6	26	20	73	19	6	7	48	14	232
An. funestus	3	2	12	9	0	2	0	0	1	0	29
An. mascarensis	27	2	3	6	0	0	0	0	0	0	38
An coustani	0	2	2	0	0	5	0	0	0	0	9
Other *Anopheles* spp.	0	0	7	12	13	11	0	16	0	51	110
Other *Genus*	23	6	21	77	44	23	58	107	0	56	415
Total	**66**	**18**	**71**	**124**	**130**	**60**	**64**	**130**	**49**	**121**	**833**

Table 9: Results of *An. gambiae* s.l. Susceptibility Tests

	Ampasimpotsy			Marofarihy			Mahambo			Vavatenina			Andasy			Besakoa 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	N# tested	24h % * Mortality	Resistance status
Bendiocarb	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
DDT	NC			100	100	S	NC			100	94	P	100	99	S	100	100	S
Deltamethrin	100	100	S	100	100	S	100	46	R	NC	55	R	100	99	S	100	94	P
Lambda-Cyhalothrin	NC			NC			NC	53	R	NC	67	R	100	99	S	100	100	S
Permethrin	NC			NC	92	P	100	60	R	100	60	R	100	100	S	100	100	S
Alphacypermethrin	NC	100	S	100	100	S	NC	29	R	100	21	R	100	99	S	100	100	S
Pirimiphos-Methyl	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Clothianidin	NC	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Chlorfenapyr	NC	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S

* Up to 7 days for clothianidin

	Tsaragiso			Bataindambo			Manakaravavy			Irina			Mahasoa		
	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
Bendiocarb	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
DDT	100	99	S	100	100	S	NC			100	99	S	100	100	S
Deltamethrin	100	98	S	100	90	P	100	100	S	100	100	S	100	100	S
Lambda-Cyhalothrin	100	98	S	100	87	R	100	100	S	100	100	S	100	100	S
Permethrin	100	97	P	100	92	P	100	100	S	100	100	S	100	100	S
Alphacypermethrin	100	78	R	NC	84	R	100	100	S	100	C	S	100	100	S
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

* Up to 7 days for clothianidin