THE PMI VECTORLINK MADAGASCAR FINAL ENTOMOLOGICAL MONITORING REPORT

JUNE 2018 – MAY 2019

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CONTENTS

List of Figures

List of Tables

ACRONYMS

CDC	Centers for Disease Control and Prevention
CS	Capsule Suspension
CSP	Circumsporozoite
DDT	Dichlorodiphenyltrichloroethane
ELISA	Enzyme-Linked Immunosorbent Assay
HLC	Human Landing Catch
HBR	Human Biting Rate
IRS	Indoor Residual Spraying
KD	Knockdown
LLIN	Long-Lasting Insecticidal Net
ODC	Outdoor Collection
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
WG	Wettable Granulation
WHO	World Health Organization

Executive Summary

Background

In Madagascar, indoor residual spraying (IRS) is an important component of the malaria control strategy, as included in the current National Strategic Plan. Madagascar is receiving support from both the USPresident's Malaria Initiative (PMI) and Global Fund (GF) for the implementation of successful IRS campaigns through provision of funds, staff and technical guidance.

During the 2018 spray campaign, the PMI VectorLink Madagascar Project has covered over 36 communes in three districts in the East Coast (Brickaville, Toamasina II and Fenerive East districts), 111 communes in four districts in the South East (Mananjary, Manakara, Vohipeno and Farafangana districts) and 37 communes in two districts in the South West with blanket IRS (Tulear II and Sakaraha districts). The project team sprayed pirimiphos-methyl CS, an organophosphate insecticide and Sumishield®50 WG, a neonicotinoid insecticide. The campaign was conducted from July 23 to August 20, 2018 in Mananjary and Manakara districts, in Vatovavy Fitovinany region in the South East, from September 3, 2018 to September 29, 2018 in the East Coast (Brickaville and toamasina II, Atsinanana region and in Fenerive East, Analanjirofo region), from September 17 to October 17, 2018 in Tulear II and Sakaraha districts in the South West using pirimiphos methyl. Clothianidin (Sumishield®50 WG) was used in the districts of Farafangana (South East region) and Vohipeno (Vatovavy Fitovinany region) from July 23 to August 20, 2018.

Entomological monitoring is an integral component of the PMI VectorLink Madagascar Project. The 2018-2019 entomological monitoring activities included collection of comprehensive entomological data on vector density, species composition, seasonal patterns, biting behavior, and parity of Anopheline mosquitoes from eleven sentinel sites including seven IRS sites and four control sites (non-IRS). Data on vector species composition, density and behavior was collected using mosquito-sampling methods that included human landing catch (HLC) and adult collection 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 from both the intervention and control sites, and subsequent monthly data was collected after spraying to help understand if there was any change in the species composition, density and behavior following IRS.

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. Insecticide susceptibility data was also collected from 13 sentinel sites, including the 11 sentinel sites where comprehensive entomological monitoring were conducted, so as to inform insecticide based malaria vector control programming. Additionally, susceptibility to chlorfenapyr was also conducted in nine sentinel sites using CDC bottle assay. Furthermore, sub species of *An. gambiae* s.l. and *An. funestus* samples from the 11 sites surveyed were molecularly identified using polymerase chain reaction (PCR) methods and plasmodium infections of both species detected using enzyme-linked immunosorbent assay (ELISA) methods. The insecticide resistance mechanisms including both Kdr and ace-1 were characterized from *An. gambiae* s.l. used for susceptibility testing.

Results

Vector density and seasonality: A total of 10,033 female Anopheline and 11,692 culicine mosquitoes were collected during the monitoring period. The most abundant vector species was *An. gambiae* s.l., representing 28.6 percent (n=2,874) of the total Anopheline mosquitoes collected. The other two Anopheline species, *An. funestus* and *An. mascarensis,* which are also vectors of malaria in Madagascar, accounted for 11.7 percent (n=1,172) and 6.8 percent (n=681) of the collected species respectively. *Anopheles coustani,* reported as a probable vector in one area of Madagascar (Nepomichene *et al.*), was present in all the sites. A total of 3,041 (30.3 percent) 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* represented 22.6% (n= 2,265) of the total Anopheline collected.

A total of 84 *An. gambiae* s.l., 54 *An. funestus,* and one *An. mascarensis* were collected indoors using the Prokopack aspiration method. Also, 346 *An. gambiae* s.l., 66 *An. funestus* and 46 *An. mascarensis,* were collected outdoors with mouth aspirators and Prokopack, mostly from artificial pit shelters. The team collected 91.3 percent (n=19,838) of the mosquitoes (all genus included) through human landing catch: 35.3 percent (n=6,994 (1,705 indoor and 5,289 outdoor)) are known or possible malaria vectors. It is apparent that the number of vectors collected resting both indoors and outdoors was very low to draw conclusions about any changes in resting habits of the vectors or to assess the impact of IRS on indoor resting density.

Feeding time and location: At the baseline before indoor residual spraying (IRS), *An. gambiae* s.l. indoor human biting rates (HBR) ranged from 0.0 bites per person per night (b/p/n) in Vohitrambato (Toamasina II District), Manambotra Sud (Farafangana District) and Mahatsinjo (Mananjary District) to 3.2 bites per person per night in Ambodifaho (Brickaville district). The outdoor human biting rates ranged from 0.0 bites per person per night in Lanivo (Vohipeno district) to 4.2 bites per person per night in Ampasimpotsy (Manakara district). In all sentinel sites, *An. gambiae* s.l. exhibited exophagic tendencies before IRS. No change in the feeding habit of *An. gambiae* s.l. was observed after IRS, compared to pre-IRS; however, it has been noted that the vector prefers feeding more outdoors than indoors in both the intervention and control sites. The indoor man biting rates of the control and sprayed sites before the campaign were 1.03 and 0.7 b/p/n (bites per person per night) respectively, while the post spray were 1.1 and 0.9 b/p/n for the control and sprayed sites respectively. The same trends were observed for the outdoor man biting rates with 0.6 and 1.4 b/p/n before IRS, 1.8 and 3.3 b/p/n after spray for the control site and sprayed sites respectively. A significant difference was observed between the mean HBR indoors and outdoors ($p < 0.0001$) of all the malaria vector species. The low mean biting rates noted at baseline as compared to after 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 of biting between sites.

Quality of spray and residual life: The results of wall bioassays indicated good spray quality in all sites with 100 percent 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. Pirimiphos-methyl CS killed more than 80 percent of the mosquitoes tested over seven months after spraying in the East Coast and six months in the South East. Clothianidin, used for IRS in two districts of the South East, Farafangana and Vohipeno was effective for seven months.

Susceptibility tests: The results of the vector susceptibility tests indicated full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl, clothianidin, chlorfenapyr and bendiocarb in all areas where the tests were conducted. Susceptibility was also recorded for DDT in all sites except in Vavatenina where possible resistance was suspected.

The test results also showed that *Anopheles gambiae* s.l. is resistant to permethrin in Ambodifaho, Vavatenina, Vohitrambato, Mahambo and Betaindambo, to alpha-cypermethrin in Ambodifaho, Vavatenina and Vohitrambato, to deltamethrin in Vohitrambato, Mahambo, Vavatenina and Betaindambo and to lambda-cyhalothrin in Vohitrambato.Resistance was suspected for deltamethrin in Ambodifaho and for permethrin in Manambotra Sud. *An. funestus An. mascarensis* and *An. coustani,* were susceptible to all insecticides tested including pirimiphos-methyl and deltamethrin in all sites, permethrin in Mahatsinjo, Manambotra Sud and Tsaravary, lambda-cyhalothrin, bendiocarb, and DDT in Tsaravary and Manambotra Sud.

Molecular analysis:

The molecular analysis showed that *An. gambiae* s.s. and *An. arabiensis* were both present in all 11 sites, with predominance of *An. gambiae* s.s. (Table 11). The *kdr*-east mutation was found in the six sites tested. *An. funestus* represented the only species of the *An. funestus* group of Madagascar in eight sites. The ELISA circumsporozoite (CSP) tests detected *Plasmodium falciparum* in *An. gambiae* in five sites, *An. funestus* in two sites and *Anopheles mascarensis* in one of the 11 sites investigated.

1. INTRODUCTION

In Madagascar, malaria is endemic within 90 percent of the population of the country. 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, delivering high-quality IRS campaigns and gathering the most comprehensive vector control entomological data in several countries around the world and mostly in Africa. The 2018 Madagascar IRS campaign was the fifth round of spraying with pirimiphos-methyl (Actellic® 300 CS) in the East Coast of Madagascar (Toamasina II, Brickaville and Fenerive Est districts), the first round with SumiShield® 50 WG in the South East (Farafangana and Vohipeno districts), after three rounds with pirimiphos-methyl in Farafangana and two rounds in Vohipeno. In the South West (Tulear II and Sakaraha), pirimiphos-methyl (Actellic® 300 CS ®) was sprayed for the first time. Entomological monitoring was conducted in districts sprayed with both Actellic® 300 CS and SumiShield® 50 WG. 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.

The Madagascar 2018 entomological annual report presents data collected from 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.

The objectives of the entomological surveillance were:

- To identify the vector species composition and density
- To assess vector biting and resting behavior
- To determine the quality of spraying and insecticide decay rates following spray operations
- To assess vector susceptibility to the World Health Organization (WHO) prequalified (WHO-PQ) insecticides, including pyrethroids (alpha-cypermethrin, deltamethrin, lambda-cyhalothrin and permethrin), organophosphate (pirimiphos methyl), carbamate (bendiocarb), Organochlorine (DDT) and pyrrole (chlorfenapyr).

2. METHODOLOGY

2.1. LONGITUDINAL MONITORING

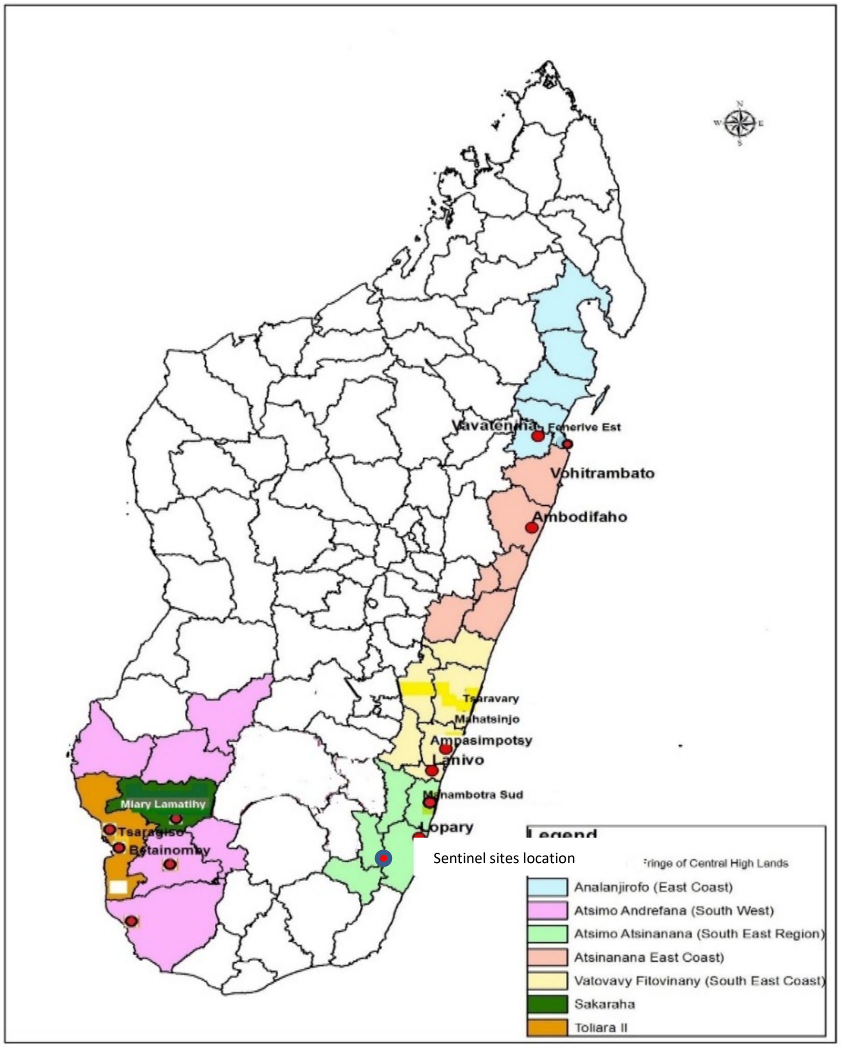
2.1.1. Study Sites

Table 1 below describes all the 2018 IRS campaign entomological surveillance sentinel sites.

Table 1: List of Sentinel Sites

Region	District	Sentinel Site Location	Status	Years as sentinel site
Antsinanana (East Coast)	Toamasina II	Vohitrambato	IRS	2014; 2015; 2016; 2017; 2018; 2019
Antsinanana (East Coast)	Brickaville	Ambodifaho	IRS	2014; 2015; 2016; 2017; 2018; 2019
Analanjirofo (East Coast)	Fenerive Est	Mahambo/ Antsikafoka	IRS	2014; 2015; 2016; 2017; 2018; 2019
Analanjirofo (East Coast)	Vavatenina	Vavatenina	Control	2014; 2015; 2016; 2017; 2018; 2019
Atsimo Antsinanana (South East)	Farafangana	Manambotra Sud	IRS	2015; 2016; 2017; 2018
Atsimo Antsinanana (South East)	Vangaindrano	Lopary	Control	2015; 2016; 2017; 2018
Vatovavy Fito Vinany	Vohipeno	Lanivo/ Anosy	IRS	2016; 2017; 2018
Vatovavy Fito Vinany	Mananjary	Mahatsinjo	IRS	2017; 2018
Vatovavy Fito Vinany	Mananjary	Tsaravary	Control	2017; 2018
Vatovavy Fito Vinany	Manakara	Ampasimpotsy	IRS	2017; 2018
Atsimo Andrefana	Tulear II	Tsaragiso	IRS	2018; 2019
Atsimo Andrefana	Sakaraha	Miary Lamatihy	IRS	2018; 2019
Atsimo Andrefana	Tulear I	Betaindambo	Control	2018; 2019

Figure 1: Map of Madagascar showing IRS entomological surveillance sites and districts boundaries



2.1.2. Methods

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), indoor resting collections by Prokopack aspiration, and outdoor resting collections using mouth aspirators and Prokopack in pit shelters, cow stables and oxen (Table 2).

The team collected adult mosquitoes from June 2018 to April 2019 in Vatovavy Fitovinany and Atsimo Atsinanana regions, and from August 2018 to May 2019 in Atsinanana, Analanjirofo and Atsimo Andrefana regions.

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

Insecticide susceptibility was conducted on two to four-day old adult female *An. gambiae* s.l. reared from field-collected larvae using WHO tube tests. The diagnostic concentrations of permethrin (0.75 percent), deltamethrin (0.05 percent), alpha-cypermethrin (0.05 percent), lambda-cyhalothrin (0.05 percent), bendiocarb (0.1 percent), pirimiphos-methyl (0.25 percent) and DDT (4 percent) were tested in the sites. The intensity of the resistance was also tested when resistance at diagnostic doses was confirmed for deltamethrin, permethrin, lambda-cyhalothrin and alpha-cypermethrin. Additionally, clothianidin was tested using the protocol designed by the VectorLink project with paper impregnated locally by the team.

CDC bottle assays were conducted using chlorfenapyr at the doses of 12.5μg/bottle, 25 μg/bottle, 50 μg/bottle, 100 μg/bottle and 200 μg/bottle. Testing and interpretation was done following the protocol of Brogdon G. *et al.* The resistance status to all the insecticides tested was determined following WHO criteria with < 9 percent as confirmed resistance, 90 percent - 97 percent as possible resistance, and >98 percent as susceptible.

2.3. SPRAY QUALITY AND RESIDUAL EFFICACY

WHO cone bioassays were used to assess spray quality and determine the residual efficacy of Actelllic and Sumishield on sprayed surfaces. Since VectorLink Madagascar does not have access to a susceptible colony in the field, wild-caught mosquitoes reared from larvae at the sentinel sites were used to determine the quality of spray and subsequently to monitor the residual efficacy of insecticides sprayed. The susceptibility of the local vector to the insecticide sprayed in the area was confirmed before mosquitoes from the same population were used for the cone bioassay testing. Bioassays were conducted within two weeks after the IRS spray campaigns started to evaluate the quality of the spray done by the operators. The residual bio-efficacy of the insecticides was then monitored monthly. Two common types of surfaces were selected from each of the different sites: thatch (Falafa), wood, or bamboo in the East and South East regions (Atsinanana, Vatovavy Fito Vinany), and mud or wood in the South West region. 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 recorded after 24 hours.

To determine the fumigant effect of Actellic sprayed in the houses, 10 female *Anopheles gambiae* s.l. were introduced in a small cage (15cmx10cm) covered with clean paper to ensure that there is no contamination of the cage with the insecticide sprayed on the wall. The cage was placed on a chair approximately 10 cm from a sprayed wall and about one meter above the floor. The mosquitoes were exposed for 30 minutes and then transferred to paper cups and fed with 10 percent glucose soaked in cotton. The knockdown effect was recorded 30 minutes post-exposure. Mortality was recorded after a 24-hour holding period. A control cage was set up 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

Insecticide resistance in mosquitoes can 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. were randomly selected per site within the WHO susceptibility tested mosquitoes and were further analysed to determine species identification and assess molecular markers of insecticide resistance. The DNA of each individual mosquito was extracted using the protocol designed by Collins et al, 1987. The presence of *kdr*-West and East was 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 was characterized following the protocol of Weill et al. (2004).

Adults *An. gambiae* s.l. and *An. funestus* from the 11 sites surveyed and collected using HLCs were further molecularly identified to sub-species as *An. gambiae* s.s. *An. coluzzii* or *An. arabiensis* or members of *An. funestus* group for both complex of species. The Short Interspersed Element (SINE) protocol described by Santolamazza et al, 2008 was used to differentiate the *An. gambiae* s.l. sub species, while the protocol of Koekemoer et al, 2002 was used for the *An. funestus* group.

The sporozoite infection status of a subsamples of mosquitoes collected from each site by HLC was determined using the Enzyme Linked Immunosorbent Assay (ELISA) protocol for identification of *Plasmodium falciparum* circumsporozoite infection.

3. RESULTS

3.1. LONGITUDINAL MONITORING

3.1.1. Vector Species Composition, Densities and Behavior Observed during the Surveillance Period

A total of 21,725 mosquitoes (all genus included) were collected from all the sentinel sites between June 2018 and April 2019 in the South East, and between August 2018 and December 2019 in the East and South West of Madagascar, using HLC, indoor Prokopack aspirations and outdoor resting collection with mouth aspirators and/or Prokopack. Listed below are the total number and proportion of mosquitoes collected per sampling method:

- HLC: 19,838 (91.3 percent)
- Indoor Collection with Prokopack: 514 (2.4 percent)
- ODC: 1,373 (6.3 percent)

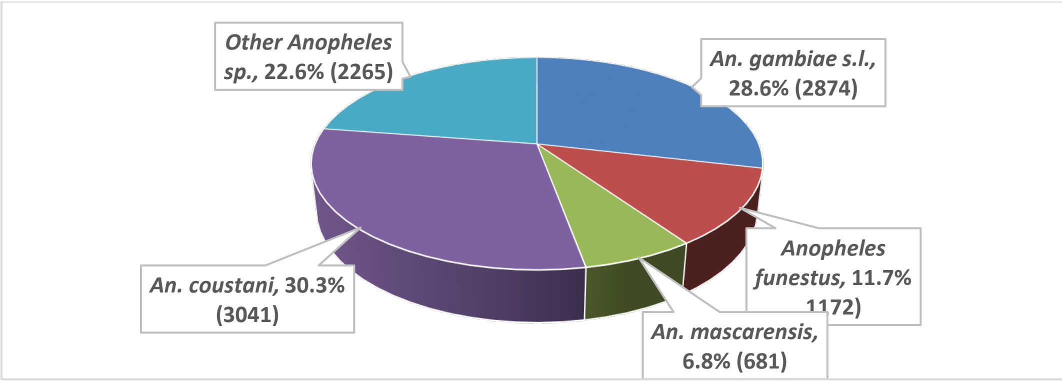
The proportion of Anopheline collected per method was:

- HLC: 9,192 (91.6 percent)
- Indoor Collection with Prokopack: 145 (1.5 percent)
- ODC: 696 (6.9 percent)

A total of 10,033 (46.2 percent of the total mosquitoes) mosquitoes collected were Anophelines and 7,768 (77.4 percent) represented known or potential malaria vectors in Madagascar: *Anopheles gambiae* s.l., *Anopheles funestus* group, *Anopheles mascarensis*, and *Anopheles coustani* (Figure 1).

The distribution of vector species varied by sentinel sites (Table 9): *Anopheles gambiae* s.l. and *An. coustani* were collected at all sentinel sites; *An. funestus* were collected at all sentinel sites except in Betaindambo. *An. gambiae* s.l. was observed as the primary and predominant vector species in the IRS and control areas. *Anopheles mascarensis* was collected in Ambodifaho, Vohitrambato, Vavatenina, Mahatsinjo, Tsaravary, Ampasimpotsy and Manambotra Sud (Table 8). Other Anopheles species, including *An. brunnipes, An. fuscicolor, An. flavicosta, An. maculipalpis, An. pauliani, An. rufipes, An. ranci,* and *An. Squamosus,* were collected in different sites and represented about 22.6% of the Anopheline mosquitoes (Figure 2).

Figure 2: Species composition of *Anopheles*



3.1.2. Result of human landing catches (HLCs)

The number of malaria vectors and potential vectors collected from eleven sentinel sites using HLCs (Tables 3 and 5) consisted of:

- 2,444 *An. gambiae* s.l.
- 1,052 *An. funestus*
- 634 *An. mascarensis*
- 2,864 *An. coustani*

The vectors showed an exophagic tendency in all IRS sites. When HLC data from all the localities surveyed were combined, the proportion of vectors and potential vectors collected outdoors was significantly higher than indoors (p<0.0001) (Table 3). For control sites, all the vectors showed also an exophagic tendency except *An. funestus* whose outdoor biting rate was not significantly different from indoors (Table 9).

Table 3: Indoor vs outdoor landing mosquitoes at IRS Sites

Vector	# indoor	# outdoor	Exophagy index	P value
An. gambiae s.l.	343	1,507	81.5%	<0.0001
An. funestus	240	496	67.4%	<0.0001
An. mascarensis	18	118	86.8%	<0.0001
An. coustani	131	1,845	93.4%	<0.0001

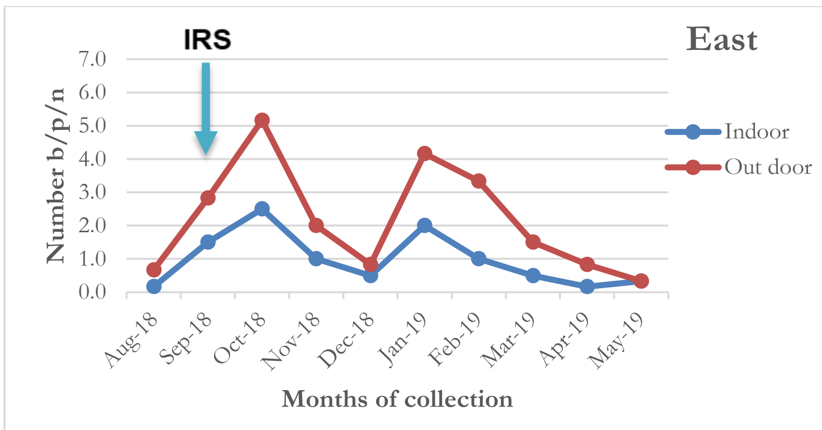
Table 4: Indoor vs outdoor landing mosquitoes at control sites

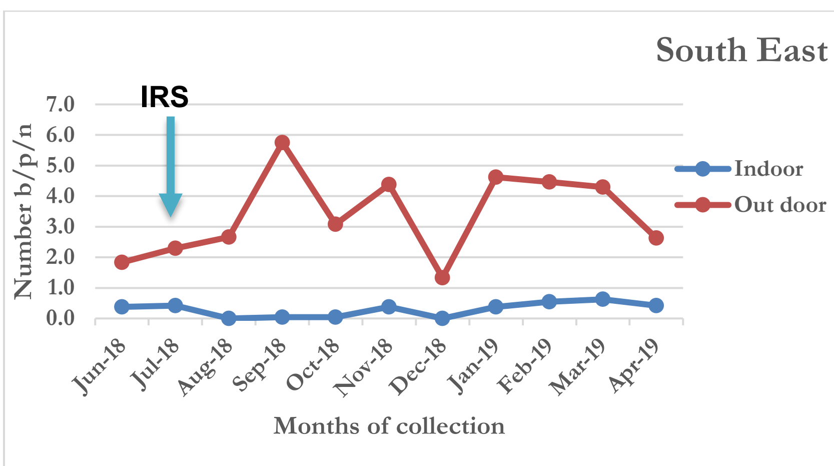
Vector	# indoor	# outdoor	Exophagy index	P value
An. gambiae s.l.	220	374	63.0%	<0.0001
An. funestus	132	184	58.2%	0.902
An. mascarensis	211	287	57.6%	<0.0001
An. coustani	410	478	53.8%	<0.0001

The mean human biting rate (HBR) was higher outdoors compared to indoors in both the sprayed and control sites. In the East, the HBR was higher in the sprayed sites than in the control site within the two first months after spraying. This period coincided with the peak of rainfall in the sprayed areas, which may be the cause of the trend observed.

In the South East and South West, indoor HBRs were lower in sprayed sites compared to the control sites. In the South East, outdoor HBRs were higher in sprayed sites compared to the control sites. In the South West, HBRs were low due to the scarce rainfall. Figures 2 and 3 below show also the monthly exophagic tendency of *An. gambiae* s.l. in both sprayed and control sites.

Figure 3: Monthly distribution of indoor and outdoor of mean human biting rates (bites/person/night: b/p/n) for *An. gambiae* s.l. at the sentinel sites in sprayed sites





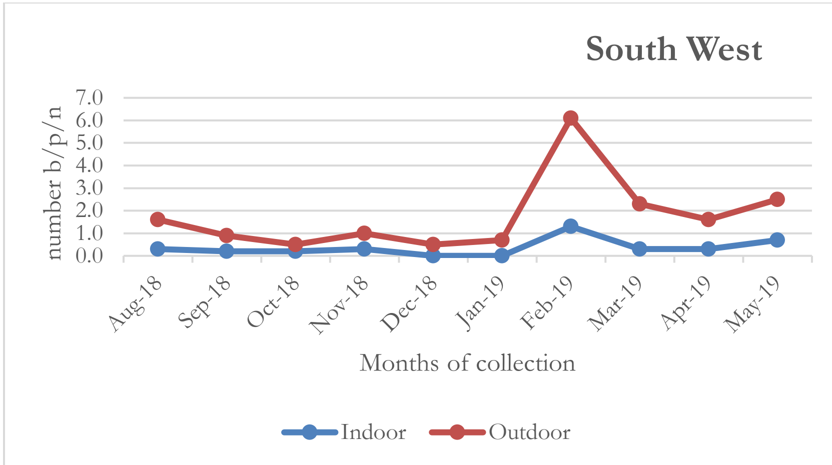
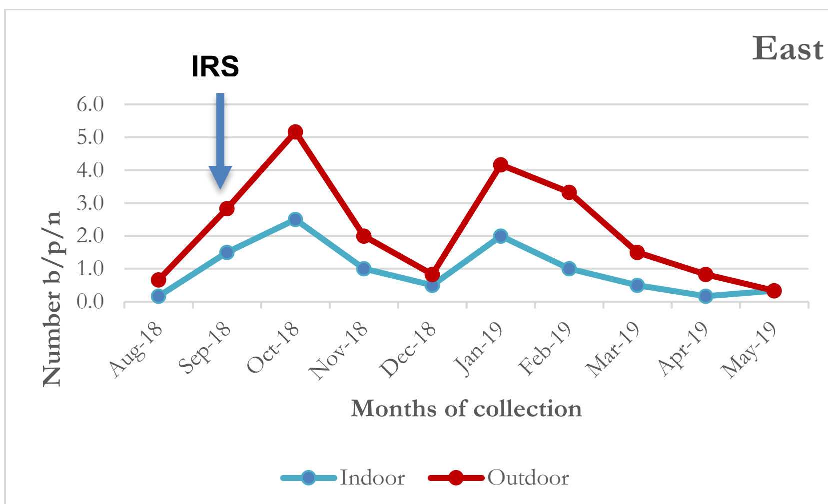
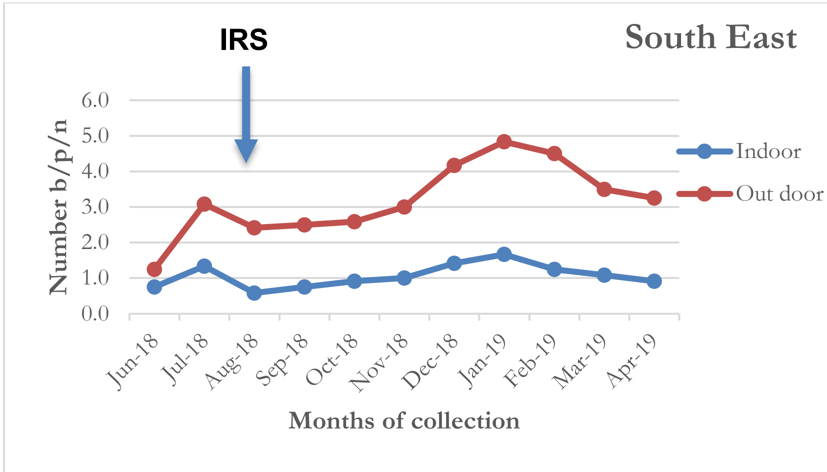
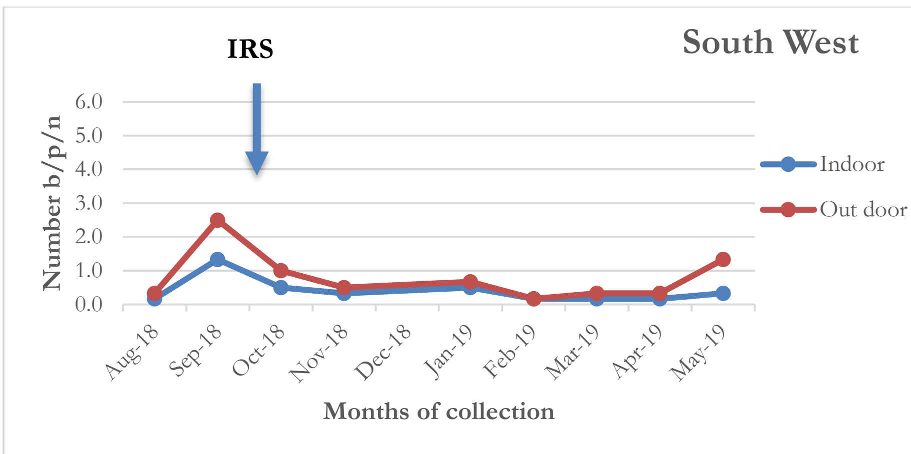


Figure 4: Monthly distribution of indoor and outdoor of mean human biting rates (bites/person/night: b/p/n) for *An. gambiae* s.l. at the sentinel sites in unsprayed (control) sites







Peak Biting Time

For *Anopheles gambiae s.l,* in the sprayed areas, peak indoor biting activity was observed from 8.00pm-01.00 am without a distinct peak in the East, both indoor and outdoor; in the South East, the peak was observed between 10.00 pm and 11.00 pm. The same trend is observed in the unsprayed area of the East and South East (Figures 4 and 5).

It was difficult to draw a conclusion on the feeding habits of *Anopheles gambiae* s.l. in the South West or *Anopheles funestus* and *Anopheles mascarensis* in all sites, based on the current data, either due to the absence of a consistent biting pattern or the small number of mosquitoes collected.

Figure 5: *Anopheles gambiae* s.l. biting hours at sprayed sites in the East

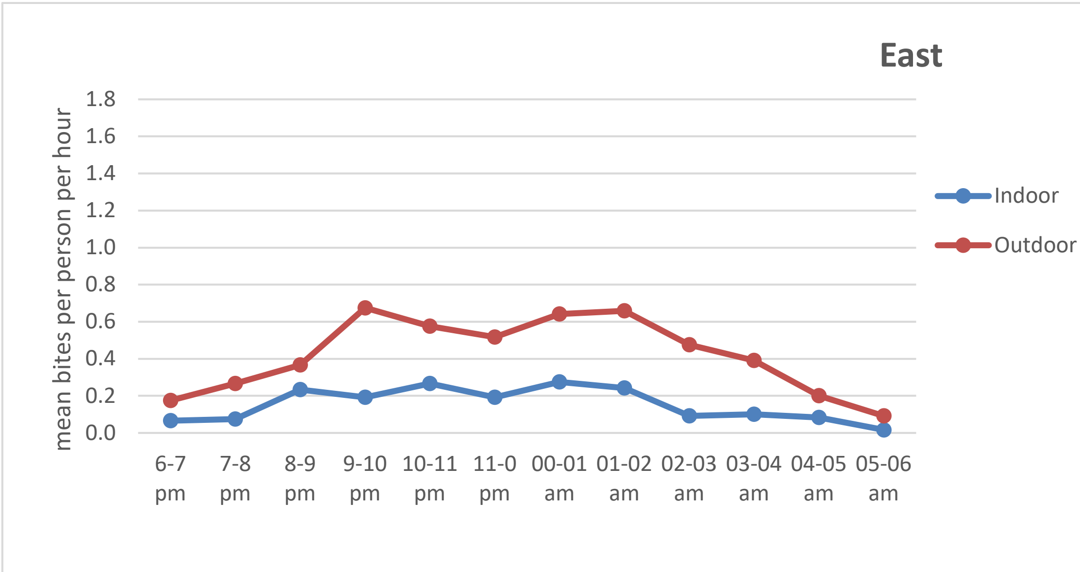


Figure 6: *Anopheles gambiae* s.l. biting hours at sprayed sites in the South East

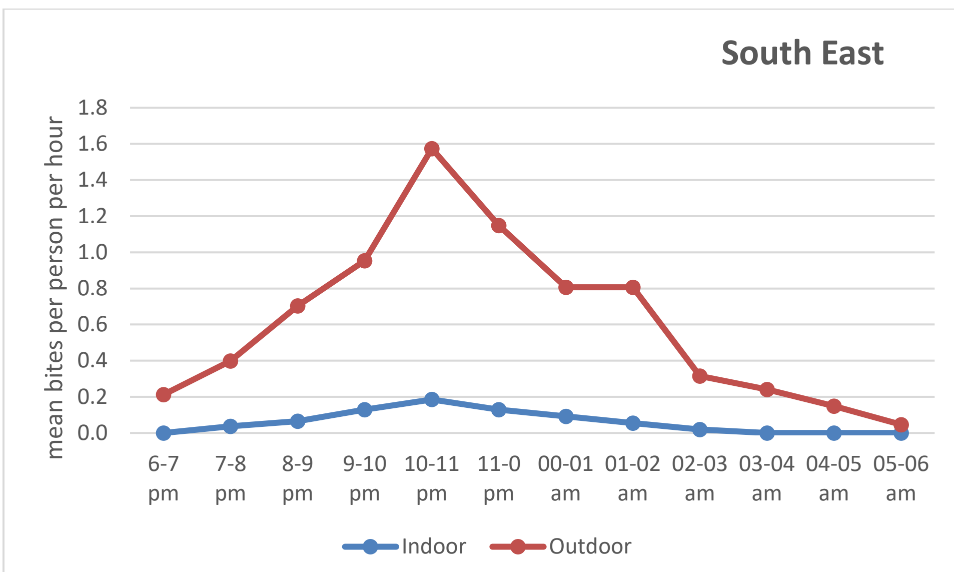


Figure 7: ***Anopheles gambiae*** **s.l. biting hours at unsprayed sites (control) in the East**

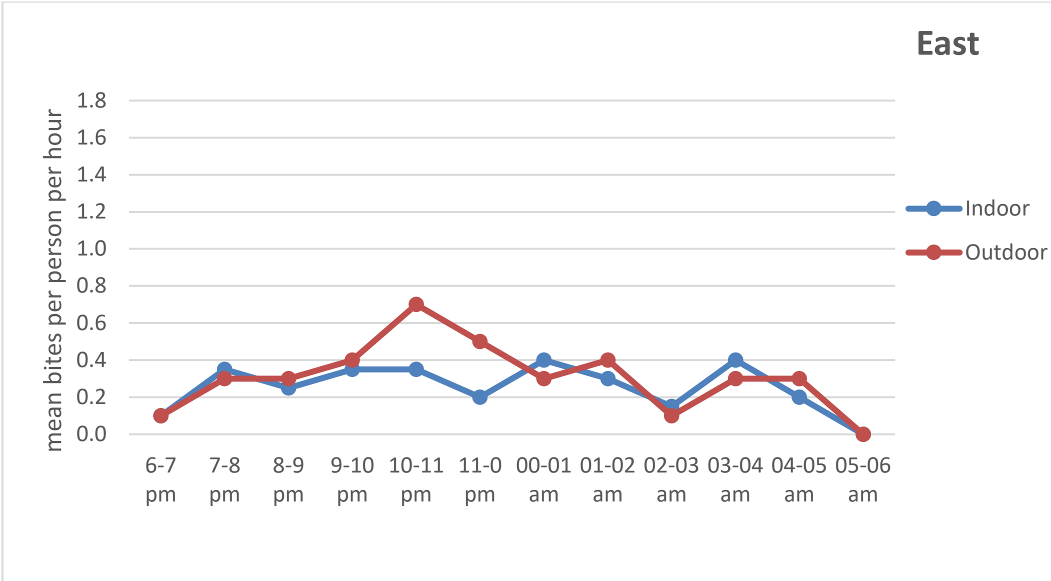
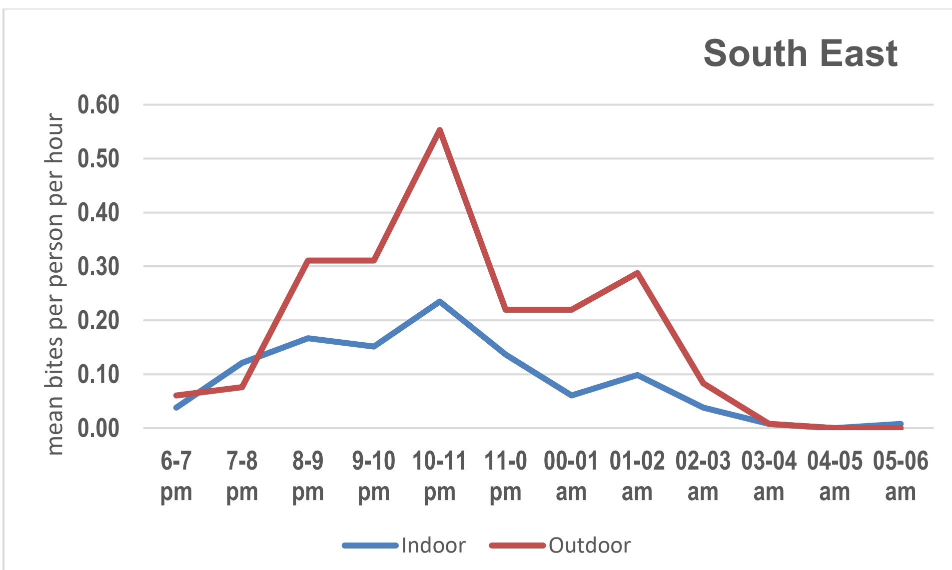


Figure 8: ***Anopheles gambiae*** **s.l. biting hours at unsprayed sites (control) in the South East**



3.1.3. Indoor Resting Density

The indoor resting density of vectors at sprayed sites was low, with 0 to 0.3 *An. gambiae* s.l per room per day in the East, 0 to 0.1 in the South East, 0.5 in the South West, 0 to 0.7 *An. funestus* in the South East and 0.3 in the South West collected at the baseline, and 0 to 0.2 *An. gambiae* s.l. in the East, 0.1 to 0.4 in the South East and 0.1 *An. funestus* in the East, 0 to 0.6 in the South East per room per day during the post-spray period. In the control sites, the indoor resting density was 0.1 *Anopheles gambiae* s.l. per room per day in the South East and South West, 0 to 0.7 *Anopheles funestus* in the South East while no vector was found in the East, at the baseline. During the post spraying period, 0.1 *Anopheles gambiae* s.l. per room per day in the East, 0.2 to 0.4 in the South East, 0.1 in the South West and 0.1 to 0.2 *Anophees funestus* were collected resting indoor. The resting habit and impact of IRS on indoor resting density could not be confirmed using this indicator. In most sites, the indoor resting density was zero or close to zero. Table 11 provides more information on the indoor resting density at each sentinel site. A total of 514 mosquitoes were collected resting indoor using Prokopack from eleven sites during the monitoring period.

3.1.4. Results of Outdoor Collections

A total of 1,373 mosquitoes were collected from resting outdoors in natural and pit shelters using aspirators from eleven sites in the South East, East and South West of Madagascar. This included 346 (25.2 percent) *An. gambiae* s.l. and 66 (4.8 percent) *An. funestus* collected in nine sites (Ambodifaho, Vohitrambato, Vavatenina, Manambotra Sud, Lopary, Lanivo, Tsaravary, Mahatsinjo and Ampasimpotsy), 46 (3.4 percent) *An. mascarensis* collected from three sites (Vohitrambato, Vavatenina and Ampasimpotsy), and 170 (12.4 percent) *An. coustani* collected from eight sites (Vohitrambato, Vavatenina, Manambotra Sud, Lopary, Lanivo, Tsaravary, Mahatsinjo and Ampasimpotsy) (Table 12).

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 (82.5%; n=106) in the sites to be sprayed. In the control sites, the parity rate was 70.8% (n=27) (Figure 9).

During the seven months post IRS, which represents the effective period of the insecticide used in the area, there was a reduction of the average parity rates, but in an irregular manner within sites and months.

Figure 9: Monthly parity of *An. gambiae* s.l. in the East Coast of Madagascar during the investigation period (bars represent 5% percentage error bars)

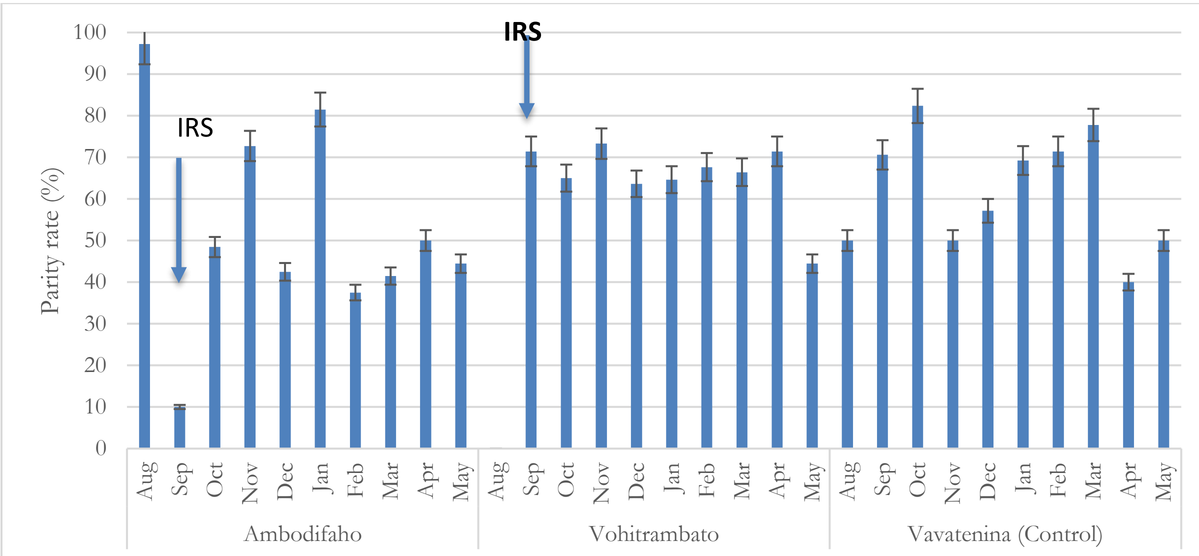


Figure 10: Monthly parity of *An. gambiae* s.l. in the South East of Madagascar during the investigation period.

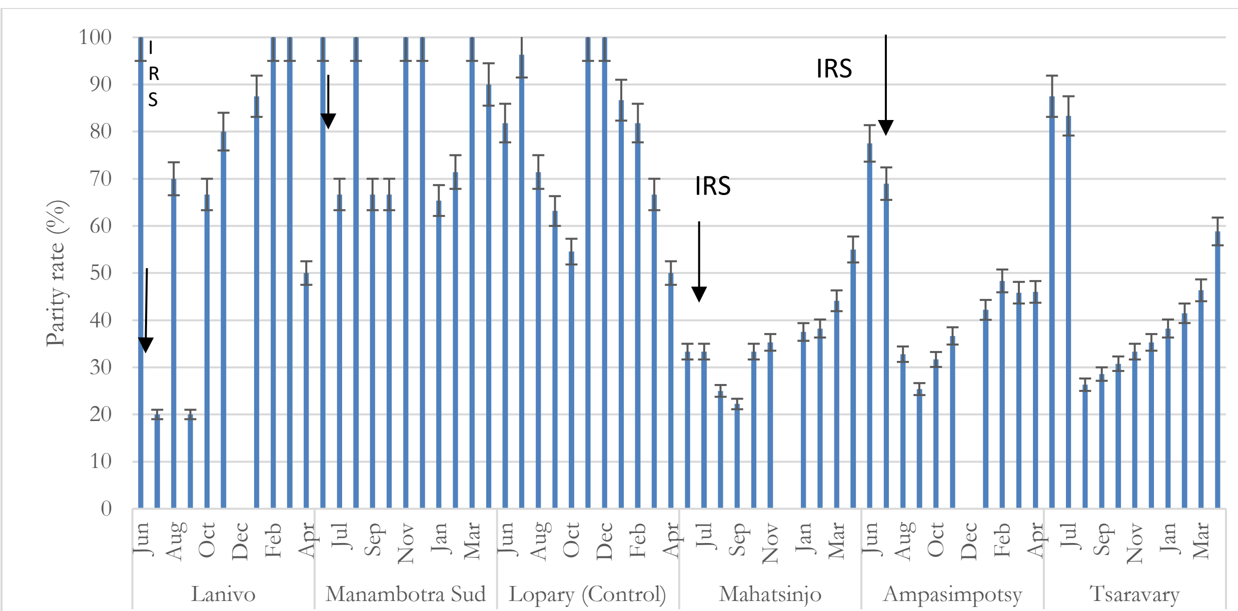
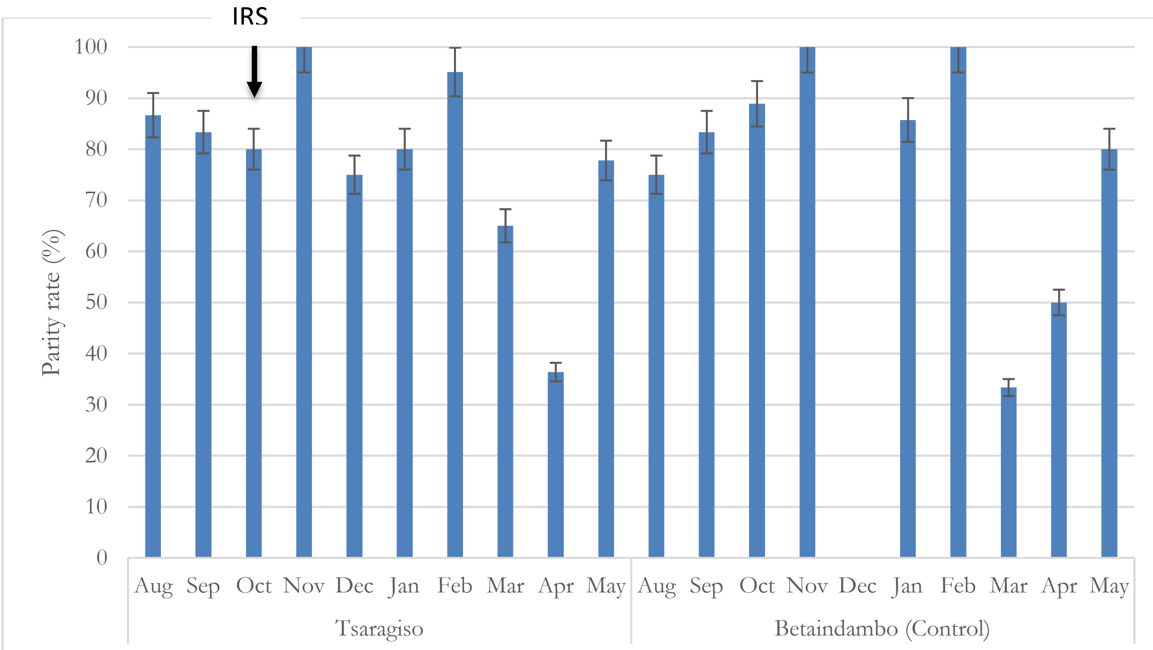


Figure 11: Monthly parity of *An. gambiae* s.l. in the South West of Madagascar during the investigation period.



3.3. INSECTICIDE SUSCEPTIBILITY TEST RESULTS

3.3.1. WHO Susceptibility Test

The results of the vector susceptibility tests (Figures 12, 13, 14 and Table 13) indicated full susceptibility of *An. gambiae* s.l. to pirimiphos-methyl, clothianidin and bendiocarb in all areas where the tests were conducted. Susceptibility to DDT was also recorded in all sites surveyed, except in Vavatenina where few survivals were observed.

The test results also showed that *An. gambiae* s.l. is resistant to permethrin in Ambodifaho, Vavatenina, Vohitrambato, Mahambo and Betaindambo, to alpha-cypermethrin in Ambodifaho, Vavatenina and Vohitrambato, to deltamethrin in Vohitrambato, Mahambo, Vavatenina and Betaindambo and to lambdacyhalothrin in Vohitrambato.

Resistance was suspected for deltamethrin in Ambodifaho and for permethrin in Manambotra Sud.

Anopheles funestus was susceptible to all insecticides tested, specifically:

- Pirimiphos-methyl in the districts of Farafangana, Mananjary, Manakara, Fenerive East, Brickaville, Toamasina II and Vavatenina
- Bendiocarb in the districts of Mananjary and Manakara
- Permethrin in the districts of Mananjary and Manakara
- DDT in the districts of Farafangana, Mananjary and Fenerive Est

Anopheles mascarensis and *An. coustani,* were susceptible to all insecticides (pirimiphos-methyl, bendiocarb, deltamethrin, permethrin, lambda-cyhalothrin, alpha-cypermethrin, DDT) tested in all sites.

Resistance intensity assays using WHO susceptibility test kits:

Intensity assays performed with permethrin at five times (5x) the diagnostic dose, yielded 100 percent mortality in Ambodifaho and Mahambo, and 99 percent in Vavatenina, showing that resistance is low in these three sites. In Vohitrambato and Betaindambo (Tulear I), 94 percent and 78 percent mortality were respectively recorded at the same 5x permethrin dose. The subsequent concentration at 10 times (10x) the diagnostic dose of permethrin tested in those two sites recorded 100 percent mortality, showing that the resistance is moderate in Vohitrambato and Betaindambo. Deltamethrin 5x yielded 100 percent mortality in Betaindambo and Mahambo, indicating a low resistance intensity, 92 percent in Vavatenina and 93 percent in Vohitrambato. Deltamethrin at 10x resulted in 100 percent mortality in Vavatenina and Vohitrambato showing that the resistance is moderate.

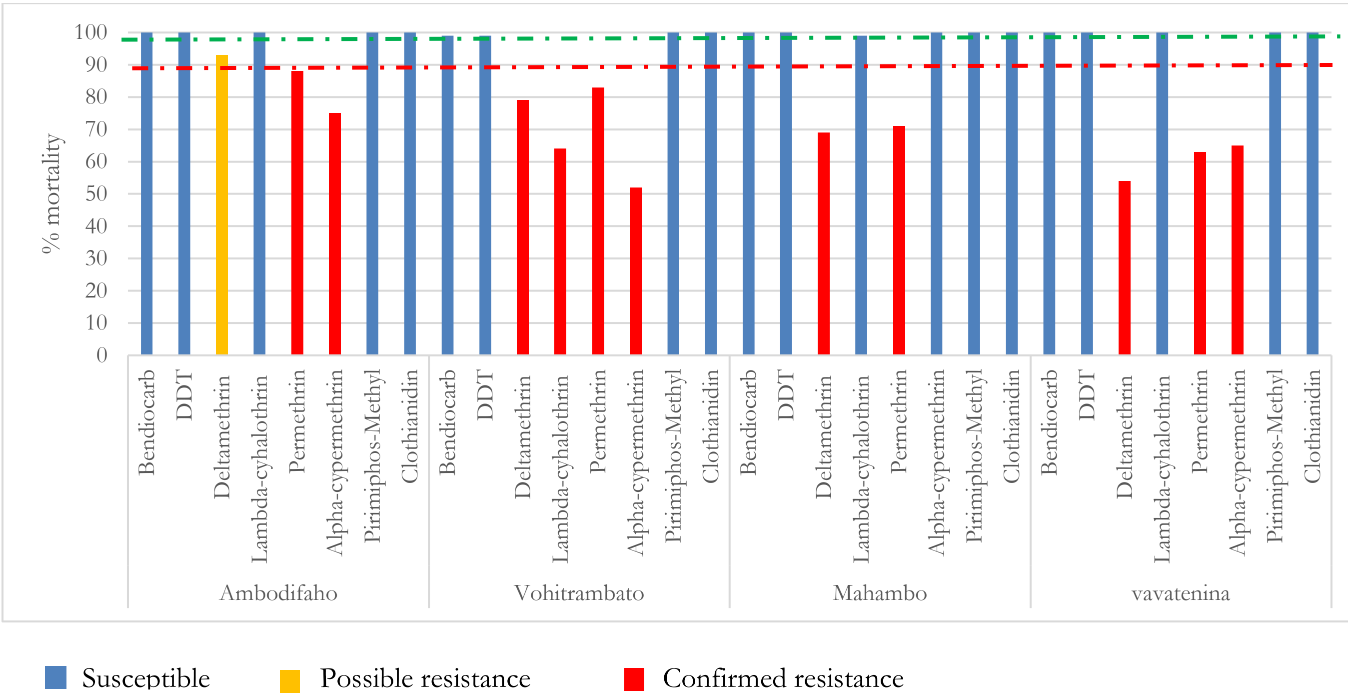
The resistance was low for alpha-cypermethrin in Ambodifaho with 5x the diagnostic dose, yielding 100 percent mortality. At the same 5x alpha-cypermethrin dose, 64 percent and 87 percent mortality were recorded in Vohitrambato and Vavatenina respectively. In Vavatenina, the resistance was moderate for alpha-cypermethrin with 100 percent mortality at 10x but high in Vohitrambato with 88 percent mortality at 10x. Lambda-cyhalothrin 5x yielded 100 percent mortality in Vohitrambato, showing low resistance.

Synergist assays using WHO susceptibility test kits:

Pre-exposure to PBO (4%) resulted in increased susceptibility to deltamethrin in four sites (Vavatenina, Vohitrambato, Mahambo and Betaindambo).

Pre-exposure to PBO (4%) resulted increased susceptibility to Permethrin in five sites (Ambodifaho, Vohitrambato, Vavatenina, Mahambo and Betaindambo), alpha-cypermethrin in three sites (Ambodifaho, Vavatenina and Vohitrambato), and lambda-cyhalothrin in one site (Vohitrambato). In all sites, PBO restored full susceptibility (Figure12).

Figure 12: Results of insecticide susceptibility tests against *An. gambiae* s.l. using the WHO Tube Test in the East coast of Madagascar.



The red and green dashed lines in all figures represent the resistance and susceptibility thresholds respectively using WHO susceptibility test kits.

Figure 13: Results of insecticide susceptibility tests against *An. gambiae* s.l. using the WHO Tube Test, in the South East of Madagascar

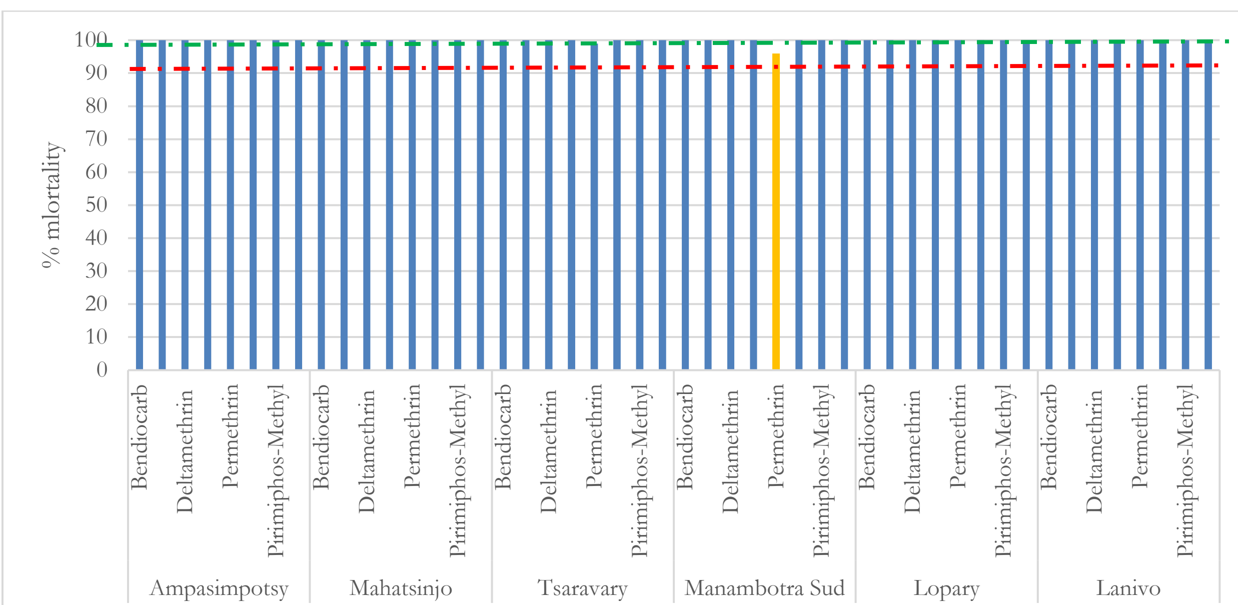
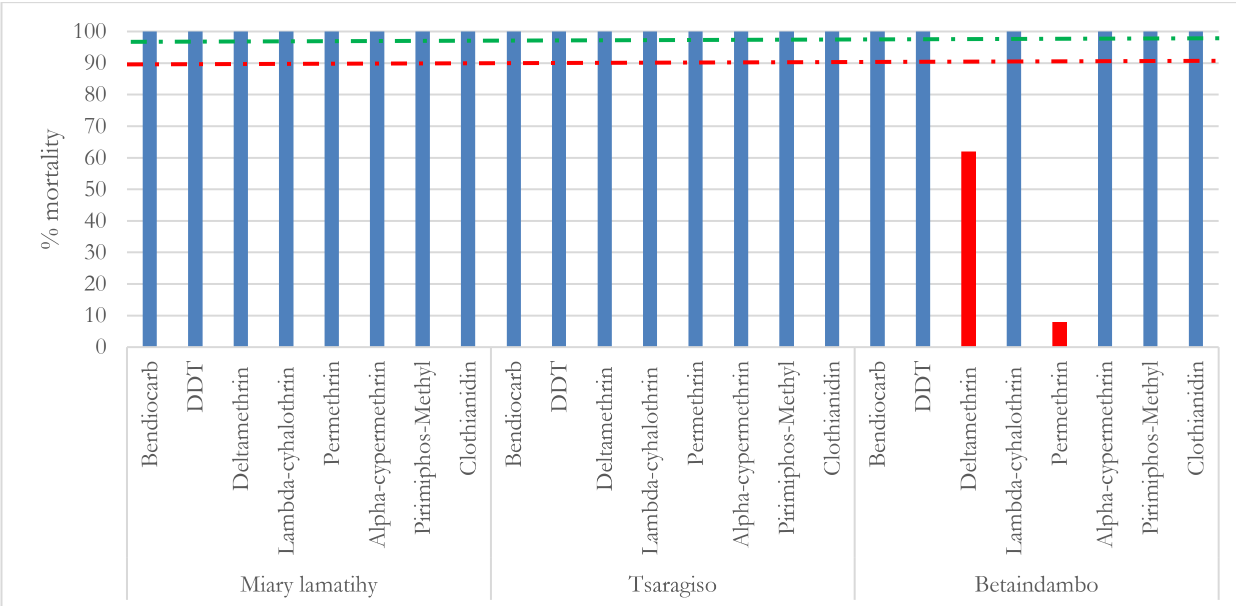


Figure 14: Results of insecticide susceptibility tests against *An. gambiae* s.l. using the WHO Tube Test, in the South East of Madagascar

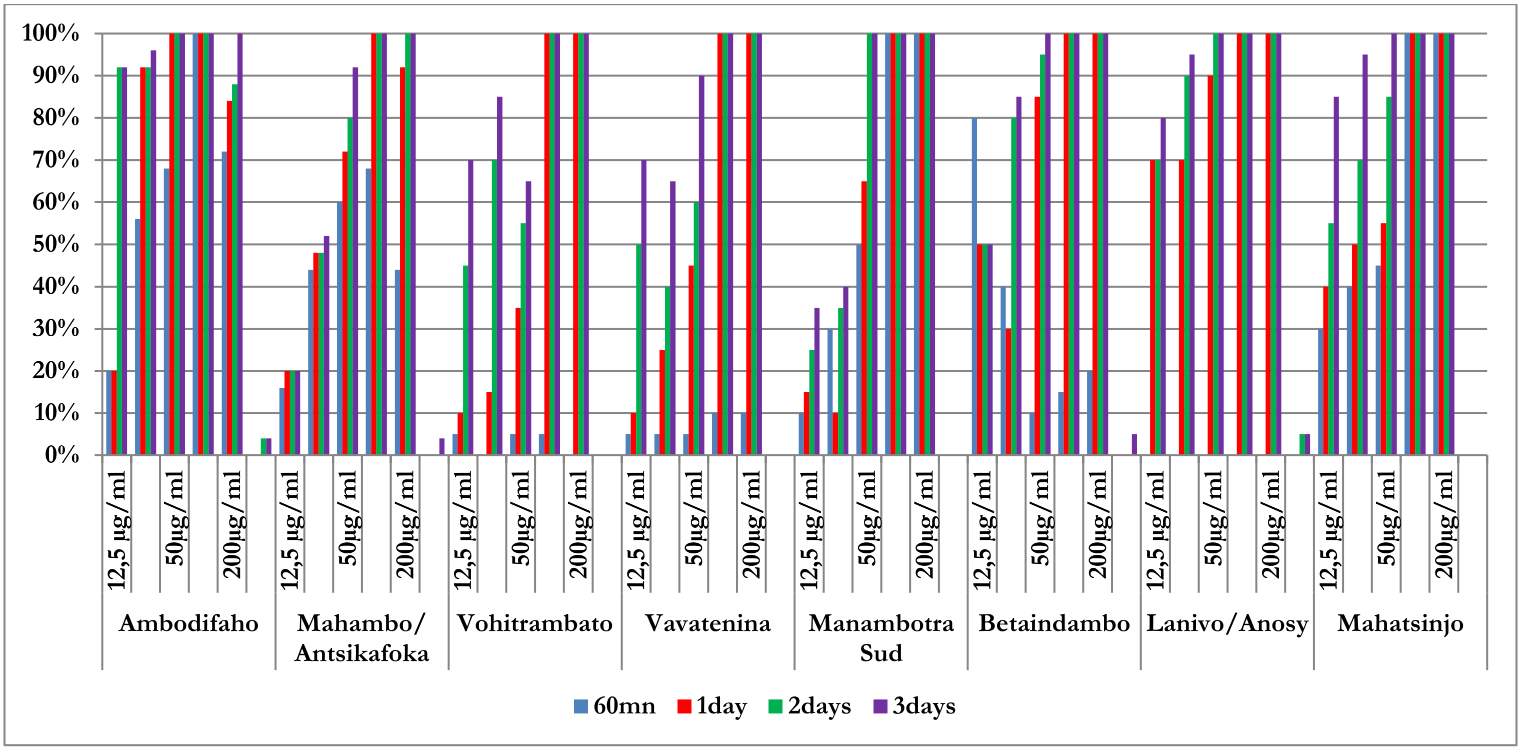


3.3.2. CDC bottle assay using chlorfenapyr against wild *Anopheles gambiae* s.l.

In 2018, chlorfenapyr susceptibility testing yielded (Figure 13 and Table 12):

- 100 percent mortality of *An. gambiae* s.l., one day after a 60-minute exposure to chlorfenapyr at 50µg/bottle in Ambodifaho
- 100 percent mortality of *An. gambiae* s.l., two days after a 60-minute exposure to chlorfenapyr at 50µg/bottle in Manambotra Sud, Lanivo.
- 100 percent mortality of *An. gambiae* s.l., three days after a 60-minute exposure to chlorfenapyr at 50µg/bottle in Betaindambo, Mahatsinjo and Ampasimpotsy.
- 100 percent mortality of *An. gambiae* s.l., recorded in all the sites after a 60-minute exposure to chlorfenapyr at 100µg/bottle and 200µg/bottle.

Figure 15: Results of chlorfenapyr CDC bottle assays



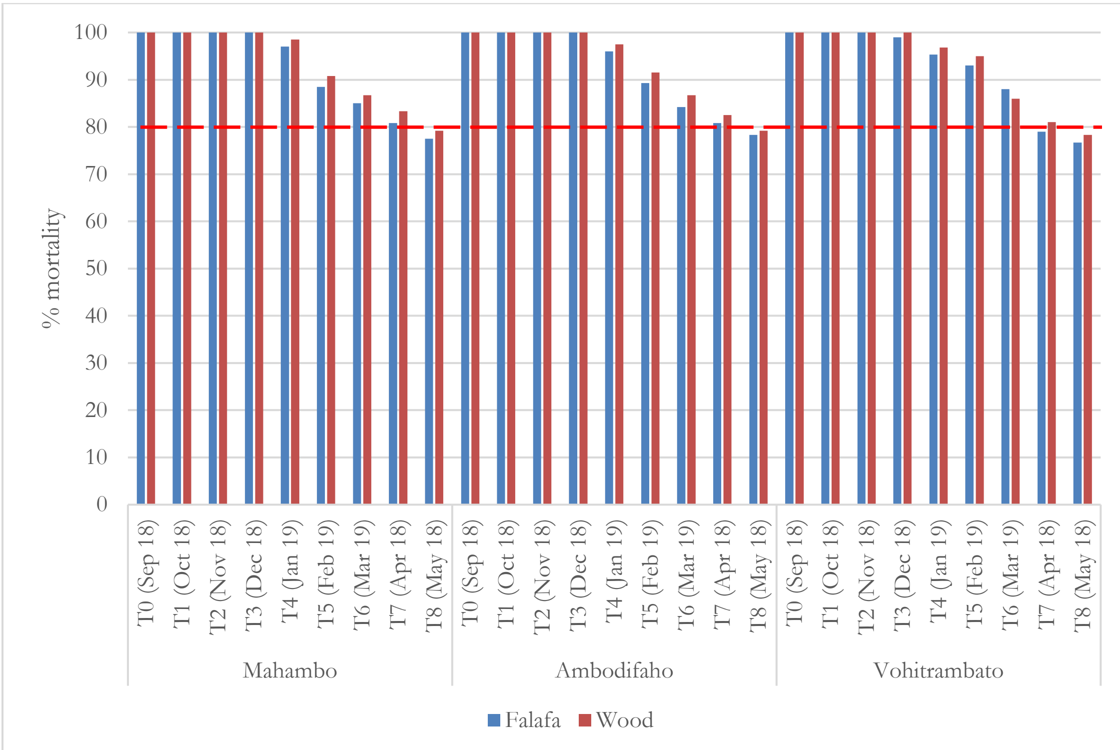
3.4. Spray Quality and Residual Efficacy

3.4.1. Cone Bioassay on Sprayed Surfaces

Cone bioassays completed after the spray campaign have already been reported within the 2018 End of Spray Report (EOSR). The results indicated good spray quality in the East Coast, South East and South West with 100 percent mortality recorded for all the structures bio assayed at T0 and T1 (Figures 16, 17, 18, 19 20).

In the East Coast sites (Ambodifaho, Brickaville; Vohitrambato, Toamasina II; Mahambo, Fenerive East) and the South East sites (Manambotra Sud, Farafangana; Lanivo/Anosy, Vohipeno, Ambohimiarina II, Mananjary), most houses are made of wood or falafa (branches of traveler's palm), while houses in the South West are made of mud or wood.

Figure 16: Residual effectiveness observed for pirimiphos-methyl (Actellic® 300 CS) in the East Coast of Madagascar



Red line represents WHO threshold of *efficacy using*

Figure 17: Residual effectiveness observed for pirimiphos-methyl (Actellic® 300 CS) in the South East Coast of Madagascar.

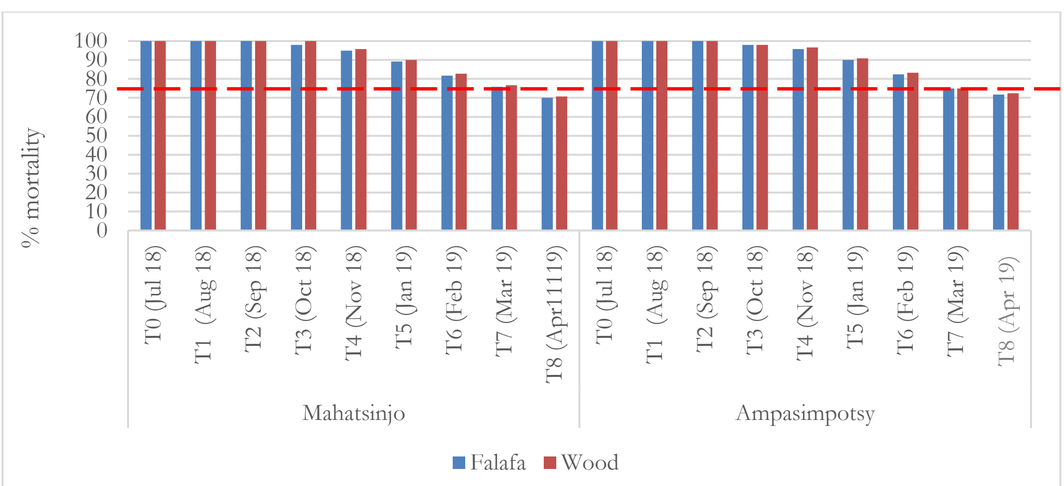


Figure 18: Residual effectiveness observed for pirimiphos-methyl (Actellic® 300 CS) in the South West Coast of Madagascar

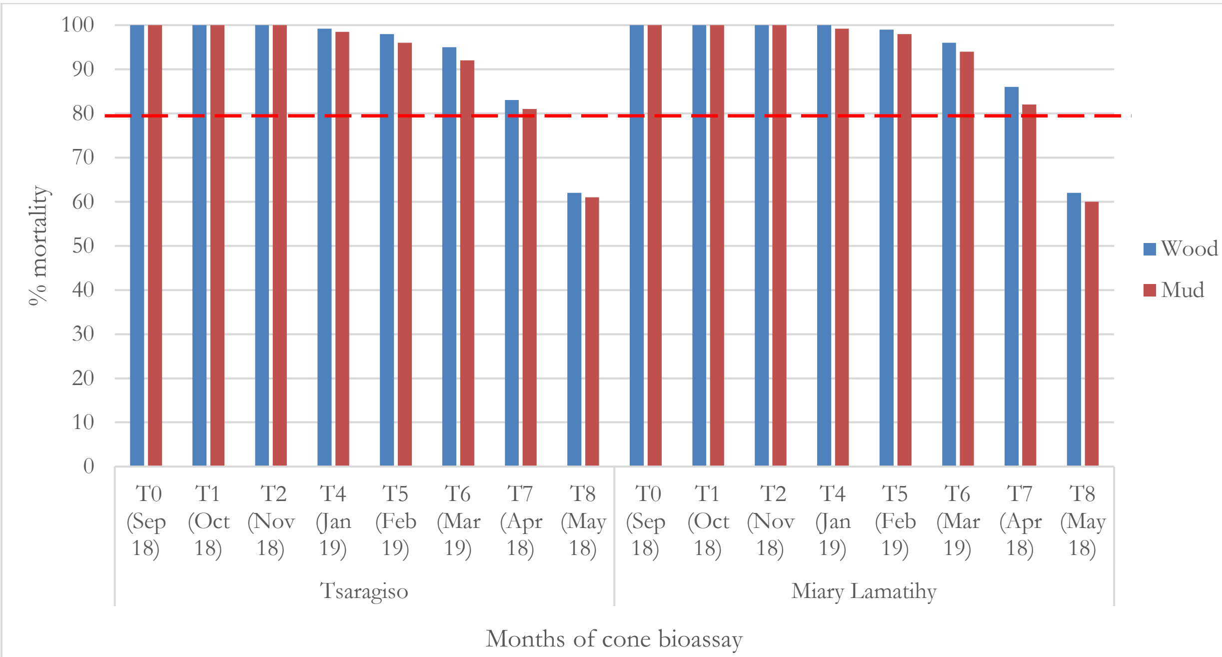


Figure 19: Residual effectiveness observed for clothianidin (SumiShield® 50 WG) in Lanivo/Anosy, Vohipeno district, in the South East of Madagascar.

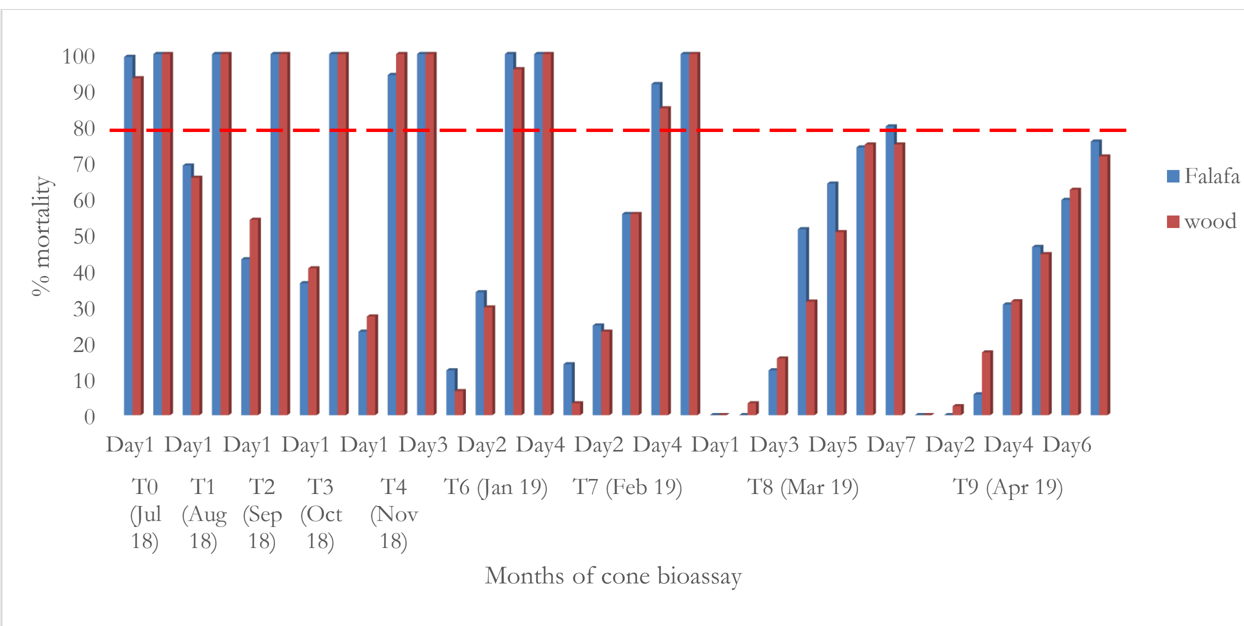
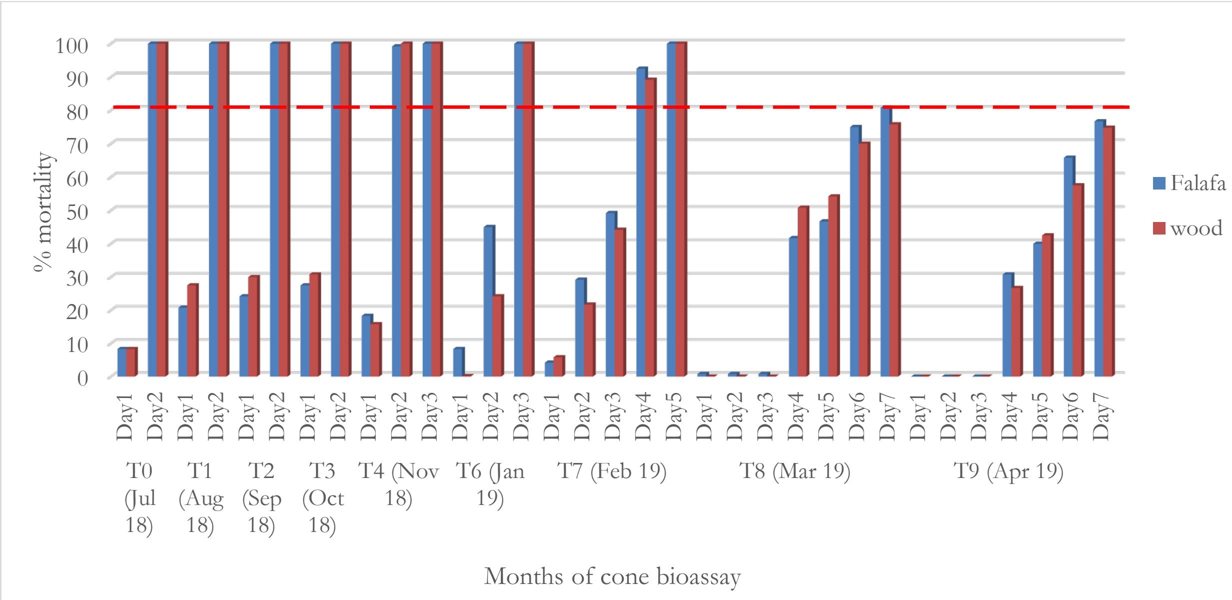


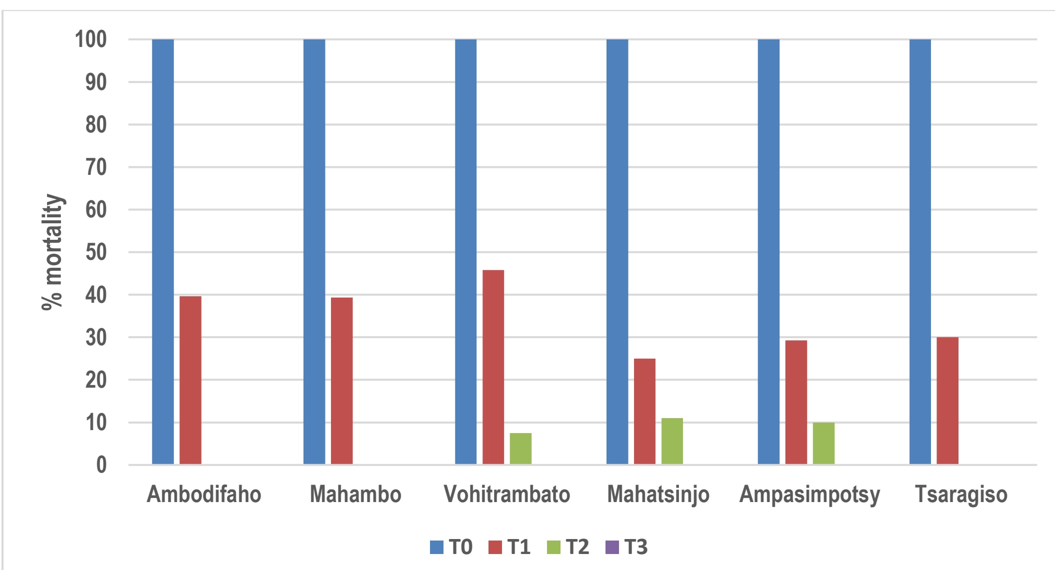
Figure 20: Residual effectiveness observed for clothianidin (SumiShield® 50 WG) in Manambotra Sud, Farafangana district, in the South East of Madagascar.



3.4.2. Fumigant effect of pirimiphos-methyl (Actellic® 300 CS)

The results of the fumigant effect of pirimiphos-methyl (Actellic® 300 CS) showed that mortality was high (100 percent) within one week after spraying in all the IRS sites. The mortality dropped under 12 percent (0 to 11 percent) two months after spraying. No fumigant effect was observed at all sites after three months post spray (Figure 21).

Figure 21: Fumigant effect of pirimiphos-methyl



3.5. Molecular Analysis

3.5.1. Species Identification within the *An. Gambiae* Complex

A total of 1,373 *An. gambiae* s.l. were sampled from the 11 sites surveyed and species identified. *Anopheles gambiae*, *An. arabiensis and Anopheles merus* were the three species found in the different sites with *An. gambiae* being the predominant species in 10 of the sites surveyed. The proportion of *An. gambiae* varied from 81.7 percent in the locality of Tsaravary to 99 percent in Lopary. The lowest proportion was recorded in Betaindambo (50%). Only Tsaragiso recorded 100 percent *An. arabiensis* population.

Table 5: Distribution of *An. gambiae* s.l. species

Regions	Districts	Localities	# tested	*An. gambiae*	*An. arabiensis*	*An. merus*
Atsinanana	Toamasina II	Vohitrambato	189	183	6	0
Atsinanana	Brickaville	Ambodifaho	148	134	14	0
Analanjirofo	Vavatenina	Vavatenina	78	73	5	0
Atsimo Atsinanana	Vangaindrano	Lopary	105	104	1	0
Atsimo Atsinanana	Farafangana	Manambotra Sud	75	64	11	0
Vatovavy Fitovinany	Mananjary	Mahatsinjo	75	73	2	0
Vatovavy Fitovinany	Mananjary	Tsaravary	213	174	39	0
Vatovavy Fitovinany	Manakara	Ampasimpotsy	288	271	17	0
Vatovavy Fitovinany	Vohipeno	Lanivo	66	65	1	0
Atsimo andrefana	Tulear II	Tsaragiso	78	0	78	0
Atsimo andrefana	Tulear I	Betaindambo	58	29	17	12
Total			**1,373**	**1,170**	**191**	**12**

3.5.2. Species Identification of the *An. Funestus* Group

A total of 599 *An. funestus* group was identified to sibling species. Only one specie*, An. funestus* was identified in all the sites tested (100 percent).

3.5.3 ELISA CSP

Out of 2,242 *Anopheles* including 1,170 *An. gambiae*, 191 *An. arabiensis*, 12 *An. merus*, 599 *An. funestus*, and 270 *An. mascarensis*, 11 *were* tested positive for *Plasmodium falciparum*. Among the *An. gambiae*, eight mosquitoes were positive for *Plasmodium falciparum*, representing an average sporozoite rate of 1.3 percent. *Anopheles gambiae* of six sites (Manambotra sud, Tsaravary, Ampasimpotsy, Lanivo, Tsaragiso and Betaindambo) out of the 11 surveyed did not record any mosquito carrying the sporozoite infections. The sporozoite rates recorded in the five remaining sites varied from 0.5 percent in Vohitrambato to 2.9 percent in Lopary.
On the other hand, two *An. funestus* out of the 599 and one *An. mascarensis* out of 270 analyzed carried sporozoites and were recorded in the localities of Lanivo, Vavatenina and Vohitrambato. The mean sporozoite rates were 2.4 percent for *An. funestus* and 1.3 percent for *An. mascarensis* (Table 6).

Table 6: Sporozoite rate across sentinel sites

Regions	Localities	*An. gambiae*			*An. arabiensis*			*An. merus*			*An. funestus*			*An. mascarensis*		
		# tested	CS+	%CS+	# tested	CS+	%CS+	# tested	CS+	% CS	# tested	CS+	%CS+	# tested	CS+	%CS+
Atsinanana	Ambodifaho	134	1	0.7	14	0	0	0	0	0	0	0	0	0	0	0
Atsinanana	Vohitrambato	183	1	0.5	6	0	0	0	0	0	76	0	0	80	1	1.3
Analanjirofo	Vavatenina	73	2	2.7	5	0	0	0	0	0	37	1	2.7	140	0	0
Atsimo Atsinanana	Lopary	104	3	2.9	1	0	0	0	0	0	102	0	0	0	0	0
Atsimo Atsinanana	Manambotra Sud	64	0	0	11	0	0	0	0	0	95	0	0	0	0	0
Vatovavy Fitovinany	Mahatsinjo	73	1	1.4	2	0	0	0	0	0	56	0	0	0	0	0
Vatovavy Fitovinany	Tsaravary	174	0	0	39	0	0	0	0	0	73	0	0	0	0	0
Vatovavy Fitovinany	Ampasimpotsy	271	0	0	17	0	0	0	0	0	112	0	0	50	0	0
Vatovavy Fitovinany	Lanivo	65	0	0	1	0	0	0	0	0	48	1	2.1	0	0	0
Atsimo andrefana	Tsaragiso	0	0	0	78	0	0	0	0	0	0	0	0	0	0	0
Atsimo andrefana	Betaindambo	29	0	0	17	0	0	12	0	0	0	0	0	0	0	0
Total		**1170**	**8**	**0.7%**	**191**	**0**	**0%**	**12**	**0**	**0%**	**599**	**2**	**0.3%**	**270**	**1**	**0.4%**

3.5.4. Entomological Inoculation Rate (EIR)

The average *An. gambiae* Human Biting Rate (HBR) was respectively 2.9, 0.5, 1.2, 1.1 and 0.7 bites per person per night in Ambodifaho, Vohitrambato, Vavatenina, Lopary, and Mahatsinjo. The EIR representing the product of the HBR x (sporozoite rate) was equal 2.03 infectious bites/person/night in Ambodifaho, 0.25 infectious bites /person/night in Vohitrambato, Vavatenina and Lopary respectively while Mahatsinjo recorded 0.99 infectious bites /person/night.

For *Anopheles funestus*, the EIR was 2.97 and 5.04 infectious bites per person per night in Vavatenina and Lopary respectively.

For *Anopheles mascarensis*, in Vohitrambato, the only site recording a positive *Plasmodium falciparum* mosquito, the EIR is 0.78 infectious bites per person per night.

Table 7: EIR of malaria vectors collected using HLC

		An. gambiae s.l.			*An. funestus*			*An. mascarensis*		
Region	**Locality**	**HBR**	**SR**	**EIR**	**HBR**	**SR**	**EIR**	**HBR**	**SR**	**EIR**
Atsinanana	Ambodifaho	2.9	0.7	**2.03**	0.1	0	**0**	0.02	0	**0**
Atsinanana	Vohitrambato	0.5	0.5	**0.25**	0.8	0	**0**	0.6	1.3	**0.78**
Analanjirofo	Vavatenina	1.2	2.7	**0.25**	1.1	2.7	**2.97**	1.1	0	**0**
Atsimo Atsinanana	Lopary	1.1	2.9	**0.25**	0.9	0	**0**	0.9	0	**0**
Atsimo Atsinanana	Manambotra Sud	1.08	0	**0**	2.0	0	**0**	2	0	**0**
Vatovavy Fitovinany	Mahatsinjo	0.7	1.4	**0.99**	1.5	0	**0**	1.5	0	**0**
Vatovavy Fitovinany	Tsaravary	1.3	0	**0**	1.9	0	**0**	1.9	0	**0**
Vatovavy Fitovinany	Ampasimpotsy	3.7	0	**0**	10.9	0	**0**	10.9	0	**0**
Vatovavy Fitovinany	Lanivo	1.03	0	**0**	2.4	2.1	**5.04**	2.4	0	**0**
Atsimo andrefana	Tsaragiso	0.9	0	**0**	1.4	0	**0**	0.1	0	**0**
Atsimo andrefana	Betaindambo	0.6	0	**0**	0.8	0	**0**	0.1	0	**0**

3.5.5. Molecular Markers of Resistance

All 425 *An. gambiae* s.l, analyzed for *kdr*-west were homozygous susceptible (SS) in all localities showing that the mutation does not exist in the area. In contrast, the *kdr*-east mutation characterization showed only heterozygotes (RS) alleles in the different localities. The allelic frequency of the *kdr*-east mutation varied from 2 percent in Vavatenina, Lanivo and Betaindambo to 5 percent in Mahambo, for *Anopheles gambiae*. No *kdr*-east resistance allele was observed in the localities of Vohitrambato, Vavatenina and Betaindambo. Only two *An. arabiensis* tested in Ambodifaho and Mahambo were *kdr*-east heterozygous giving respectively 0.13 and 0.25 allele frequency. The *Ace*-1R mutation was absent in all localities. The distribution of the species of *An. gambiae* and *An. arabiensis*, as well as the allelic frequencies of the *kdr*-west, *kdr*-east and *Ace*-1 mutations by locality are presented in Table 8.

Table 8: Frequency of *Kdr* (West and East) and *Ace-1*

Regions	Districts	Localities	Species	# tested	*Kdr-West*				*Kdr-East*				*Ace-1*			
					RR	RS	SS	F (*Kdr-w*)	RR	RS	SS	F (*Kdr-E*)	RR	RS	SS	F (Ace-1)
Atsinanana	Brickaville	Ambodifaho	*An. gambiae*	71	0	0	71	0.00	0	6	65	0.04	0	0	36	0.00
			An. arabiensis	4	0	0	4	0.00	0	1	3	0.13	0	0	3	0.00
Analanjirofo	Fenerive East	Mahambo	*An. gambiae*	48	0	0	48	0.00	0	5	43	0.05	0	0	23	0.00
			An. arabiensis	2	0	0	2	0.00	0	1	1	0.25	0	0	1	0.00
Atsinanana	Toamasina II	Vohitrambato	*An. gambiae*	99	0	0	99	0.00	0	6	93	0.03	0	0	51	0.00
			An. arabiensis	1	0	0	1	0.00	0	0	1	0.00				
Analanjirofo	Vavatenina	Vavatenina	*An. gambiae*	122	0	0	122	0.00	0	4	118	0.02	0	0	46	0.00
			An. arabiensis	3	0	0	3	0.00	0	0	3	0.00	0	0	2	0.00
Fitovinany	Vohipeno	Lanivo	*An. gambiae*	25	0	0	25	0.00	0	1	24	0.02	0	0	14	0.00
Atsimo Andrefana	Tulear I	Betaindambo	*An. gambiae*	49	0	0	49	0.00	0	2	47	0.02	0	0	23	0.00
			An. arabiensis	1	0	0	1	0.00	0	0	1	0.00	0	0	1	0.00
Total				425	0	0	425	0.00	0	26	399	0.03	0	0	200	0.00

See the monthly EIR breakdown EIR in Annex Table 15.

4. CONCLUSIONS

The data collected indicate that the known malaria vectors *An. gambiae* s.l., *An. funestus* group, and *An. mascarensis*, and potential vector, *An. coustani*, were present in several localities of Madagascar and at different proportions according to each site. *Anopheles gambiae* s.l. is the most common mosquito collected in all the areas: East, South East and South West. The sub-species of the *An. gambiae* s.l. complex of Madagascar were composed of *An. gambiae* and *An. arabiensis* that were found in all the localities surveyed. A single species of *An. funestus* was recorded in the sites were *An. funestus* was collected.

The average human biting rate (HBR) of all the vectors was very low for all vectors in all the localities surveyed. The HBR of *An. gambiae* s.l. ranged from 0.5 bites/person/night at Vohitrambato to the maximum of 2.9 bites/person/night at Ambodifaho. However, the EIR yielded after the few sporozoites detected was very high with about two infectious bites/person/night at Ambrodifaho. *Anopheles funestus* recorded the highest EIR (three and five infectious bites/person/night) within both sites where infected mosquitoes were observed. This represents a concern and implies closer monitoring to understand the trend of the malaria transmission in the country. In addition, the multiplicity of vector recorded across the country call for concrete vector surveillance throughout the year.

Anopheles gambiae s.l. was susceptible to pirimiphos-methyl and clothianidin in all sprayed areas and susceptibility to chlorfenapyr was also observed in all the sites at 100 and 200µg/bottle.

The synergist assay results could have an important implication for ITN decision making in Madagascar, so that ITN incorporating PBO could be introduced in one district.

Anopheles gambiae s.s. and *An. arabiensis* represented the main species collected across the country for susceptibility testing. However, the frequency of the only insecticide resistance mutation (*kdr-East*) observed within the different population is still low and the mosquito populations were heterozygous. Deep investigation and close monitoring will be required to delimitate the extent of the resistance mechanism so that anticipated measures could be planned to manage any impact on the different vector control tools implemented in the country. Investigation on the susceptibility status could also target the additional vectors such as *An. Funestus,* which contributes to the transmission of the disease.

Cone bioassay tests conducted during the first week of the IRS campaign indicated good spray quality in all the IRS sites. One hundred percent mortality (100 percent) was recorded after 24 hour post exposure to all structures tested. The results were confirmed one month after the structures were sprayed with 100 percent mortality recorded for all structures while the airborne effect of the insecticide decreased significantly. The monthly monitoring of the insecticide decay rate for Actellic® 300 CS used in the East, South West and both districts of the South East, and Sumishield sprayed in two districts of the South East showed that both insecticides remained effective for seven months in the East and South west and six months in the South East.

Per the results of the entomological surveys, it could be suggested that a rotation of Actellic CS and SumiShield WG for IRS campaigns in the sentinels sites of Madagascar to enable insecticide resistance management. It was shown that both insecticides lasted long enough to cover the peak transmission season. Also, Fludora Fusion, which is a mixture of clothianidin and deltamethrin insecticides, could be an additional option of choice.

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.

5. Annex

Table 9: Number of mosquitoes collected at each sentinel site between June 2018 and April 2019 in the South East and between August 2018 and May 2019 in the East and South West

	Ambodifaho	Vohitrambato	Vavatenina (Control - East)	Manambotra Sud	Lanivo	Lopary (Control - South East)	Betaindambo (Control South West)	Tsaragiso	Mahatsinjo	Tsaravary (Control - South East Vatovavy F)	Ampasipotsy	Total	%
An. gambiae s.l.	424	521	148	121	88	200	61	135	185	329	662	2,874	13.2%
Anopheles funestus	16	224	60	106	54	197	0	24	72	104	315	1,172	5.4%
An. mascarensis	4	75	522	3	0	0	0	0	11	10	56	681	3.1%
An. coustani	3	1,073	589	240	31	298	4	41	61	53	648	3041	14.0%
Other *Anopheles* sp.	5	932	774	26	22	18	18	66	30	48	326	2,265	10.4%
Other *Genus*	426	801	877	745	530	856	2,430	1,623	818	1,431	1,155	11,692	53.8%
Total	878	3,626	2,970	1,241	725	1,569	2,513	1,889	1,177	1,975	3,162	21,725	100.0%

Table 10: Number of mosquitoes collected by HLC and average Human Biting Rates (bites/person/night = b/p/n) between June 2018 and April 2019 in the South East and between August 2018 and May 2019 in the East Coast and the South West

Sites	Months	*Anopheles gambiae s.l.*				*Anopheles funestus*				*Anopheles mascarensis*				*Anopheles coustani*				Other *Anopheles*			
		Total Indoor	Average Indoor b/p/n	Total Outdoor	Average Outdoor b/p/n	Total Indoor	Average Indoor b/p/n	Total Outdoor	Average Outdoor b/p/n	Total Indoor	Average Indoor b/p/n	Total Outdoor	Average Outdoor b/p/n	Total Indoor	Average Indoor b/p/n	Total Outdoor	Average Outdoor b/p/n	Total Indoor	Average Indoor b/p/n	Total Outdoor	Average Outdoor b/p/n
Ambodifaho, Brickaville	Aug, 2018*	19	3.2	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
	Sept 18-May 19	127	2.4	217	4	4	0.1	11	0.2	2	0.04	2	0.04	0	0.0	3	0.1	2	0.04	3	0.1
Vohitrambato, Toamasina II	Aug, 2018*	0	0.0	2	0.3	1	0.2	3	0.5	4	0.7	7	1.2	2	0.3	15	2.5	0	0.0	9	1.5
	Sept 18-May 19	98	0.6	394	1.1	46	1.0	155	1.5	11	0.1	43	0.5	36	0.6	1008	1.8	86	0.2	835	0.8
Betaindambo, Toliara I (control site for south west)	Aug, 2018*	1	0.2	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Sept 18-May 19	21	0.7	20	1.3	0	1.3	0	1.8	0	0.1	0	0.4	2	0.7	2	2.0	4.0	0.2	7.0	0.9
Tsaragiso, Toliara II	Aug, 2018*	2	0.3	8	1.3	4	0.7	9	1.5	0	0.0	0	0.0	3	0.5	6	1.0	2	0.3	11	1.8
	Sept 18-May 19	20	0.7	77	1.2	3	1.4	5	2.0	0	0.1	0	0.4	6	0.9	26	2.0	7	0.2	35	0.6
Vavatenina (control site for East)	Aug, 2018*	57	0.7	69	1.3	20	1.3	30	1.9	164	0.1	235	0.4	197	0.8	293	1.9	356	0.1	379	0.6
	Sept 18-May 19	9	1.5	8	1.3	3	0.5	3	0.5	5	0.8	16	2.7	8	1.3	18	3.0	1	0.2	7	1.2
Manambotra Sud, Farafangana	June, 2018*	0	0.0	7	1.2	1	0.2	5	0.8	0	0.0	1	0.2	3	0.5	22	3.7	0	0.0	2	0.3
	Jul 18-Apr 19	10	0.7	78	2.4	21	3.0	64	4.1	0.2	0.0	2	0.15	41	0.8	119	2.6	4	0.3	12	1.1
Lanivo/ Anosy Vohipeno	June, 2018*	4	0.7	0	0.0	7	1.2	5	0.8	0	0.0	0	0.0	0	0.0	0	0.0	3	0.5	5	0.8
	Jul 18-Apr 19	9	0.7	59	2.7	11	3.2	26	4.4	0.3	0.004	9.0	0.2	5	0.9	18	2.9	2	0.25	12	1.1
Lopary, Vangaindrano,	June, 2018*	7	1.2	3	0.5	2	0.3	3	0.5	0	0.0	0	0.0	3	0.5	5	0.8	0	0.0	1	0.2
	Jul 18-Apr 19	77	1.3	76	1.3	92	1.5	78	1.3	0	0.0	0	0.0	159	2.7	114	1.9	7	0.1	10	0.2
Mahatsinjo, Mananjary	June, 2018*	0	0	3	0.5	9	1.5	24	4	0	0	0	0	4	0.7	8	1.3	0	0	0	0
	Jul 18-Apr 19	22	0.4	123	2.05	3	0.05	27	0.45	1	0.02	10	0.2	1	0.02	27	0.5	0	0	19	0.3
Tsaravary, Mananjary	June, 2018*	2	0.3	3	0.5	8	1.3	32	5.3	0	0	0	0	0	0	0	0				
	Jul 18-Apr 19	54	0.9	199	3.3	10	0.2	41	0.7	1	0.02	9	0.2	1	0.02	25	0.4	0	0	1	0.03
Ampasipotsy, Manakara	June, 2018*	5	0.8	25	4.2	121	20.2	135	22.5	0	0	0	0	18	3	49	8.2	7	1.2	11	1.8
	Jul 18-Apr 19	27	0.5	500	9.3	9	0.2	27	0.5	0	0	53	1.0	12	0.2	544	10.1	24	0.4	284	5.3

Table 11: Total number of mosquitoes collected by Prokopack aspirator and Indoor Resting Density between June 2018 and April 2019 in the South East and between August 2018 and May 2019 in the East and South West

Species	Month	Ambodifaho		Vohitrambato		Vavatenina		Manambotra Sud		Lopary		Lanivo		Mahatsinjo		Betaindambo		Tsaragiso		Tsaravary		Ampasipotsy	
		#	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.	June 2018*							1	0.1	1	0.1	0	0	0	0					1	0.1	0	0
	July 18-April 19							1	0.01	19	0.2	2	0.02	4	0.04					7	0.4	7	0.4
	August 2018*	3	0.3	0	0	0	0									1	0.1	5	0.5				
	September 18-May 19	14	0.2	0	0.0	1	0.01									10	0.1	2	0.0				
An. funestus	June 2018*							2	0.2	0	0	3	0.3	6	0.6					7	0.7	7	0.7
	July 18-April 19							6	0.03	28	0.14	0	0	0	0					1	0.01	0	0
	August 2018*	0	0	0	0	0	0									0	0	3	0.3				
	September 18-May 19	1	0.1	5	0.1	3	0.03									0	0	0	0				

*Baseline data

Table 12: Total number of mosquitoes collected by outdoor collection with aspirator (ODC) method, between June 2018 and April 2019 in the South East and between August 2018 and May 2019 in the East and South West

Species	East			South East						South West			
	Ambodifaho	Vohitrambato	Vavatenina	Manambotra Sud	Lopary	Lanivo	Mahatsinjo	Ampasipotsy	Tsaravary	Betaindambo	Tsaragiso	Total	%
An. gambiae s.l.	30	27	17	24	17	14	33	100	56	7	21	346	25.2%
An. funestus	1	14	7	10	8	2	3	16	5	0	0	66	4.8%
An. mascarensis	0	9	34	0	0	0	0	3	0	0	0	46	3.4%
An. coustani	0	12	12	55	11	8	21	24	27	0	0	170	12.4%
Other *Anopheles* sp.	0	2	2	8	0	0	11	0	26	7	11	67	4.9%
Other *Genus*	0	9	13	118	28	83	93	70	112	55	97	678	49.4%
Total	31	73	85	215	64	107	161	213	226	69	129	1373	100.0%

Table 13: Results of *An. gambiae* s.l. susceptibility tests

	Ambodifaho (Brickaville			Vohitrambato (Toamasina II)			Mahambo			Vavatenina			Manambotra Sud			Lopary (Vangaindrano)		
	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	99	S	100	100	S	100	100	S	100	100	S	100	100	S
DDT	100	99	S	100	99	S	100	100	S	100	93	S	100	100	S	100	100	S
Deltamethrin	100	93	P	100	79	R	100	69	R	100	54	R	100	100	S	100	100	S
Lambda-Cyhalothrin	100	100	S	100	64	R	100	99	S	100	100	S	100	100	S	100	100	S
Permethrin	100	88	R	100	83	R	100	71	R	100	63	R	100	96	P	100	100	S
Alphacypermethrin	100	75	R	100	52	R	100	100	S	100	65	R	100	100	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	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S

	Mahatsinjo			Tsaravary (Manajanry)			Ampasimpotsy			Miary Lamatihy			Tsaragiso			Bataindambo		
	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	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Deltamethrin	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	62	R
Lambda-Cyhalothrin	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S
Permethrin	100	100	S	100	99	S	100	100	S	100	100	S	100	100	S	100	8	R
Alphacypermethrin	100	100	S	100	100	S	100	100	s	100	100	S	100	100	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	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S	100	100	S

Table 14: Mortality observed for *An. gambiae* s.l. exposed to various concentrations of chlorfenapyr after 60-minute exposure

	Time	**KD / mortality in Bottle1 (12,5 µg/ml)**	**KD / mortality in Bottle2 (25µg/ml)**	**KD / mortality in Bottle3 (50µg/ml)**	**KD / mortality in Bottle4 (100µg/ml)**	**KD / mortality in Bottle5 (200µg/ml)**	**KD / mortality in Control**
Ambodifaho	Nb tested	25	25	25	25	25	25
	60 mn	5	14	17	25	18	0
	1 day	5	23	25	25	21	0
	2 days	23	23	25	25	22	1
	3 days	23	24	25	25	25	1
Mahambo/ Antsikafoka	Nb tested	25	25	25	25	25	25
	60 mn	4	11	15	17	11	0
	1 day	5	12	18	25	23	0
	2 days	5	12	20	25	25	0
	3 days	5	13	23	25	25	1
Vohitrambato	Nb tested	20	20	20	20	20	20
	60 mn	1	0	1	1	0	0
	1 day	2	3	7	20	20	0
	2 days	9	14	11	20	20	0
	3 days	14	17	13	20	20	0
Vavatenina	Nb tested	20	20	20	20	20	20
	60 mn	1	1	1	2	2	0
	1 day	2	5	9	20	20	0
	2 days	10	8	12	20	20	0
	3 days	14	13	18	20	20	0
Manambotra Sud	Nb tested	20	20	20	20	20	20
	60 mn	2	6	10	20	20	0
	1 day	3	2	13	20	20	0

	Time	KD / mortality in Bottle1 (12,5 µg/ml)	KD / mortality in Bottle2 (25µg/ml)	KD / mortality in Bottle3 (50µg/ml)	KD / mortality in Bottle4 (100µg/ml)	KD / mortality in Bottle5 (200µg/ml)	KD / mortality in Control
	2 days	5	7	20	20	20	0
	3 days	7	8	20	20	20	0
Betaindambo	Nb tested	20	20	20	20	20	20
	60 mn	16	8	2	3	4	0
	1 day	10	6	17	20	20	0
	2 days	10	16	19	20	20	0
	3 days	10	17	20	20	20	1
Lanivo/Anosy	Nb tested	20	20	20	20	20	20
	60 mn	0	0	0	0	0	0
	1 day	14	14	18	20	20	0
	2 days	14	18	20	20	20	1
	3 days	16	19	20	20	20	1
Mahatsinjo	Nb Tested	20	20	20	20	20	20
	60 mn	5	7	10	20	20	0
	1 day	8	15	16	20	20	0
	2 days	10	17	18	20	20	0
	3 days	16	18	20	20	20	0
Ampasimpotsy	Nb tested	20	20	20	20	20	20
	60 mn	6	8	9	20	20	0
	1 day	8	10	11	20	20	0
	2 days	11	14	17	20	20	0
	3 days	17	19	20	20	20	0

Table 15: Monthly EIR break down

An.gambiae	Month	Lopary			Mahatsinjo			Ambodifaho			Vavatenina			Vohitrambato		
		Sporozoite index	HBR	EIR	Sporozoite index	HBR	EIR	Sporozoite index	HBR	EIR	Sporozoite index	HBR	EIR	Sporozoite index	HBR	EIR
	Jun-18	0	0.8	0	0	0.3	0									
	Jul-18	0	2.1	0	0	0.6	0									
	Aug-18	0	1.1	0	0	0.5	0	0	2.8	0	0	0.3	0	0	0.2	0

	Sep-18	0.05	0.9	0.05	0	0.6	0	0	0.6	0	0	1.4	0	0	0.5	0
	Oct-18	0	0.8	0	0	1.0	0	0.03	4.6	0.14	0	2.6	0	0.04	1.7	0.07
	Nov-18	0	1.0	0	0.09	1.3	0.03	0	0.8	0	0	1.0	0	0	11.9	0
	Dec-18	0.2	1.7	0.33	0	0.0	0	0	9.0	0	0	0.4	0	0	2.8	0
	Jan-19	0	2.5	0	0	1.7	0	0	2.2	0	0	2.1	0	0	6.8	0
	Feb-19	0	1.8	0	0	2.5	0	0	0.4	0	0.06	1.7	0.10	0	2.8	0
	Mar-19	0	0.5	0	0	2.7	0	0	5.8	0	0.11	0.8	0.08	0	11.4	0
	Apr-19	0	0.5	0	0	1.3	0	0	3.6	0	0	0.4	0	0	1.8	0
	May-19							0	1.8	0	0	0.2	0	0	1.5	0

An funestus		**Lanivo**			**Vavatenina**		
	Month	Sporozoite index	HBR	EIR	Sporozoite index	HBR	EIR
	Jun-18	0	1.0	0			
	Jul-18	0.13	0.3	0.04			
	Aug-18	0	0	0	0	0.3	0
	Sep-18	0	0	0	0	1.4	0
	Oct-18	0	0	0	0.06	2.6	0.16
	Nov-18	0	0	0	0	1.0	0
	Dec-18	0	0	0	0	0.4	0
	Jan-19	0	0	0	0	2.1	0
	Feb-19	0	0	0	0	1.7	0

	Mar-19	0	0	0	0	0.8	0
	Apr-19				0	0.4	0
	May-19				0.6	0.2	0

An. mascarensis		Vohitrambato		
	Month	Sporozoite index	HBR	EIR
	Aug-18	0	0.9	0
	Sep-18	0	0.4	0
	Oct-18	0	0.4	0
	Nov-18	0	0.2	0
	Dec-18	0	0.0	0
	Jan-19	0	0.1	0
	Feb-19	0	0.1	0
	Mar-19	0	1.2	0
	Apr-19	0	0.8	0
	May-19	0.06	1.4	0.09