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THE PMI VECTORLINK PROJECT

ZIMBABWE ANNUAL ENTOMOLOGICAL REPORT

MARCH 2020 – FEBRUARY 2021

Recommended Citation: The PMI VectorLink Project. May 2021. The PMI VectorLink Project *Zimbabwe Annual Entomological Monitoring Report: March 1, 2020 through February 28, 2021*. Rockville, MD. Abt Associates.

Contract: AID-OAA-I-17-00008

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

Submitted to: United States Agency for International Development/The U.S. President's Malaria Initiative (PMI)

Submitted: May 28, 2021

Approved: September 14, 2021

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CONTENTS

List of Tables:

List of Figures:

ACRONYMS

ACE	Acetylcholinesterase
AChE	Insensitive Acetylcholinesterase
AU	Africa University
CDC	U.S. Centers for Disease Control and Prevention
CSP	Circumsporozite protein
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FF	Fludora Fusion
HBI	Human Blood Index
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
ITN	Insecticide-treated Nets
kdr	knock-down rate
NIHR	National Institute of Health Research
NMCP	National Malaria Control Program
PCR	Polymerase Chain Reaction
PM	Pirimiphos-methyl
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Collection
s.l.	*Sensu lato*
s.s.	*Sensu stricto*
SOP	Standard Operating Procedure
USAID	United States Agency for International Development
VGSC	Voltage-Gated Sodium Channel
WHO	World Health Organization

EXECUTIVE SUMMARY

Through support from the U.S. President's Malaria Initiative (PMI), the PMI VectorLink Project implemented indoor residual spraying (IRS) with pirimiphos-methyl in Mudzi District and with Fludora Fusion in Mutoko District in Mashonaland East Province in 2019. During the 2020 IRS campaign, the project used Fludora Fusion in both districts.

In addition, PMI VectorLink is implementing entomological monitoring for malaria vector control in Zimbabwe, in partnership with the National Institute of Health Research, National Malaria Control Program (NMCP), and Provincial Medical Directorates. Monthly longitudinal vector surveillance was conducted at six sites: four sites (Arcturus, Dendera, Kawere, and Makarara) in Mashonaland East Province and two (Burma Valley and Vumba) in Manicaland Province. The residual efficacy of pirimiphos-methyl and Fludora Fusion was monitored at the PMI-supported districts in Mashonaland East and of dichlorodiphenyltrichloroethane (DDT) at NMCP/Global Fund-supported districts in Manicaland. In addition, insecticide resistance tests were conducted in both Mashonaland East (deltamethrin, clothianidin, alpha-cypermethrin, and DDT) and Manicaland (DDT) sites.

Anopheles funestus s.l. and *An. gambiae* s.l. are the main malaria vectors in Zimbabwe. *An. funestus* s.l. was the predominant species at two of the six longitudinal monitoring sites (Burma Valley and Vumba), whereas *An. gambiae* s.l. was predominant at Kawere and Makarara. *An. coustani* was the predominant species at Arcturus while *An. rufipes*, *An. pretoriensis* and *An. funestus* s.l. were the most abundant at Dendera. All species were found in low densities due to the drought in 2020. Additionally, densities were underestimated because of the six-month disruption of monitoring due to COVID-19 in 2020 and 2021.

Five sibling species of the *An. funestus* s.l. were identified at Burma Valley, namely, *An. funestus* s.s., *An. leesoni*, *An. parensis*, *An. rivulorum,* and *An. rivulorum*-like. The presence of *An. funestus* s.l. sibling species varied at the other five sites as follows: *An. funestus* s.s. and *An. rivulorum* at Dendera, *An. funestus* s.s., *An. parensis* and *An. rivulorum*-like at Makarara, *An. parensis* and *An. rivulorum* at Arcturus, *An. leesoni* and *An. parensis* at Kawere and *An. funestus* s.s. and *An. leesoni* at Vumba. *An. gambiae* s.l. sibling species were recorded at five of the longitudinal monitoring sites: *An. arabiensis* and *An. gambiae* s.s. at Burma Valley, *An. arabiensis, An. gambiae* s.s. and *An. quadriannulatus* at Makarara, *An. arabiensis* at Dendera, and *An. quadriannulatus* only at Arcturus and Kawere. *An. gambiae* s.l. was absent at Vumba. Other potential secondary vectors found included *An. coustani, An. squamosus, An. maculipalpis,* and *An. rufipes;* however, their propensity to feed on humans was low, and the ubiquitous *An. pretoriensis,* which is considered a non-vector, showed no tendency to feed on humans.

Mosquito densities at all sentinel sites in Mashonaland East and Manicaland using all collection methods were generally low, which did not allow for definitive conclusions to be made about vector behavior. However, U.S. Centers for Disease Control and Prevention (CDC) light trap collections and pit shelters indicated higher numbers of malaria vectors (both *An. funestus* s.l. and *An. gambiae* s.l.) outdoors versus indoors at all sites and might indicate a preference to feed and rest outdoors. The low human blood index in both the two main vectors and other species suggests an opportunistic feeding behavior.

Wall bioassays were conducted monthly following the 2019 IRS campaign at 2 sites in Mashonaland East showed a residual efficacy of Actellic 300CS and Fludora Fusion of at least four months; however, monitoring was disrupted from T5 to T8 at Dendera and not measured after T4 for Fludora Fusion at Kawere where bioassay rooms were resprayed in May. DDT (sprayed by the NMCP) was monitored at Burma Valley in Manicaland and showed residual efficacy of up to nine months (monitoring disrupted from T5 to T8 but resumed thereafter). Residual efficacy varied for DDT by wall surface type, with greater residual efficacy on mud and brick walls than on cement and painted walls. Residual efficacy of Fludora Fusion was less varied in the observations completed.

The results from the 2020 spray campaign showed that the residual efficacy of Fludora Fusion at Dendera and Kawere was still above the 80% cut-off point five months after spraying despite the interruption of cone bioassay due to COVID-19. Residual efficacy of DDT in Burma Valley also remained above the 80% cut-off point five months after spraying.

The primary vectors *An. gambiae* s.l. and *An. funestus* s.l. remain susceptible to clothianidin and DDT at most sites but possible resistance to deltamethrin for *An. gambiae* s.l. at Dendera (February and March 2020) and resistance to alpha-cypermethrin for *An. gambiae* s.l. in Hwedza was found. Resistance tests done later indicate *An. gambiae* s.l. from Dendera (March and April 2021) is susceptible to deltamethrin, and from Hwedza (April 2021) is susceptible to alpha-cypermethrin. In Mashonaland East, *An. gambiae* s.l. was susceptible to clothianidin, DDT, and deltamethrin in Mudzi and Mutoko districts. Laboratory tests indicated absence of knockdown resistance and insensitive acetylcholinesterase (Ace-1) genes among the *An. gambiae* s.l. tested.

I. INTRODUCTION

Malaria is heterogeneously distributed in Zimbabwe, with the vast majority of cases reported from three of the eight rural provinces: Manicaland, Mashonaland Central, and Mashonaland East. In Zimbabwe, malaria is transmitted by *Anopheles arabiensis*, *An. gambiae* s.s., and *An. funestus* s.s. More than 98% of cases are caused by *Plasmodium falciparum*, while *Plasmodium malariae* and *Plasmodium ovale* are responsible for the remainder. Despite concerted efforts by stakeholders to prevent and control malaria transmission, and relatively high rainfall, the disease burden increased from 29/1000 population in 2015 to 32/1000 in 2020 (NMCP 2020). Malaria remains one of the most important public health challenges in some parts of the country. To control malaria, Zimbabwe's National Malaria Control Program (NMCP) relies on two core vector control strategies: deployment of indoor residual house-spraying (IRS) and distribution of insecticide-treated nets (ITNs). Though these two strategies are known to contribute immensely to the prevention, control, and elimination of malaria in most settings, their effectiveness depends greatly on the behavior and resistance status of local primary vectors to insecticides as well as on human behavior. The country rotates insecticides used for IRS on a two-year basis as guided by the country's Insecticide Resistance Management Plan.

Regular entomological monitoring is important for ensuring the evidenced-based selection and deployment of insecticides for IRS and ITNs because monitoring provides timely key information on vector species composition and their distribution, resting and feeding behavior, and susceptibility to insecticides. Additionally, these investigations are helpful for monitoring emerging and re-emerging primary and secondary malaria vectors and assessing the role they play in disease transmission. Information collected through entomological monitoring helps program managers and implementers understand the spatial and temporal changes in vector species, quality of IRS application, residual efficacy of insecticides on sprayed surfaces, and effectiveness of vector control interventions deployed to interrupt malaria transmission.

The President's Malaria Initiative (PMI) supported IRS and entomological surveillance under the Africa Indoor Residual Spraying Project from 2013 to February 2018. This support continues the PMI VectorLink project, which started in March 2018. Prior to 2018, PMI supported IRS in four districts in Manicaland Province (Chimanimani, Mutare, Mutasa, and Nyanga), but in 2018 transitioned support to two districts in Mashonaland East Province (Mutoko and Mudzi). This report focuses on activities completed from March 2020 to February 2021 under the PMI VectorLink Project. The objectives include the following:

1. Monitor spray quality and residual efficacy of pirimiphos-methyl (Actellic 300CS) used in Mashonaland East in the 2019 IRS campaign and of clothianidin-deltamethrin (Fludora Fusion) combination IRS used there in the 2019 and 2020 campaigns.
2. Monitor spray quality and residual efficacy of dichlorodiphenyltrichloroethane (DDT) in Manicaland in the two spray campaigns implemented by the NMCP in 2019 and 2020.
3. Perform annual insecticide susceptibility testing at six sites in Mashonaland East (four sites) and in Manicaland (two sites) to inform vector control decision making.
4. Continue monthly vector bionomics monitoring at six sites in Mashonaland East (four sites) and in Manicaland (two sites) to monitor the impact of IRS.

The project continued the collaboration with Africa University (AU), which provided support in analyzing mosquitoes to determine species identification, parasite infection (sporozoite rate), host choice, and target site resistance mechanisms. AU also provided susceptible colony of *Anopheles arabiensis* KGB strain that are required for bioassay tests to monitor the residual efficacy of insecticides. The National Institute of Health Research (NIHR) also provided the same strain of colony mosquitoes for the bioassay tests.

2. MATERIALS AND METHODS

2.1 SITES

Entomological surveillance was conducted initially at four sites in March 2020 in Mashonaland East Province (IRS sites of Arcturus, Dendera, and Kawere, and control site of Makarara) and two sites in Manicaland Province (IRS site of Burma Valley and control site of Vumba) in March 2020. The number of sites were reduced to three in Mashonaland East Province (Dendera, Kawere, and Makarara) and one in Manicaland Province (Burma Valley) from April 2020 onwards, following budgetary limitations in 2020. Because of the COVID-19-related national lockdown, no activities were conducted at any site between April and July 2020; in January 2021, activities were again disrupted at Dendera and Kawere sites and in February 2021 activities were not conducted at any of the four sites.

Insecticide susceptibility tests were conducted in both provinces, and wall bioassay tests were conducted to monitor residual efficacy of pirimiphos-methyl and Fludora Fusion in Mashonaland East, sprayed during the 2019 IRS campaign, and efficacy of DDT in Manicaland Province. Separate wall bioassay tests were also conducted to monitor residual efficacy of Fludora Fusion in Mudzi and Mutoko, and DDT in Mutare during the 2020 IRS campaign. Activities accomplished are outlined in Table 1 and Figure 1.

TABLE 1. SENTINEL SITES BY GEOGRAPHIC LOCATIONS AND ACTIVITIES MARCH 2020-APRIL 2021

Province	District	Site	VC intervention	Ento activity	M	A	M	Jn	Ju	A	S	O	N	D	J	F	M	A
Manicaland	Mutare	Burma Valley*	2019 & 2020: IRS with DDT	VB						X	X	X	X	X				
				CB	X					X	X	X	X	X			X	
				IR				X			X							
		Vumba	2019 & 2020: None	VB	X													
				IR				X			X							
Mash East	Goromonzi	Arcturus*	2018 & 2019: IRS - DDT	VB	X													
	Mudzi	Dendera**	2019: IRS- pirimiphos-methyl & 2020: IRS - Fludora Fusion	VB	X					X	X	X	X	X	X			
				CB	X					X			X	X			X	X
				IR	X		X									X		
	Mutoko	Kawere**	2019 & 2020: IRS - Fludora Fusion	VB	X					X	X	X	X	X	X			
				CB	X								X	X	X		X	X
				IR	X		X									X		
	Hwedza	Makarara	2019 & 2020: ITNs	VB	X					X	X	X	X	X				
				IR	X		X	X										

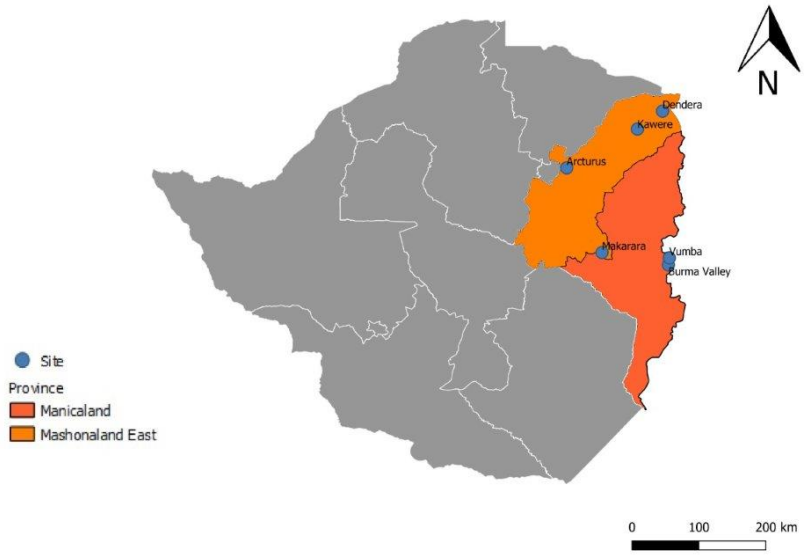
Note: VC=vector control, IR=insecticide resistance testing, VB=vector bionomics, CB=cone bioassays

*Non-PMI supported spray districts

**PMI-supported spray districts in 2019 and 2020

Gray shading	Activity not done because of Covid-19	Orange shading	Activity terminated with new workplan	Vertical grid	Challenge with availability of colony mosquitoes _ Covid-19	X	Activity completed

FIGURE 1. MAP OF ZIMBABWE SENTINEL SITES, MARCH 2020-FEBRUARY 2021



2.2 ROUTINE VECTOR BIONOMICS MONITORING

Mosquito collections were done to monitor vector bionomics at sites in both Mashonaland East (Arcturus, Dendera, Kawere, and Makarara) and Manicaland (Burma Valley and Vumba) provinces. Pyrethrum spray collections (PSCs), pit shelters, and U.S. Centers for Disease Control and Prevention (CDC) light traps as proxies for human landing catches (HLCs) were used to assess the following indicators (Table 2):

1. Vector species composition
2. Indoor and outdoor resting densities
3. Indoor and outdoor human biting rates
4. Sporozoite infection rates

TABLE 2. SUMMARY OF SAMPLING METHODS

Collection method	Collection time	Frequency	Sample
PSC	6:00 am to 9:00 am	Three days per site per month	25 houses per site
CDC light trap as HLC proxy	6:00 pm to 6:00 am, hourly	Two nights per site per month	Two houses per site per night , using four CDC light traps: two indoor, two outdoor
CDC light trap	6:00 pm to 6:00 am collection	Two nights per site per month	Two houses per site (two houses per night), using four CDC light traps: two indoor, two outdoor
Pit shelters	6:00 am to 9:00 am	Three days per site per month	Five pits at each site

All entomological monitoring at each sentinel site was conducted by teams consisting of staff from NIHR, the Provincial Medical Directorates, and PMI/VectorLink.

2.2.1 Estimating Indoor Resting Densities Using PSCs

Indoor resting mosquitoes were sampled from 25 houses (1 sleeping room per house) per month at each of the vector bionomics monitoring collection sites following Standard Operating Procedure (SOP) #3[1]. Mosquitoes collected from the different rooms were transferred to separate petri dishes. Each petri dish was labeled with the following information: location, household name, method of collection, and date. The abdominal stage of all female *Anopheles* was recorded as unfed, blood-fed, half-gravid, or gravid. Data on the number of people who slept in the house the previous night, the type of house and walls, and the number of ITNs present were recorded on appropriate forms. All *Anopheles* mosquitoes collected were identified morphologically and preserved individually in silica gel for laboratory analysis.

2.2.2 Estimating Outdoor Resting Densities Using Pit Shelter Collections

Outdoor resting mosquitoes were sampled at all the six sites using five pit shelters per site. The pit shelters were dug at least 10m away from nearest household and fenced for human and animal safety. Each pit shelter had a depth of 2m, a 1.5m x 1.5m opening, and at least eight holes (2 holes x 4 sides) on its walls for mosquitoes to rest. Mosquito sampling was conducted following SOP #13[1]. The abdominal stage of all female *Anopheles* mosquitoes was recorded. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

2.2.3 Estimating Indoor and Outdoor Densities Using CDC Light Traps

A total of four battery-operated CDC light traps per site per month were used to collect mosquitoes from 6:00 p.m. to 6:00 a.m. for two consecutive nights in each of the six sites following SOP #1[1]. Two sentinel houses were randomly selected, with one trap placed indoors and one trap placed outdoors at each house. Households selected for PSC collections were excluded from the sampling pool. The same houses were used for collections throughout the reporting period. Both indoor and outdoor CDC light traps were placed 1m above a person sleeping under a mosquito net. Outdoor CDC light traps were about 10m away from the house and, when possible, in a shaded area with a person sleeping under an untreated mosquito net. Traps were set at 6:00 p.m. and mosquitoes were collected from each of the traps at around 6:00 a.m. the following morning. The persons did not swap positions, from indoor to outdoor or vice versa, at hourly intervals due to COVID-19 regulations. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

2.2.4 Estimating Biting Behavior Using CDC Light Traps

PMI VectorLink used CDC light traps with human bait as a proxy for HLCs to evaluate human-vector contact including the place, time, and seasonal distribution of the vectors estimated through hourly mosquito collections from CDC light traps set indoor and outdoor alongside a human bait protected by a mosquito net. The procedure is a modification of SOP #1. Houses used for PSCs and CDC light traps for density estimation were excluded from the sample. Collections were done over two consecutive nights at each site. In both indoor and outdoor collections, the light trap was set at the feet of a volunteer sleeping under an untreated mosquito net. For outdoor placement, light traps were set about 10m from the house. The persons did not swap positions, from indoors to outdoors or vice versa, at hourly intervals due to COVID-19 regulations. Mosquitoes were collected from each trap hourly from 6:00 p.m. to 6:00 a.m. Mosquito collections were conducted indoors and outdoors simultaneously, using the same set-up, to compare vectors host-seeking activity inside and outside houses. The teams recorded temperature, relative humidity, wind status, and precipitation hourly during the night. All *Anopheles* mosquitoes collected were identified morphologically and preserved in silica gel for laboratory analysis.

[1] Complete SOPs can be found here: https://pmivectorlink.org/resources/tools-and-innovations/

2.3 Measuring Quality of Spray and Insecticide Decay

The quality of IRS application and insecticide decay rate of DDT at Burma Valley in Mutare District and clothianidin-deltamethrin combination IRS (Fludora Fusion) in Mutoko and Mudzi districts was measured using SOP #9[1] following IRS at the three sites in October and November 2020, respectively. Bioassays were conducted within 24 hours after spraying to assess the spray quality of the IRS operation and then monthly until average mortality rates fell below 80% for two consecutive months to determine residual efficacy. Susceptible *An. arabiensis* (KGB strain), from insectaries at AU in Mutare and NIHR in Harare, were used to conduct the cone bioassays. Ten rooms were tested at each site per month. Additionally, a continuation of monthly bioassays from the 2019 spray campaign was completed in this reporting period (before the 2020 IRS campaign started), and full results are presented in this report. The number of houses from each sentinel site by wall surface types, and insecticide sprayed are summarized in Table 3.

Table 3. Summary of Wall Types Tested with Cone Bioassays, November 2019-April 2021

Province	District	Sentinel site	Year sprayed	Insecticide sprayed	Type of wall	Number of houses
Mashonaland East	Mutoko	Kawere	2019	Fludora Fusion	Mud	2
					Brick	2
					Cement	5
					Painted	1
			2020	Fludora Fusion	Mud	4
					Brick	1
					Cement	4
					Painted	1
	Mudzi	Dendera	2019	Pirimiphos-methyl	Mud	2
					Brick	2
					Cement	4
					Painted	2
			2020	Fludora Fusion	Mud	2
					Brick	2
					Cement	3
					Painted	3
Manicaland	Mutare	Burma Valley	2019	DDT	Mud	2
					Brick	4
					Cement	1
					Painted	3
			2020	DDT	Painted brick	6
					Cement	1
					Painted	3

2.3.1 Wall Cone Bioassay Tests

For the wall cone bioassays, 10 unfed, two- to five-day-old female susceptible *An. arabiensis* (KGB strain) mosquitoes were exposed on the treated walls per cone following SOP #9. Three cones were randomly positioned per room at 0.5, 1.0, and 1.5 meters above the floor. These positions were marked and used in all subsequent tests. Mosquitoes were exposed for 30 minutes, after which they were aspirated to a holding paper cup and provided with 10% sugar solution. Knockdown rates were also recorded at 30 minutes and 60 minutes before mortality was recorded after a 24-hour observation period for DDT and pirimiphos-methyl, whereas a 120-hour observation period was observed for Fludora Fusion. Controls were run concurrently with the wall cone bioassays with mosquitoes exposed to unsprayed surfaces in unsprayed room. Temperature and relative humidity were recorded during the exposure and the subsequent 24-hour holding period for DDT, and up to the 120-hour holding period for Fludora Fusion.

2.3.2 Bioassay Tests to Assess Fumigant Effect of Insecticide

Bioassays to assess the fumigant effect of Fludora Fusion were conducted in each room where wall cone bioassay tests were done. Ten two- to-five-day-old unfed female mosquitoes placed in one paper cup per room were exposed for 30 minutes at the same time as the wall bioassay tests. The paper cup was placed on a wire support, designed so it was 10 centimeters from a sprayed wall and 1m above the floor. Mosquitoes were removed after 30 minutes and knockdowns recorded. They were then transferred to holding paper cups using a clean aspirator and provided with 10% sugar solution during the holding period. Mortality was recorded up to 120 hours. Controls for the bioassays were conducted simultaneously using a similar set-up, but in an unsprayed room.

2.4 Insecticide Resistance Monitoring

Insecticide susceptibility testing was conducted at Dendera, Kawere, Makarara, and Burma Valley from March 2020 to April 2021 (Table 1, above). The insecticides tested were:

1. Deltamethrin (1X)
2. DDT (1X)
3. Clothianidin (2%)
4. Alpha-cypermethrin (1X)

Insecticide susceptibility tests were performed using *An. gambiae* s.l. raised from larvae for all sites except Burma Valley, where adult *An. funestus* s.l. collected from pit shelters and inside rooms were used to raise F1 mosquitoes that were then tested, since it is difficult to get the larval stages of this species (Table 4). The susceptibility of *An. funestus* s.l. to DDT was determined at Burma Valley as this was the insecticide the NMCP sprayed in this district. Alpha-cypermethrin was tested on mosquitoes from Makarara The number of insecticides tested at any given site was determined by the availability of mosquitoes. There is no IRS in Hwedza; LLINs distributed in Hwedza are all treated with alpha-cypermethrin as the major vector control strategy in the district, hence the prioritization of this insecticide in the susceptibility tests. Going forwards, other pyrethroids used in public health will be tested if the availability of local mosquitoes is sufficient to include these extra tests.

Table 4. Summary of Insecticides Tested in Monitoring Sites, February 2020-April 2021

Province	District	Sentinel site	Anopheles species	Deltamethrin	DDT	Clothianidin	Alpha-cypermethrin
Mashonaland East	Mutoko	Kawere	*An. gambiae* complex	X	X	X	
	Mudzi	Dendera		X	X	X	
	Hwedza	Makarara					X
Manicaland	Mutare	Burma Valley	An. funestus s.l.		X		

CDC bottle assays (SOP #4) were used to test all insecticides except clothianidin, for which the World Health Organization (WHO) tube test (SOP #6) method was used. For WHO tests, female adult mosquitoes were exposed for 60 minutes, transferred to holding tubes, and monitored for seven days. Treated clothianidin papers were prepared by the PMI VectorLink entomologist. Exposure tests were accompanied by negative control tests in which mosquitoes were exposed to filter papers impregnated with solvent only. Four replicates of 25

An. gambiae s.l. for most sites were exposed to the interim diagnostic concentration of 2% clothianidin. Knockdown was recorded at the end of the 60-minute exposure period. Clothianidin is a slow-acting insecticide, hence mosquito mortalities were monitored every 24 hours after exposure in holding tubes for a period up to seven days, or until 100% mortality was observed.

For CDC bottle assays, four replicates of 25 female *An. gambiae* s.l. were exposed for 60 minutes to the diagnostic dose of each insecticide. Mortality was recorded at the diagnostic time – 30 minutes for pyrethroids and 45 minutes for DDT.

2.5 LABORATORY ANALYSES

All laboratory analyses of mosquito specimens were conducted following established protocols at the AU molecular laboratory.

2.5.1 MOLECULAR IDENTIFICATION OF *ANOPHELES* SPECIES

Anopheles mosquitoes collected from all six sentinel sites were analyzed for species identification using polymerase chain reaction (PCR) methods performed at the AU laboratory. Briefly, DNA samples were extracted from either single mosquitoes or available parts of mosquitoes using standard extraction protocols and amplified through PCR. Extracted DNA was analyzed based on morphological identification of the mosquito specimen done initially by the PMI VectorLink team and verified by the AU team. The protocol for *An. gambiae* s.l. is described by Wilkins et al. (2006), while the protocol for *An. funestus* s.l. is described by Koekemoer et al. (2002).

2.5.2 IDENTIFICATION OF BLOOD MEALS

Mosquitoes collected and recorded as freshly fed or half-gravid were tested for the blood meal source using PCR (Kent and Norris 2005).

2.5.3 SPOROZOITE RATE

Mosquitoes collected during the reporting period were tested for sporozoite rate using enzyme-linked immunosorbent assay (ELISA). Specimens positive for circumsporozoite protein (CSP) with ELISA were subsequently processed by the boiling method (Durnez et al. 2011) and further analyzed by PCR to confirm *Plasmodium* infection.

2.5.4 KDR ASSAYS

Target-site mutations encoding the voltage-gated sodium channel (VGSC) cause pyrethroid resistance and confer cross-resistance to the organochlorine DDT. The VGSC mutations are referred to as 'knockdown resistance' (kdr). The MR4[2] protocol was used for detecting kdr in *An. gambiae* s.l.

2.5.5 ACE-1 ASSAYS

The presence of insensitive acetylcholinesterase (AChE) was determined in *An. gambiae* s.l. mosquitoes. The analysis detects the G119S mutation in the acetylcholinesterase (Ace-1) gene, a target site mutation that is associated with resistance to carbamates and organophosphates. The MR4 protocol was followed for the Ace-1 analysis.

[2] MR4: The Malaria Research and Reference Reagent Resource Center

3. RESULTS

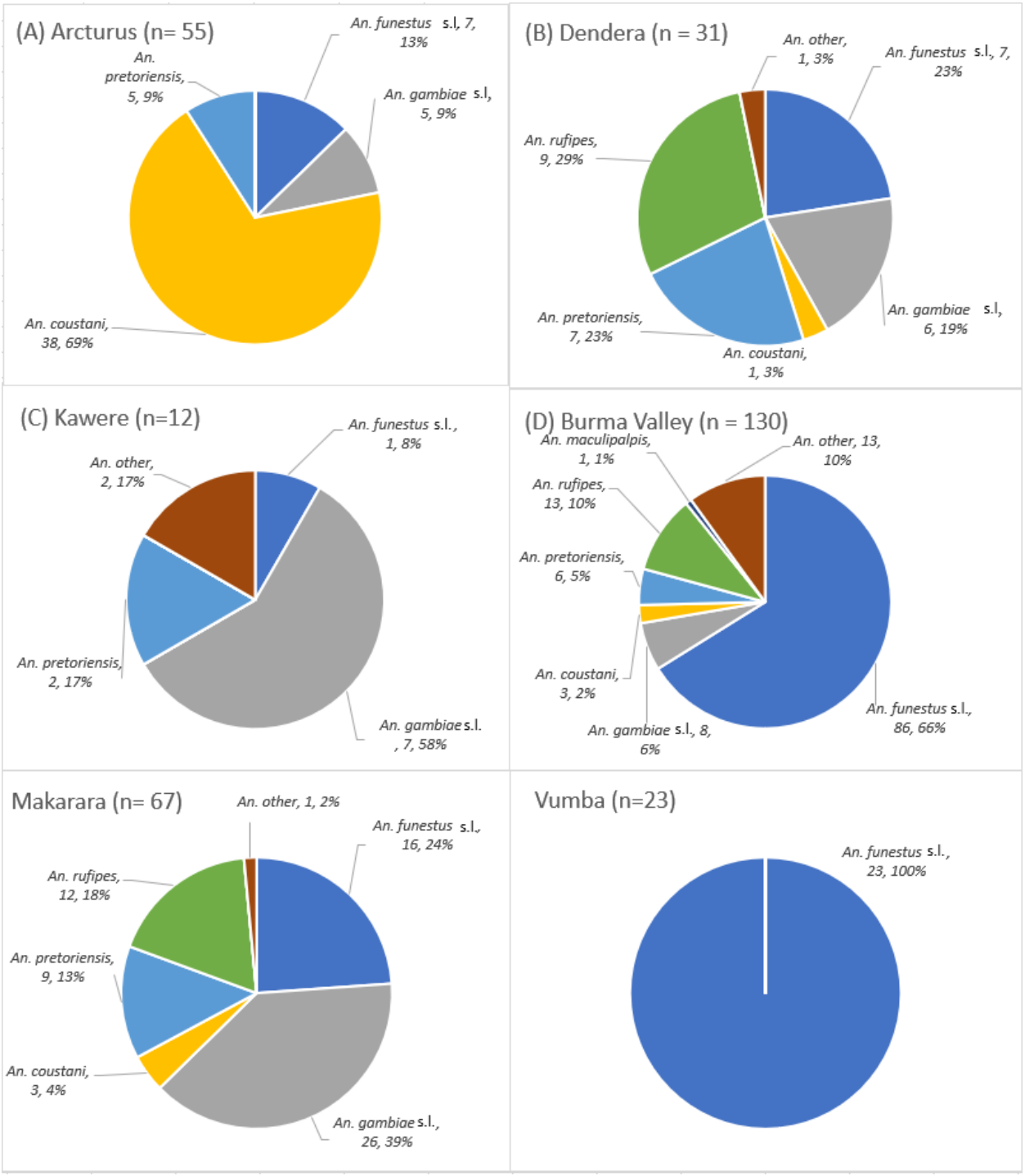
3.1 ROUTINE VECTOR BIONOMICS MONITORING

Dendera was sprayed with pirimiphos-methyl during the 2019 IRS campaign, and with Fludora Fusion during the 2020 IRS campaign. Kawere was sprayed with Fludora Fusion during both the 2019 and 2020 campaigns. Two sites were sprayed with DDT (Arcturus and Burma Valley) during the 2019 and 2020 IRS campaigns, while two sites were unsprayed (Makarara and Vumba). Longitudinal monitoring was suspended for four months in 2020 – April, May, June, and July, and subsequently in January and February 2021 – during the lockdown resulting from the coronavirus pandemic. Thus, the longitudinal data presented are for six months at four sites (Burma Valley, Dendera, Kawere, and Makarara) and one month for two sites (Arcturus and Vumba).

3.1.1 VECTOR COMPOSITION

A total of 318 female *Anopheles* mosquitoes were collected – using PSC, pit shelters, and human-baited CDC light traps as HLC proxy and CDC light traps that were collected once in the morning instead of hourly – between March 2020 and February 2021 at all sites except for Arcturus and Vumba, which were surveyed in March only as these two sites were thereafter excluded from routine surveillance during the reporting period. *An. funestus* s.l. was the predominant species collected by all methods at two of the six sentinel sites: Burma Valley and Vumba (Figures 2D and 2F), while *An. gambiae* s.l. was the most common species at Kawere and Makarara (Figures 2C and 2E). These two species are the major malaria vectors in Zimbabwe. Two other species, *An. coustani* (dominant at Arcturus, 2A) and *An. rufipes* (found at 3 sites) are considered secondary malaria vectors. *An. pretoriensis* was caught at Arcturus, Dendera, Kawere, Burma Valley, and Makarara, but is not considered as a malaria vector. At all sites except for Vumba (100% *An. funestus* s.l.) four or more *Anopheles* species were collected.

FIGURE 2. ANOPHELES SPECIES MORPHOLOGICAL COMPOSITION AT SENTINEL SITES FROM ALL COLLECTION METHODS (ARCTURUS (A), DENDERA (B), KAWERE (C), AND BURMA VALLEY (D)) AND UNSPRAYED (MAKARARA (E) AND VUMBA (F)) SITES IN MASHONALAND EAST AND MANICALAND PROVINCES WHERE N = NUMBER OF ANOPHELES MOSQUITOES PER SITE, MARCH 2020-FEBRUARY 2021.



3.1.2 Indoor Resting Densities

PSC collections at six sites indicated very few mosquitoes rested indoors in both sprayed and unsprayed sites, making it difficult to draw conclusions. An average of 0.8 *An. funestus* s.l. were collected per house per night from Vumba, an unsprayed area, in one month at the start of the reporting period (Table 5). An average 0.08 and 0.01 *An. gambiae* s.l. mosquitoes were collected per house per night from Makarara and Burma Valley, respectively. No other species were collected indoors during the reporting period. Too few mosquitoes were collected to discern a clear seasonal trend, or the impact of the vector control intervention.

Table 5. Indoor Vector Densities (based on PSC) at Sentinel Sites (PM/FF = Dendera, FF = Kawere, DDT = Burma Valley and Arcturus), Unsprayed (ITNs = Makarara, No Intervention = Vumba) Sites in Mashonaland East and Manicaland Provinces, March 2020-February 2021

Site	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	Other species
Arcturus	0	0	0	0	0
Dendera	0	0	0	0	0
Kawere	0	0	0	0	0
Makarara	0.08	0	0	0	0
Burma Valley	0.01	0	0	0	0
Vumba	0	0.8	0	0	0

Note: PM-pirimiphos-methyl, FF=Fludora Fusion

3.1.3 Outdoor Resting Densities

The number of *Anopheles* mosquitoes collected outdoors with the pit shelter collection (Table 6) was more than from indoors with PSC. More *An. funestus* s.l. were collected resting outdoors than indoors at Burma Valley and Makarara with an average 0.5 and 0.1 mosquitoes per trap per day at these sites, respectively. More *An. gambiae* s.l. per trap per day were collected than *An. funestus* s.l. at Dendera (0.07), Kawere (0.07), and Makarara (0.13). *An. gambiae* s.l. was relatively abundant with an average mosquitoes per trap per day of 0.07, 0.07 and 0.13, respectively at Dendera, Kawere, and Makarara but was absent at Burma Valley, where only *An. funestus* s.l. was collected from pits. The higher outdoor resting densities of *An. funestus* s.l. from the pit shelter collections than the indoor resting densities from the PSCs at Makarara and Burma Valley might indicate that the vector tends to rest outdoors. The same was the trend for *An. gambiae* s.l. at Dendera, Kawere, Makarara, and for *An. funestus* s.l. at Burma Valley. Other species collected outdoors from pit shelters included *An. rufipes* (Dendera, Makarara, and Burma Valley) and *An. pretoriensis* at Burma Valley.

Table 6. Outdoor Vector Densities (based on pit shelter collections) In Sprayed (PM/FF = Dendera, FF = Kawere, DDT = Burma Valley) and Unsprayed (ITNs = Makarara, No intervention = Vumba) Sites in Mashonaland East and Manicaland Provinces, March 2020-February 2021

Site	Total number of days sampled per pit	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	*An. maculipalpis*	*An. rufipes*	Other species
Arcturus	3	0	0.1	0	0	0	0	0
Dendera	18	0.07	0	0	0	0	0.1	0

Site	Total number of days sampled per pit	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	*An. maculipalpis*	*An. rufipes*	Other species
Kawere	18	0.07	0	0	0	0	0	0
Makarara	18	0.13	0.1	0	0	0	0.1	0
Burma Valley	18	0	0.5	0.1	0	0	0.2	0.1
Vumba	3	0	0	0	0	0	0	0

Note: PM-pirimiphos-methyl, FF=Fludora Fusion

3.1.4 Indoor and Outdoor Densities from CDC Light Trap Collections

CDC light traps set outdoors collected a higher number of mosquitoes than traps set indoors (Table 7). This was observed at Arcturus where the average number of mosquitoes per trap per night was greater for four species (*An. funestus* s.l., *An. gambiae* s.l., *An. pretoriensis* and *An. coustani*) and Burma Valley for *An. funestus* s.l., *An. gambiae* s.l. and *An. coustani*.. Both *An. gambiae* s.l. and *An. funestus* s.l. were collected at Arcturus and Burma Valley.

Table 7. Indoor and Outdoor Densities of *Anopheles* Mosquito Vectors as Collected by the CDC Light Traps* at Four Sentinel Sites in Mashonaland East and Two Sentinel Sites in Manicaland, March 2020-February 2021

Site	In/Out	No. of months sampled	*An. gambiae* s.l.	*An. funestus* s.l.	*An. pretoriensis*	*An. coustani*	Other species
Arcturus	In	1	0.25	0.3	0.3	0.5	0
	Out	1	0.5	0.8	0.8	7	0
Dendera	In	6	0	0	0	0	0
	Out	6	0	0	0.3	0	0
Kawere	In	6	0	0	0	0	0
	Out	6	0	0	0	0	0
Makarara	In	6	0	0	0	0	0
	Out	6	0	0.1	0	0	0
Burma Valley	In	6	0	0.3	0	0	0
	Out	6	0.15	0.5	0	0.1	0.2
Vumba	In	1	0	1	0	0	0
	Out	1	0	0	0	0	0

* CDC-light traps were set alongside human bait under an untreated mosquito net indoors and outdoors.

3.1.5 Hourly Biting Rates of *An. funestus* s.l. and *An. gambiae* s.l.

Too few mosquitoes were collected during the night at most sites to depict clear biting behavior. At Arcturus, both *An. funestus* s.l. and *An. gambiae* s.l. were collected outdoors, with biting occurring at 1-2 a.m. and 4-5 a.m. for the two vector species, respectively (Figure 3A). At Dendera, *An. funestus* s.l. was collected biting indoors at 3-4 a.m. (Figure 3B). Biting activity was from midnight to 1 a.m. for *An. funestus* s.l., followed by *An. gambiae* s.l. at 3-4 a.m. indoors at Kawere (Figure 3C). At Makarara, both *An. funestus* s.l. and *An. gambiae* s.l. were collected, with the two species collected outdoors at 10-11 p.m.; only *An. funestus* s.l. was collected indoors, from 1 a.m. to 2 a.m. and 3 a.m. to 4 a.m., and only *An. gambiae* s.l. indoors from 3 a.m. to 4 a.m. (Figure 3D). At Burma Valley, *An. funestus* s.l. was collected outdoors at 7-11 p.m., and indoors at 1-2 a.m., and 3-4 a.m., and *An. gambiae* s.l. was collected outdoors at 7-8 p.m. and 9-10 p.m. and indoors at 5-6 a.m. (Figure 3E). At Vumba, only *An.*

funestus s.l. was collected outdoors from 5 a.m. to 6 a.m. (Figure 3F). The summary of peak biting times of the two major malaria vectors are indicated in Table 8.

FIGURE 3. AVERAGE INDOOR AND OUTDOOR HOURLY BITING RATES OF AN. FUNESTUS S.L. AND AN. GAMBIAE S.L. AS DETERMINED BY CDC LIGHT TRAP COLLECTIONS AS PROXY TO HLCS AT ARCTURUS (A), DENDERA (B), KAWERE (C), AND MAKARARA (D) IN MASHONALAND EAST AND BURMA VALLEY (E) AND VUMBA (F) IN MANICALAND, MARCH 2020-FEBRUARY 2021

TABLE 8. PEAK BITING TIMES FOR *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* S.L. ACROSS ALL SITES

Site	*An. gambiae* s.l.		*An. funestus* s.l.	
	Indoor	Outdoor	Indoor	Outdoor
Arcturus		4-5am		1-2am
Burma Valley	5-6am	7-8pm, 9-10pm	10-11pm, 1-2am, 3-4am, 5-6am	7-11pm, 1-5am,
Dendera			3-4am	
Kawere	3-4am			12-1am
Makarara	3-4am	10-11pm	1-2am, 3-4am	10-11pm, 12-1am
Vumba				5-6am

3.2. IRS SPRAY QUALITY AND RESIDUAL EFFICACY

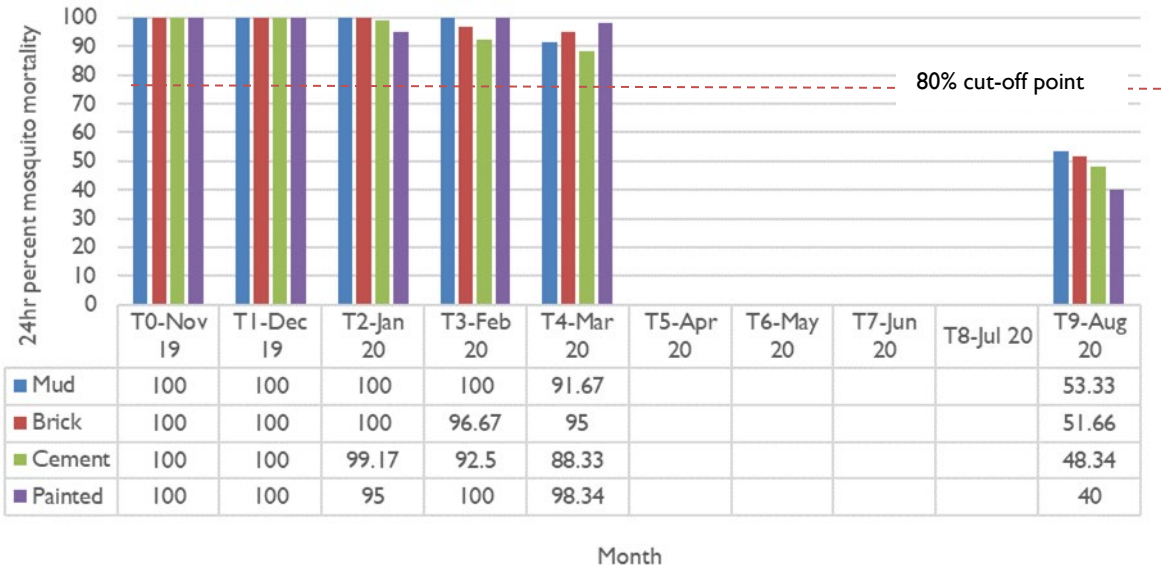
For the 2019 IRS campaign, the team monitored the spray quality, the residual efficacy on sprayed wall surfaces, and the fumigant effect of pirimiphos-methyl (Dendera) from November 2019 until August 2020, Fludora Fusion (Kawere) from November 2019 until March 2020, and DDT (Burma Valley) from November until September 2020. For the 2020 IRS campaign, the team monitored Fludora Fusion (Dendera and Kawere) from November 2020 to April 2021 and for DDT from October 2020 until March 2021 (Burma Valley). Monitoring was discontinued from April to July 2020 at all three sites due to the COVID-19 lockdown, but resumed in August at Dendera, and Burma Valley; monitoring could not be resumed at Kawere since the province sprayed the bioassay rooms with a different insecticide to Fludora Fusion in May 2020.

3.2.1 CONE BIOASSAY TESTS

2019 IRS CAMPAIGN

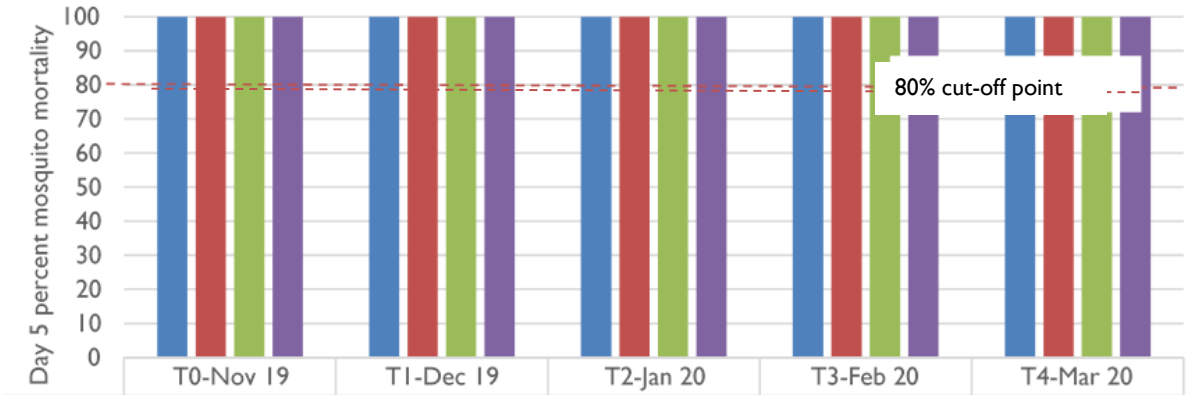
At all three sites that were monitored (10 rooms each site), the quality of spray was acceptable for all wall surface types. Residual efficacy of pirimiphos-methyl varied among wall surface types, for which mosquito mortality remained >80% for at least four months at Dendera but was much below the 80% cut-off point nine months after spray (Figure 4) when the tests were resumed after the COVID-19 lockdown period. The residual efficacy of Fludora Fusion was good (100% mosquito mortality) for all wall surface types for at least four months at Kawere (Figure 5), but the endpoint remained undetermined due to the lockdown disruption. The residual efficacy of DDT was at least four months, extending to nine months at Burma Valley where the NMCP sprayed DDT (Figure 6). The mud houses in the test were destroyed during the coronavirus pandemic to drive out unofficial residents from the farm compound, thus only three surface types were assessed during August and September 2020 at Burma Valley. An early decline in mosquito mortality was observed for DDT on brick and cement wall surfaces two months post-spray at Burma Valley but was still above the 80% cut-off point nine months post-spray for cement and paint, and 79.1% for brick.

Figure 4. Residual Efficacy of Pirimiphos-Methyl in Dendera (Gatakata/Champion villages), Mudzi District, Reported as *An. arabiensis* (KGB strain) Mortality After 24-hour Holding Period in WHO Cone Bioassays, November 2019-August 2020



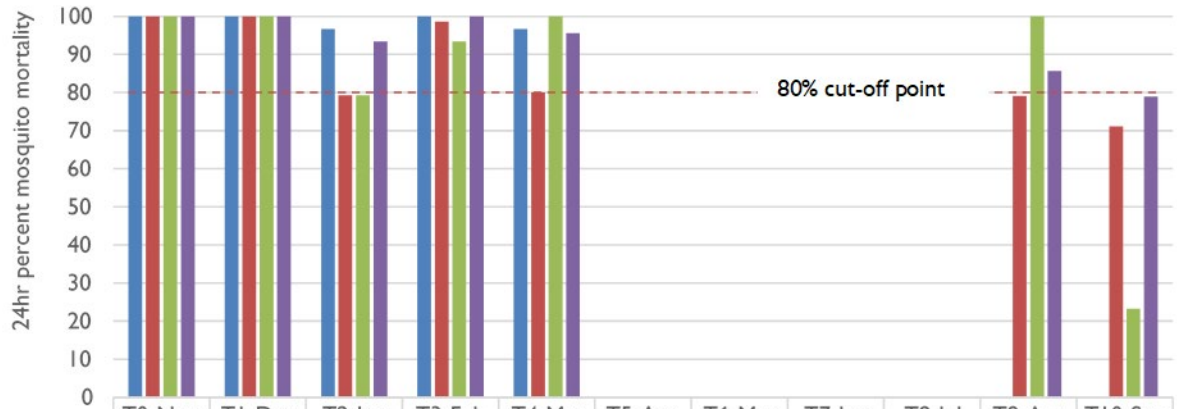
	T0-Nov 19	T1-Dec 19	T2-Jan 20	T3-Feb 20	T4-Mar 20	T5-Apr 20	T6-May 20	T7-Jun 20	T8-Jul 20	T9-Aug 20
Mud	100	100	100	100	91.67					53.33
Brick	100	100	100	96.67	95					51.66
Cement	100	100	99.17	92.5	88.33					48.34
Painted	100	100	95	100	98.34					40

Figure 5. Residual Efficacy of Fludora Fusion in Kawere (Kapondoro/Chakanetsa villages), Mutoko District, Reported as *An. arabiensis* (KGB strain) Mortality After Five-Day Holding Period in WHO Cone Bioassays, November 2019-March 2020



	T0-Nov 19	T1-Dec 19	T2-Jan 20	T3-Feb 20	T4-Mar 20
Mud	100	100	100	100	100
Brick	100	100	100	100	100
Cement	100	100	100	100	100
Painted	100	100	100	100	100

FIGURE 6. RESIDUAL EFFICACY OF DDT IN BURMA VALLEY, MUTARE DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS, NOVEMBER 2019-SEPTEMBER 2020

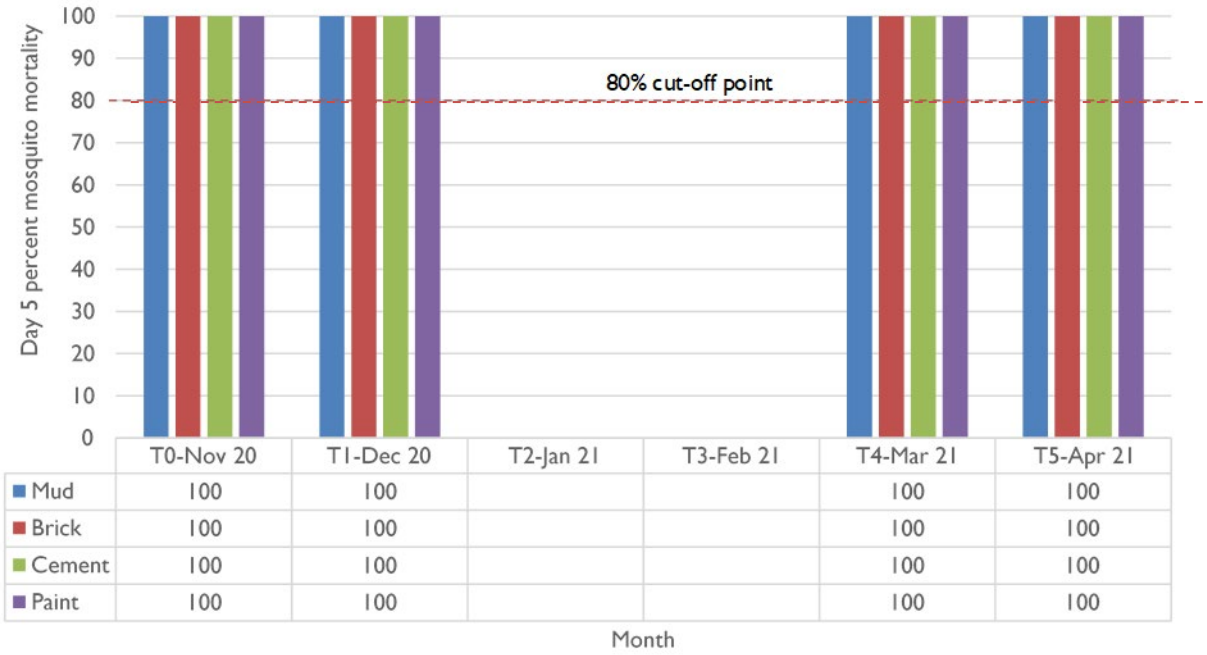


	T0-Nov 19	T1-Dec 19	T2-Jan 20	T3-Feb 20	T4-Mar 20	T5-Apr 20	T6-May 20	T7-Jun 20	T8-Jul 20	T9-Aug 20	T10-Sep 20
Mud	100	100	96.67	100	96.67						
Brick	100	100	79.25	98.67	80					79.1	71.1
Cement	100	100	79.25	93.34	100					100	23.3
Painted	100	100	93.33	100	95.6					85.6	78.9

2020 IRS CAMPAIGN

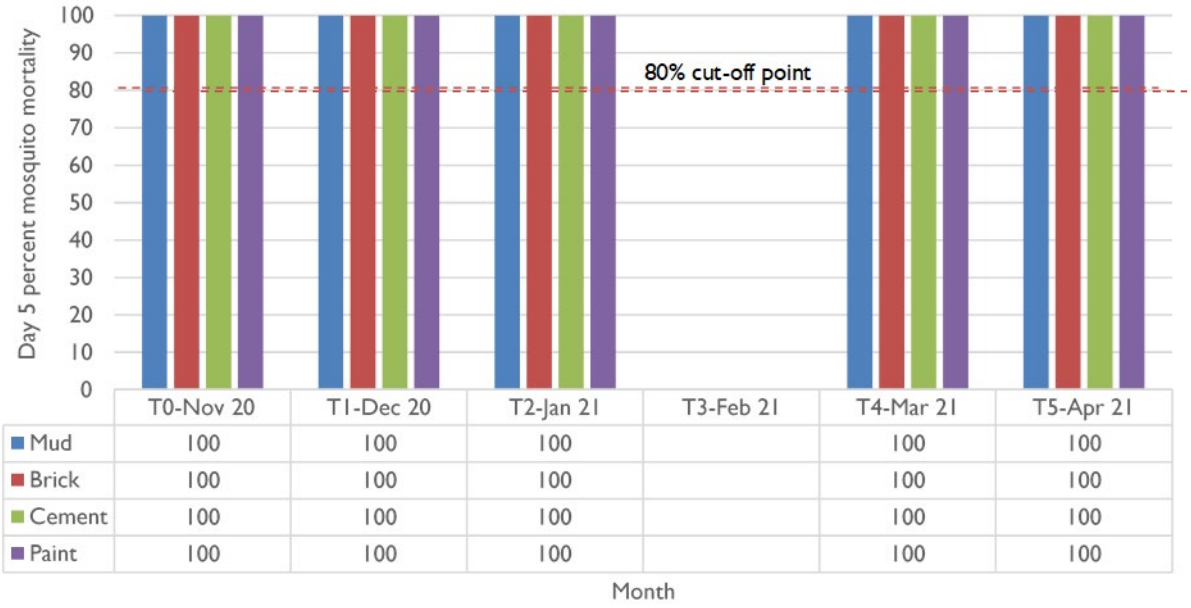
Quality of spray was acceptable at all three sites sprayed with Fludora Fusion at Dendera (Tizora Village) and Kawere (Katiyo and Machona villages) and DDT at Burma Valley. Residual efficacy of Fludora Fusion at Dendera and Kawere was good with mosquito mortality at 100% after five months after spray (Figure 7 and 8). Residual efficacy of Fludora Fusion at Kawere and Dendera was not determined in February because of the lockdown due to the coronavirus pandemic, and further in January at Dendera because of a lack of colony mosquitoes compounded by the lockdown. Residual efficacy of DDT at Burma Valley was good with mosquito mortality still above the 80% cut-off point for painted brick, cement, and painted cement five months after spray (Figure 9). No bioassay tests were done in January and February due to the pandemic-related lockdown. The team will continue monitoring until mosquito mortality falls below the 80% cut-off point at the stipulated observation period at Dendera and Kawere sites but will hand over the monitoring to the district team at Burma Valley.

Figure 7. Residual Efficacy of Fludora Fusion in Dendera (Tizora Village), Mudzi District, Reported as *An. arabiensis* (KGB Strain) Mortality After Five-Day Holding Period in WHO Cone Bioassays, November 2020-April 2021



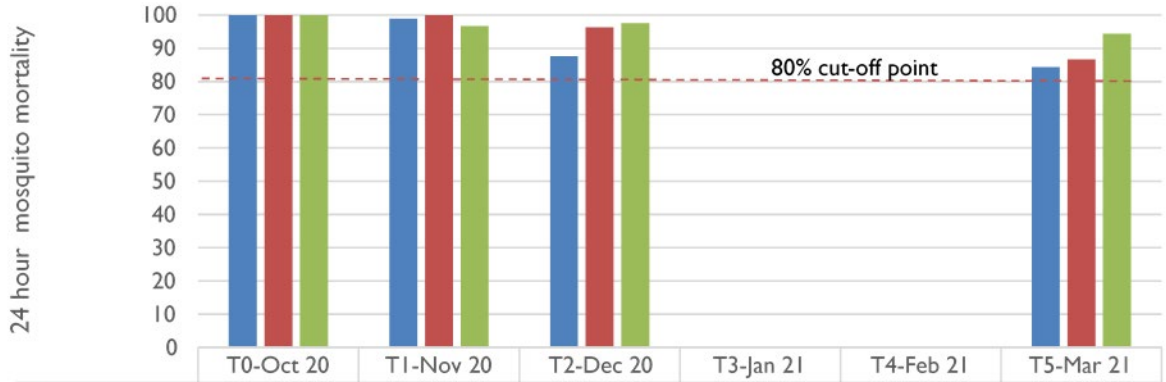
	T0-Nov 20	T1-Dec 20	T2-Jan 21	T3-Feb 21	T4-Mar 21	T5-Apr 21
Mud	100	100			100	100
Brick	100	100			100	100
Cement	100	100			100	100
Paint	100	100			100	100

Figure 8. Residual Efficacy of Fludora Fusion in Kawere (Katiyo/Machona Villages), Mutoko District, Reported as *An. arabiensis* (KGB Strain) Mortality After Five-Day Holding Period in WHO Cone Bioassays, November 2020-April 2021



	T0-Nov 20	T1-Dec 20	T2-Jan 21	T3-Feb 21	T4-Mar 21	T5-Apr 21
Mud	100	100	100		100	100
Brick	100	100	100		100	100
Cement	100	100	100		100	100
Paint	100	100	100		100	100

FIGURE 9. RESIDUAL EFFICACY OF DDT AT BURMA VALLEY, MUTARE DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD IN WHO CONE BIOASSAYS, OCTOBER 2020-MARCH 2021



	T0-Oct 20	T1-Nov 20	T2-Dec 20	T3-Jan 21	T4-Feb 21	T5-Mar 21
Painted brick	100	98.9	87.7			84.4
Cement	100	100	96.3			86.7
Painted cement	100	96.7	97.6			94.4

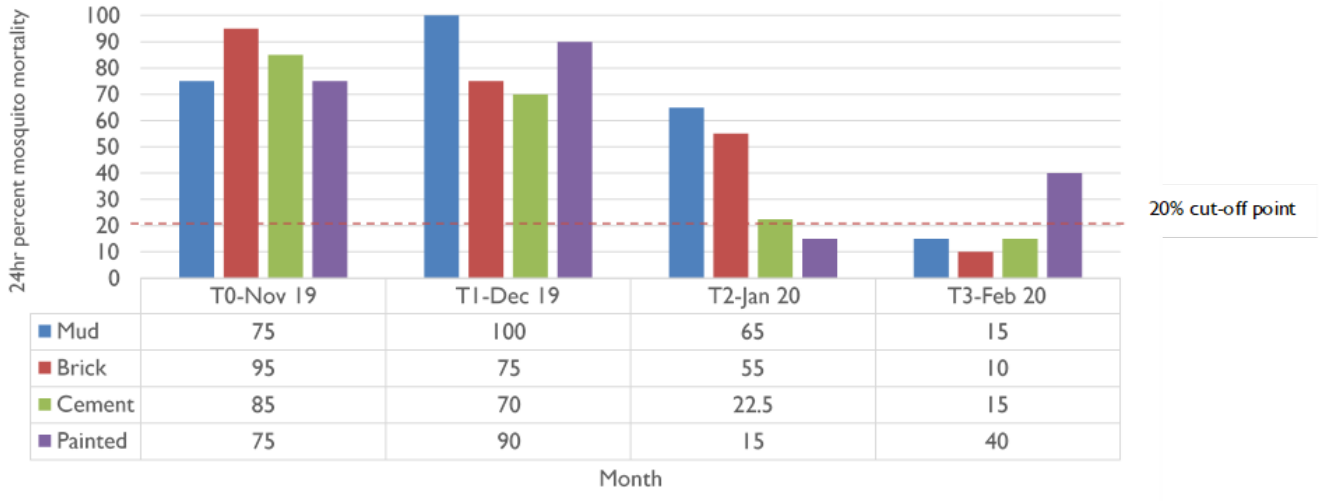
3.2.2 FUMIGANT EFFECT

The fumigant effect for pirimiphos-methyl and Fludora Fusion was assessed for the 2019 IRS campaign, whereas for the 2020 campaign it was done for Fludora Fusion and not for DDT.

2019 IRS CAMPAIGN

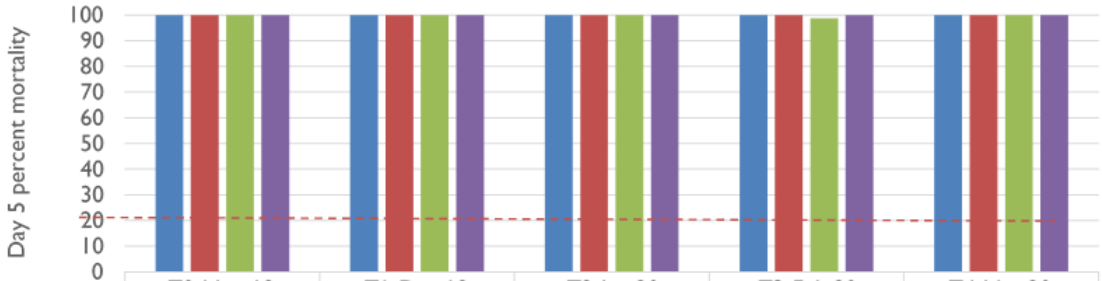
The fumigant effect of insecticide was monitored at Dendera and Kawere in 2019 (Figure 10 and 11). The tests for fumigant effect at Dendera were discontinued after February 2020, partly due to the COVID-19 national lockdown and because mosquito mortality had gone below 20% that month for most wall surface types (mud, brick, and cement). Mosquito mortality remained 100% four months post IRS at Kawere but the tests were discontinued in March 2020 due the COVID-19 lockdown. The T0 fumigant effect tests were not done at Burma Valley in November 2019 due to a shortage of mosquitoes.

FIGURE 10. FUMIGANT EFFECT OF PIRIMIPHOS-METHYL IN DENDERA (GATAKATA/CHAMPION VILLAGES), MUDZI DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER 24-HOUR HOLDING PERIOD, NOVEMBER 2019-FEBRUARY 2020



	T0-Nov 19	T1-Dec 19	T2-Jan 20	T3-Feb 20
Mud	75	100	65	15
Brick	95	75	55	10
Cement	85	70	22.5	15
Painted	75	90	15	40

Figure 11. Fumigant Effect of Fludora Fusion in Kapondoro, Mutoko District, Reported as *An. arabiensis* (KGB strain) Mortality After Five-Day Holding Period, November 2019-March 2020



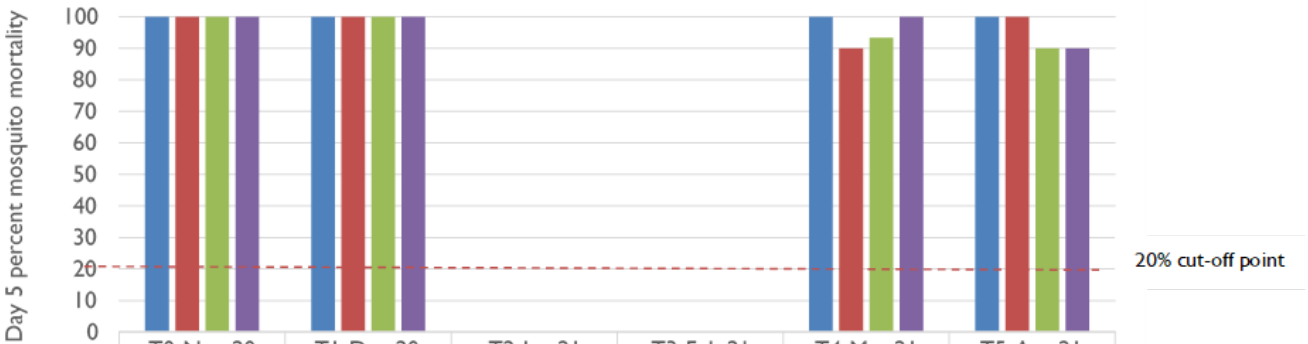
	T0-Nov 19	T1-Dec 19	T2-Jan 20	T3-Feb 20	T4-Mar 20
Mud	100	100	100	100	100
Brick	100	100	100	100	100
Cement	100	100	100	98.75	100
Painted	100	100	100	100	100

Month

2020 IRS Spraying Campaign

The fumigant effect was monitored for Fludora Fusion at Dendera and Kawere during the 2020 IRS campaign. Mosquito mortality was still above the 20% cut-off point five months post IRS at both sites (Figure 12 and 13). The team will continue monitoring the fumigant effect at the sites until the mosquito mortality falls below the 20% cut-off point.

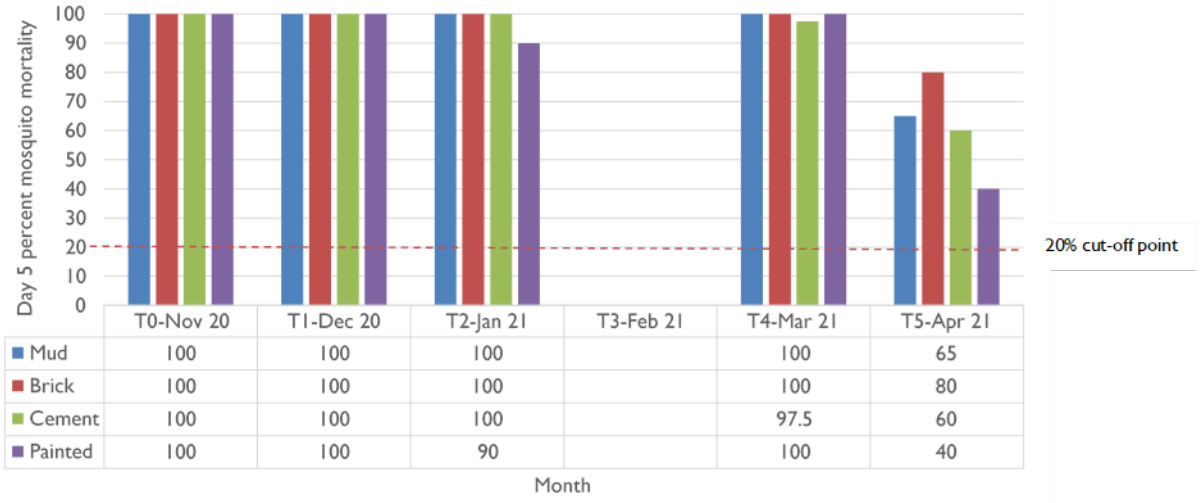
Figure 12. Fumigant Effect of Fludora Fusion in Dendera (Tizora Village), Mudzi District, Reported as *An. arabiensis* (KGB strain) Mortality After Five-Day Holding Period, November 2020-April 2021



	T0-Nov 20	T1-Dec 20	T2-Jan 21	T3-Feb 21	T4-Mar 21	T5-Apr 21
Mud	100	100			100	100
Brick	100	100			90	100
Cement	100	100			93.33	90
Painted	100	100			100	90

Month

FIGURE 13. FUMIGANT EFFECT OF FLUDORA FUSION IN KAWERE, MUTOKO DISTRICT, REPORTED AS *AN. ARABIENSIS* (KGB STRAIN) MORTALITY AFTER FIVE-DAY HOLDING PERIOD, NOVEMBER 2020-APRIL 2021



	T0-Nov 20	T1-Dec 20	T2-Jan 21	T3-Feb 21	T4-Mar 21	T5-Apr 21
Mud	100	100	100		100	65
Brick	100	100	100		100	80
Cement	100	100	100		97.5	60
Painted	100	100	90		100	40

3.3 INSECTICIDE RESISTANCE MONITORING

The insecticide susceptibility of *An. gambiae* s.l. collected from localities in Mashonaland East Province was tested using the CDC bottle bioassay method for deltamethrin, DDT and alpha-cypermethrin, and using the WHO tube method for clothianidin (Table 9). Few mosquitoes were collected from the sites due to the drought that extended into the 2020 rainy season.

An. gambiae s.l. was susceptible (100% mortality) to clothianidin and to DDT at Dendera and Kawere but indicated possible resistance (95% mortality) for deltamethrin in mosquitoes collected from Dendera Irrigation, Musau, and Nyamapanda Dam. *An. gambiae* s.l. collected from Kawere was susceptible (100%) to deltamethrin. *An. gambiae* s.l. collected from Makarara (Samuriwo, Makuwaza, and Chakanyuka in Hwedza District) were resistant to alpha-cypermethrin (53.3% mortality) in tests done in 2020 but were susceptible (100% mortality) for mosquitoes collected from Chikurumadziwa locality, Makarara site, in 2021 despite the small sample tested.

TABLE 9. RESULTS OF INSECTICIDE SUSCEPTIBILITY TESTS ON *AN. GAMBIAE* S.L. CONDUCTED AT SITES IN MASHONALAND EAST PROVINCE

District	Sentinel site	Larval collection locality	Date of test	Insecticide tested (Dose)	Test method	Total # mosquitoes tested	Resistance status (mosquito mortality)
Mudzi	Dendera	Dendera Spillway, Meza, Musau, and Nyamapanda Dam	Feb and May 2020	Clothianidin (2%)	WHO	51	S (100%)
		Dendera Irrigation, Musau, and Nyamapanda Dam	Feb and Mar 2020	Deltamethrin (1X)	CDC	184	PR (95%)
		Musaruro Village and Karonga Dam	May 2020	Clothianidin (2%)	WHO	11	S (100%)
		Dendera Irrigation, Meza, Kotwa, and Chijaka	Mar and Apr 2021	DDT (1X)	CDC	121	S (100%)
		Dendera Irrigation, Meza, Kotwa, and Chijaka	Mar and Apr 2021	Deltamethrin (1X)	CDC	97	S (100%)
Mutoko	Kawere	Kawere Pool, Hunda, and Ndemera	Feb and Mar 2020	Deltamethrin (1X)	CDC	235	S (98%)
		Lot Highway stream	Feb 2020	Deltamethrin (1X)	CDC	13	S (100%)
		Chitora	May 2020	Clothianidin (2%)	WHO	8	S (100%)
		Kawere	Mar and Apr 2021	DDT (1X)	CDC	53	S (100%)
Hwedza	Makarara	Samuriwo, Makuwaza, and Chakanyuka	Mar, May and Jun 2020	Alpha-cypermethrin (1X)	CDC	70	R (69%)
		Chikurumadziwa	Apr 2021	Alpha-cypermethrin (1X)	CDC	8	S (100%)

Anopheles funestus s.l. from Burma Valley (and Vumba) in Mutare District were susceptible to DDT (Table 10).

TABLE 10. RESULTS OF INSECTICIDE SUSCEPTIBILITY TESTS ON *AN. FUNESTUS* S.L. CONDUCTED AT BURMA VALLEY IN MANICALAND PROVINCE

District	Sentinel site	Collection locality	Date of test	Insecticide Tested (Dose)	Test	Total number of mosquitoes tested	Resistance status (mosquito mortality)
Mutare	Burma Valley	Brandhill Farm and Zonwe Dam (Vumba)	Jun 2020	DDT (1X)	CDC	52	S (100%)

3.4 RESULTS OF LABORATORY ANALYSIS

3.4.1 MOLECULAR IDENTIFICATION OF *ANOPHELES* SPECIES

A total of 318 *Anopheles* mosquitoes collected in 2020-2021 were assayed for species identification in the AU laboratory as follows: 130 from Burma Valley, 31 from Dendera, 67 from Makarara, 55 from Arcturus, 12 from Kawere, and 23 from Vumba (Table 11). At Burma Valley, 61/130 were *An. funestus* s.l. (46.9%), which were identified to five sibling species whose relative proportion within the group were: *An. funestus* s.s. (19.7%), *An. leesoni* (49.2%), *An. parensis* (14.7%), *An. rivulorum* (13.1%), and *An. rivulorum*-like (3.3%). *An. gambiae* s.l. constituted 3.1% (4/130) of the total *Anopheles* collected from Burma Valley that were identified as *An. arabiensis* (2/4) and *An. gambiae* s.s. (2/4). The other species were mainly *An. rufipes* (13/20) and *An. pretoriensis* (6/20) constituting 15.4%. A total of 34 (26.1%) *Anopheles* mosquitoes from Burma Valley did not amplify, while 11/130 specimens were not subjected to the molecular analysis as there is no appropriate protocol.

At Dendera 22.5% (7/31) were *An. funestus* s.l., out of which 71.4% (5/7) were identified as *An. funestus* s.s. *An. parensis* (14.3% (1/7) and 14.3% (1/7) *An. rivulorum*. The other species found were *An. rufipes* (11/31) and *An. pretoriensis* (5/31). Six *An. gambiae* were collected, with 16.7% (1/6) *An. arabiensis* and the remainder 83.3% *An. quadriannulatus* (5/6) specimens identified. Two out of 31 specimens did not amplify.

Out of 67 *Anopheles* mosquitoes from Makarara, 19.4% (13/67) were *An. funestus* s.l. and 43.3% (29/67) *An. gambiae* s.l. Most of the *An. funestus* s.l. were identified as *An. parensis* 69.2% (9/13) and the remainder two each of *An. funestus* s.s. (15.4%) and *An. rivulorum*-like (15.4%). 55.2% of the *An. gambiae* s.l. from Makarara were *An. arabiensis* (16/29), 41.4% (12/29) were *An. quadriannulatus* with only 3.4% (1/29) *An. gambiae* s.s. Twenty of the *Anopheles* from Makarara were other species, namely, *An. rufipes* (75%; 15/20) and *An. pretoriensis* (25%; 5/20). Two out of the 67 (3.0%) of the specimens did not amplify while three (4.5%) were not subjected to molecular analysis.

An. gambiae s.l. (6/12) was the dominant species at Kawere; all six of which were *An. quadriannulatus*. The single *An. funestus* s.l. (8.3%) was identified as *An. leesoni*.

An. funestus s.l. was predominant (22/23) at Vumba, with two species identified by PCR, namely, *An. funestus* s.s. (95.4%; 21/22) and *An. leesoni* (4.5%; 1/22). No *An. gambiae* s.l. was collected from Vumba. Only 1/23 (4.3%) did not amplify.

Less than a quarter of the *Anopheles* from Arcturus that was identified by PCR were *An. funestus* s.l. (14.5%; 78/55), mostly *An. parensis* (7/8) and *An. rivulorum*-like (1/8). Only one *An. gambiae* s.l. was identified as *An. quadriannulatus* (1.8%). Twenty percent (11/55) were other species that were identified as *An. rufipes* (54.5%; 6/11), *An. squamosus* (36.4%; 4/11) and *An. pretoriensis* (9.1%; 1/11). It must be noted that morphologically identified *An. coustani* that constituted most of the adult collection (33/55) were excluded from the above analysis as they were not applicable for the analysis. Only two specimens did not amplify.

TABLE 11. MAIN *ANOPHELES* MOSQUITOES COLLECTED AT BURMA VALLEY, DENDERA, MAKARARA, ARCTURUS, KAWERE, AND VUMBA IDENTIFIED WITH PCR, 2020

Site	Collection method	*An. funestus* s.l.					*An. gambiae* s.l.				*Other*				No amplification	Not Applicable	Total
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*An. rivulorum*	*An. rivulorum*-like	*An. arabiensis*	*An. gambiae* s.s.	*An. quadriannulatus*	*An. merus*	*An. rufipes*	*An. maculipalpis*	*An. squamosus.*	*An. pretoriensis*			
Burma Valley	CDC in	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6
	CDC out	0	6	1	0	2	1	0	0	0	0	0	0	0	6	2	18
	HLC Proxy-In	2	1	0	2	0	1	0	0	0	0	0	0	0	1	0	7
	HLC Proxy-Out	5	14	0	5	0	0	0	0	0	1	1	0	0	11	5	42
	Pit shelter	0	8	8	1	0	0	1	0	0	12	0	0	6	15	4	55
	PSC	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	2
	Total	12	30	9	8	2	2	2	0	0	13	1	0	6	34	11	130
Dendera	CDC in	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2
	CDC out	0	0	0	0	0	0	0	0	0	1	0	0	5	1	0	7
	Pit shelter	0	0	1	0	0	1	0	5	0	9	0	0	0	1	0	17
	PSC	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	HLC Proxy-In	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	Total	5	0	1	1	0	1	0	5	0	11	0	0	5	2	0	31
Makarara	CDC in	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
	CDC out	0	0	1	0	2	0	0	0	0	1	0	0	2	1	0	7
	HLC Proxy-In	2	0	0	0	0	2	0	0	0	0	0	0	1	0	0	5
	HLC Proxy-Out	0	0	1	0	0	0	0	1	0	2	0	0	0	1	3	8
	Pit shelter	0	0	7	0	0	3	1	11	0	12	0	0	1	0	0	35
	PSC	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	11
	Total	2	0	9	0	2	16	1	12	0	15	0	0	5	2	3	67
Arcturus	CDC in	0	0	1	0	0	0	0	0	0	0	0	1	0	0	3	5
	CDC out	0	0	4	0	1	0	0	1	0	3	0	2	1	2	22	36
	HLC Proxy-Out	0	0	1	0	0	0	0	0	0	3	0	1	0	0	8	13
	Pit shelter	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
	Total	0	0	7	0	1	0	0	1	0	6	0	4	1	2	33	55
Kawere	HLC Proxy-Out	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	2
	Pit Shelter	0	0	0	0	0	0	0	6	0	0	0	0	3	1	0	10
	Total	0	1	0	0	0	0	0	6	0	0	0	0	3	2	0	12
Vumba	CDC in	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
	PSC	18	0	0	0	0	0	0	0	0	0	0	0	0	1	0	19
	HLC Proxy-Out	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Total	21	1	0	0	0	0	0	0	0	0	0	0	0	1	0	23

Two *An. gambiae* s.s. were classified by molecular method as *An. gambiae* (former 'S' molecular form), while *An. coluzzii* was absent. One of the *An. gambiae* s.s. was collected from Makarara and the other from Burma Valley.

3.4.2 Results of Blood Meal Analysis

A total of 108 blood-fed mosquitoes collected in the six sites were analyzed by PCR to determine the blood meal sources. Only two out of 14 *An. funestus* s.l. at Burma Valley had fed on humans, both of them *An. funestus* s.s., one of which *An. funestus* s.s. had fed on human and cow blood. None of the *An. leesoni* and *An. parensis* had fed on human blood. None of the other species had human blood; some had cow and goat separately (*An. rufipes*) or cow (*An. leesoni, An. rufipes* and *An. pretoriensis*). The single *An. arabiensis* from Burma Valley had fed on human blood.

At Arcturus, none of the three *An. funestus* s.l. or the two other species that did not amplify) had fed on humans. One of the two *An. funestus* s.s. had fed on goat, while the other did not amplify. At Kawere, none of the specimens had human blood. *An. quadriannulatus* had fed on either cow or goat. At Makarara, the bloodmeal from the single *An. funestus* s.s. did not amplify, while *An. parensis* had fed on cow (5/6) and human plus cow (1/6). *An. rufipes* had fed on either cow or goat with four bloodmeals that did not amplify. More than half (7/13) *An. arabiensis* had fed on human, dog (2/13) and four that did not amplify. *An. funestus* s.s. at Vumba had the highest proportion with human blood meal (16/18), while one had cow (1/18) and one one did not amplify. At Dendera, the two *An. funestus* s.s. had fed on humans, while *An. arabiensis* had fed on dog, and 4/5 *An. quadriannulatus* had fed on dog and one on mixed human-goat-dog. All five *An. rufipes* had fed on cow (Table 12).

Table 12. Results of the Blood Meal Analyses of *Anopheles* Species from Burma Valley, Dendera, Makarara, Arcturus, Kawere, and Vumba

Site	Blood meal source	Species										Total
		An. funestus s.l.				*An. gambiae* s.l.		Other			No amplification	
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*An. rivolum*	*An. arabiensis*	*An. quadriannulatus*	*An. rufipes*	*An. squamous*	*An. pretoriensis*		
Burma Valley	Cow	0	5	0	0	0	0	2	0	1	3	11
	Cow & human	1	0	0	0	0	0	0	0	0	0	1
	Dog	1	0	0	0	0	0	0	0	0	0	1
	Goat	0	0	0	0	0	0	1	0	0	0	1
	Human	1	0	0	0	1	0	0	0	0	1	3
	No amplification	0	0	1	0	0	0	3	0	3	1	8
	Total	3	5	1	0	1	0	6	0	4	5	25
Dendera	Cow	0	0	0	0	0	0	5	0	0	0	5
	Human	2	0	0	0	0	0	0	0	0	0	2
	Dog	0	0	0	0	1	4	0	0	0	0	5
	Human, goat&dog	0	0	0	0	0	1	0	0	0	0	1
	Total	2	0	0	0	1	5	5	0	0	0	13
Makarara	Cow	0	0	5	0	0	3	2	0	2	0	12
	Goat	0	0	0	0	0	0	1	0	0	1	2
	Dog	0	0	0	0	2	1	0	0	0	0	3
	Cow & goat	0	0	0	0	0	0	0	0	1	0	1
	Human & goat	0	0	0	0	1	0	0	0	0	0	1
	Human & cow	0	0	1	0	0	0	0	0	0	0	1
	Human	0	0	0	0	6	0	0	0	0	0	6
	No amplification	1	0	0	0	4	0	4	0	0	1	10
	Total	1	0	6	0	13	4	7	0	3	2	36

Site	Blood meal source	Species										Total
		An. funestus s.l.				*An. gambiae* s.l.		Other			No amplification	
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*An. rivolum*	*An. arabiensis*	*An. quadriannulatus*	*An. rufipes*	*An. squamous*	*An. pretoriensis*		
Arcturus	Goat	1	0	0	0	0	0	0	0	0	2	3
	No amplification	1	0	0	1	0	0	0	0	0	2	4
	Total	2	0	0	1	0	0	0	0	0	4	7
Kawere	Cow	0	0	0	0	0	3	0	0	1	0	4
	Goat	0	0	0	0	0	2	0	0	0	0	2
	No amplification	0	1	0	0	0	0	0	0	0	2	3
	Total	0	1	0	0	0	5	0	0	1	2	9
Vumba	Cow	1	0	0	0	0	0	0	0	0	0	1
	Human	16	0	0	0	0	0	0	0	0	0	16
	No amplication	1	0	0	0	0	0	0	0	0	0	1
	Total	18	0	0	0	0	0	0	0	0	0	18

3.4.3 Sporozoite Infection Rate

A total of 230 *Anopheles* mosquitoes collected from six sentinel sites by various methods were analyzed by ELISA for CSP for *P. falciparum*: *An. funestus* s.l. (n=117), *An. gambiae* s.l. (n=45), and other *Anopheles* (n=68). Dendera had four species that initially tested positive, namely, 2/4 *An. funestus* s.s., , 1/2 *An. rivulorum,* and 1/5 *An. pretoriensis*. while Burma Valley had 1/10 *An. parensis* that was positive (Table 13). However, the CSP-positive specimens were all negative when subjected to the boiling method that is used to exclude false positives.

Table 13. Sporozoite Infection Rate*

Province	Sentinel site	*An. funestus* s.l.					*An. gambiae* s.l.			Other *Anopheles*		
		An. funestus s.s.	*An. leesoni*	*An. parensis*	*An. rivulorum*	*An. rivulorum* -like	*An. gambiae* s.s.	*An. arabiensis*	*An. quadriannulatus*	*An. rufipes*	*An. pretoriensis*	*An. squamosus*
Mashonaland East	Arcturus	0	0	0/7	0/1	0	0	0	0/1	0/6	0/1	0/4)
	Dendera	2/4 both from PSC	0	0/1	1/2 from CDC IN	0	0	0/1	0/5	0/11	1/5 from CDC OUT	0
	Kawere	0	0/1	0	0	0	0	0	0/6	0	0	0
	Makarara	0/2	0	0/9	0	0/2	0/1	0/16	0/12	0/15	0/5	0
Manicaland	Burma Valley	0/12	0/29	1/11 from pit shelter	0/9	0/2	0/1	0/2	0	0/13	0/6	0
	Vumba	0/23	0/2	0	0	0	0	0	0	0	0/2	0
Total		2/41	0/32	1/28	1/12	0/4	0/2	0/19	0/24	0/45	1/19	0/4

*The numerator denotes the number of specimens positive by ELISA out of the total number tested (denominator).
None of the specimens were positive by the confirmatory boiling procedure, which was done as described by Durnez et al, (2011)

3.4.4 Results of kdr Assays

A total of 101 *An. gambiae* s.l. were tested for the kdr mutation; Leu – Ser (kdr East) and Leu – Phe (kdr West). Only 5.9% (5/101) had the heterozygote allele for kdr East, whereas most (95%; 96/101) were susceptible homozygous (Table 14). All 149 specimens were susceptible homozygous for kdr West gene. The *An. gambiae* s.l. analyzed were from routine surveillance (43/101) and from insecticide resistance samples (58/101).

Table 14. Results of KDR Assays

Province	District	Site	Number of species tested	Kdr East			Kdr West		
				SS	RS	RR	SS	RS	RR
Manicaland	Mutare	Burma Valley	3	3	0	0	3	0	0
Mashonaland East	Mudzi	Dendera	24	23	1	0	24	0	0
	Mutoko	Kawere	36	32	4	0	36	0	0
	Goromonzi	Makarara	37	37	0	0	37	0	0
	Hwedza	Arcturus	1	1	0	0	1	0	0
Total			101	96	5	0	101	0	0

Note: SS=Susceptible homozygous, RS=Resistant heterozygous, RR=Resistant homozygous

3.4.5 Results of Ace-1 Assays

A total of 63 *An. gambiae* s.l. were analyzed for insensitive AChE gene by molecular method. All 63 *Anopheles* were susceptible for Ace-1: 20 from Mudzi, 33 from Mutoko, and 10 from Hwedza districts (Table 15).

Table 15. Results of Ace-1 Assays

Province	District	Site	Insecticide	Ace-1 Status		
				*SS	RS	RR
Mashonaland East	Mudzi	Dendera	Deltamethrin	20	0	0
	Hwedza	Makarara	Alpha-cypermethrin	10	0	0
	Mutoko	Kawere	Deltamethrin	33	0	0
Total				63	0	0

Note: SS=Susceptible homozygous, RS=Resistant heterozygous, RR=Resistant homozygous

4. DISCUSSION

Entomological monitoring results from March 2020 to February 2021 show variation in the species composition at the six sites. *An. funestus* s.l. is the major malaria vector species in Burma Valley and Vumba in Manicaland. *An. gambiae* s.l. was dominant at Kawere and Makarara even though *An. funestus* s.l. was evident at Makarara, Arcturus, and Kawere. The *An. coustani* predominance at Arcturus needs to be taken with caution as this occurrence was based on one month of surveillance (March 2020). In this reporting period, *An. funestus* s.l. was the dominant species at two sites: Burma Valley (n = 84/128; 66%) and Vumba (n = 25; 100%).

Historically, four members of *An. funestus* s.l. have been collected: *An. funestus* s.s., *An. leesoni, An. parensis,* and *An. rivulorum*. Both *An. funestus* s.s. and *An. rivulorum* are known vectors. The role of the other species within *An. funestus* s.l. such as *An. rivulorum*-like is not well documented, but these are considered potential secondary vectors where they occur. The low mosquito densities are attributed to the severe 2020 drought that affected *An. gambiae* s.l., which typically breeds in temporary rainwater pools, as compared to *An. funestus* s.l., which breeds in semi-permanent water bodies that abound at Burma Valley and Vumba. The longitudinal monitoring was conducted mostly under drought conditions, and hence the low number of mosquitoes collected. Further, longitudinal entomological surveillance was affected by the COVID-19 lockdown for four consecutive months in 2020 and two months in 2021.

Few *Anopheles* mosquitoes were collected resting indoors at all six sites except at Makarara and Vumba, both of which are not under routine spraying. More *An. funestus* s.s. were collected indoors at Vumba (by PSC and light trap), at Burma Valley (by HLC proxy and light trap), at Dendera (by PSC and light trap) and at Makarara (by HLC proxy). Some *An. funestus* s.l. and *An. gambiae* s.l. were collected resting outdoors (in pits) and from light traps and HLC proxy outdoors. This suggests a vector population that prefers resting outdoors and/or the impact of residual insecticide from routine IRS on indoor resting mosquitoes. However, this observation is based on very low mosquito densities in the areas. Other species, *An. pretoriensis* and *An. rufipes,* were relatively more abundant outdoors than indoors. CDC light traps set outdoors also collected more mosquitoes than those set indoors.

Too few mosquitoes were collected to determine hourly biting rates and preferred biting location. Fewer *An. gambiae* s.l. were collected than *An. funestus* s.l. Most *An. gambiae* s.l. were observed biting outdoors before midnight with limited biting during early morning, 4-5 a.m. In contrast, *An. funestus* s.l. was observed biting both indoors and outdoors, starting before midnight and extending outdoors until 6 a.m. Most biting behavior data for *An. funestus* s.l. are from Burma Valley and Makarara.

Laboratory analysis provided insights on species occurrence at all six sites. Data from Burma Valley show the occurrence of five sibling species of *An. funestus* s.l. (Table 10). *An. leesoni* (46.6%) was found in greater abundance than the main vector *An. funestus* s.s. (22.4%), followed by *An. parensis* (17.2%), *An. rivulorum* (10.3%), and *An. rivulorum*-like (3.4%). *An. funestus* s.s. was generally more abundant than its sibling species at Dendera and Vumba, but *An. leesoni* was more at Burma Valley, and *An. parensis* more at Makarara, Arcturus, and Kawere, where *An. parensis*. *An. gambiae* s.l. was represented by the major malaria vectors *An. arabiensis* and *An. gambiae* s.s. but overshadowed by *An. quadriannulatus*. *An. merus* was conspicuous by its absence at the six sites among the species identified by PCR during this reporting period.

Other species found include *An. rufipes*, *An. squamosus,* and *An. pretoriensis*. While *An. rufipes* and *An. squamosus* are considered potential malaria vectors, *An. pretoriensis* is probably not a vector although the species has been reported positive for sporozoites in Eastern Zambia that borders with Mozambique. The low human blood index (HBI) in most of the other species suggests it is unlikely they feed on humans to the extent of transmitting malaria.

That none of the 230 mosquito samples analyzed were confirmed positive for *Plasmodium* infection is attributed to the small sample size. The few specimens that were initially positive were not confirmed by either the additional step of boiling or by PCR.

An. funestus s.s. showed overall high HBIs following the analysis of blood-fed mosquitoes collected from the six sites (Table 12). HBI, the proportion of blood meals that are of human origin, is a key determinant of malaria transmission. HBI of 58.9% for *An. funestus* s.s. from Vumba was the highest recorded during the reporting period. However, some of the same species had fed on goat, cow, and one mixed dog and goat. At Burma Valley, *An. funestus* s.s. had a HBI of 14.3%, and it also fed on dog and on mixed dog and goat (1/14, each). A substantial proportion (83.3%; 5/6) of the *An. leesoni* from Burma Valley had fed on cow, suggesting either a preference for domestic animals or the influence of where these specimens were collected. Only one out of eight *An. rivulorum* from Burma Valley had human blood, and none had fed on the available domestic animals that were tested, namely cow, goat, and dog. The single *An. arabiensis* collected from Burma Valley had human blood. One out of nine *An. pretoriensis* had human blood in this species that is generally regarded as a non-vector that prefers feeding on domestic animals. *An. parensis* from Makarara showed a preference for cow (4/5; 80%) although one had had a mixed human and cow blood meal. Preference for domestic animal blood for *An. parensis* was also evident at Arcturus, with 95.5% of the species having fed on cow (7/22), goat (4/22), dog (9/22), and mixed cow and goat (1/22). Some specimens with human blood could not be identified to species level in the laboratory: 23/46 at Vumba, 5/51 at Burma Valley, 2/7 at Dendera, and 1/18 at Makarara.

Entomological monitoring yielded low numbers of mosquitoes overall, limiting the ability to identify clear seasonal trends yet highlighting the need for an assessment and potential consideration around other mosquito collection methods such as using animal- or human-baited tent traps. The pit shelter has proven to be an affordable but productive outdoor collection method in vector surveillance.

Results of insecticide susceptibility tests on *An. gambiae* s.l. and *An. funestus* s.l. were encouraging since there was generally no resistance to clothianidin, deltamethrin, and DDT. *An. gambiae* s.l. from Mudzi and Mutoko were susceptible to clothianidin, a constituent insecticide in Fludora Fusion. The species was also susceptible to deltamethrin in Mutoko and Mudzi except for possible resistance observed on *An. gambiae* s.l. from Dendera Irrigation, Musau, and Nyamapanda Dam. *An. funestus* s.l. from Burma Valley and Vumba were 100% susceptible to DDT. *An. gambiae* s.l. from some localities in Hwedza were resistant to alpha-cypermethrin in 2020, but tests on the species from another locality showed 100% susceptibility in 2021.

Laboratory tests for insecticide resistance confirm the absence of either kdr or Ace-1 mutations. Only 4% of the specimens analyzed for kdr had the heterozygous allele for kdr East. None of the specimens had kdr West resistance. None of the *An. gambiae* s.l. had Ace-1 resistance although these tests were based on small sample sizes. This augurs well for insecticide use in vector control although monitoring should be done on a wider scope geographically.

For the 2019 IRS campaign, the residual efficacy of pirimiphos-methyl was at least four months at Dendera, but monitoring was disrupted by the coronavirus pandemic in 2020. Mosquito mortality had declined below the 80% cut-off point nine months post spray. Fludora Fusion had a residual life of at least four months at Kawere before this was also disrupted by the lockdown and later when the bioassay houses were sprayed in May 2020. DDT residual efficacy declined substantially 10 months post spray at Burma Valley. Fumigant effect declined rapidly for pirimiphos-methyl at Dendera but remained relatively high at Kawere up to the point the monitoring was disrupted.

Current monitoring following the 2020 IRS campaign shows the residual efficacy of Fludora Fusion at both Dendera and Kawere remains good five months after spray based on the 120-hour holding period for the insecticide. Fumigant effect remains high at both Dendera and Kawere. In Burma Valley, DDT residual efficacy is still above the 80% cut-off point. The team will continue monitoring residual efficacy at the two Fludora Fusion sites at Dendera and Kawere but will discontinue and transfer the responsibility of bioassay tests at Burma Valley to the district due to budgetary constraints.

5. RECOMMENDATIONS

Based on the data presented and discussed in this report, the following recommendations and next steps should be considered going forward:

- *Anopheles gambiae* s.l. is susceptible to clothianidin, deltamethrin, alpha-cypermethrin, and DDT at the sites tested in Mashonaland East Province, and therefore it is recommended that Fludora Fusion continue to be considered as the insecticide of choice for IRS in those areas.
- The sample size for testing DDT resistance *of An. funestus* s.l. was low. PMI VectorLink and the NMCP should ensure adequate sample size by increasing *An. funestus* s.l. collections in that area.
- PMI VectorLink and the NIHR should continue to inform NMCP and sensitize the Vector Control Technical Sub-Committee on insecticide resistance to guide policy and action.
- PMI VectorLink in collaboration with the NIHR should evaluate alternative collection methods to potentially increase mosquitoes collected at sentinel sites, specifically the Furvela and the Ifakara tent traps.
- In collaboration with the NMCP, PMI VectorLink should continue training staff at the NIHR and AU laboratories to improve capacity for morphological identification of *Anopheles* mosquitoes for focused molecular and immunodiagnostic analyses and build similar morphological identification capacity at sentinel sites.
- The NMCP and partners, in collaboration with CDC, AU, and NIHR, should establish an *An. funestus* s.s. colony for reference in bioassay and resistance tests for the 3rd and 4th quarter 2021.VL in collaboration with NMCP and NMCP should determine the role of the now prevalent *An. funestus* s.l., and other species in malaria transmission and investigate approaches to control residual transmission

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