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ENTOMOLOGICAL ASSESSMENT ANNUAL REPORT: YEAR 3

September 29, 2021

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ENTOMOLOGICAL SURVEILLANCE ANNUAL REPORT: YEAR 3

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REVOLUTIONARY GOVERNMENT OF ZANZIBAR

MINISTRY OF HEALTH, SOCIAL WELFARE, ELDERLY, GENDER AND CHILDREN

ZANZIBAR MALARIA ELIMINATION PROGRAMME

Entomological Monitoring Report for 2020

January 2021

1. ENTOMOLOGICAL MONITORING AT 10 SENTINEL SITES

1.1 Objectives of entomological surveillance

- To assess the quality of IRS operation and insecticide decay rate
- To determine insecticide susceptibility of malaria vectors to insecticide, intensity and mechanisms of resistance
- To assess malaria vector density, species, behavior (feeding and resting) and seasonality.

1.2 Sentinel sites

Entomological information at sentinel sites was collected from October 2019 to September 2020 whilst IRS quality assessment and insecticide residual efficacy were carried out from February 2020 for six months. Ten entomological (10) sentinel sites are used for entomological monitoring. These sites are located in ten different districts i.e. one site per district. There were six (6) sites in Unguja and four (4) in Pemba. The selection of entomological sentinel sites considered the following criteria:

-Disease incidence/ prevalence
-Topography of the area
-Agricultural practices (Rain fed rice, Irrigation etc)
-Urban or rural setting

Location	District	sentinel site	IRS status in 2020	Entomological parameters collected
Unguja	Mjini	Stone town	Not sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Kaskazini B	Bumbwini	Sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Kaskazini A	Donge	Sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate, IRS quality assessment and insecticide decaying rate
	Magharibi	Mwera	sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Kusini	Muyuni	Sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Kati	Cheju	Sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate. IRS quality assessment and insecticide decaying rate
		Shakani	Sprayed	IRS quality assessment and

				insecticide decaying rate
Pemba	Micheweni	Tumbe	Sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Wete	Bopwe	Not sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Chake Chake	Uwandani	Not sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Mkoani	Wambaa	sprayed	Vector abundance, species distribution, seasonality, feeding time and location and infection rate
	Micheweni	Tumbe	sprayed	IRS quality assessment and insecticide decaying rate
	Micheweni	Makangale	sprayed	IRS quality assessment and insecticide decaying rate
	Wete	Gando	Sprayed	IRS quality assessment and insecticide decaying rate

1.3 Vector bionomics

Entomological monitoring was done to assess the impact of IRS at sentinel sites with regards to entomological parameters that are potential for malaria transmission including vector abundance, species, seasonality, density, infection rate, feeding and resting behavior and susceptibility of vectors to insecticides. Methods used for vector sampling include Pyrethrum spray catches, Human landing catches, CDC light traps and Pit traps.

2.0 METHOD USED FOR VECTOR SAMPLING AT SENTINEL SITES

Method	**Purpose**	**Sentinel site**	**≠ of Households**	**Days/month**	**Time**	**Sample processing**
HLC	Indoor & outdoor biting behavior	10	2 house/ site	2 days/site	1800-0600	Species, sporozoite rate
PSC/Prokopack	Indoor resting behavior	10	5 houses/site	2 days/site	0600-0800	Species, sporozoite rate, HBI
CDC light trap	Indoor abundance	10	2 houses/site	2 days/site	1800-0600	Species, sporozoite rate
PTC	Outdoor resting behavior	10	2 pits/site	2 days/site	0600-0800	Species, sporozoite rate,HBI

2.1 Human landing catches (HLC)

Human-landing catches (HLC) (WHO, 1975) were conducted between 18.00 hrs – 06.00 hrs outdoors and indoors twice per month at each site. Catches from the

collections were kept in paper cup/hour/collectors. Two collectors were collecting mosquitoes outdoor and the other two were doing the same indoor in two houses per site. They were then kept in a cool box until sorted, counted and recorded in the following morning.

2.2 Pyrethrum Spray Catches (PSC)

Pyrethrum spray catch was carried out in five houses during each mosquito collection morning twice per month. White sheets were laid on the entire floor and over the furniture within one room where people slept in previous night in each selected house. White sheets facilitate visibility of the knocked down mosquitoes. The doors and windows of the houses were shut then the rooms sprayed with pyrethrum (0.3%) synergized with piperonyl-butoxide (PBO) as described by Gimnig et al. (2003). Briefly, a collector outside the house sprayed around the eaves with insecticide to prevent the mosquitoes inside the houses from escaping and another collector sprayed the roofs and the walls inside the house. The houses were then closed for 10-15 minutes. The white sheets were removed from all the rooms of the houses and the knocked down mosquitoes collected using forceps. Knocked down mosquitoes for each room were recorded and then transferred onto moist filter paper inside labelled petri dishes indicating the date and house number. The same procedure was repeated for all the five houses and collected mosquitoes put in a cool box and transported to the laboratory for further processing.

2.3 Pit Trap Catches (PTC)

A rectangular pit was dug in the ground (1.5 m in depth, 1.2 m in length and 1 m in width) within 10 m of each selected residential house. In each of the four vertical sides, about 50–60 cm and 90–100 cm from the bottom of the pit, 5-8 little cavities were dug into a depth of about 30 cm. The main pits were then shaded by an artificial framework thatched with locally available coconut palms on top to provide shade. Resting mosquitoes were sampled from 06:00 to 09:00 hrs inside the cavities by using hand-held mouth aspirators and an intensive visual search. Collected mosquito samples were kept in paper cups before being processed accordingly in the laboratory. The collection was done twice per month at each site.

2.4 CDC light trap

Indoor host-seeking mosquitoes were collected using Centre for Disease Control and Prevention (CDC) light traps (John W. Hock Ltd, Gainesville, FL., USA). In every mosquito trapping night, mosquitoes were collected from **2 randomly selected houses from a single collection site.** In each house, light traps were hanged a meter off the ground and approximately 150 cm from an occupied untreated mosquito net. A single trap was set per room and a total of two traps were set each sampling night/HOUSE. These traps were positioned at the head side of the sleeper. Light traps were switched on at 1800 hrs and switched off at 0600 hrs the next morning. Mosquitoes were then collected in the morning. Live mosquitoes were aspirated into clearly labelled paper cups and transported to the laboratory for further processing.

2.5 Morphological identification of mosquitoes

Morphological identification was done using the taxonomic keys of Gillies and Coetzee (1987) at laboratory once the samples are arrived from field. Based on morphological characteristics, *Anophelines* were sorted to generally known species i.e. *An. gambiae* s.l., *An. funestus, An. coustani, An. pharoensis*, etc.

2.6 Detection of sporozoites in mosquitoes by CSP-ELISA technique

A sandwich ELISA schematic principle for malaria vector infection rate

Enzyme-linked immunosorbent assays (ELISAs) were developed to detect *Plasmodium falciparum*, *P. vivax*-210, and *P. vivax*-247 circumsporozoite proteins (CSP) in malaria-infected mosquitoes. The sensitivity and specificity of the ELISAs are based on the monoclonal antibodies (Mabs) used. The ELISAs detect CS proteins, which can be present in the developing oocysts, dissolved in haemolymph, and on sporozoites present in the haemocoel or in the salivary glands.

The ELISA assays were carried out using dried mosquitoes. The specimens were processed and preserved in Eppendorf tubes with silica gel to keep them dry. The specimens were grinded using grinding solution containing Blocking Buffer (BB) and Igepal CA-630, combination was mixed properly so that the Igepal to dissolve in BB.

Mosquito grinding

Each mosquito sample was ground separately (one mosquito per well), only the head-thorax part was placed in a labelled 1.5ml micro centrifuge grinding tube. Grinding buffer was then added at this point for the specimen to be well grinded. The pestles were rinsed with Grinding Solution; to make sure that the rinses were held in the tube containing mosquito triturate.

The "sandwich" begun by absorption of the capture Monoclonal antibodies (Mab) to the wells of a micro plate. After the capture Mab had bound to the plate, the well contents were aspirated, and the remaining binding sites were blocked with blocking buffer containing IGEPAL CA- 630 and an aliquot was tested. Positive and Negative Control were also added to specific well at this time. If circum-sporozoite (CS) antigen is present it will form AgAb complex with the capture Mab. After 2hrs incubation at room temperature, the mosquito triturate was aspirated, and the wells were washed. Peroxidase – linked Mab was then added to the wells, completing the formation of the "sandwich". After 1hr, the well contents were aspirated, the plate was washed again, and the clear peroxidase substrate solution was added. As the peroxidase enzyme reacts with the substrate, a dark green product is formed; the intensity of the colour is proportional to the amount of CS antigen present in the test sample.

2.7 Detection of blood meal sources by ELISA

The blood meal origins of freshly fed anopheline mosquitoes collected inside houses were determined using a direct enzyme-linked immunosorbent assay (ELISA) following the method of Beier *et al.* (1988) using human and bovine antibodies. Each mosquito abdomen was crushed in 50-µl phosphate buffered saline (PBS) solution (pH 7.4), which was further diluted by adding 950 µl PBS. Fifty microliters of sample added to each well in a 96-well microtitre plate and incubated overnight at room temperature. Each well was washed twice with PBS containing Tween-20 solution, and 50-µl host specific conjugate (either human or bovine) was added to each well and incubated for one hour. After one hour, each well was washed three times with a PBS–Tween-20 solution. Finally, 100 µl of peroxidase substrate was added to each

well and after 30 minutes the absorbance at 405 nm was recorded with an ELISA plate reader. Each blood meal sample was considered positive if the absorbance value exceeded the mean plus three times the standard deviation of the four negative controls (from a laboratory colony of *An. gambiae* Kisumu strain adults not fed with blood). Positive controls contained human and bovine blood.

2.8 Quality of IRS programme and insecticide decay rate

Quality assurance of IRS programs of ***Sumishield 50WG*** was done on the first seven days of the operation in order to assess the spray quality and sprayer performance which was then followed by monitoring insecticide decay rate on a monthly basis. Six shehias, were involved in both Unguja and Pemba. The shehias were selected randomly per district. Fifteen houses per shehias built up using common wall surfaces namely mud, cement, oil paint, water paint and stone block were selected for the quality assay and decay rate assessment. Three houses made of each wall surface type were tested in each shehia.

2.9 Test procedures for assessment of IRS quality and insecticide decay rate

- Bioassays were conducted in accordance with WHO (2006) guidelines. A susceptible colony of laboratory-reared *An. gambiae* s.s. (R-70 strain) was used for the cone bioassays on the different wall surface types.
- Two to five days old, non-blood fed females of *An. gambiae* s.s. R-70 from the ZAMEP insectary were exposed to sprayed wall surfaces in batches of ten mosquitoes for 30 minutes under WHO cones.
- Cones were attached to walls at two different heights in each of the two rooms sampled in a house: at a lower level (1.0-meter-high) and an upper level (1.5 meters high).
- At the end of the test, mosquitoes were transferred to paper cups and supplied with cotton pads soaked with sugar solution.
- First knockdown was scored after 30 minutes followed by 60 minutes post exposure and mortality scored and recorded after 24 hours holding period for seven days. Moribund and dead mosquitoes were counted as dead.
- Negative control bioassays were conducted on unsprayed surfaces covered with manila sheet layers. When control mortality was scored between 5% and 20%, experimental mortality was corrected using Abbott's formula (Abbott, 1925). If the mortality score was more than 20%, the experiment was discarded.

2.10 Insecticide susceptibility test

The entomology team assessed the susceptibility of local vectors against common insecticides thus guiding the IRS campaign on insecticide to be applied. The test was carried out across the sentinel sites in 10 districts. (six in Unguja and 4 in Pemba). The tests used WHO discrimination doses of Permethrin (0.75%), Deltamethrin (0.05%) and Bendiocarb (0.01%) Alpha-cypermethrin 0.05% Clothianidin 2% and Pirimiphos methyl 0.25%.

Strength/intensity of pyrethroid resistance against malaria vectors was determined at concentrations of 5x to 10x of Alpha-cypermethrin deltamethrin Permethrin and lambda-cyhalothrin in WHO impregnated paper in Pemba.

2.11 Mosquito collection

Wild *Anopheles* larvae and pupae were collected in various breeding sites from established entomological surveillance sites ranging from home yards, cultivated land and rice fields. Therefore, mosquitoes obtained could be representative of the vectors available in the areas. Mosquito larvae were collected with a 350 ml dipper and transferred into plastic containers, which were then loosely capped to allow aeration. These were transported in cool boxes to the ZAMEP insectary in both Unguja and Pemba where they were reared at 27-30°C. Larvae collected from several breeding sites in the same village were pooled together for rearing and testing. The larvae were fed with Tetramin® fish food. The development of the larvae was monitored regularly and all those that pupated were transferred into shallow plastic cups/small beakers using Pasteur pipettes, and then placed in appropriately labelled cages for adult emergence. Female adult mosquitoes aged 2-5 days were used for WHO susceptibility tests and PBO synergy testing.

2.12 Insecticide Resistance Test procedures

The susceptibility tests were carried out using the World Health Organization test kits for adult mosquitoes (WHO, 2016). Two to five-day old female F1 generation mosquitoes were tested using standard WHO insecticide susceptibility procedures with four replicates of 25 wild adult female mosquitoes per test tube. Mosquitoes were exposed to papers impregnated with the WHO-recommended discriminating concentrations of deltamethrin (0.05%), Bendiocarb (0.1%), Permethrin (0.75%) Alpha alpha-cypermethrin (0.05%), Pirimiphos-methyl (0.25%) and Clothianidin (2%) .

At the end of the exposure period, mosquitoes were transferred into holding tubes (lined with untreated papers) by gently blowing them through the open space between the exposure tube and the holding tubes. Cotton soaked in 10% sugar was placed on top of the holding tube. This is to avoid death by starvation. The mortality was scored 24 hours post-exposure except for clothianidin whereby the score and record continued for seven days. The susceptibility status was evaluated based on the WHO criteria i.e. 98-100% mortality indicate susceptibility; 90-97% mortality required confirmation and less than 90% mortality indicate resistance (WHO, 2016). When the control mortality between 5% and 20% was recorded, the mean observed mortality was corrected using Abbott's formula (Abbott, 1925). All tested mosquitoes were preserved with *silica gel* in 1.5 ml eppendorf tubes and transported to ZAMEP molecular laboratory for further laboratory analyses (molecular species identification and detection of mechanisms of insecticide resistance).

2.13 PBO – Synergist Bioassays

The aim of this test was to ascertain the involvement of mixed function oxidases in the observed phenotypic resistance. In this test, 2–5 days old F1 adult mosquitoes

were pre-exposed to 4% Piperonyl butoxide (PBO) paper for 1 h and immediately exposed to 0.75% Permethrin, or 0.05% Alpha alpha-cypermethrin for 1 h. Two controls were used during this experiment: control constituted mosquitoes exposed to clean papers neither with insecticides nor with PBO, while control 2 constituted mosquitoes exposed to papers treated with PBO only. Mortalities were later assessed after exposure; the PBO synergized group was compared to the un-synergized group after 24 h post-exposure. This comparison was used to evaluate the potential role of Monooxygenase enzyme in the observed resistance.

2.14 Molecular identification of *An. gambiae* s.l. and *An. funestus*

Anopheles gambiae sibling species identification was carried out according to the standard polymerase chain reaction (PCR) method (Scott *et al.*, 1993). Five oligonucleotide primers, GA, ME, AR, QD and UN designed from the DNA sequences of the intergenic spacer region of complex ribosomal DNA (rDNA) were used to amplify species-specific DNA sequences. The UN-primer is universal and anneals to the same position on the rDNA sequences of all five species, the GA anneals specifically to *Anopheles gambiae s.s.*, the ME anneals to either *Anopheles merus* or *Anopheles melas*, AR to *Anopheles arabiensis* and the QD to *Anopheles quadriannulatus*. The PCR reaction mix of 25 µl contained 1 X PCR buffer (constituents), 200 µM of each of the deoxyribonucleotide triphosphates (dNTPs), 20 µM of oligonucleotide primers, 0.125 units of Taq Polymerase enzyme (Sigma, USA) and 0.5 µl of the extracted genomic DNA. Sterile double distilled water was added to make up the volume to 25 µl. The reaction mix was spun down briefly at 14,000 rpm and overlaid with mineral oil to avoid evaporation and refluxing during thermo-cycling.

The amplification reactions were carried out using PTC 100 thermal cycler (MJ Research Inc., USA) and the cycling parameters were as follows: 3 minutes at 94ºC (initial denaturation), followed by 35 cycles with denaturation at 94ºC for 30 seconds, annealing at 50ºC for 30 seconds and extension at 72ºC for 60 seconds and ended with a final cycle at 94ºC for 30 seconds, annealing at 50ºC for 30s and extension at 72ºC for 10 minutes. For each reaction, a positive control containing 0.5 µl of PCR products of *An. gambiae s.s* as template DNA and a negative control that contained no DNA template were included.

The amplified products were analysed by agarose gel electrophoresis. Ten micro-litres of each PCR product were added to 1µl of 10x Orange-G loading dye and electrophoresed in 2% agarose gel stained with 0.5 µg/ml of ethidium bromide. The electrophoresis was run in 1X Tris acetate-EDTA (TAE) buffer at 100V for one hour and were visualized and photographed over a UVP dual intensity trans-illuminator at short wavelength using a digital camera fitted with an orange filter and a hood. The amplified PCR product was identified to the sibling species on the basis of the diagnostic band size determined by comparison with the mobility of a standard 100bp DNA ladder (Sigma, USA).

2.15 Detection of target site resistance mechanisms

The PCR-based standard protocol used for the detection of the L1014S or L1014F kdr alleles was adapted from the protocols developed (Martinez-Torres, Chandre et al. 1998; Ranson, Jensen et al. 2000). All wild-caught *An. gambiae* s.l. from each of the above-mentioned sentinel sites were tested for KDR mutation.

Amplification was performed in a 25 µl reaction containing 2 µl of template DNA, GoTaq 5X PCR Buffer (containing 15 mM MgCl2), MgCl2 (25mM), dNTP (2-2.5 mM mix), dNTP (2-2.5 mM, and 1 5 U/µl of Go-Taq DNA polymerase, 2.5 pmol/µl for both IPCF and IPCR, 2.5 pmol/µl East primer and 8.8 pmol/µl for West primer.

The *kdr* genotyping of susceptible and resistant individuals was possible after amplifying the DNA template from mosquitoes following the PCR conditions of 95°C for 5 minutes (initial denaturation), followed by 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds. For the East Africa *kdr* amplification, there was a final extension cycle of 72°C for 5 minutes followed by 4°C coolingFor west African *kdr* amplification, the PCR conditions involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 59°C for 30 seconds and 72°C for 30 seconds. There was a final extension cycle of 72°C for 5 minutes followed by cooling at 4°C. The products were electrophoresed through 2% agarose gel with ethidium bromide stain and visualized under UV light. Knock down resistant (*kdr*) genotypes of both the susceptible and resistant individuals were then recorded. All successful reactions should contain a band of 285 bp. In addition, a band of 210 bp indicates the susceptible (wild type) allele and one of 188 bp the resistant allele.

3.RESULTS

3.1 Insecticide Residual efficacy monitoring

Clothianidin 50WG efficacy residue against susceptible *Anopheles gambiae s.s* R70 in WHO cone wall bioassays

Quality assurance of IRS programme was done in the first week of the operation to assess the quality of indoor residual spraying and spray team performance. This was followed by insecticide decaying rate assessment monthly.

≥ 80% mosquito mortality at the age of 270 days of Clothianidin deposit is detected at various wall surfaces, indicating that insecticide deposit efficacy can cover the long transmission period. However, the killing power of insecticide for 24 - 48 hours is losing as the age increases

Figure 1: Clothianidin efficacy residue at the age of 270 in Pemba

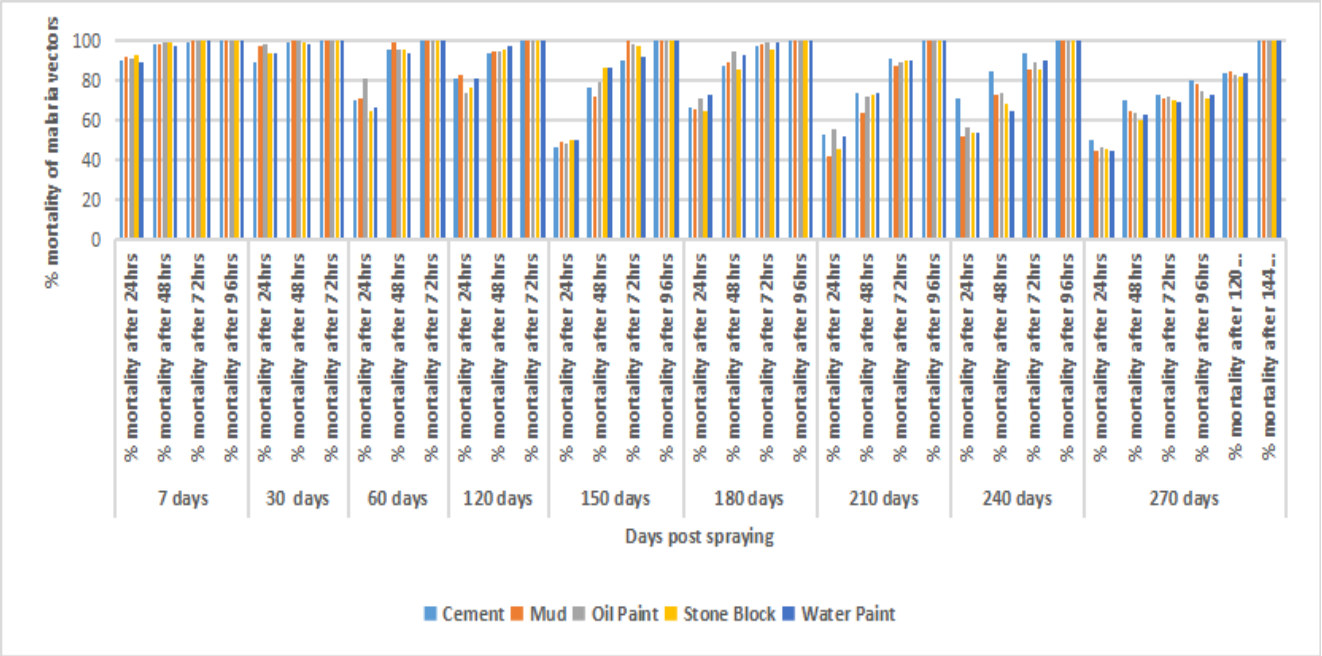
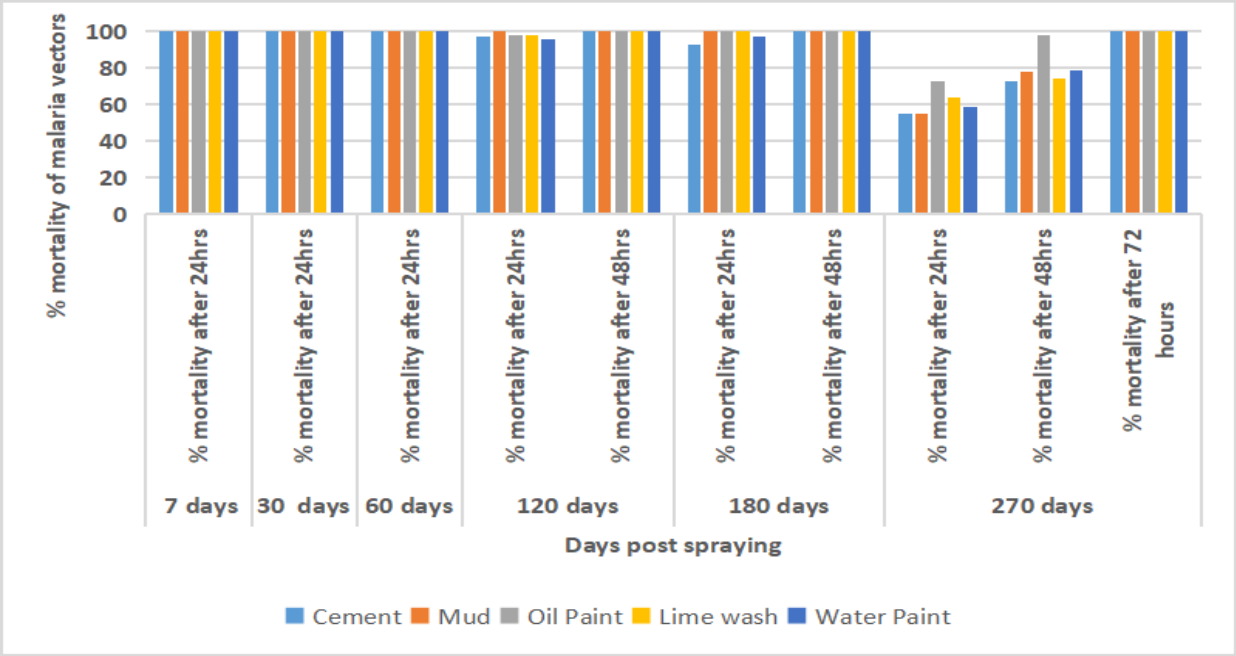


Figure 2: Clothianidin efficacy residue at the age of 270days in Unguja



3.2 Insecticide resistance monitoring in Zanzibar

3.2.1 Susceptibility test of *An gambiae* complex

The results of the WHO susceptibility tests indicated that *Anopheles gambiae* s.l. from both sprayed and non-sprayed sentinel sites in Pemba and Unguja were fully susceptible to Pirimiphos-methyl, Clothianidin and Bendiocarb. However, the vectors were found to be resistant to Permethrin, deltamethrin, lambda cyhalothrin and alpha-cypermethrin across all the tested sites.(see table below)

Table 1: Susceptibility test results of *An. gambiae complex* against various insecticides - Pemba

Site	Percentage mortality (95% CI)						
	Permethrin	Deltamethrin	Alphacypermethrin	Lambda cyhalothrin	P-methyl	Bendiocarb	Clothianidin
Bopwe	43 (36 - 49)	22 (6 - 38)	34 (10 - 55)	27 (16 - 38)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Tumbe	52 (40 - 63)	35 (28 - 41)	49 (34 - 63)	48 (38 - 58)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Uwandani	46 (42 - 50)	15 (10 - 21)	10 (2 - 15)	40 (30 - 50)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Wambaa	60(46 - 74)	77 (58 - 96)	30 (21.78 - 38.2)	68 (58.9 - 77)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)

Key Possible resistance 90 - 97% Resistance <90% Susceptible ≥ 98%

Table 2: Susceptibility test results of *An. gambiae complex* against various insecticides - Unguja

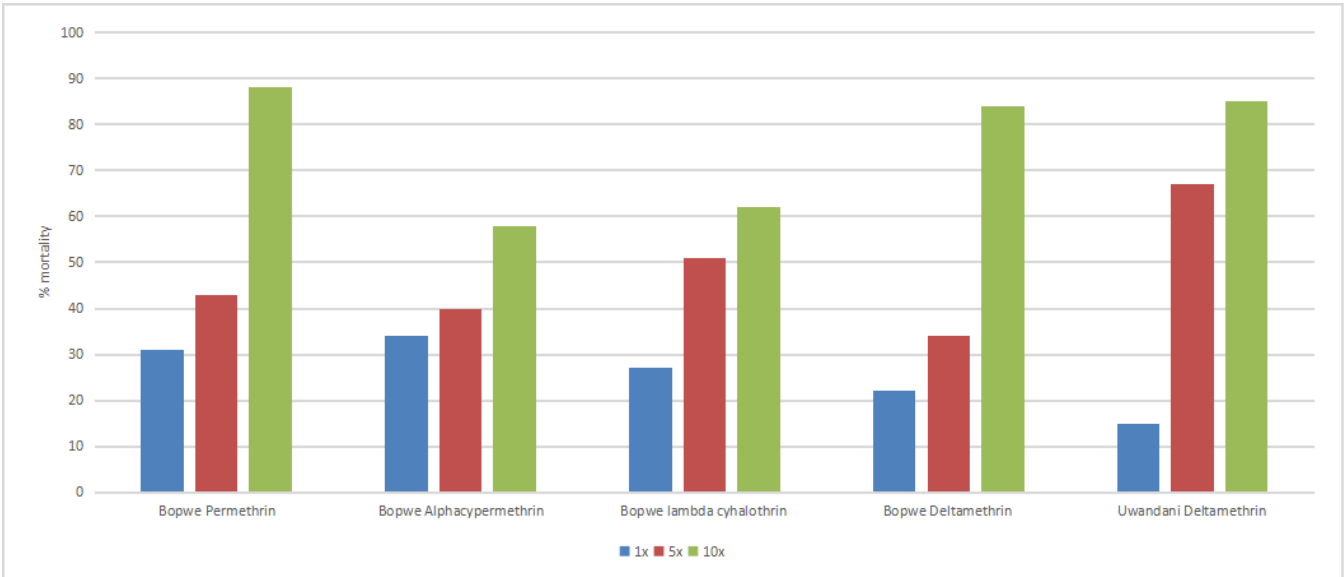
Site	Percentage mortality (95% CI)						
	Permethrin	Deltamethrin	Alphacypermethrin	Lambda cyhalothrin	P-methyl	Bendiocarb	Clothianidin
Mwera	95 (89- 100)	98 (90 -100)	86 (71-100)	81 (75 - 100)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Donge	90(79 - 100)	81(75 - 100)	86 (70 - 100)	87 (80- 95)	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Cheju	46 (42 - 50)	Not tested	66(58 - 74)	Not tested	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Muyuni	60(46 - 74)	89 (83 - 95)	89(83 - 95)	Not tested	100 (100 - 100)	100 (100 - 100)	100 (100 - 100)
Bumbwini	89 (83 - 95)	96 (90 - 100)	82 (70 -95)	87 (75 -95)	97(90 - 100)	100 (100-100)	100 (100-100)
Stonetown	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested	Not tested

Key Possible resistance 90 - 97% Resistance <90% Susceptible ≥ 98%

3.2.2 Strength of pyrethroid resistance

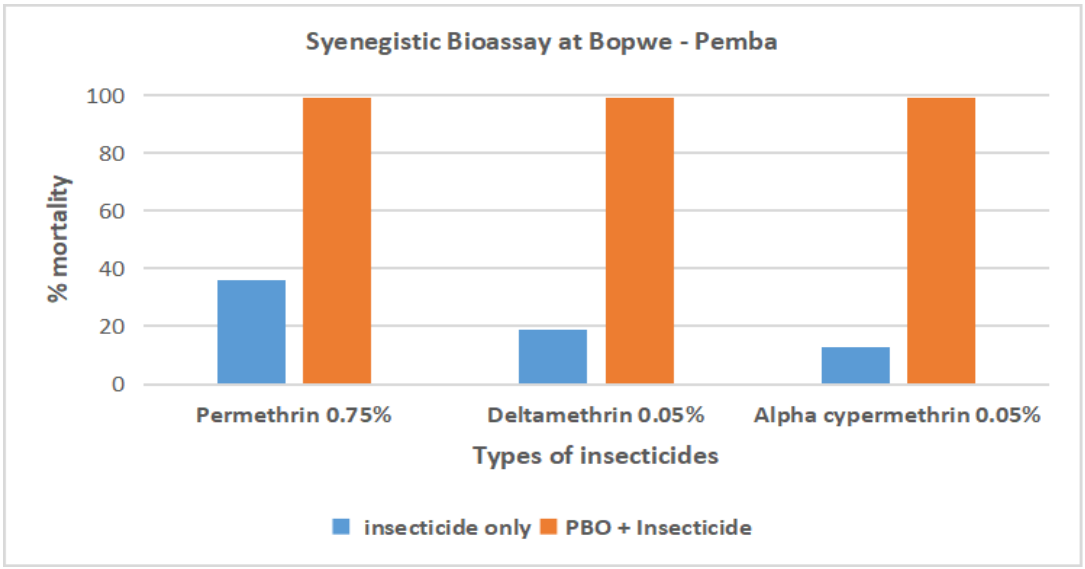
The results obtained from Bopwe and Uwandani - Pemba indicate that, *An. gambiae* s.l. mosquitoes had high resistance to 5× and 10x diagnostic dose of alpha-cypermethrin, Permethrin, Deltamethrin and lambda cyhalothrin as shown in graph below. The team in Unguja did not manage to conduct this test due to shortage of mosquitoes.

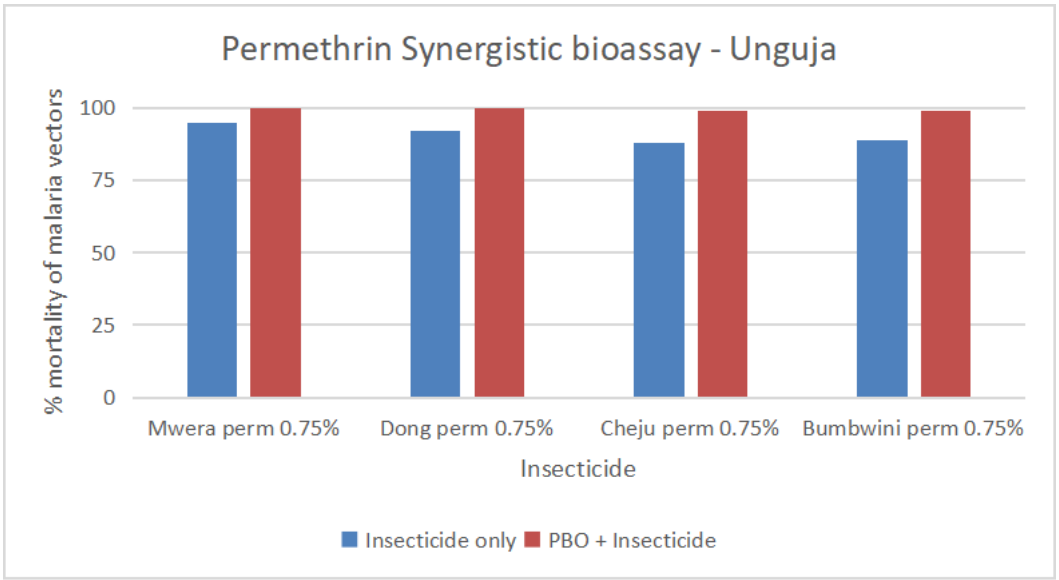
Figure 3: Pyrethroid intensity assay results – Pemba 2020

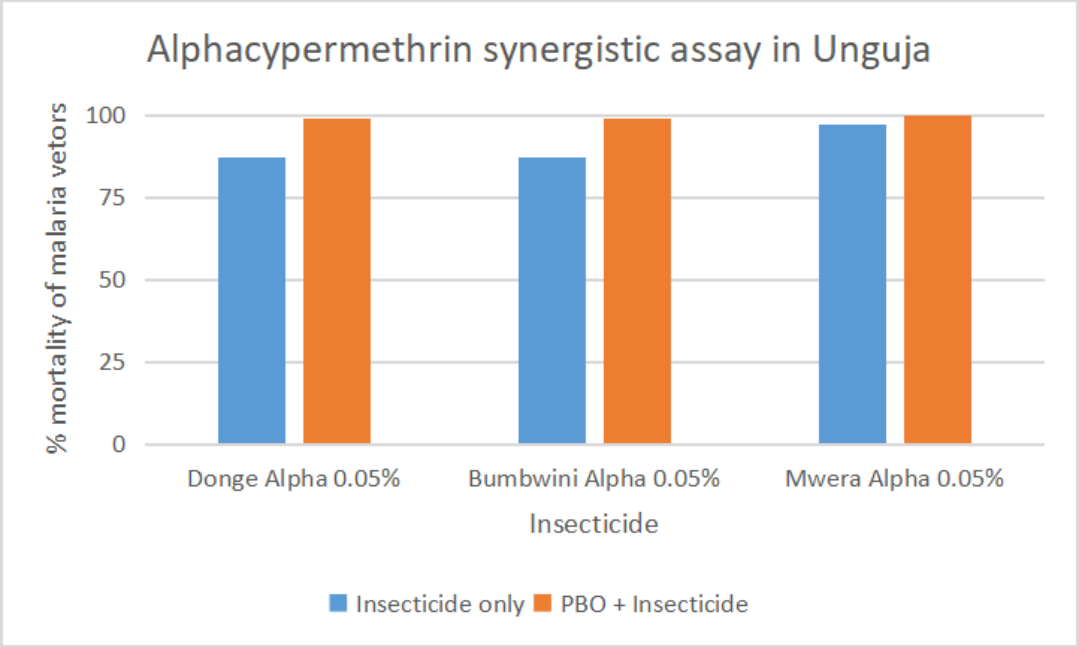


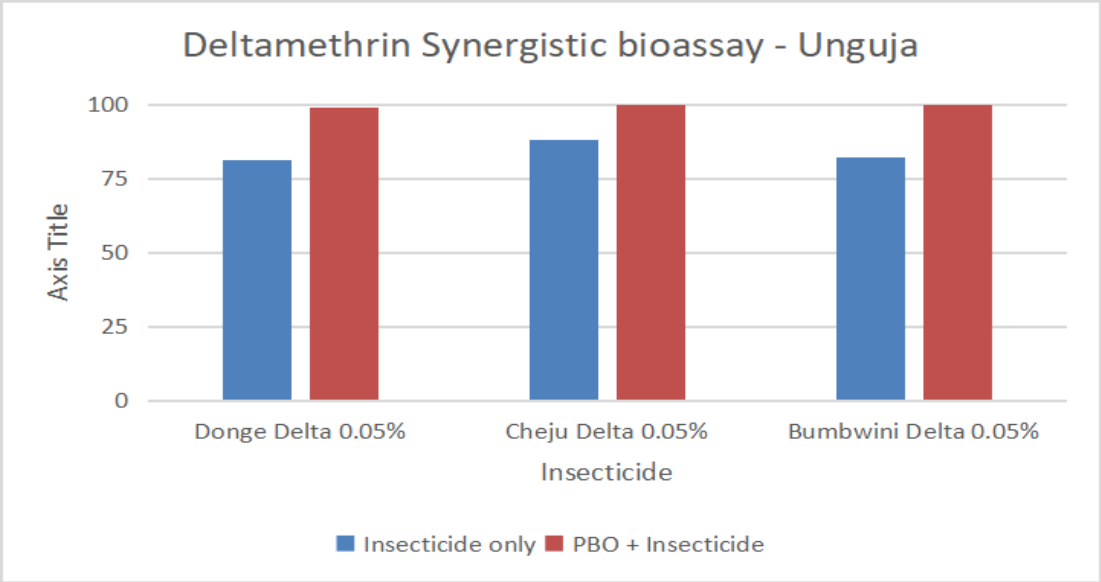
3.2.3 Mechanism of pyrethroid resistance to *An. gambiae complex*

The graph below shows, complete restoration of susceptibility (mitigation of resistance) after exposing *An. gambiae complex* from Bopwe site in Pemba to PBO followed by Permethrin 0.75%, Deltamethrin 0.05%, Alpha cypermethrin 0.05%, implies that a monooxygenase-based resistance mechanism fully accounts in vector population. The introduction of PBO nets within the community could have additional value in malaria protection









3.2.4 Species demonstrated in resistance samples and target site

The Pemba team sent Six hundred and twelve (612) vectors survived in susceptibility test, the strength of resistance and synergistic test experiment for knock-down resistance test (KDR) and species composition. The results revealed that 96% and 4% were *An. arabiensis and An merus* relatively. We failed to conduct KDR as the facility had no reagents.

3.3 Vector bionomics in Zanzibar

Abundance, distribution and species composition in Zanzibar

In a one year time (October 2019 - September 2020), entomology team collected a total of 2781 female Anopheles mosquitoes (1651) in Pemba and 1130 in Unguja). of the total amount collected in Pemba 1543(93.4%) were morphologically identified as *An gambiae complex, 43 (3%) An funestus, 36 (2%) An coustani, 16(0.96%) An Ziemani, 10 (0.6%) An rufipes, 2 (0.1%)An maculipalpis and 1 (0.06%)An pretoriensis*. In Unguja 100% were morphologically identified as *An gambiae complex*.

HLC method was the most capable sampling technique by collecting 82% and, 50.5% of the total *Anopheles* catches in Pemba and Unguja respectively followed by Pit trap method. However, other techniques succeeded to collect in Pemba as sequentially arranged as Prokopack aspirator/PSC followed by LTC
An. gambiae s.l. was the predominant vector species sampled by all collection methods in each sentinel site, although other areas had a small number of mosquitoes including stone town and Muyuni in Unguja.

Table 3: Morphological identification of malaria vectors in Pemba October 2019 to September 2020

Sentinel site	IRS status 20	Morphological ID	LTC	HLC	Prokop	PTC	Grand Total
		An gambiae complex	6	342	9	16	373
Bopwe		*An ziemani*	0	6	0	0	6
	NO	*An rufipes*	0	1	0	0	1
		An funestus group	0	4	0	3	7
		An maculipalpis	0	1	0	0	1
		An coustani	0	4	1	0	5
Bopwe Total			**6**	**358**	**10**	**19**	**393**
		An costani	1	13	1	1	16
	Yes	*An gambiae complex*	9	461	20	64	554
		An, ziemani	1	6	0	1	8
Tumbe		*An. funestus group*	0	3	0	10	23
		An rufipes	0	0	0	6	6
Tumbe Total			**11**	**483**	**21**	**82**	**597**
Uwandani	No	*An gambiae complex*	0	13	11	66	90
		An coustani	0	0	0	7	7
		An funestus	0	0	2	7	9
		An ziemani	0	0	0	1	1
		An maculipalpis	0	0	0	1	1

Uwandani Total			**0**	**13**	**13**	**82**	**108**
		An coustani	1	7	0	0	8
		An gambiae complex	23	488	11	14	536
Wambaa		*An rufipes*	0	3	0	0	3
		An funestus group	0	4	0	0	4
		An pretoriensis	0	1	0	0	1
Wambaa Total			**24**	**503**	**11**	**14**	**544**
Grand Total							**1651**

Table 4: Morphological identification of malaria vectors in Unguja October 2019 to September 2020

Sentinel site	IRS status 20	Morphological ID	LTC	HLC	PIT	PSC	Grand Total
BUMBWINI	Yes	*An gambiae* complex	0	90	0	0	90
BUMBWINI Total			**0**	**90**	**0**	**0**	**90**
CHEJU	Yes	*An gambiae* complex	0	411	0	0	411
CHEJU Total			**0**	**411**	**0**	**0**	**411**
DONGE	Yes	An gambiae complex	0	65	0	0	65
DONGE Total			**0**	**65**	**0**	**0**	**65**
MUYUNI	Yes	*An gambiae* complex	0	3	0	0	3
MUYUNI Total			**0**	**3**	**0**	**0**	**3**
MWERA	Yes	*An gambiae* complex	0	1	0	559	560
MWERA Total			**0**	**1**	**0**	**559**	**560**
STONE TOWN	No	*An gambiae* complex	0	1	0	0	1
STONE TOWN Total			**0**	**1**	**0**	**0**	**1**
General Total							**1130**

3.4 Molecular analysis for mosquito species composition and sporozoite rate

The team analyzed all samples of females *Anopheles* identified as *An gambiae complex and Anopheles funestus group* from both Unguja and Pemba regardless of the spraying status of sentinel sites. Note that in Unguja five sentinel sites out of six had positive spray status whilst in Pemba, we sprayed two surveillance sites out of four
In Pemba, of the 1,617 samples analyzed for the species identification, 86% were *An arabiensis* followed by 6% *An merus* and 8% is a combination of *An parensis*, *An gambiae s.s*, *An revulorum*, *An lessoni* and *An veneedine*.
In Unguja 1054 sample analyzed for species composition 87% were *An arabiensis*, 6% is a combination of *An merus, An lessoni*, *An gambiae s.s*, *An parensis*, *An lessoni*, *An revulorum* and 7% did not amplify. *Anopheles arabiensis* was the most abundant species in all sites regardless of their spraying status. See the proportions of species heterogeneousness in all sprayed and unsprayed sites in the table below

Table 5: Molecular species identification in Pemba (October 2019 - September 2020)

site	IR status 20	No tested	*An arabiensis*	*An gambiae*	*An merus*	*An parensis*	*An lessoni*	*An veneedine*	*An revulorum*
Tumbe	Yes	583	*464 (80%)*	*9*	*75*	*1*	*28*	*1*	*5*
Bopwe	NO	386	*374 (90%)*	*0*	*5*	*0*	*9*	*0*	*0*
Uwandan	No	98	*84 (86%)*	*0*	*5*	*5*	*4*	*0*	*0*
wambaa	Yes	550	*469 (85%)*	*0*	*7*	*0*	*4*	*0*	*0*
Total		**1617**	***1391***	***9***	***92***	***6***	***45***	***1***	***5***

Table 6: Molecular species identification in (Unguja October 2019 - September 2020)

site	IR status 20	No tested	*An arabiensis*	*An gambiae*	*An merus*	*Not amplified*	*An lessoni*	*An veneedine*	*An revulorum*
Bubwini	Yes	89	*62 (70%)*	*0*	*12*	*2*	*14*	*0*	
Cheju	Yes	383	*346 (90%)*	*1*	*17*	*25*	*18*	*0*	*1*
Donge	Yes	65	*44 (68%)*	*0*	*15*	*4*	*2*	*0*	*0*
Mwera	Yes	513	*462 (90%)*	*0*	*1*	*41*	*2*	*2*	*5*
Muyuni	Yes	3	*1 (33%)*		*1*	*0*	*1*	*0*	*0*
Stone twon	No	1	*1 (100%)*	*0*	*0*	*0*	*0*	*0*	*0*
Total		**1054**	***916***	***1***	***46***	***72***	***37***	***2***	***6***

3.5 Malaria vector infectivity

The average sporozoite rate in Pemba was 1.3%, varied across the surveillance sites ranging from 0% - 2.6%. Wambaa had higher sporozoite rate (2.6%), followed by Tumbe (0.83%), Bopwe (0.5%) and lastly Uwandani with 0%.

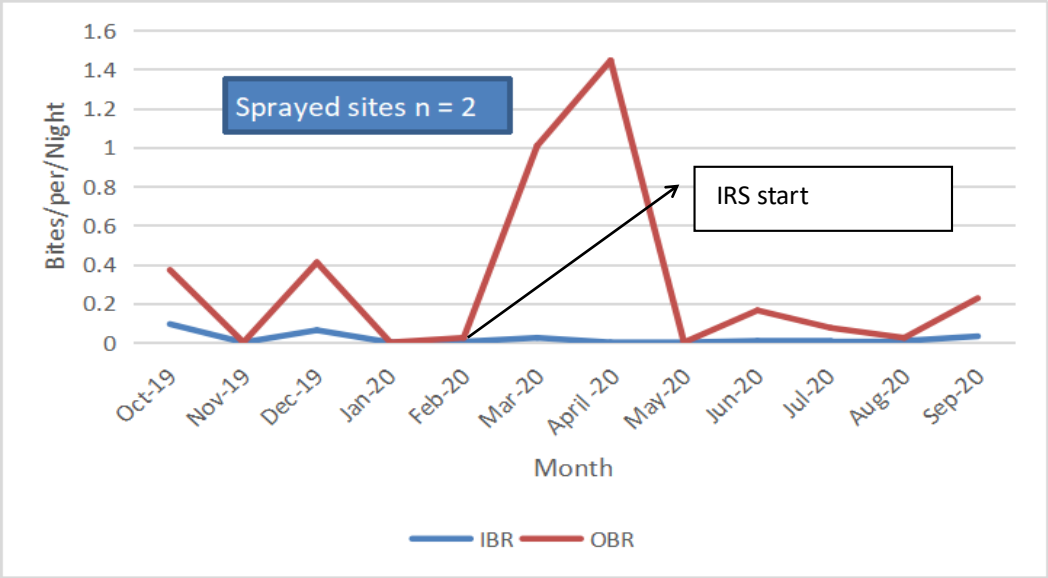
The mean sporozoite rate in Unguja was 0.5%, ranging from 0% - 1.2% in the surveillance site. Cheju is the only site with sporozoite positive vectors. Plasmodium infection within the vectors corresponds to a few malaria cases in Zanzibar. However, Unguja reports over 90% of the cases. Further analysis indicated that *An merus* had higher sporozoite rate in Pemba 7.6% (n = 92) followed by *An arabiensis* 1% (n = 1391).

In Unguja, the analysis indicated that *An gambiae s.s* had higher sporozoite rate of 100% (n = 1) followed by *An arabiensis* 0.4% (n = 916). Moreover, of the twenty collected sporozoite vectors in Pemba, 15 or (75%) were collected using HLC outdoor whilst Unguja team 100% (n = 4) collected Positive vectors out of door using HLC. The findings suggest outdoor transmission of malaria in Zanzibar.

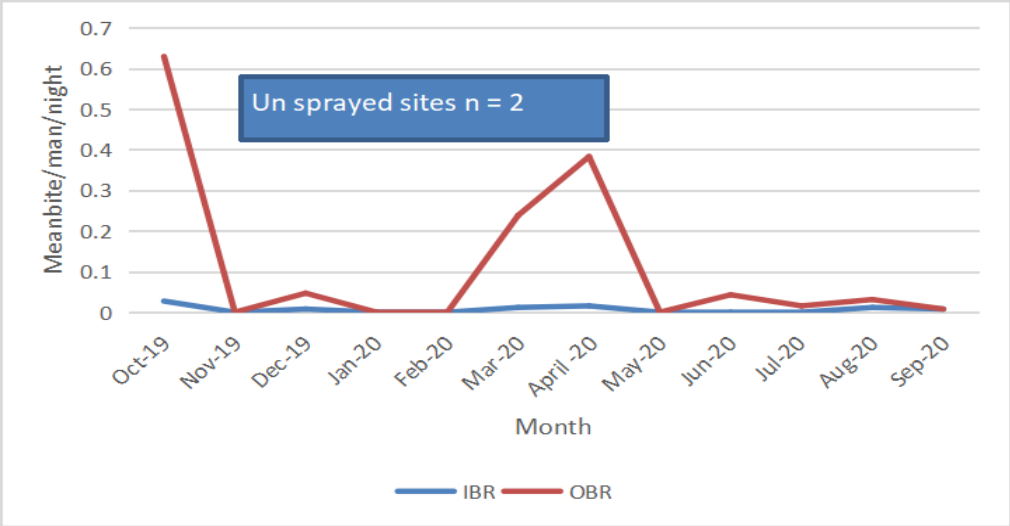
3.6 Biting density of *An gambiae complex* as expressed by HLC

The outdoor biting density of *An gambiae s.l* is significantly higher throughout the year in both sprayed and unsprayed surveillance sites. However, the indoor biting of the vectors in Unguja seems to be close with outdoor biting, as shown in the graph. Indoor biting of *An gambiae s.l* is maintained at the lower level, suggesting a high risk of contracting malaria when you stay outdoor of the house at night hours.

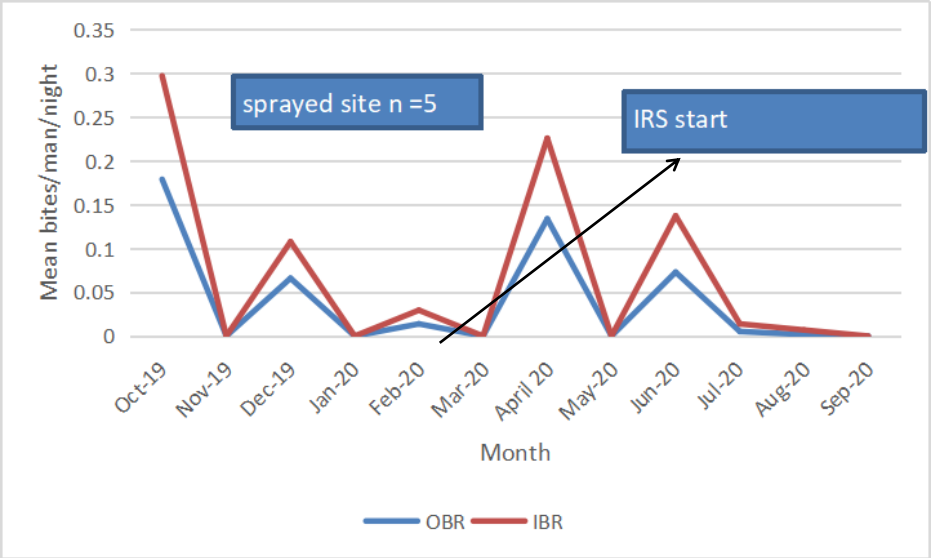
Mean indoor and outdoor bite of *An gambiae s.l* in sprayed sites in Pemba



Mean indoor and outdoor bite of *An gambiae s.l* in non-sprayed sites in Pemba



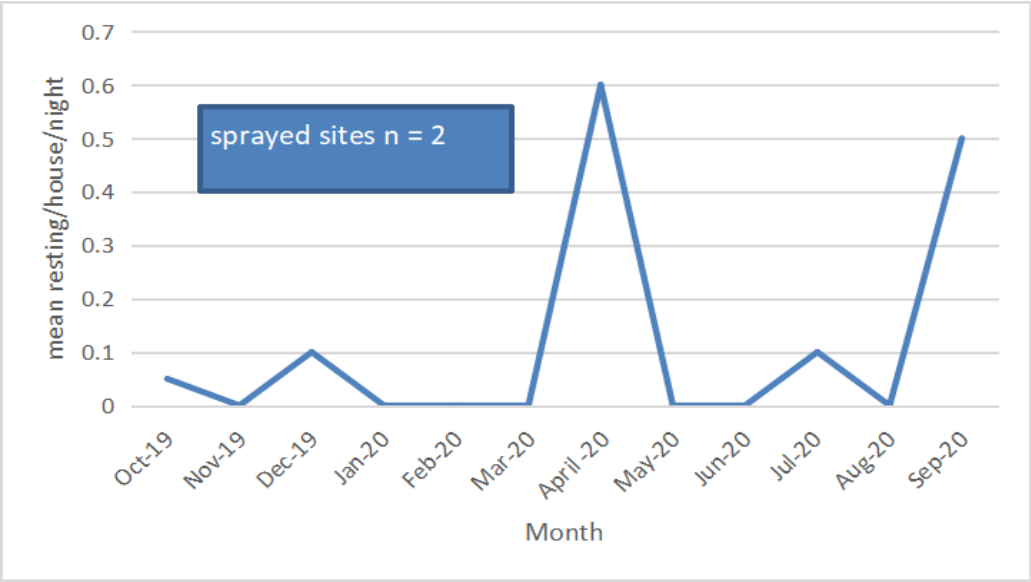
Mean indoor and outdoor bite of *An gambiae s.l* in sprayed sites in Unguja



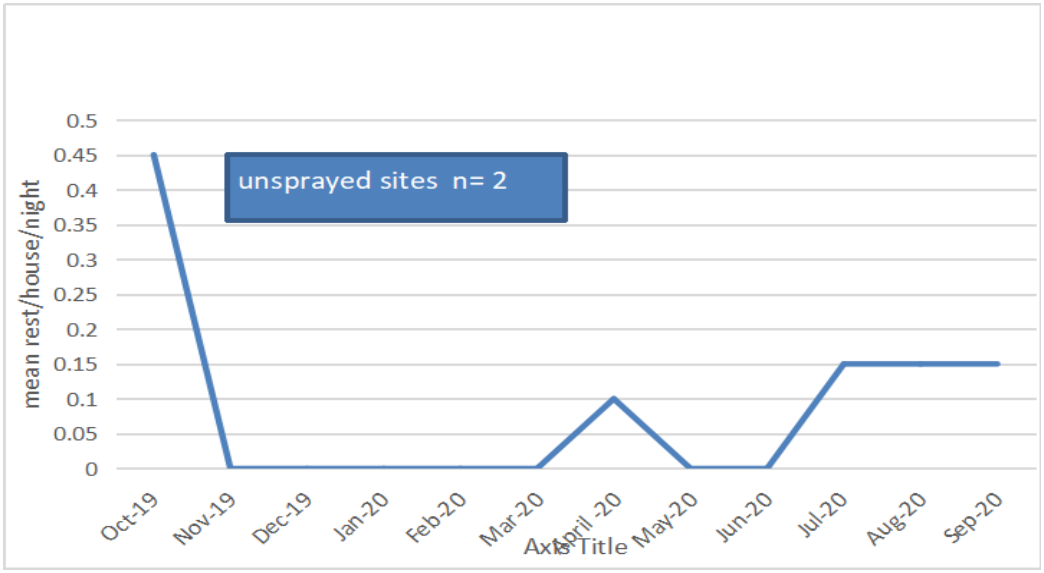
3.7 Indoor resting density of female *Anopheles gambiae s.l* in Pemba

Indoor Resting densities of vectors is expressed as the number of females per house per night collected using PSC/ Prokopack. Generally, the indoor resting density of *An gambiae s.l* was very low in both sprayed or unsprayed sites. However, we have noted the highest peak of indoor resting of *An gambiae s.l* in April at 0.6 per house/ night in chemical sprayed surveillance sites. The possible cause of the situation is high coverage of LLINs at the household level, which repels vectors outside.
We did not calculate Indoor resting density of An gambiae s.l in Unguja because the entomology team did not manage to collect the indoor resting vectors.

Average indoor resting density of *An gambiae sl* at sprayed sites in Pemba

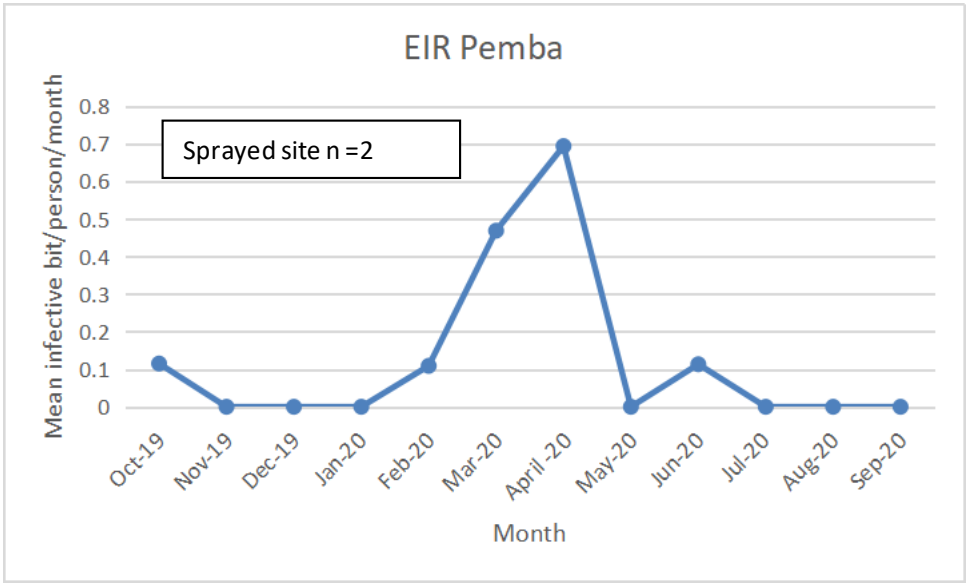


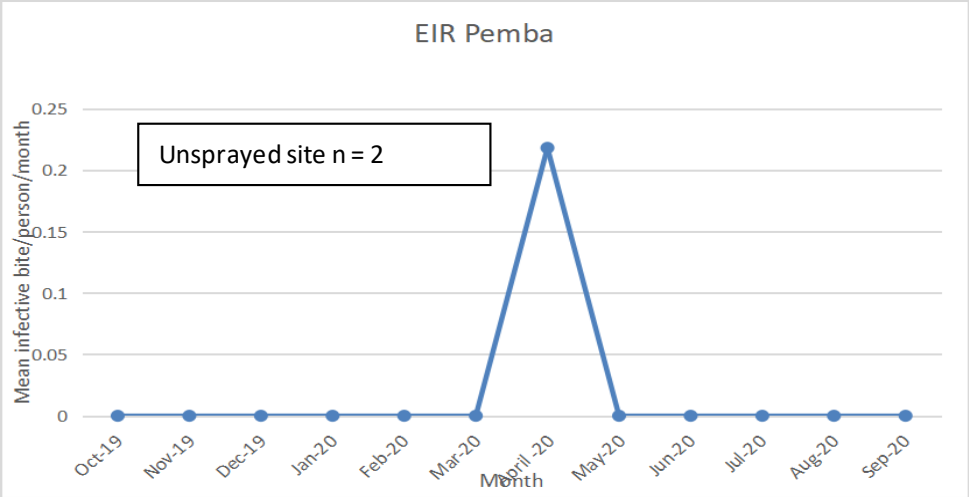
Average indoor resting density of *An gambiae sl* at unsprayed sites in Pemba

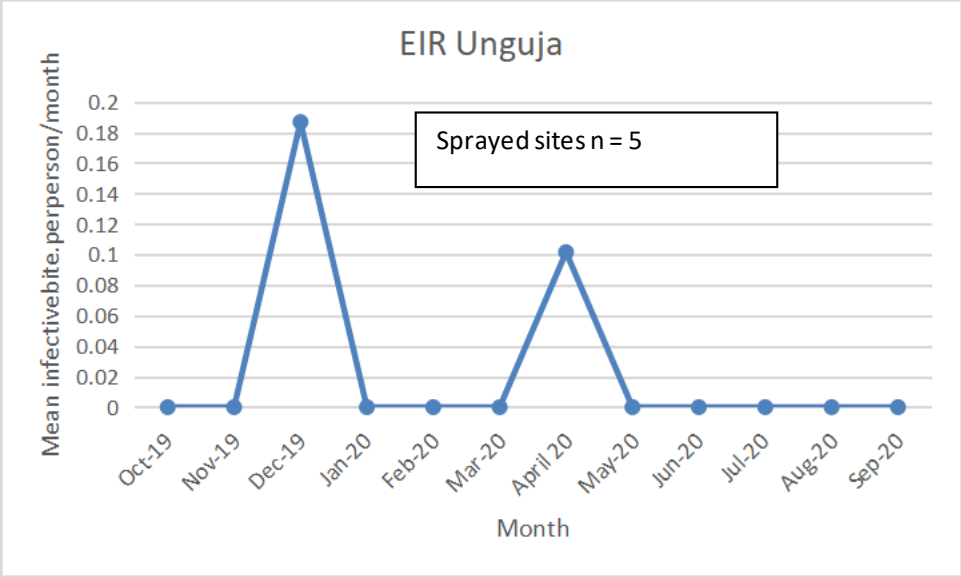


3.8 Entomological inoculation rate (EIR) of *An gambiae s.l* in Zanzibar

The EIR is the number of infectious bites per person per unit time, usually measured or expressed per year. It is the product of the human biting rate and the sporozoite rate It measures the intensity of malaria transmission and has a Direct reflection of vector control and antigametocytocidal drugs. Entomological inoculation rate in both sprayed and unsprayed sites is below one (1) which correspond to very low malaria infections on the islands. We are using IRS and LLINs as preventive tools for many years with disease prevalence and EIR are maintained to less than one. It is possible to push EIR to zero if we can supplement larval source management method to boost IRS and LLINs







4. DISCUSSION POINTS

- *An arabiensis* is still predominant vector exhibiting exophagic behaviour
- EIR is gradually increasing- giving an indication of the presence of indigenous malaria cases
- *An merus* should not be ignored, they might be good vectors even if the population density is low.
- Complete restoration of susceptibility (mitigation of resistance) after exposing *An. gambiae* complex to PBO followed by pyrethroids (Permethrin 0.75%, Deltamethrin 0.05%, Alpha cypermethrin 0.05%), implies that a

monooxygenase-based resistance mechanism are responsible for the observed phenotypic pyrethroid resistance in vector population.

- Introduction of LLINs with pyrethroids + PBO within the community could have an additional value in malaria protection. Nevertheless, outdoor biting vectors might not be affected by these new generation LLINs..
- The local malaria vectors are fully susceptible to pirimiphos-methyl, bendiocarb and clothiadin.
- *An gambiae* abundance and infection rate corresponds with the rainfall patterns.
- In the presence of outdoor biting *An arabiensis* calls for supplementary vector control methods so as to push the EIR to zero.

Challenges

- Failed to conduct blood meals analysis of mosquito vectors and detect knock down resistance mechanism in collected mosquito samples due to lack of reagents.
- Covid 19 interfered with the smooth implenation entomological monitoring activities.
- The flow of entomological monitoring funds is not certain, leading to delay in implementation