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PMI VECTORLINK ETHIOPIA PROJECT
FINAL ENTOMOLOGY REPORT
MAY 2019-MARCH 2020

Recommended Citation: The PMI VectorLink Project. June 2020. PMI VectorLink Ethiopia Project Final Entomology Report May 2019-March 2020. 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/PMI

Submitted on: 30 June 2020

Approved on: 14 September 2020

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS

CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
HLC	Human Landing Catch
IRS	Indoor Residual Spraying
ITN	Insecticide treated net
ND	Not Done
NMCEP	National Malaria Control and Elimination Program
P.	*Plasmodium*
PBO	Piperonyl-Butoxide
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
SNNPR	Southern Nations Nationalities and Peoples Region
WHO	World Health Organization

EXECUTIVE SUMMARY

BACKGROUND

The President's Malaria Initiative (PMI) VectorLink Ethiopia Project conducted monthly entomological monitoring from May 2019 to March 2020 in seven sentinel sites, namely Abaya, Lare, Bambasi, Jabitehnan, Harbu, Metema, and Benatsemay, to determine *Anopheles* species composition, density and seasonal variation, biting and resting habits and habitats, sporozoite infection rates, and blood meal sources. Mosquitoes were collected using human landing catches and pyrethrum spray catches from all sites and additionally CDC light traps in Bambasi. Structures that could serve as resting sites for *An. arabiensis*, the major malaria vector were investigated in Lare and Pawi. In addition, monthly longitudinal surveillance of adults *An. stephensi* was conducted in the towns of Dire Dawa and Kebridehar from June to December and in Awash and Metehara from August to December 2019, and a cross-sectional survey of *An. stephensi* was conducted in 11 towns, namely Metehara, Meki, Zeway, Hawassa, Negelle Borena, Yabello, Jimma, Gambela, Assosa, Bahirdar, and Shire. Adult *An. stephensi* were raised from wild collected larvae and identified to species. Circumsporozoite and blood meal enzyme-linked immunosorbent assays (ELISAs) were applied to examine sporozoite infections in *An. arabiensis*, *An. pharoensis* and *An. stephensi* and blood meal sources of *An. arabiensis* and *An. stephensi*. Polymerase chain reaction (PCR) was used to identify members of *An. gambiae* s.l and *An. funestus* group. Insecticide resistance monitoring through susceptibility tests was done on *An. arabiensis* in 21 sentinel sites and on *An. stephensi* in five sites. Resistance intensity assays were conducted in seven sites, and synergist piperonyl-butoxide (PBO) assays were conducted in 11 sites. The decay rate of Actellic 300CS applied during the 2019 indoor residual spraying (IRS) campaign was evaluated in Abaya, Lare, and Bambasi, through cone bioassays. Additional evaluations on the airborne effect of the insecticide were also carried out through parallel fumigant assays.

RESULTS

A total of 10,706 *Anopheles* and 33, 478 culicines were collected. At least eight species of *Anopheles* occurred in the sentinel sites: *An. arabiensis*, *An. pharoensis*, *An. funestus* group, *An. coustani*, *An. ziemanni*, *An. squamosus/cydippis*, *An. tenebrosus,* and *An. demeilloni*. Of all the *Anopheles* sampled *An. arabiensis* and *An. pharoensis* comprised 43.0% and 20.2%, respectively. These two species are considered the principal and secondary malaria vectors in Ethiopia. Another secondary vector, *An. funestus*, constituted only 4.3% of all collections. Polymerase chain reaction assays on 155 specimens morphologically identified as *An. funestus* group from Bambasi showed 7.8% were *An. funestus* s.s., 91.0% were *An. parensis,* and 0.6% each were *An. rivolurum* like and *An. leesoni*. Past and present molecular studies established *An. arabiensis* to be one of the two members of *An. gambiae* s.l. that are widely distributed throughout the country. The identity of *An. arabiensis* was confirmed from 319 specimens (97.3%) out of 328 tested, from Benatsemay, Bambasi, Pawi, Lare, and Harbu. The remaining nine (2.7%) specimens didn't amplify.

In general, resting densities of *An. arabiensis* in houses were low, less than 1.0 *An. arabiensis*/house/day in most of the months. The highest density, 21.8 *An. arabiensis*/house/day, was recorded in Benatsemay in June, and the second largest, 4.5 *An. arabiensis*/house/day, was from Jabitehnan in August. The impact of IRS could not be assessed on the vector in Bambasi due to the small collections in May (pre-IRS) and in Abaya because data collection was suspended in July due to security problems.

Most vector-host contact occurred outdoors in Abaya, Lare, Bambasi, Harbu, Benatsemay, and Metema and indoors in Jabitehnan. *An. arabiensis* occurred in most months, with variable night biting rates. This species was active indoors and outdoors searching for a blood meal throughout the night. Most of the human hourly biting rate peaks were before midnight.

The overall sporozoite infection rates of *Plasmodium (P.) falciparum* and *P. vivax* in *An. arabiensis* was equal and it was 0.05% for each. The rate of *P. falciparum* infection in *An. pharoensis* was 0.11%. *An. stephensi* was found infected with *P. vivax* in Dire Dawa and Kebridehar, with infection rates of 0.5% and 0.3%, respectively.

Animal shelters were the preferred resting structures for *An. arabiensis* in both Pawi and Lare. *An. arabiensis* was also found to rest in kitchens (indoors) in Pawi.

Anopheles stephensi prevailed throughout the surveillance period in Metehara, Awash, Dire Dawa, and Kebridehar towns and most of the collections were made from animal shelters and horse stables through hand collections. Surveys in 2019 proved the presence of *An. stephensi* in Metehara, Meki, and Zeway towns; bringing the total number of sites where *An. stephensi* has been detected to 13.

Populations of *An. arabiensis* were susceptible (98-100% mortality) to pirimiphos-methyl and propoxur in all 21 sites, and to bendiocarb in 20 of the sites. *An. arabiensis* is susceptible to clothianidin in Abobo, Amibara, Omonada, Bambasi, Halaba, Dubti, and Zeway-Dugda. Susceptibility to chlorfenapyr was also detected in six of eight sites. As has been the case for at least the past eight years, *An. arabiensis* remains highly resistant to pyrethroids in all the monitoring sites. Moderate to high alpha-cypermethrin, deltamethrin, and permethrin resistance intensities have manifested in six, six, and three sites tested, respectively. Pre-exposure to PBO restored susceptibility to alpha-cypermethrin in seven of the 11 sites, to deltamethrin in eight of the sites, and to permethrin in five sites. Partial restoration of susceptibility to alpha-cypermethrin, deltamethrin, and permethrin was also observed in the remaining test sites. *An. stephensi* was resistant to all pyrethroid, carbamate, and organophosphate insecticides tested except pirimiphos-methyl and propoxur in Semera.

The residual bio-efficacy of Actellic 300CS was three months in Lare and Bambasi. The fumigant effect of the insecticide persisted for more than five months in Lare and four months in Bambasi, killing more than 20% of test mosquitoes. In Abaya, fumigant effect monitoring was stopped five months after spraying in July due to security issues and results of cone bioassay test turned out to be below 80% threshold.

CONCLUSIONS

Anopheles arabiensis and *An. pharoensis* remain the predominant malaria vectors in Ethiopia. Their increased tendency to feed and rest outdoors requires the need to search for/adapt other vector control interventions to supplement the insecticide-treated nets and IRS currently in use. Although *An. arabiensis* was susceptible to the two insecticides (pirimiphos-methyl and propoxur) used in IRS, it is important that Ethiopia consider adopting pre-emptive rotation of these insecticides with others to preserve the efficacy of these insecticides. The finding of sporozoite-infected *An. stephensi* suggests its role in the transmission of malaria in towns in eastern Ethiopia. Vector surveillance and control, therefore, should include this species.

I. INTRODUCTION

The President's Malaria Initiative (PMI) VectorLink Project supports 24 African countries to implement quality vector control interventions, build technical capacities, and undertake monitoring and evaluation as well as collect entomological data that will be used to inform decision making.

In Ethiopia, the project conducts indoor residual spraying (IRS) operations in 44 districts in three regional states, Gambela, Benishangul-Gumuz, and Oromia. The project also does entomological surveillance and monitors insecticide resistance in order to generate data on key entomological indices that help guide the selection of appropriate vector control interventions (in addition to IRS, Ethiopia distributes insecticide treated nets (ITNs). The information is also valuable for assessing the entomological impacts of vector control interventions.

VectorLink Ethiopia conducted monthly entomological monitoring from May 2019 through March 2020 in seven selected sentinel sites in project-supported and non-project regions. The methods of mosquito sampling were mainly human landing catches (HLCs) and pyrethrum spray catches (PSCs). In addition, insecticide resistance monitoring was conducted through susceptibility tests in 26 sentinel sites (15 out of the 25 were the NMCEP sentinel sites), resistance intensity assays in seven sites, and piperonyl-butoxide (PBO) synergist assays in 13 sites.

This report discusses the aims and achievements of the different activities, which were to:

- Assess malaria vector density and species composition in seven sentinel sites. Three of the sites (Lare, Bambasi, and Abaya) are the PMI VectorLink project IRS sites and two (Metema, and Jabitehnan) are government-supported IRS sites; and the remaining two (Harbu, and Benatsemay) are non-IRS sites.
- Understand vector feeding times and locations (indoors/outdoors);
- Monitor the quality of insecticide application and decay rates in three PMI-supported IRS sites (Lare, Bambasi, and Abaya);
- Determine sporozoite rates of malaria vectors;
- Investigate structures used for resting of *An. arabiensis* in Lare and Pawi;
- Conduct insecticide resistance tests, resistance intensity assays, and synergist assays to measure the response of *An. arabiensis* and *An. stephensi* populations to insecticides and get data on mechanisms of resistance;
- Continue investigating the occurrence of *An. stephensi* in 11 new urban sites (started in 2018) to obtain additional information on the extent of its distribution in the southern, western, central, and northern parts of the country; and;
- Conduct longitudinal surveillance of *An. stephensi* to measure density, behavior, and sporozoite infection rates in four sites (Dire Dawa, Kebridehar, Awash Sebat Kilo (Awash), and Metehara) and also determine the human and animal blood meal indices.

2. METHODOLOGY

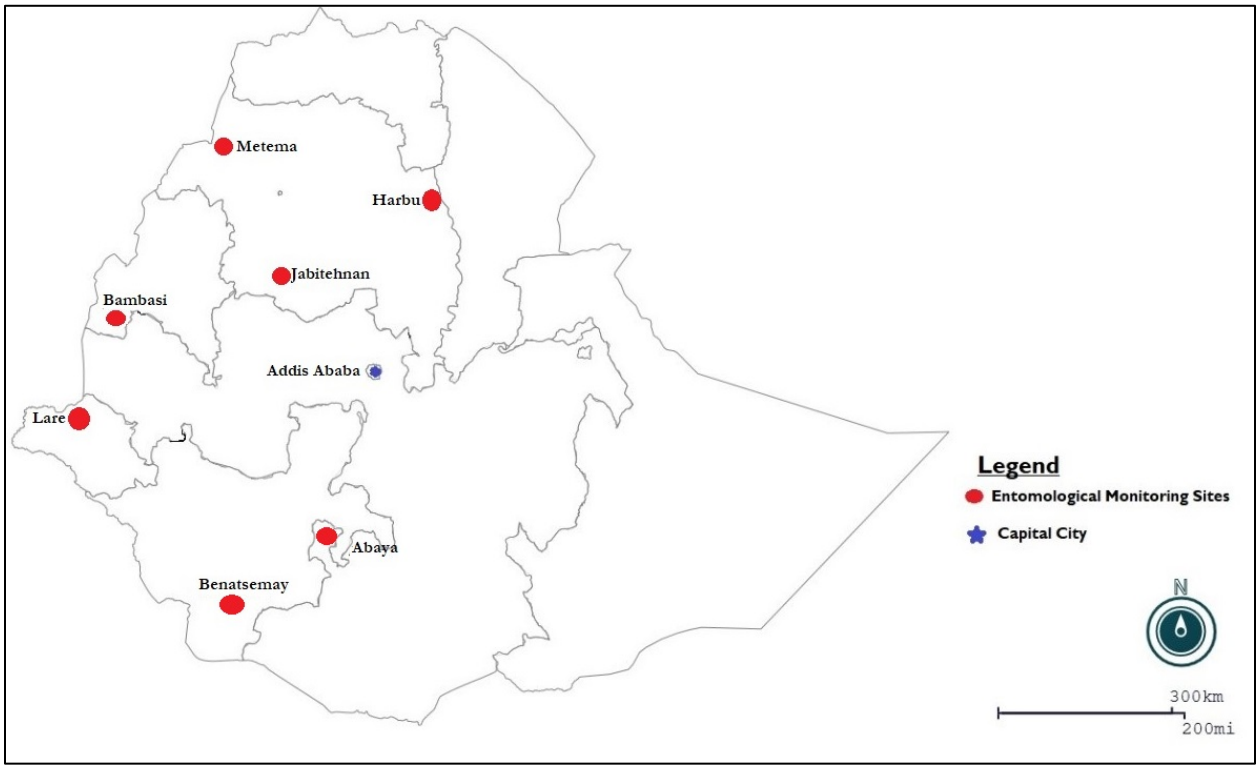
2.1 LONGITUDINAL ENTOMOLOGICAL MONITORING

Monthly entomological monitoring to assess density, behavior, sporozoite infection rates, and blood meal sources were conducted from May 2019 to March 2020 in a total of seven sites: three PMI VectorLink Ethiopia project sites and four non-PMI project sentinel sites (Figure 1). The project sites were Abaya in Oromia Region, Lare in Gambela, and Bambasi in Benishangul-Gumuz. The non-project sites were Harbu, Jabitehnan, and Metema in Amhara Region and Benatsemay in the Southern Nations Nationalities and Peoples Region (SNNPR). Entomological monitoring was conducted for 11 months in six of the seven sites. In Abaya site, the time was shortened by one month, July, because of security concerns.

2.1.1 ENTOMOLOGICAL SURVEILLANCE SITES

Entomological surveillance has been in place in the three project sites since 2017. The four non-project sites are among the 25 sentinel sites of the National Malaria Control and Elimination Program (NMCEP) and were added in fiscal year 2019/20 upon the recommendation of the Ethiopian Public Health Institute. The PMI VectorLink project sites were sprayed with Actellic 300CS from May to July 2019. The Ministry of Health conducted IRS in Metema and Jabitehnan from the last week of September to October 2019 using propoxur. IRS was not conducted in Harbu and Benatsemay. ITNs that were distributed between 2017 and 2019 were observed in all the sites.

FIGURE 1. ENTOMOLOGICAL MONITORING SENTINEL SITES, 2019



2.1.2 Mosquito Sampling Methods

Two mosquito surveillance methods, HLCs and PSCs, were used in all seven sites. In addition to HLCs and PSCs, Center for Disease Control and Prevention (CDC) light traps were used in Bambasi because this trap type, used in the 2018/19 entomological monitoring exercise, were more efficient at capturing *An. funestus* than other collection methods. Mosquitoes were sampled following the methods described in the PMI VectorLink Standard Operating Procedures (SOPs).

Frequency of *Anopheles* sampling in the sentinel sites is depicted in Table 1.

Human Landing Catches

Mosquitoes that bite humans indoors and outdoors were sampled following the method described in PMI VectorLink SOP #2. In each site, three houses were randomly selected and each month, mosquitoes were collected by collectors who spent two consecutive nights inside and outside of each house for a total of six nights per site. Over the 11 months, this totaled 66 HLC-nights per site (6 nights/month x 11 months) with the exception of Abaya, with 60 HLC-nights (6 nights/month x 10 months). The data collected from the HLCs was used to determine species composition, seasonality, preferred feeding locations (indoors/outdoors) for human night biting rates, hourly biting patterns, and sporozoite infection rates. The same houses were used to sample mosquitoes monthly during the monitoring period.

Pyrethrum Spray Catches

Mosquitoes resting in human dwellings were sampled in 20 randomly selected houses in each site, using PSC in accordance with the protocol in PMI VectorLink SOP #3. During the mosquito sampling period, a total of 200 PSC attempts were made in Abaya and 220 in each of the remaining six sites. Collections of *An. arabiensis* from PSCs were used to determine the daily resting density with seasonal variability, proportion of abdominal feeding stages (blood unfed, fresh fed, half gravid, and gravid), sporozoite infection rates, and origins of blood meals. The same houses were used to sample mosquitoes monthly during the monitoring period.

CDC Light Trap Catches

Mosquito collections using CDC light traps were conducted in Bambasi in accordance with PMI VectorLink SOP #1. Each month, traps were hung in 12 randomly selected houses for two nights. The sampling period was August 2019 to May 2020 (8 months) and the same houses were used every month.

TABLE 1. FREQUENCY OF *ANOPHELES* SAMPLING; MAY 2019-MARCH 2020

Type of Collection	Time	Frequency	Sample
HLC	6:00 pm to 6:00 am	3 houses per site and 2 collection nights per site per month	6 indoor and 6 outdoor collection nights per site per month
PSC	6:00 am to 8:00 am	Once a month	20 houses per site (total 140)
CDC light trap	6:00 pm to 6:00 am	Once a month	Only in Bambasi; 12 houses, 2 nights in each house (24 trap-nights/month)

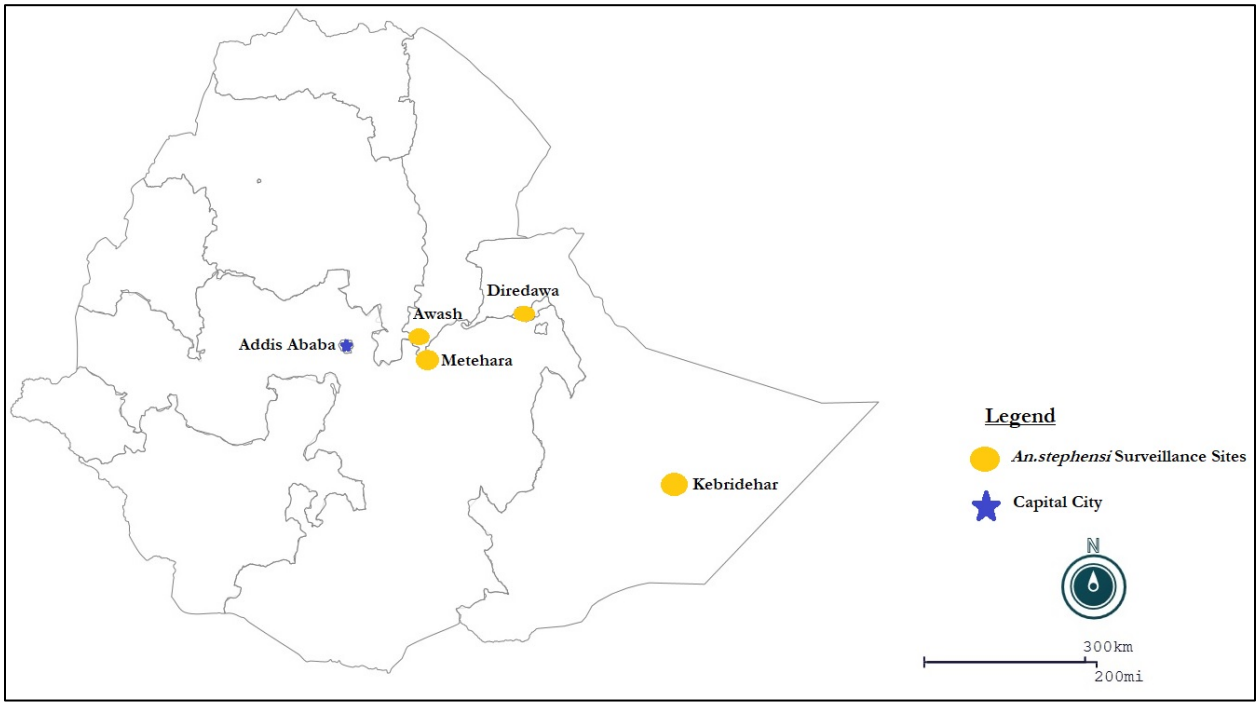
2.1.3 Mosquito Identification, Labelling, and Preservation

Mosquitoes were sorted into *Anopheles* and culicines, and males and females. *Anopheles* mosquitoes were identified to the species using the morphological identification key of Gillies and Coetzee (1987). Female *An. arabiensis* from collections of PSC and CDC light traps were categorized as unfed, freshly fed, half gravid, or gravid. Individual specimens were labelled, preserved in Eppendorf tubes over silica gel (desiccant), and shipped to the laboratories of Jimma and Arbaminch universities for molecular species identification, and investigation of sporozoite infections and blood meal origins.

2.2 *AN. STEPHENSI* SURVEILLANCE

Anopheles stephensi surveillance was carried out in Dire Dawa and Kebridehar towns from June to December 2019 (7 months) and in Awash and Metehara towns from August to December 2019 (5 months). Figure 2 shows the location of the study sites.

FIGURE 2. *AN. STEPHENSI* SURVEILLANCE SITES, 2019



2.2.1 *AN. STEPHENSI* SURVEILLANCE METHODS AND FREQUENCIES

Anopheles stephensi were sampled through HLCs indoors and outdoors in three houses for two nights per month, PSCs in 20 houses, CDC light traps in six houses for two nights, animal-baited tent traps for three nights, and hand collections from animal shelters and horse stables. In addition, collection attempts were made using black boxes in Dire Dawa and Kebridehar. The black boxes were placed in the compound of HLC houses. In addition, boxes were placed near a horse stable in Dire Dawa.

The standard PMI VectorLink SOPs were used when collecting mosquitoes by HLC, PSC, and CDC light trap. For black box resting traps, packing paper carton boxes were used to make black boxes by lining the interior with black cloth sheets. The black boxes were placed outdoor in the compound of residential houses. For animal-baited tent traps, a cow or ox was tethered inside a tent and mosquitoes were collected from the wall of the tents with mouth aspirators. Horse stable, goat, and cattle shelters were also searched and resting mosquitoes were collected using mouth aspirators and paper cups. The horse stable is closed on two sides with brick walls and has corrugated room. Goat and cattle shelters are enclosures with walls in all sides with either corrugated or thatched roof.

The frequency of *An. stephensi* collection is shown in Table 2.

TABLE 2. FREQUENCY OF *AN. STEPHENSI* SAMPLING, JUNE-DECEMBER 2019 AND AUGUST-DECEMBER 2019

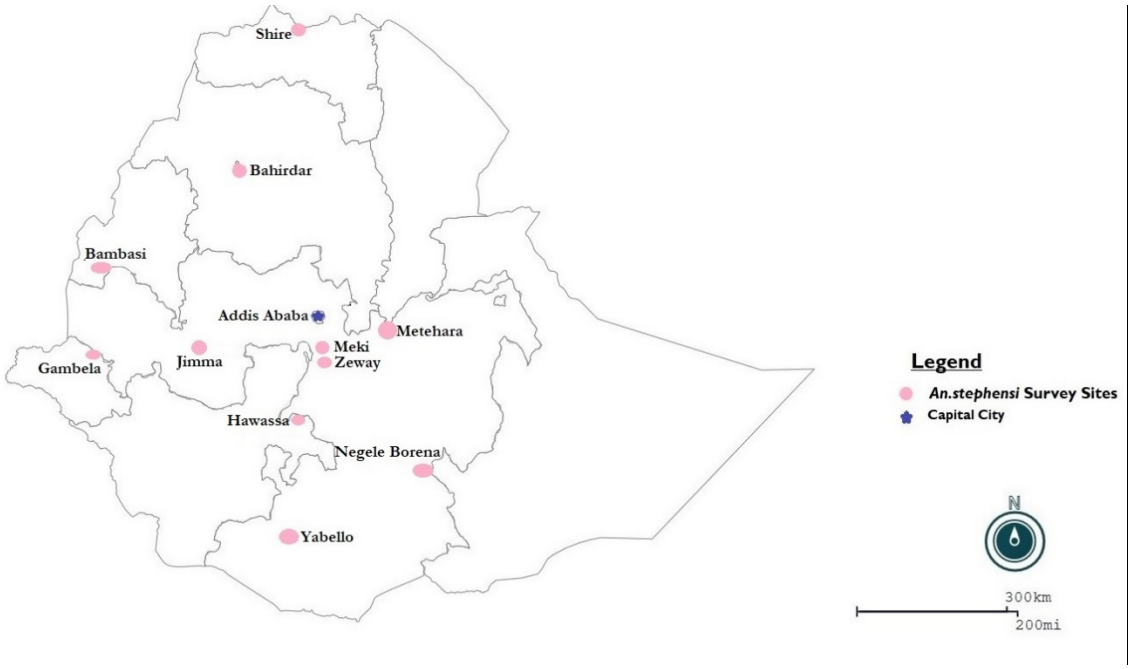
Type of Collection	Time	Frequency	Sample
HLC	6:00 pm to 6:00 am	3 houses per site and 2 collection nights per site per month	6 indoor and 6 outdoor collection nights per site per month
PSC	6:00 am to 8:00 am	Once a month	20 houses per site (total 80)
CDC (baited with humans sleeping under many times washed treated nets)	6:00 pm to 6:00 am	6 houses per site and 2 collection nights per site per month	12 indoor and 12 outdoor collections nights per site per month
Black box resting trap	6:00 am to 8:00 am	One box/6 collection nights/month	6 box-nights per site per month
Animal-baited tent trap	6:00 pm to 6:00 am	3 nights/month	3 collection nights per site per month
Hand collection from animal sheds	6:00 am to 8:00 am	As available	Depended on the number of available sheds (2-20)

Adult female mosquitoes were identified to species using Gillies and Coetzee (1987) and Coetzee (2020). The same method of labelling, preservation, and shipping described under Section 2.3.1 was applied. Preserved specimens were submitted to Jimma University and Armauer Hansen Research Institute for laboratory examination of sporozoite infection and blood meal analysis.

2.3 *AN. STEPHENSI* SURVEYS IN URBAN SITES

In a follow-up to the 2018 surveys, one-time cross-sectional surveys of *An. stephensi* was done in 11 urban localities in five regional states: Oromia (Metehara, Meki, Zeway, Jimma, Negele Borena, and Yabello), SNNPR (Hawassa), Gambela (Gambela town), Benishangul-Gumuz (Bambasi), Amhara (Bahirdar), and Tigray (Shire) to map the species' geographical distribution across the country (Figure 3).

FIGURE 3. *AN. STEPHENSI* SURVEY IN URBAN SITES, 2019



Larvae and pupae were collected from artificial and natural breeding habitats in the 11 sites, raised to adults, and identified to species using morphological keys of Gillies and Coetzee (1987) and Coetzee (2020).

2.4 *An. arabiensis* Collections from Different Structures in Pawi and Lare

To find out the preferred resting structures of *An. arabiensis,* sampling was done in Lare (Gambela) and Pawi (Benishangul-Gumuz). A backpack aspirator was used to collect mosquitoes from houses (human dwellings), indoor kitchens, animal shelters, and latrines. The mosquitoes then were identified to species, preserved, and shipped to laboratories for molecular ID using polymerase chain reaction (PCR).

2.5 Molecular and Immunological Assays

2.5.1 Species ID PCR

Specimens identified morphologically as *An. gambiae* s.l. were subjected to species identification PCR as described by Scott et al. (1993). The method developed by Koekmeoer et al. (2002) was used to identify members of the *An. funestus* group.

2.5.2 Sporozoite ELISA

The Enzyme-Linked Immunosorbent Assay (ELISA) method described by Wirtz et al. (1992) was used to examine specimens of *An. arabiensis*, *An. pharoensis,* and *An. stephensi* for circumsporozite proteins. Mosquitoes with all abdominal stages including blood unfed, feds, half-gravids and gravids were tested.

2.5.3 Blood meal ELISA

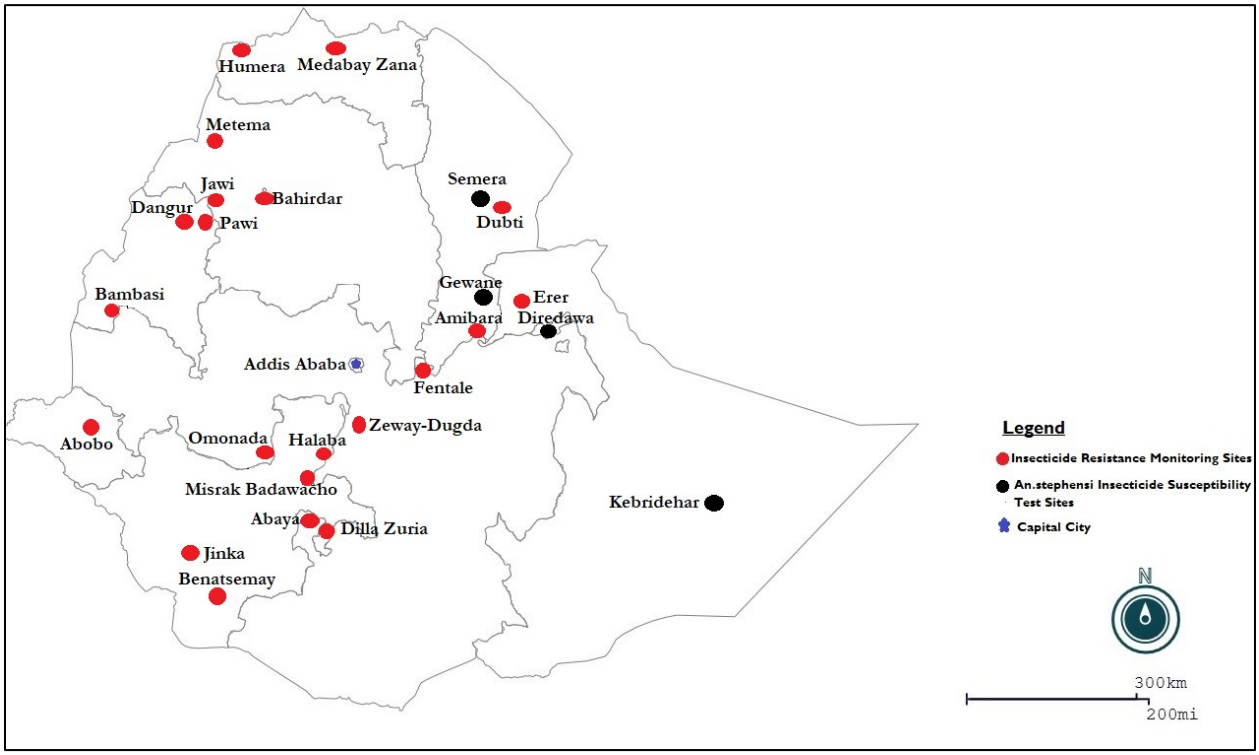
Blood meal sources of *An. arabiensis*, *An. stephensi,* and *An. funestus* group were investigated by conducting blood meal direct ELISA as described in Biere et al. (1988).

2.6 Insecticide Resistance Monitoring and Mechanism of Resistance

Insecticide resistance monitoring, which included susceptibility tests with discriminating doses, resistance intensities, and synergist tests of PBO, were done on populations of *An. arabiensis* in 21 sites.

Figure 4 shows the 2019 insecticide resistance monitoring sentinel sites: Amibara and Dubti in Afar; Bahirdar, Metema, and Jawi in Amhara; Bambasi, Dangur, and Pawi in Benishangul-Gumuz; Abobo in Gambela; Abaya, Fentale, Omonada, and Zeway-Dugda in Oromia; Benatsemay, Dilla Zuria, Halaba, Jinka, and Misrak Badawacho in SNNPR; Erer in Somali; and Humera and Medabay Zana in Tigray. Four of these sites namely Abaya, Bambasi, Benatsemay and Metema also serve as longitudinal entomological surveillance sites.

FIGURE 4. INSECTICIDE RESISTANCE MONITORING SENTINEL SITES, 2019



In addition, populations of *An. stephensi* from Dire Dawa city, Kebridehar in Somali, and Gewane, Semera, and Awash in Afar were investigated for their susceptibility to insecticides.

2.6.1 INSECTICIDE SUSCEPTIBILITY TESTS

As described in PMI VectorLink SOP #6, the World Health Organization (WHO) tube test was used to measure the susceptibility/resistance status of populations of *An. arabiensis* to 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl, 0.5% alpha-cypermethrin, 0.5% deltamethrin, and 0.75% permethrin in all 21 sites; that of *An. stephensi* was measured in five sites. All insecticide-impregnated papers were obtained from the University Sans Malaysia. The WHO method was also used to test 2% clothianidin-impregnated papers donated by Sumitomo Chemicals. Clothianidin tests were conducted in Abobo, Amibara, Omonada, Bambasi, Halaba, Dubti, and Zeway-Dugda.

The CDC bottle bioassay method (PMI VectorLink SOP #4) was used to test chlorfenapyr at a dose of 100 micrograms/bottle. Bottles were impregnated by VectorLink staff. *An. arabiensis* from Abaya, Abobo, Bahirdar, Dubti, Fentale, Halaba, Omonada, and Zeway-Dugda were tested against chlorfenapyr.

All tests including susceptibility to diagnostic concentration, resistance intensity and PBO synergist were conducted on 2–5-day-old females raised from wild-collected larvae and pupae.

The results of the susceptibility tests, resistance intensity assays, and PBO synergist assays were interpreted as described in the PMI VectorLink SOP #6.

2.6.2 RESISTANCE INTENSITY ASSAYS

The level of resistance intensity (PMI VectorLink SOP #6) to the pyrethroid insecticides (alpha-cypermethrin, deltamethrin, and permethrin) at the concentrations of 1X, 5X, and 10X was assessed on the populations of *An. arabiensis* from Abaya, Abobo, Amibara, Omonada, Zeway-Dugda, Halaba, and Pawi. Similar tests were carried out on *An. stephensi* from Awash.

2.6.3 PBO Synergist Assays

The PMI VectorLink SOP #6 method was used to carry out PBO synergist tests. *Anopheles arabiensis* was pre-exposed to PBO and then to alpha-cypermethrin, deltamethrin, and permethrin in Abaya, Abobo, Amibara, Bambasi, Omonada, Dangur, Humera, Jawi, Medabay Zana, Pawi, and Zeway-Dugda to assess if PBO restores susceptibility to the three insecticides. PBO synergist assays were also conducted on *An. stephensi* from Dire Dawa against the three pyrethroids and from Awash against deltamethrin.

2.7 Entomological Assessment of Quality and Residual Efficacy of Actellic 300CS

WHO cone wall bioassays were conducted in 12 houses per site in Lare, Bambasi, and Abaya using an insectary susceptible colony of *An. arabiensis* as per PMI VectorLink SOP #9. The houses used for quality assurance tests and subsequently for residual bio-efficacy monitoring were randomly selected from houses treated as part of the IRS campaign. In parallel to the cone bioassays, fumigant effects of pirimiphos-methyl were assessed following the project protocol. The bioassays were done from May through August 2019 except in Abaya, where the tests were discontinued in July because of security issues.

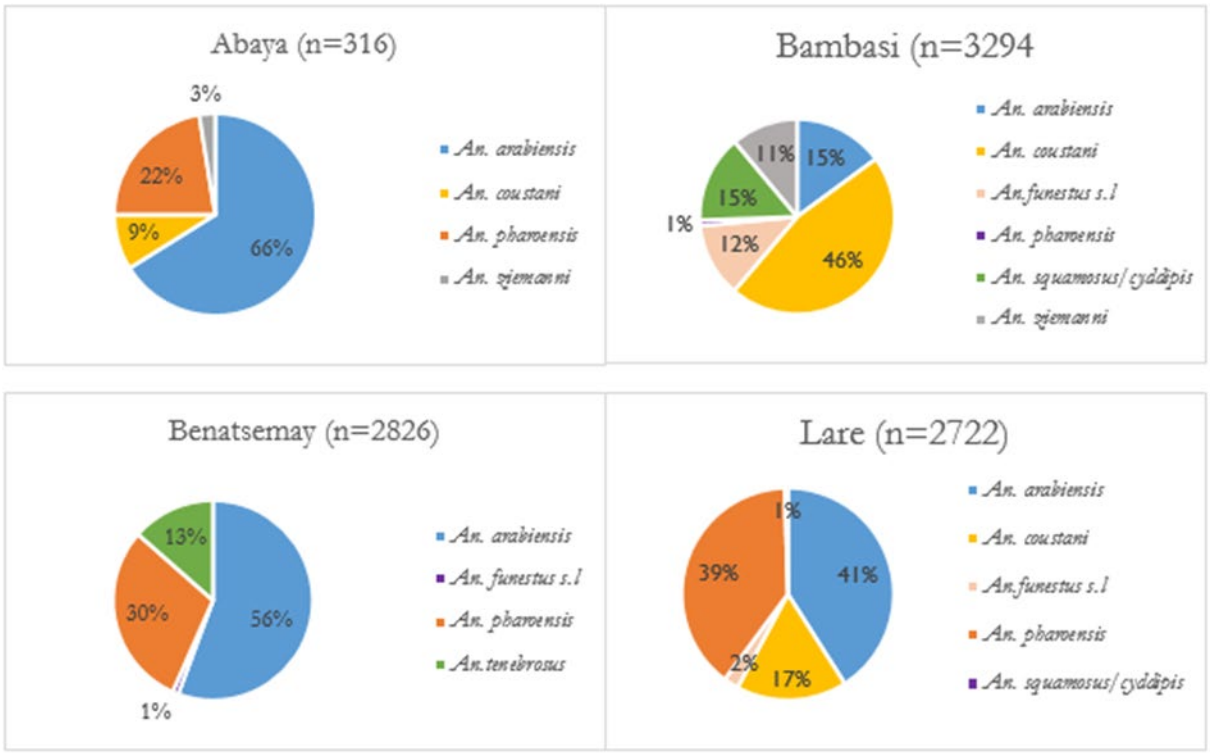
3. RESULTS

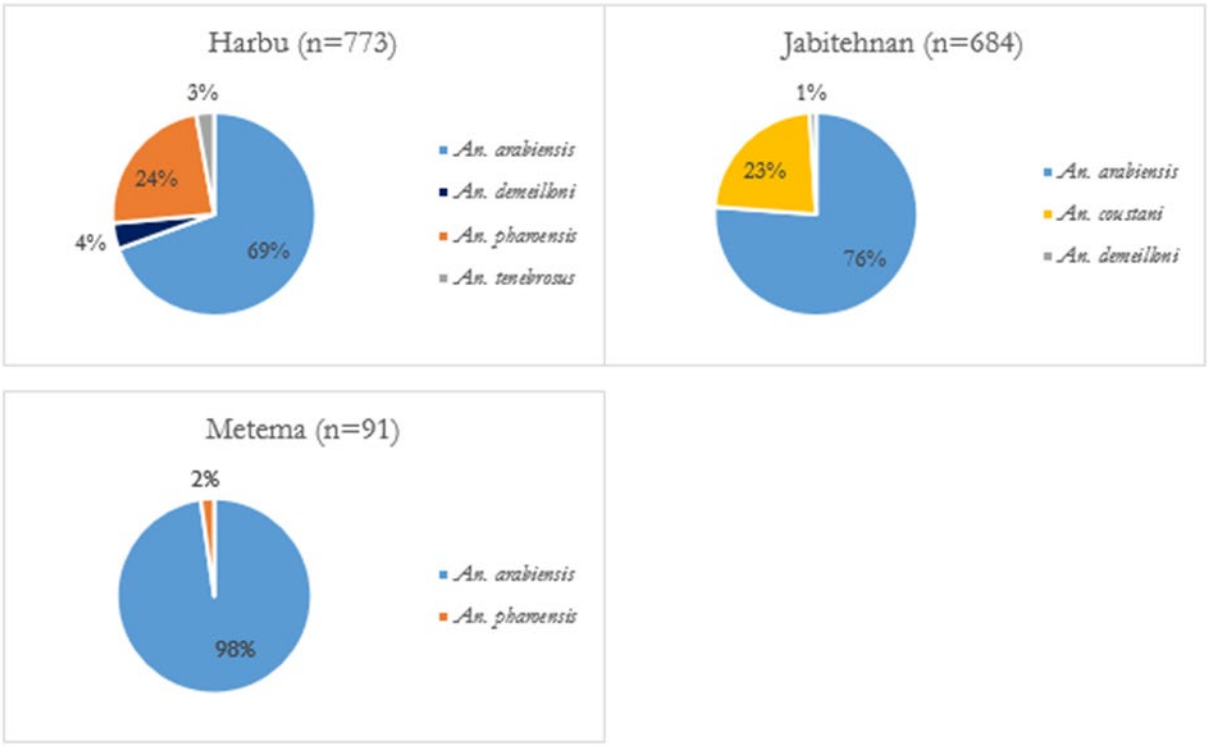
3.1 SPECIES COMPOSITION AND ABUNDANCE

A total of 10,706 *Anopheles* and 33,478 culicines were collected. The *Anopheles* species were *An. arabiensis*, *An. pharoensis* , *An. funestus* group, *An. coustani*, *An. ziemanni*, *An. squamosus/cydippis*, *An. tenebrosus*, and *An. demeilloni*. Of the *Anopheles*, 43% (n=4606) were *An. arabiensis* and 20.2% (n=2160) were *An. pharoensis*, the species considered the principal and secondary vectors in Ethiopia.

Anopheles arabiensis was the predominant species in Abaya (66%, n=209), Benatsemay (56%, n=1819), Harbu (69%, n=537), Jabitehnan (76%, n=521), Lare (41%, n=935), and Metema (98%, n= 89), but not in Bambasi (15%, n=496). In Bambasi, *An. coustani* (47%, n=1535) was predominant. *An. pharoensis* was the second most predominant species in Abaya, Benatsemay, Harbu, and Lare. The *An. funestus* group was prevalent in low proportions in Bambasi (12%, n=377), Lare (2%, n=52), and Benatsemay (1%, n=31) (Figure 5).

FIGURE 5. PROPORTION OF *ANOPHELES* IN SENTINEL SITES (MAY 2019-MARCH 2020)





3.2 SPECIES BY METHOD OF COLLECTION

The largest proportion of *Anopheles* in six of the sentinel sites was sampled through HLCs. CDC light traps were the most productive in Bambasi.

Of the *Anopheles* collections made in each site, HLC constituted 94.0%, 91.8%, 83.0%, 78.0%, 73.6%, and 56.6% in Lare, Abaya, Harbu, Metema, Benatsemay, and Jabitehnan, respectively. In Bambasi CDC light traps and HLCs captured 56.2% and 41.4% of *Anopheles,* respectively. The majority of *An. arabiensis* in all the sites were sampled through HLCs but PSCs were also effective in Benatsemay 46.3% (n=843), Jabitehnan 51.6% (n=269), and Harbu 19.4% (n=104). From 97% to 100% of *An. pharoensis* in Harbu, Benatsemay, and Abaya were collected using HLCs.

CDC light traps captured 89% (n=336) of *An. funestus* group in Bambasi where as 96.2% (n=50) from Lare were collected using HLCs (Table 3).

TABLE 3. PROPORTION OF *ANOPHELES* BY METHOD OF COLLECTION (MAY 2019-MARCH 2020)

A) PMI project sites

Species	Abaya			Bambasi				Lare		
	HLC	PSC	Total	HLC	PSC	CDC	Total	HLC	PSC	Total
An. arabiensis	184 (88.0)	25 (12.0)	209 (100)	282 (56.9)	33 (6.7)	181 (36.4)	496 (100)	857 (91.7)	78 (8.3)	935 (100)
An. pharoensis	71 (100)	0	71 (100)	5 (16.7)	1 (3.3)	24 (80)	30 (100)	847 (94.2)	52 (5.8)	899 (100)
An. funestus s.l.	-	-	-	17 (4.5)	24 (6.4)	336 (89.1)	377 (100)	50 (96.2)	2 (3.8)	52 (100)
An. coustani	28 (100)	0	28 (100)	794 (51.7)	13 (0.9)	728 (47.2)	1535 (100)	386 (100)	0	386 (100)
An. ziemanni	7 (87.5)	1 (12.5)	8 (100)	198 (54.0)	5 (1.4)	164 (44.6)	367 (100)	-	-	-
An. squamosus/cydippis	-	-	-	65 (13.5)	3 (0.6)	414 (85.9)	482 (100)	9 (100)	0	9 (100)
Overall	290 (91.8)	26 (8.2)	316 (100)	1361 (41.4)	79 (2.4)	1847 (56.2)	3287 (100)	2149 (94.0)	132 (6.0)	2281 (100)

Highlighted figures indicates the method that was successful for each species.

B) Non-PMI project sites

Species	Benatsemay			Harbu			Jabitehnan			Metema		
	HLC	PSC	Total	HLC	PSC	Total	HLC	PSC	Total	HLC	PSC	Total
An. arabiensis	976 (53.7)	843 (46.3)	1819 (100)	433 (80.6)	104 (19.4)	537 (100)	252 (48.4)	269 (51.6)	521 (100)	69 (77.5)	20 (22.5)	89 (100)
An. pharoensis	974 (99.8)	2 (0.2)	976 (100)	176 (96.7)	6 (3.3)	182 (100)	-	-	-	2 (100)	0	2 (100)
An. funestus s.l.	14 (45.2)	17 (54.8)	31 (100)	-	-	-	-	-	-	-	-	-
An. tenebrosus	440 (99.8)	1 (0.2)	441 (100)	19 (86.4)	3 (13.6)	22 (100)	-	-	-	-	-	-
An. coustani	-	-	-	-	-	-	135 (86.5)	21 (13.5)	156 (100	-	-	-
An. demeilloni	-	-	-	14 (43.8)	18 (56.2)	32 (100)	0	7 (100)	7 (100)	-	-	-
Overall	2404 (73.6)	863 (26.4)	3267 (100)	642 (83.0)	131 (17.0)	773 (100)	387 (56.6)	297 (43.4)	684 (100)	71 (78.0)	20 (22.0)	91 (100)

3.3 INDOOR RESTING DENSITY OF AN. ARABIENSIS

3.3.1 AN. ARABIENSIS ABUNDANCE INDOORS

A total of 1372 *An. arabiensis* were collected resting in human dwellings. The greatest number (61.4%, n=843,) were collected in Benatsemay, followed by Jabitehnan (19.6%, n=269,) and Harbu (7.6%, n=104,). The remaining (11.4%, n=156) were collectively from Lare, Bambasi, Abaya, and Metema (Table 4).

TABLE 4. NUMBER AND PROPORTION OF *AN. ARABIENSIS* COLLECTED FROM INSIDE HUMAN DWELLINGS (MAY 2019-MARCH 2020)

Site	Number (%)
Benatsemay	843 (61.4)
Jabitehnan	269 (19.6)
Harbu	104 (7.6)
Lare	78 (5.7)
Bambasi	33 (2.4)
Abaya	25 (1.8)
Metema	20 (1.5)
Total	1372 (100)

3.3.2 *Abdominal Blood Feeding Stages*

The results of the abdominal blood feeding stages of *An. arabiensis* from PSCs in Benatsemay revealed that 93.4% were fresh blood-fed mosquitoes, suggesting that exophily exceeds endophily in the site's vector population. Although it is not comparable, the proportion of fresh fed was higher (69.2%) than gravid in Harbu. On the other hand, 66.9% of those collected from Jabitehnan were gravid, showing the tendency of *An. arabiensis* to rest indoors (Table 5).

TABLE 5. ABDOMINAL FEEDING STATUS OF *AN. ARABIENSIS* (MAY 2019-MARCH 2020)

Site	Unfed N (%)	Fed N (%)	Half Gravid N (%)	Gravid N (%)	Total
Benatsemay	54 (6.4)	787 (93.4)	1 (0.1)	1 (0.1)	843 (100)
Jabitehnan	49 (18.2)	40 (14.9)	10 (3.7)	170 (63.2)	269 (100)
Harbu	8 (7.7)	72 (69.2)	19 (18.3)	5 (4.8)	104 (100)
Lare	22 (28.2)	33 (42.3)	19 (24.4)	4 (5.1)	78 (100)
Bambasi	10 (30.3)	13 (39.4)	6 (18.2)	4 (12.1)	33 (100)
Abaya	4 (16.0)	8 (32.0)	10 (40.0)	3 (12.0)	25 (100)
Metema	1 (5.0)	8 (40.0)	4 (20.0)	7 (35.0)	20 (100)
Overall	148 (10.8)	961 (70.0)	69 (5.0)	194 (14.2)	1372 (100)

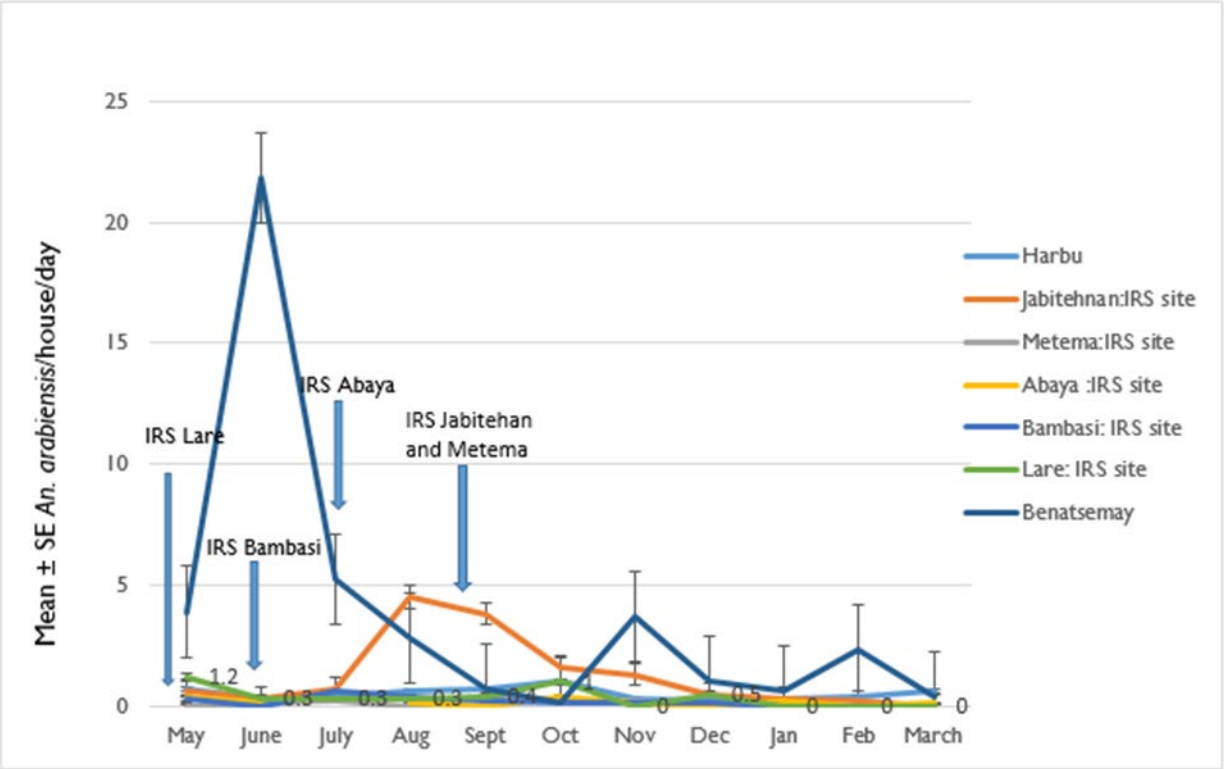
3.3.3 *Indoor Resting Density of An. arabiensis*

The indoor resting density (as determined from PSC) of *An. arabiensis* throughout the study period was very low, less than 1.0 *An. arabiensis*/house/day in Harbu, Metema, Abaya, and Bambasi. The other three sites, Jabitehnan, Lare, and Benatsemay, had more than 1.0 *An. arabiensis*/house/day in both the wet and dry months, from two months in Lare to seven months in Benatsemay. The greatest density in Lare, 1.2 *An. arabiensis*/house/day, was recorded in May during small rainy season, and the second greatest, 1.0 *An. arabiensis*/house/day, was in October at the end of the main rainy season. In Jabitehnan, the daily peak density was 4.5 *An. arabiensis*/house/day in August (main rainy season); in the three following months, density was 3.8,

1.6, and 1.3. In Benatsemay, the highest peak, 21.8 *An. arabiensis*/house/day, was in June and the second highest, 5.2 *An. arabiensis*/house/day, was in July (Figure 6).

There was a difference in the mean resting density of *An. arabiensis* in Abaya and Bambasi before (0.3 and 0.75 mosquitoes per house per day, respectively) and after (0.13 and 0.3 mosquitoes per house per day respectively) IRS with Actellic 300CS was modest, but the density in Lare dropped from 1.2 *An. arabiensis*/house/day before spraying to close to 0.3 for the four months following the spraying. In Jabitehnan, the resting density of *An. arabiensis* in August and September was high before IRS with propoxur but gradually declined from October onwards. In Benatsemay, where IRS was not conducted, the density of *An. arabiensis* peaked in June, sharply declined in July and persisted indoors throughout the 11 months with slight fluctuations from month to month. *Anopheles arabiensis* was totally absent from indoor PSCs in Bambasi, Lare, and Metema from January to March (Figure 6).

FIGURE 6. MONTHLY INDOOR RESTING DENSITY OF *AN. ARABIENSIS*, BY SITE (MAY 2019-MARCH 2020)



3.4 BITING BEHAVIORS OF AN. ARABIENSIS AND AN. PHAROENSIS

3.4.1 FEEDING LOCATION

The abundance of *An. arabiensis* collected outdoors was greater than indoors in all sentinel sites with the exception in Jabitehnan, where the indoor collection was higher than the outdoor one. The ratio of host-seeking *An. arabiensis* outdoors to that of indoors varied from site to site. In Abaya, more than twice *An. arabiensis* were collected outdoors than indoors (ratio 2.4:1). The ratio in Bambasi and Harbu was close to 2:1. *An. pharoensis* showed similar trends of searching human hosts outdoors (Table 6).

A number of entomological studies in Ethiopia, including those conducted with PMI support, showed the tendency of *An. arabiensis*, *An. pharoensis,* and *An. funestus* s.l. to feed more frequently outdoors than indoors.

TABLE 6. FEEDING LOCATION AND PROPORTION OF *AN. ARABIENSIS* AND *AN. PHAROENSIS*

Sites	*An. arabiensis*			*An. pharoensis*		
	Indoors N (%)	Outdoors N (%)	Ratio Outdoors/ Indoors	Indoors N (%)	Outdoors N (%)	Ratio Outdoors/ Indoors
Abaya	54 (29.3)	130 (70.7)	2.4:1	20 (28.2)	51 (71.8)	2.6:1
Bambasi	95 (33.7)	187 (66.3)	1.96:1	-	-	-
Lare	350 (40.8)	507 (59.2)	1.5:1	372 (43.9)	475 (56.1)	1.3:1
Benatsemay	447 (45.8)	529 (54.2)	1.2:1	450 (46.1)	526 (53.9)	1.2:1
Harbu	150 (34.6)	283 (65.4)	1.9:1	67 (38.2)	108 (61.8)	1.6:1
Jabitehnan	140 (55.8)	111 (44.2)	0.8:1	-	-	-
Metema	30 (43.5)	39 (56.5)	1.3:1	-	-	-
Overall	1266 (41.5)	1786 (58.5)	1.4:1	909 (43.9)	1160 (56.1)	1.3:1

3.4.2 MONTHLY NIGHT BITING RATES OF AN. ARABIENSIS, AN. PHAROENSIS, AND AN. FUNESTUS GROUP

This section discusses the night biting rates of *An. arabiensis* from all the seven sites, *An. funestus* group from Lare, and *An. pharoensis* from Abaya, Lare, Benatsemay, and Harbu, where the collections for which the night biting rates were estimated. However, because of the small numbers collected in HLCs, this could not be done for *An. pharoensis* in Bambasi (n=30) and Metema (n=2), for *An. funestus* group from Bambasi (n=17) and Benatsemay (n=14).

The densities of *An. arabiensis*, *An. pharoensis,* and *An. funestus* group from CDC collections in Bambasi are also included in this section.

NIGHT BITING RATES OF AN. ARABIENSIS (ABAYA, BAMBASI, LARE, BENATSEMAY, HARBU, JABITEHNAN, AND METEMA)

In Abaya, *An. arabiensis* was present in the outdoor HLCs throughout the 10 months, but indoors for only eight months. February and March were the two months when *An. arabiensis* collection was zero both indoors and outdoors. The overall mean human biting rate from September to March was less than 1.0 *An. arabiensis*/person/night. The peak biting density outdoors was 7.2, 5.2, 3.2, and 2.3 *An. arabiensis*/person/night in June, May, October, and August, respectively. Indoors, the peak biting was in June at 3.3 *An. arabiensis*/person/night and in May and August at 2.2 *An. arabiensis*/person/night (Figure 7A). The interruption of entomological monitoring in July created a gap to observe the trend of the IRS impact fully, but it is notable that biting rates increased between May (before IRS) and June (after IRS). The June mosquito surveillance was conducted shortly after the IRS operation, when the insecticide had little impact on the adult mosquitoes and during the wet season. The biting rates, however, declined in the following months (Figure 7A).

Anopheles arabiensis in Bambasi was not found by HLCs in May and June outdoors or from January to March both outdoors and indoors. The mean human biting rates indoors in May and June were the same, 0.2 *An. arabiensis*/person/night, but density increased to 5.8 and 5.7 *An. arabiensis*/person/night indoors in July and August. The outdoor mean density during the respective months was 13.7 and 4.3 *An. arabiensis*/person/night. In the following months, the rate was between 1.0 and 2.0 *An. arabiensis*/person/night. Figure 7A shows that the peak *An. arabiensis* season in Bambasi was from July to September. Data from 2018/19 together with this

year's entomological monitoring data are evidence that this species is almost non-existent in May (dry season); as a result, it is impossible to evaluate the entomological impact of IRS in the absence of comparable non-IRS site (Figure 7A).

In Lare, *An. arabiensis* were collected in all 11 months of sampling, both indoors and outdoors, with the exception of indoors in March. In May, before the IRS campaign, the mean night biting rate was 1.8 *An. arabiensis*/person/night indoors and 2.7 *An. arabiensis*/person/night outdoors; in July, after the campaign, peak biting rates of 18.2 and 24.7 were recorded indoors and outdoors, respectively. Pre-IRS data were collected during the dry season in May but the post-IRS data in July were collected during the rainy season. The increase in breeding sites in July, which is favorable for the proliferation of *An. arabiensis,* might fully or partially explain the increase in vector density observed after IRS. The other biting peaks, 16.2 *An. arabiensis*/person/night indoors and 21.7 *An. arabiensis*/person/night outdoors, were in October. There was also a biting peak, albeit at a much lower level (2.5 indoors and 6.0 outdoors) in December (Figure 7B).

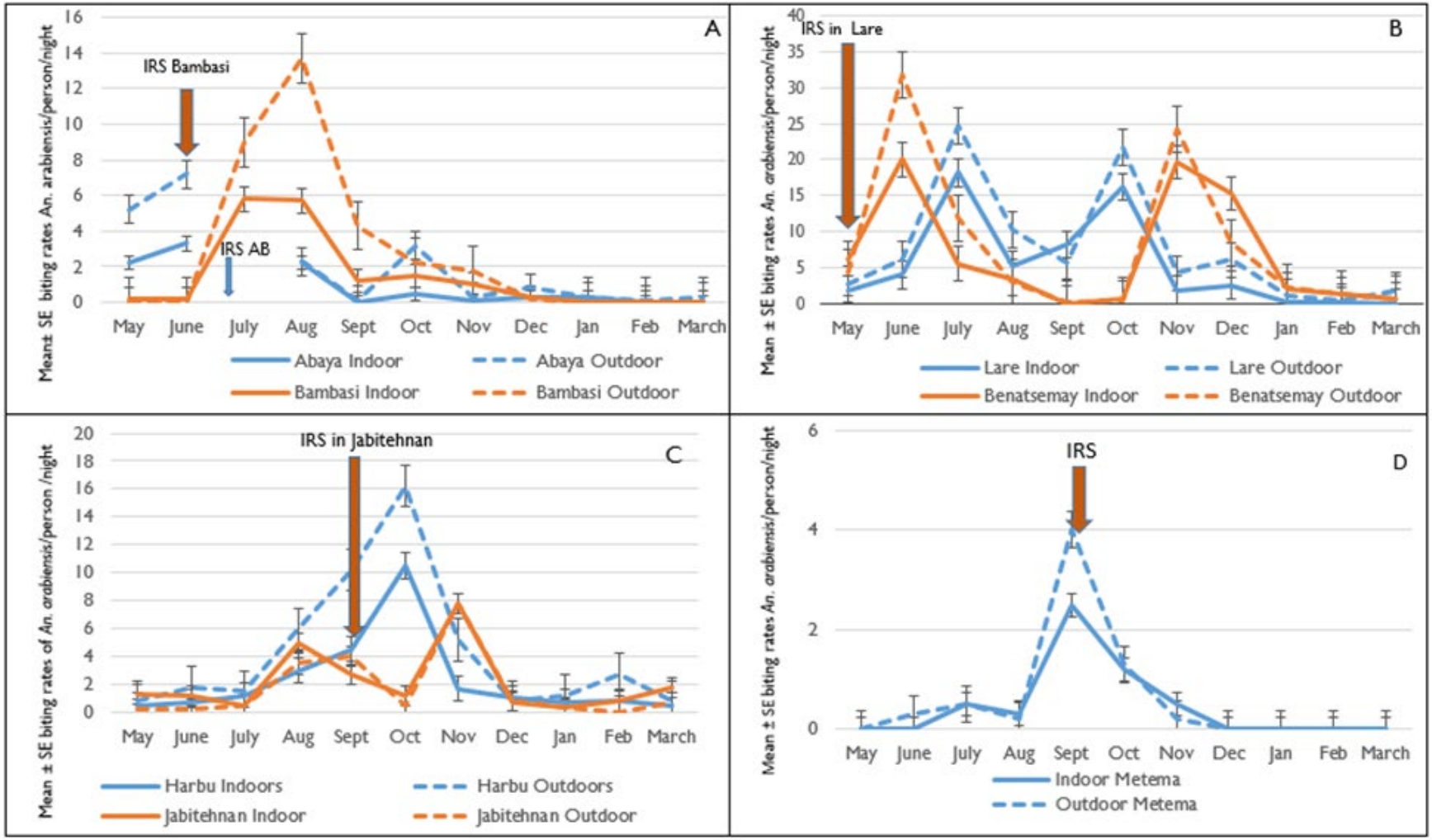
In Benatsemay, *An. arabiensis* was prevalent throughout the surveillance period with a high daily mean biting rate peak in June, at 20.0 *An. arabiensis*/person/night indoors and 31.8 *An. arabiensis*/person/night outdoors. The second biting peak, 19.7 *An. arabiensis*/person/night indoors and 24.2 *An. arabiensis*/person/night outdoors, was in November. In December, the indoor biting rate, 15.3 *An. arabiensis*/person/night, was greater than the outdoor rate of 8.5 *An. arabiensis*/person/night (Figure 7B).

In Harbu, the mean human biting rates of *An. arabiensis* were variable from May to March. The lowest biting rates, 0.5 *An. arabiensis*/person/night indoors and 0.8 *An. arabiensis*/person/night outdoors, occurred in May and March. The peak was in October, at 10.5 *An. arabiensis*/person/night indoors and 16.2 *An. arabiensis*/person/night outdoors (Figure 7C).

In Jabitehnan, *An. arabiensis* were present throughout the sampling period. In September, entomological monitoring was conducted a few days before the IRS campaign which was done at the end of the same month. The biting rate dropped from 2.7 *An. arabiensis*/person/night indoors and 4.0 outdoors in September to 1.2 indoors and 0.5 outdoors in October. In November, the biting rates increased to 7.8 *An. arabiensis*/person/night both indoors and outdoors, but in the following months, the rates remained below 1.0 *An. arabiensis*/person/night except indoors in March (1.8 *An. arabiensis*/person/night) (Figure 7C). The variation in biting rates observed in October and November might be due to a combination of IRS and other environmental factors.

In Metema, *An. arabiensis* were collected between June and November only. In general, the human biting rates were low except in September, when the rate was 2.5 *An. arabiensis*/person/night indoors and 4.0 *An. arabiensis*/person/night outdoors (Figure 7D).

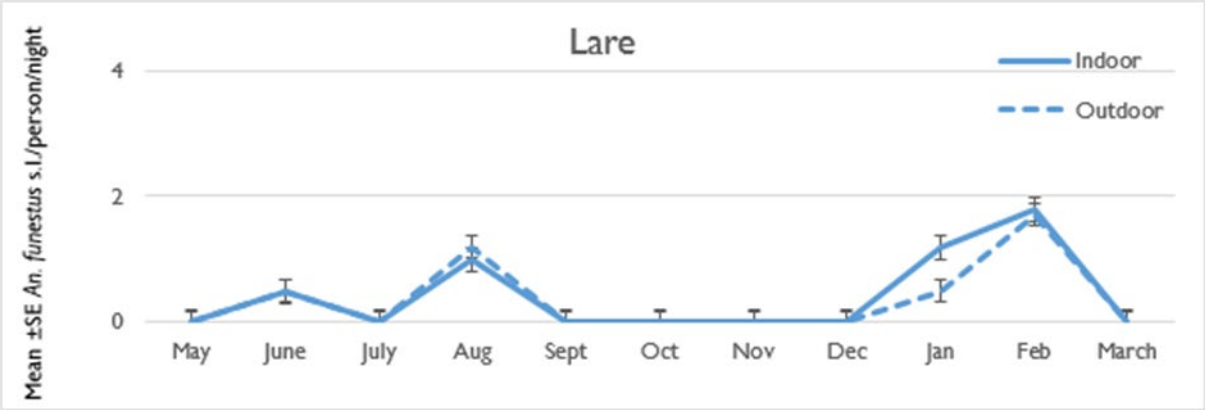
FIGURE 7. MEAN HUMAN BITING RATES OF *AN. ARABIENSIS* PER PERSON PER NIGHT FROM ABAYA AND BAMBASI (A), LARE AND BENATSEMAY (B), HARBU AND JABITEHNAN (C), AND METEMA (D) (MAY 2019-MARCH 2020)



Night Biting Rates of An. funestus Group (Lare only)

In Lare, *An. funestus* group were present during four months of the entomological surveillance period: June, August, January, and February. The human biting rate was measured from a total of 50 mosquitoes collected using HLCs. In June, the rate was 0.5 *An. funestus* s.l./person/night both indoors and outdoors. The mean indoor biting rates in August, January, and February were 1.0, 1.2, and 1.8 *An. funestus* s.l./person/night, respectively, while the outdoor rates were 1.2, 0.5, and 1.7 (Figure 8).

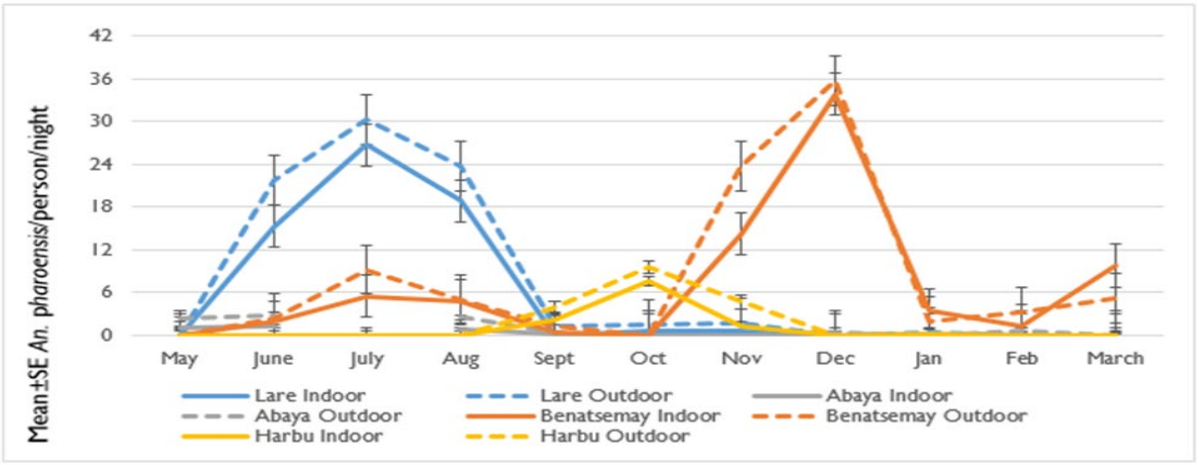
FIGURE 8. HUMAN NIGHT BITING RATES OF *AN. FUNESTUS* GROUP IN LARE (MAY 2019-MARCH 2020)



Night Biting Rates of An. pharoensis (Abaya, Benatsemay, Lare, and Harbu)

An. pharoensis appeared in Lare from June to November, and in Abaya from May to September, and December and February. This species was completely absent from HLCs in Benatsemay in May and October, but was available in the other months with variable biting density. In contrast to the other sites, in Harbu, *An. pharoensis* prevailed for only three months, September through November. Three peaks in the mean human biting rate of *An. pharoensis* were evident in Lare, in June (15.3 indoors, 21.8 outdoors), July (26.7 indoors, 30.3 outdoors), and August (18.8 indoors, 23.7 outdoors). In Benatsemay high human biting rates were observed in November (14.2 indoors, 23.7 outdoors) and December (33.8 indoors, 35.7 outdoors) (Figure 9). October was the most productive month in Harbu, with human biting rates of 7.5 and 9.5 *An. pharoensis*/person/night indoors and outdoors, respectively (Figure 9).

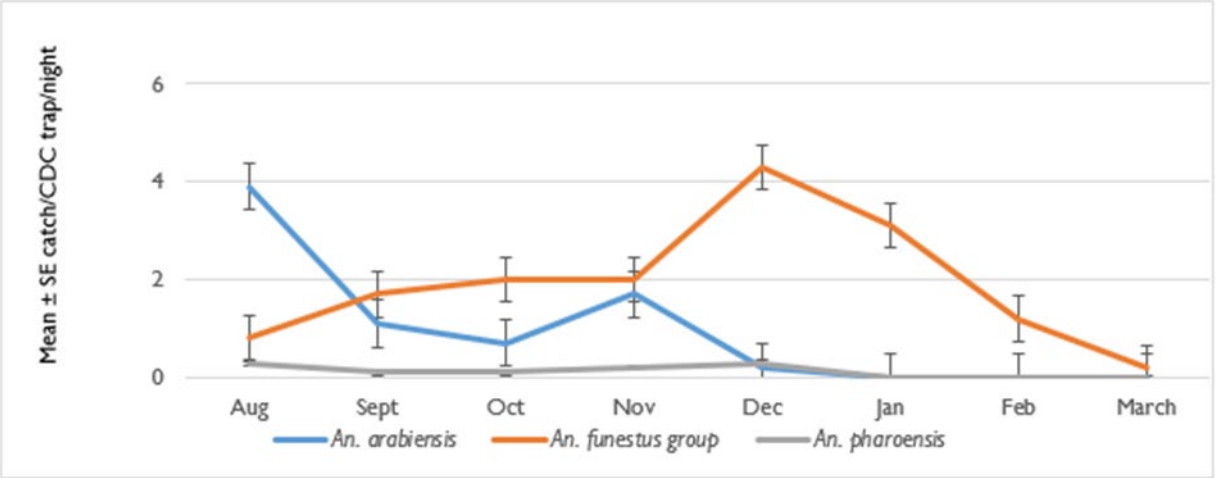
FIGURE 9. HUMAN NIGHT BITING RATES OF *AN. PHAROENSIS* (MAY 2019-MARCH 2020)



Density of An. arabiensis, An. funestus group, and An. pharoensis from CDC light trap collections (Bambasi only)

CDC light traps caught *An. funestus* group in Bambasi from August to March; no *An. arabiensis* or *An. pharoensis* were trapped from January to March. The mean nightly trap catch of *An. funestus* group ranged from the minimum of 0.8 *An. funestus* s.l./trap/night in August to the maximum of 4.3 *An. funestus* s.l./trap/night in December. The second largest catch, 3.1 *An. funestus* s.l./trap/night, was recorded in January. The density of *An. arabiensis* was 3.9 *An. arabiensis*/trap/night in August, 1.1 in September, and 1.7 in November, but less than 1.0 in October and December. The density of *An. pharoensis* in all months was less than 0.5 mosquitoes per trap/night (Figure 10).

FIGURE 10. *ANOPHELES* DENSITY FROM CDC LIGHT TRAP COLLECTIONS IN BAMBASI (AUGUST 2019-MARCH 2020)



3.4.3 *Night Biting Cycle of An. arabiensis, An. funestus group, and An. pharoensis*

The night biting cycles together with the hourly biting rates of *An. arabiensis*, *An. funestus* group, and *An. pharoensis* indoors and outdoors is presented in this section.

Summary of night biting cycle of An. arabiensis before and after midnight

In four of the seven sites, most of the indoor vector-host contact occurred before midnight. The four sites were: Lare, where the respective share of *An. arabiensis* was 62.0%, Harbu at 60.7%, Benatsemay at 56.2%, and Metema at 51.6%. The share of *An. arabiensis* biting outdoors before midnight was slightly higher than after midnight in Harbu (64.3%), Benatsemay (55.2%), Lare (54.6%), and Jabitehnan (51.4%). In Bambasi, Jabitehnan, and Abaya a slightly higher proportion of the vector was found feeding after midnight indoors than before midnight indoors (Table 7).

TABLE 7. PROPORTION OF *AN. ARABIENSIS* CAUGHT BY HLCS BEFORE AND AFTER MIDNIGHT

Site	Indoors		Outdoors	
	6pm-12 am	12am-6 am	6pm-12 am	12am-6 am
Abaya	26 (48.0)	28 (52.0)	57 (43.8)	73 (56.2)
Bambasi	44 (46.3)	51 (53.7)	81 (45.0)	100 (55.0)
Lare	217 (62.0)	133 (38.0)	277 (54.6)	230 (45.4)
Jabitehnan	64 (45.7)	76 (54.3)	57 (51.4)	54 (48.6)
Harbu	91 (60.7)	59 (39.3)	180 (64.3)	100 (35.7)
Benatsemay	257 (56.2)	200 (43.8)	292 (55.2)	237 (44.8)
Metema	16 (51.6)	15 (48.4)	14 (36.8)	24 (63.2)

Highlighted figures indicate proportion of highest biting time.

HOURLY NIGHT BITING ACTIVITIES OF AN. ARABIENSIS (ABAYA, BAMBASI, LARE, BENATSEMAY, HARBU, JABITEHNAN, AND METEMA)

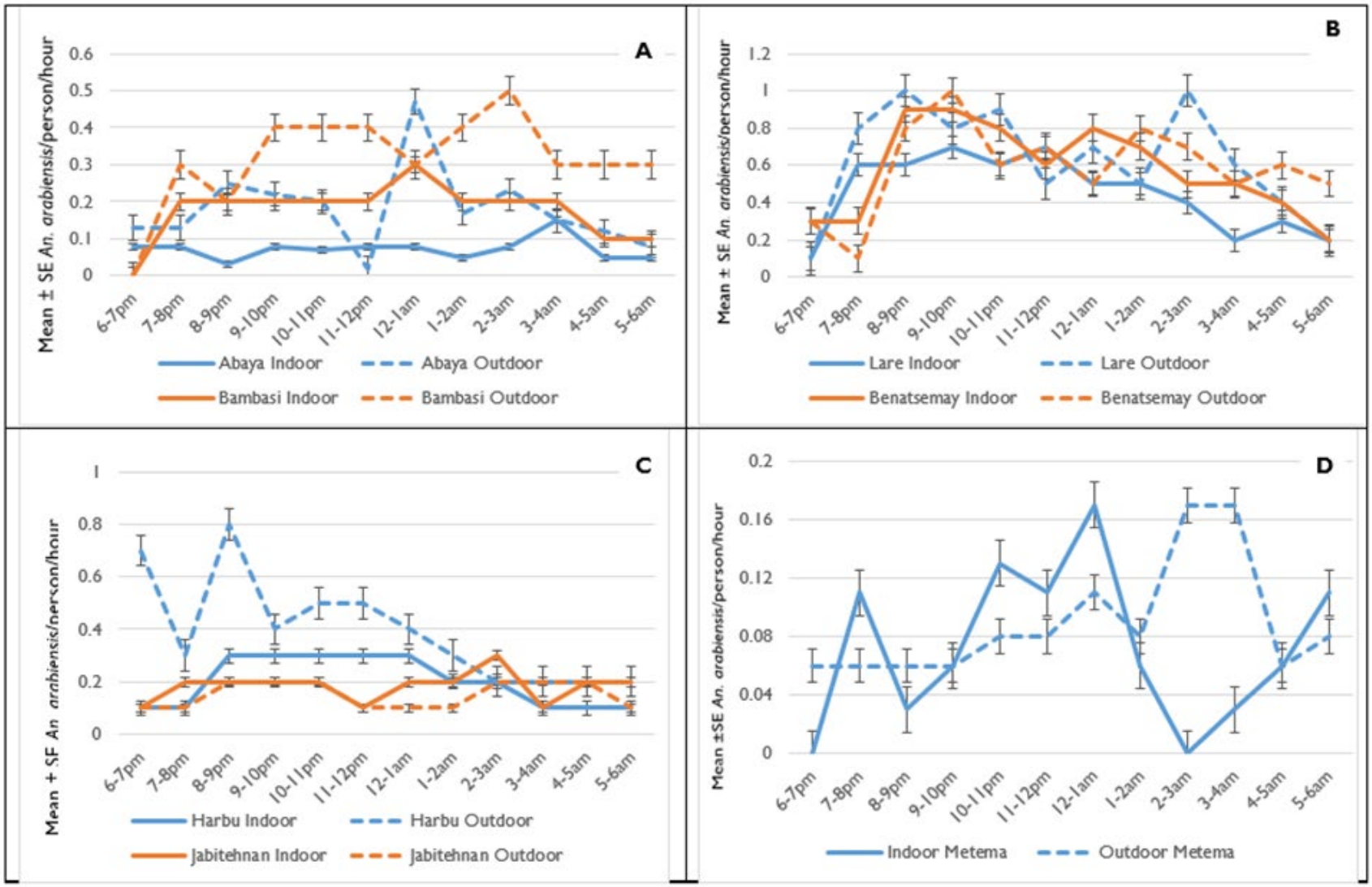
In Abaya, the mean hourly biting rates of *An. arabiensis* indoors were below 0.10 bites/person/hour for all times of the night except between 3:00 am-4:00 am which had a higher biting rate of 0.15 bites/person/hour. The mean hourly biting rate outdoors varied by time and peaked (0.47 bites/person/hour) between 12:00 am and 1:00 am. In Bambasi, the outdoor *An. arabiensis* hourly biting rate showed two major peaks, at 9:00 pm-12:00 pm and 2:00 am-3:00 am. The peak biting time indoors was immediately after midnight (12:00 am-1:00 am) (Figure 11A).

In Lare, *An. arabiensis* was most active outdoors from 8:00 pm to 11:00 pm in the period before-midnight, and from 2:00 am to 3:00 am in the after-midnight period. The indoor biting rate was consistently high between 7:00 pm and 12:00 am and gradually decreased after midnight. In Benatsemay, the peak biting hours were before midnight both indoors and outdoes between 8:00 pm and 10:00 pm (Figure 11B).

In Harbu, *An. arabiensis* had two marked peaks outdoors, at 6:00 pm-7:00 pm and 8:00 pm-9:00 pm; biting rates were higher before midnight than after. The mean hourly biting rates observed indoors in Harbu and both indoors and outdoors in Jabitehnan were consistently low, without a distinct spike, throughout the night (Figure 11C).

In Metema, the indoor biting rates of *An. arabiensis* were highest between 10:00 pm and 1:00 am. Outdoors, the biting gradually increased during the night until it peaked between 2:00 am and 4:00 am; then it sharply declined (Figure 11D).

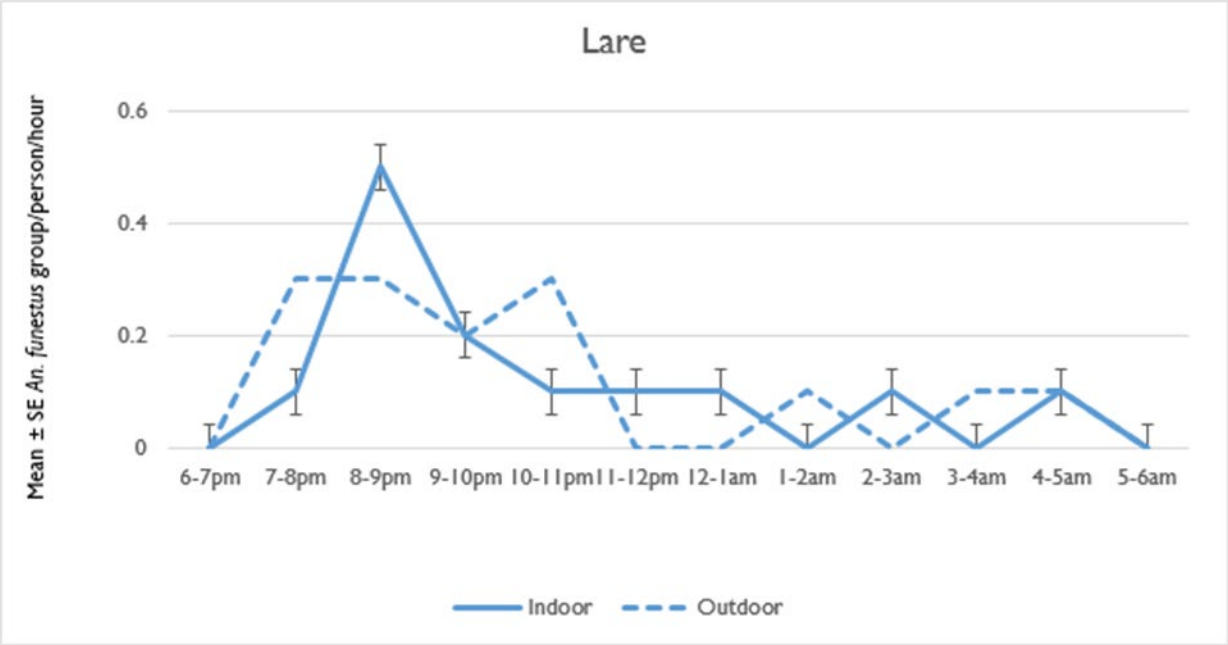
FIGURE 11. NIGHT BITING ACTIVITIES OF *AN. ARABIENSIS* IN ABAYA AND BAMBASI (A), LARE AND BENATSEMAY (B), HARBU AND JABITEHNAN (C), AND METEMA (D)



Hourly Night Biting Activities of An. funestus s.l. in Lare

In Lare, a high proportion of *An. funestus* group was actively searching for human blood mainly during the first half of the night and gradually declined after the midnight (Figure 12)

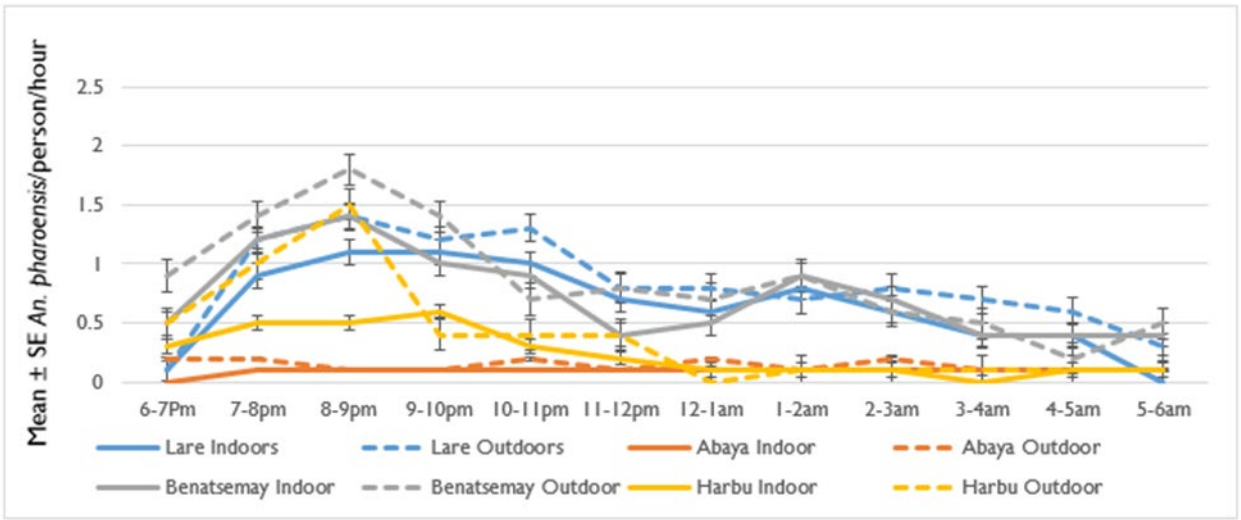
FIGURE 12. NIGHT BITING ACTIVITIES OF *AN. FUNESTUS* GROUP IN LARE



Hourly night biting activities of An. pharoensis (Abaya, Benatsemay, Harbu, and Lare)

Most of the night biting activities of *An. pharoensis* took place before midnight, with variable hourly biting rates. The peak was between 8:00 pm-9:00 pm (Figure 13).

FIGURE 13. NIGHT BITING ACTIVITIES OF *AN. PHAROENSIS*



3.5 LABORATORY TEST RESULTS

This section presents results of laboratory tests on species identification of members of *An. gambiae* and *An. funestus* complexes, sporozoite infection rates of *Anopheles* species, and blood meal sources of *An. arabiensis* and *An. funestus* group.

3.5.1 SPECIES IDENTIFICATION

A total of 328 specimens morphologically identified as *An. gambiae* s.l. from Bambasi, Harbu, Benatsemay, Pawi, and Lare were examined for molecular identification. Of these, 319 (97.3%) were found to be *An. arabiensis* (Table 8). DNA of the rest of the specimens was not amplified, and therefore, the species could not be identified

TABLE 8. *AN. ARABIENSIS* IDENTIFIED FROM ID PCR ASSAYS (2019)

Site	Number Tested	*An. arabiensis* (%)
Bambasi	102	100 (98)
Harbu	24	21 (87.5)
Benatsemay	92	92 (100)
Lare	50	48 (96.0)
Pawi	60	58 (96.7)
Total	328	319 (97.3)

Molecular species identification of 155 mosquitoes morphologically identified as *An. funestus* s.l. confirmed the presence of four species in Bambasi namely *An. parensis* 91% (n=141), *An. funestus* s.s. 7.8% (n=12), *An. rivolorum* like 0.6% (n=1), and *An. leesoni* 0.6% (n=1). The predominant species was *An. parensis,* which constituted 91% of the *An. funestus* group. This species is implicated as a malaria vector in South Africa (Burke et al. 2017) but its status is not yet known in Ethiopia and requires further studies.

3.5.2 SPOROZOITE INFECTION RATES OF ANOPHELES

A total of 2,011 *An. arabiensis* from all sentinel sites, 873 *An. pharoensis* from Lare, Abaya, and Bambasi, and 216 *An. funestus* group from Lare and Bambasi were examined for sporozoite infections. A single *Plasmodium* (*P.*) *falciparum* infection in *An. arabiensis* from 772 specimens (0.13%) from Lare was detected. Similarly, a single *An. arabiensis* from Jabitehnan was found to be infected with *P. vivax* (1/356, 0.28% infection rate). *An. pharoensis* from Lare were also infected with *P. falciparum*. The overall *P. falciparum* and *P. vivax* sporozoite infection rates in *An. arabiensis* were both 0.05%, while that of *P. falciparum* in *An. pharoensis* was 0.11% (Table 9).

TABLE 9. SPOROZOITE INFECTION RATES IN *AN. ARABIENSIS, AN. PHAROENSIS*, AND *AN. FUNESTUS* GROUP

	An. arabiensis			*An. pharoensis*			*An. funestus* group		
Site	Tested	+ for *P. falciparum* (%)	+ for *P. vivax* (%)	Tested	+ for *P. falciparum* (%)	+ for *P. vivax* (%)	Tested	+ for *P. falciparum* (%)	+ for *P. vivax* (%)
Lare	772	1 (0.13)	0	820	1 (0.12)	0	31	0	0
Benatsemay	761	0	0	0	0	0			
Harbu	48	0	0	-	0	0			
Abaya	47	0	0	51	0	0			
Jabitehnan	356	0	1 (0.28)	-	0	0			
Metema	8	0	0	-	0	0		0	0
Bambasi	19	0	0	2	0	0	185		
Total	2011	1 (0.05)	1 (0.05)	873	1 (0.11)	0	216	0	0

3.5.3 BLOOD MEAL SOURCES OF AN. ARABIENSIS IN BENATSEMAY

Blood meal sources of 176 *An. arabiensis* from Benatsemay were investigated through blood meal ELISA. All the specimens were from PSCs inside human dwellings. Species ID PCR was conducted on 78 of the 176 specimens, and all of them were *An. arabiensis*.

Human blood only was detected in 10.2%, bovine blood only in 33.0%, and mixed blood in 4.5%. The remaining 52.3% of the blood meals were non-reactive to the antibodies of human and bovine blood, implicating the presence of other hosts. Pastoralists in the area keep large flocks of goats, and hence, these animals are suspected to be the main blood meal sources of *An. arabiensis*.

Although it was not systematically documented, visual observations showed a high rate of ITNs use in the community. Families sleep outdoors protected by nets. This practice might account for the low composition of human blood (Table 10).

TABLE 10. BLOOD MEAL SOURCES OF *AN. ARABIENSIS* IN BENATSEMAY (2019)

Host	N (%)
Human	18 (10.2)
Bovine	58 (33.0)
Mixed (human and bovine)	8 (4.5)
Non-reactive	92 (52.3)
Total	176 (100)

Attempts were made to identify the blood meals of 147 *An. funestus* group specimens - 12 *An. funestus* s.s. and 135 *An. parensis*. Of the mosquitoes subjected to blood meal analysis against human and bovine antigens, the blood meals of 42.9% mosquitoes were identified. The remaining 57.1% of blood meals were not reactive to human and bovine antigens, indicating the presence of other hosts. Bovine only constituted 37% of the blood meals of *An. parensis* (Table 11).

TABLE 11. BLOOD MEAL SOURCES OF *AN. FUNESTUS* GROUP IN BAMBASI (2019)

Species	# Tested	Human	Bovine	Mixed	Unidentified*
An. funestus s.s.	12	1 (8.4)	2 (16.6)	0 (0)	9 (75.0)
An. parensis	135	4 (3.0)	50 (37.0)	6 (4.4)	75 (55.6)
Total	147	5 (3.4)	52 (35.4)	6 (4.1)	84 (57.1)

*additional antigens will be procured and blood source identification expanded to other animals in 2020.

3.6 AN. ARABIENSIS COLLECTION FROM STRUCTURES IN PAWI AND LARE TO DETERMINE THE RESTING HABIT

This section presents *An. arabiensis* collection results from Pawi and Lare.

3.6.1 AN. ARABIENSIS COLLECTIONS FROM PAWI

A total of 881 *An. arabiensis* collected in Pawi were from the interiors of houses, animal shelters, kitchens, and latrines. Out of the 881, 463 (52.6%) were from structures that had been sprayed with Actellic 300 CS, and 418 (47.4%) were from unsprayed structures. Animal shelters yielded the most mosquitoes, followed by kitchens. The mean numbers of *An. arabiensis* collected from sprayed and unsprayed animal shelters were 56.3 and 42.4, respectively, and from kitchens 12.2 and 8.1 (Table 12).The number of *An. arabiensis* collected from animal shelters vary from the lowest 0 to the largest 240. The collections were made two months after spraying.

TABLE 12. TYPE OF STRUCTURES, NUMBER, AND MEAN OF *AN. ARABIENSIS* COLLECTED FROM PAWI (SEPTEMBER 2019)

Type of Structure	Sprayed			Unsprayed		
	Number of structures	*An. arabiensis*		Number of structures	*An. arabiensis*	
		Number	Mean		Number	Mean
House	9	7	0.8	6	4	0.7
Cattle shelter	7	394	56.3	8	339	42.4
Kitchen	5	61	12.2	9	73	8.1
Latrine	5	1	0.2	8	2	0.3
Total		463			418	

The proportion of blood-fed *An. arabiensis* was greater in sprayed animal shelters (91.6%) than in unsprayed ones (82.9%) (Table 13). More fresh blood feds were also sampled in kitchens. The small number of half gravid and gravid mosquitoes compared with fresh fed implies that *An. arabiensis* completes egg development in other locations, probably hiding in vegetation, burrows and discarded containers around human habitations (Table 13).

TABLE 13. ABDOMINAL FEEDING STATUS OF *AN. ARABIENSIS* COLLECTED FROM ANIMAL SHELTERS AND KITCHENS IN PAWI (SEPTEMBER 2019)

Abdominal Feeding Status	Animal Shelter N (%)		Kitchen N (%)	
	Sprayed	Not Sprayed	Sprayed	Not Sprayed
Unfed	1 (0.3)	11 (3.2)	0	3 (4.1)
Blood fed	361 (91.6)	281 (82.9)	60 (98.4)	64 (87.7)
Half gravid	24 (6.1)	38 (11.2)	0	6 (8.2)
Gravid	8 (2.0)	9 (2.7)	1 (1.6)	0
Total	394 (100)	339 (100)	61 (100)	73 (100)

Of those *An. arabiensis* collected in Pawi, 60 specimens were tested for species ID, out of which 58 (96.7%) were *An. arabiensis*. The two specimens DNA failed to amplify probably because of the small amount of DNA in the test.

3.6.2 *AN. ARABIENSIS* COLLECTIONS IN LARE

A total of 183 *An. arabiensis* collected in Lare were from sprayed houses, sprayed animal shelters, unsprayed animal shelters, kitchens, and latrines. (Table 14). The daily mean number of *An. arabiensis* in animal shelters was 89.6, which is greater than the 6.6 mean collected from houses.

TABLE 14. *AN. ARABIENSIS* COLLECTED FROM LARE (SEPTEMBER 2019)

Type of Structure	Number Searched	*An. arabiensis*	
		Number	Mean
Sprayed houses	15	12	6.6
Sprayed animal shelters	15	164	89.6
Unsprayed animal shelter	2	24	12
Sprayed kitchens	14	3	1.6
Sprayed latrine	13	4	2.2
Total		183	

Of the 164 *An. arabiensis* caught in animal shelters, 52.4% were gravids, 6.7% were half gravids, while 40.2% were fresh feds (Table 15). The greater percentage of gravids and half gravids compared with fresh feds implies that *An. arabiensis* rests for a longer time in animal shelters in Lare than in Pawi.

TABLE 15. ABDOMINAL FEEDING STATUS OF *AN. ARABIENSIS* COLLECTED FROM ANIMAL SHELTERS IN LARE, GAMBELA (SEPTEMBER 2019)

Abdominal Feeding Status	*An. Arabiensis* N (%)
Unfed	1 (0.7)
Blood fed	66 (40.2)
Half gravid	11 (6.7)
Gravid	86 (52.4)
Total	164 (100)

3.7 AN. STEPHENSI LONGITUDINAL SURVEILLANCE: MONTHLY ABUNDANCE, SPOROZOITE INFECTION RATES, AND BLOOD MEAL SOURCES

This section discusses the results obtained from monthly surveillance of *An. stephensi* conducted in Dire Dawa, Kebridehar, Awash, and Metehara towns. Data are presented on abundance of *An. stephensi*, sporozoite infection rates, and blood meal sources.

3.7.1 ABUNDANCE OF AN. STEPHENSI

A total of 1,040 *An. stephensi* were collected from Dire Dawa (n=412), Kebridehar (n=368), Awash (n=154), and Metehara (n=106). The majority (n=585, 56.3%) were collected in animal shelters (cattle, goats, sheep, and horses) using hand collections; suggesting the most productive collection method for *An. stephensi* currently available. In the outskirts of Dire Dawa, nearly 39% (n=159) of *An. stephensi* were sampled resting in black boxes placed in the compounds of houses with horse stables. Black boxes were inefficient in the rest of the towns and in Dire Dawa proper, where the compounds sampled had no horse stables. Cattle-baited traps caught 19.0% (n=198) of all these collections. The common mosquito sampling methods, HLCs, PSCs, and CDC light traps, were less effective at collecting *An. stephensi* (Table 16, Annex B).

TABLE 16. *AN. STEPHENSI* COLLECTED, BY METHOD

Sentinel Sites	*An. stephensi* N (%)						Total
	PSC	HLC	CDC	Hand Collection from Animal Shelters	Black Box	Cattle-baited Tent Trap	
Dire Dawa	4 (1.0)	0 (0)	4 (1.0)	205 (49.8)	159 (38.5)	40 (9.7)	412 (100)
Kebridehar	29 (7.9)	5 (1.4)	3 (0.8)	212 (57.6)	0 (0)	119 (32.3)	368 (100)
Awash	3 (1.9)	7 (4.5)	0 (0)	123 (79.9)	0 (0)	21 (13.7)	154 (100)
Metehara	9 (8.5)	11 (10.4)	23 (21.7)	45 (42.5)	0 (0)	18 (17.0)	106 (100)
Overall	45 (4.3)	23 (2.2)	30 (2.9)	585 (56.3)	159 (15.3)	198 (19.0)	1040 (100)

TABLE 17. *ANOPHELES STEPHENSI* ABUNDANCE BY MONTH

Month	Awash	Dire Dawa	Kebridehar	Metehara	Total
June	ND	0	8	ND	8
July	ND	21	1	ND	22
August	24	160	13	7	204
September	47	118	41	65	271
October	ND	92	113	23	228
November	50	13	79	7	149
December	33	8	113	18	172
Overall	**154**	**412**	**368**	**106**	**1040**

ND= Not done

3.7.2 SPOROZOITE INFECTION RATES OF AN. STEPHENSI

A total of 780 *An. stephensi* specimens (412 from Dire Dawa and 368 from Kebridehar) were tested for *P. circumsporozoite* proteins. Of these, three specimens were reactive for *P. vivax*, giving infection rates of 0.5% and 0.3% in the samples from Dire Dawa and Kebridehar, respectively (Table 18).

TABLE 18. SPOROZOITE INFECTION RATES OF *AN. STEPHENSI*

Site	# Tested	Pf +ve N (%)	Pv210 +ve N (%)	Pv247 +ve N (%)	Total +ve N (%)
Dire Dawa	412	0	2 (0.5)	0	2 (0.4)
Kebridehar	368	0	0	1 (0.3)	1 (0.3)
Overall	780	0	2 (0.3)	1 (0.12)	3 (0.37)

Pf= *Plasmodium falciparum;* Pv=*Plasmodium vivax*, +ve= positive; N=number tested

3.7.3 BLOOD MEAL SOURCES OF AN. STEPHENSI

A total of 631 *An. stephensi* from Dire Dawa and Kebridehar sites were tested by ELISA for blood meal sources. One (0.25%) of the 394 *An. stephensi* from Dire Dawa and 0/237 from Kebridehar were found with human blood only. In contrast, 29.7% and 53.2% were found to have fed on goats, and 1.02% and 0.4% on cows, in the respective sites. Dog blood was identified from 2.03% of *An. stephensi* from Dire Dawa and 1.3% from Kebridehar. Mixed blood was found in 20.92% of *An. stephensi* tested. The remaining 38.4% of blood meals were not identified, indicating the presence of other hosts (Table 19).

TABLE 19. BLOOD MEAL SOURCES OF *ANOPHELES STEPHENSI* COLLECTED USING DIFFERENT SAMPLING METHODS FROM DIRE DAWA AND KEBRIDAHR,

Site	Shelter	Number tested	Blood meal source											
			Human	Bovine	Goat	Dog	B+G	B+G+D	G+D	H+B+G+D	H+D	H+G	H+G+D	Unknown
Dire Dawa	Black Box in HLC compound	22	0	0	6	1	0	0	3	0	0	0	0	12
	Black Box near horse stable	152	0	0	40	4	1	1	29	0	0	1	1	75
	Horse stable	119	0	1	27	3	1	2	13	0	0	0	0	72
	Cattle baited tent trap	36	0	1	4	0	14	1	0	0	0	0	0	16
	Goat sheds	60	0	2	37	0	1	0	0	0	0	0	0	20
	Human dwelling	5	1	0	3	0	0	0	0	0	0	0	0	1
	Total	**394**	**1(0.25%)**	**4 (1.02%)**	**117(29.7%)**	**8(2.03%)**	**17(4.3%)**	**4(1.02%)**	**45(11.4%)**	**0**	**0**	**1(.025%)**	**1(0.25%)**	**196(49.7%)**
Kebridehar	Cattle shelter	111	0	0	53	0	33	0	0	1	0	1	0	23
	Cattle baited tent trap	84	0	0	47	0	25	0	0	0	0	0	0	12
	Goat sheds	30	0	0	21	0	3	0	0	0	0	0	0	6
	Human dwelling	12	0	1	5	0	1	0	0	0	0	0	0	5
	Total	**237**	**0**	**1(0.4%)**	**126(53.2%)**	**0**	**62(26%)**	**0**	**0**	**1(0.4%)**	**0**	**1(0.4%)**	**0**	**46(19.4%)**
Total		**631**	**1(0.16%)**	**5(0.8%)**	**243(38.5%)**	**8(1.3%)**	**79(12.5%)**	**4(0.6%)**	**45(7%)**	**1 (0.16%)**	**0**	**2(0.32%)**	**1(0.16%)**	**242(38.4%)**

3.8 AN. STEPHENSI SURVEY RESULTS

The occurrence of *An. stephensi* was confirmed from adult identifications in the towns of Metehara (n=322), Meki (n=86), and Zeway (n=17), all in Oromia Region. In this survey period, *An. stephensi* was absent from surveyed sites in Negelle-Borena, Yabello, Jimma, Gambela, Assosa, Bahirdar, Hawassa, and Shire (Table 20). The present finding raised the distribution sites of *An. stephensi* to 13 as compared to 10 urban localities in eastern Ethiopia previously documented by Balkew et al. (2020).

TABLE 20. *AN. STEPHENSI* SURVEY RESULTS (2019)

Urban Site	Larval Habitat Type	# larvae	*Anopheles*			
			An. stephensi	*An. gambiae* s.l.	*An. rhodesiensis*	*An. cinereus*
Negelle-Borena	Water containers	55	-	13	0	0
	Water tanks	66	-	11	0	1
	Stagnant water pools	211	-	132	0	0
Yabello	Water tanks	39	-	13	0	0
	Stagnant water pools	55	-	23	0	0
	Cement water reservoirs	194	-	90	15	0
Jimma town	Rain pools	378	-	148	-	-
Gambela town	Rain pools	143	-	61	-	-
Assosa	Discarded tires	150	-	86	-	-
	Temporary habitats	1618	-	1266	-	-
	Natural habitats	710	-	531	-	-
Bahirdar	Tire track	1681	-	1213	-	-
	Stagnant water pools	807	-	294	-	-
Meki	Tire tracks	45	24	0	-	-
	Concrete water container	68	43	20	-	-
	Water tanks	36	19	10	-	-
	Discarded buckets	2	0	1	-	-
Zeway	Tire tracks	24	14	0	-	-
	Water drums	1	0	0	-	-
	Concrete water containers	12	3	5	-	-
Hawassa	Tire tracks	0	-	0	-	-
	Water drums	7	-	5	-	-
	Concrete water containers	6	-	4	-	-
	Waste bin	12	-	9	-	-
	Plastic bucket	4	-	4	-	-
Shire	Tire	14	-	14	-	-
	Temporary habitats	2327	-	990	-	-
	Natural habitats	208	-	130	-	-
Metehara	Water tanks	1075	322	0	-	-

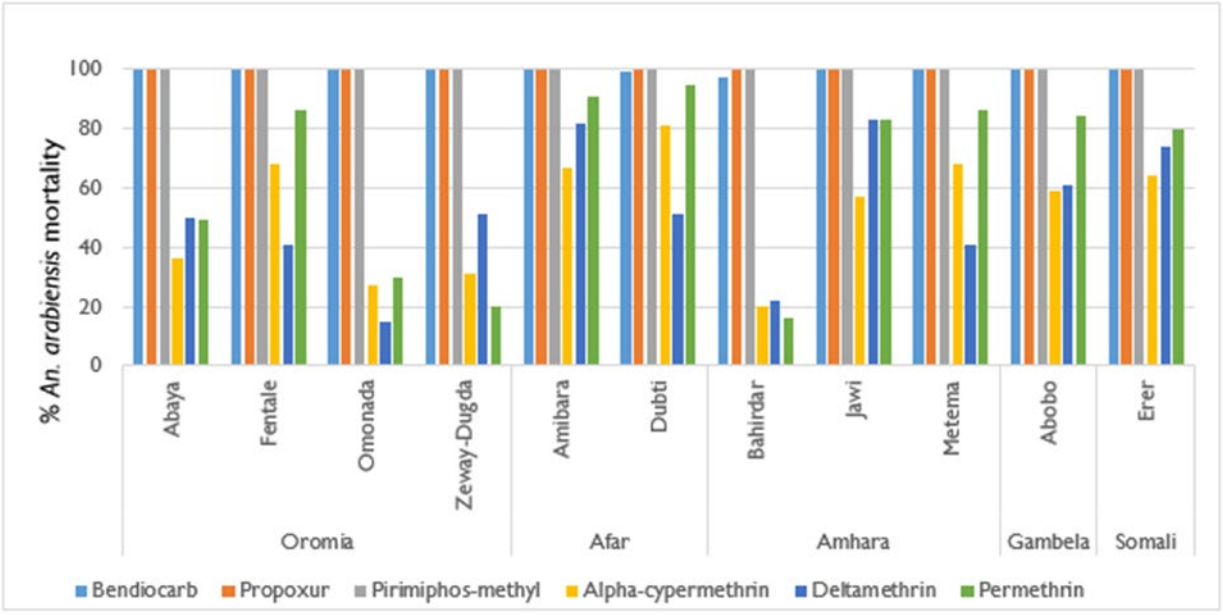
3.9 INSECTICIDE RESISTANCE MONITORING

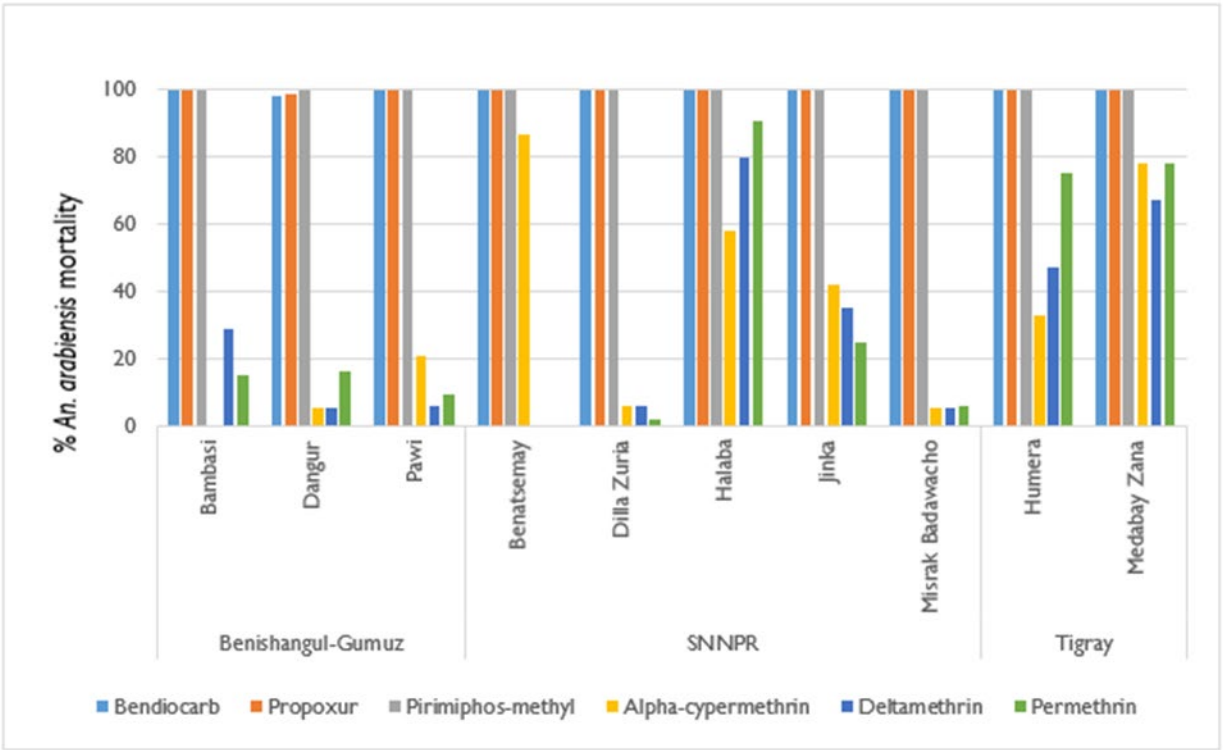
3.9.1 AN. ARABIENSIS SUSCEPTIBILITY TO INSECTICIDES

In WHO tube tests, pirimiphos-methyl and propoxur caused 98-100% mortality of the populations of *An. arabiensis* in all 21 sentinel sites after 24 hrs of holding period. Bendiocarb produced the same mortality results, except in Bahirdar, where the vector mortality was 97% (categorized as possible resistance). In contrast, as in the past, *An. arabiensis* was extremely resistant to the pyrethroid insecticides alpha-cypermethrin, deltamethrin,

and permethrin where the susceptibility tests were conducted. This resistance to the pyrethroids might have existed for a long time because of the selection pressure of ITNs together with insecticides used in the agricultural sector (Figure 14, Annex C).

FIGURE 14. *AN. ARABIENSIS* MORTALITY IN WHO TUBE TEST

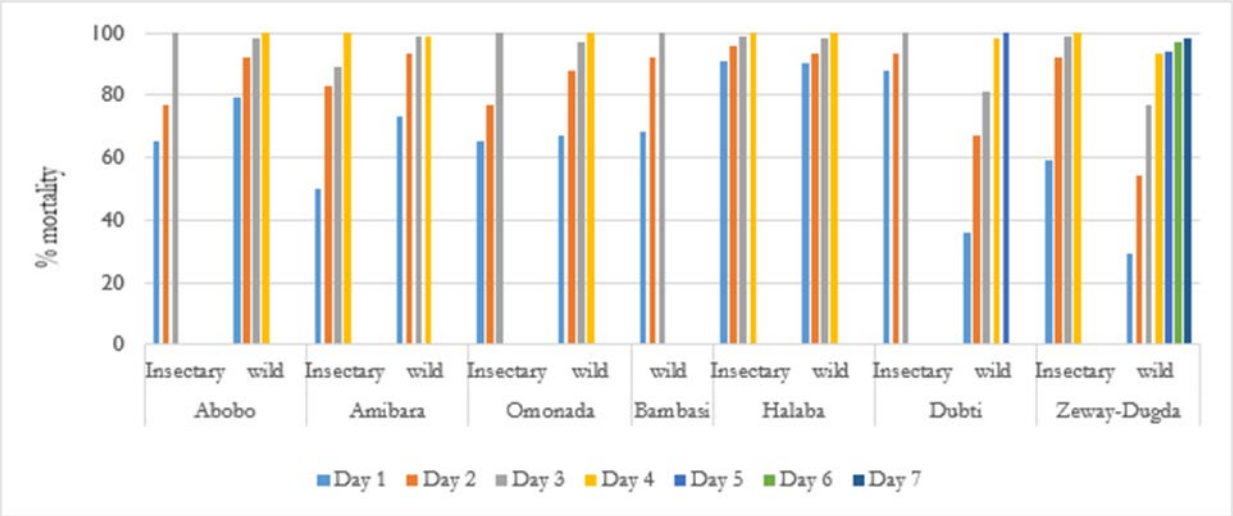




3.9.2 *AN. ARABIENSIS* SUSCEPTIBILITY TO CLOTHIANIDIN

Clothianidin killed 100% of wild *An. arabiensis* by Day 4 in five of the seven sites: Abobo, Amibara, Omonada, Bambasi, and Halaba. On Day 5, 100% mortality was recorded in the population of *An. arabiensis* from Dubti. It took seven days to kill 98% of wild *An. arabiensis* from Zeway-Dugda. All insectary *An. arabiensis* tested parallel to the wild *An. arabiensis* and died on Days 3 and 4 (Figure 15, Annex D).

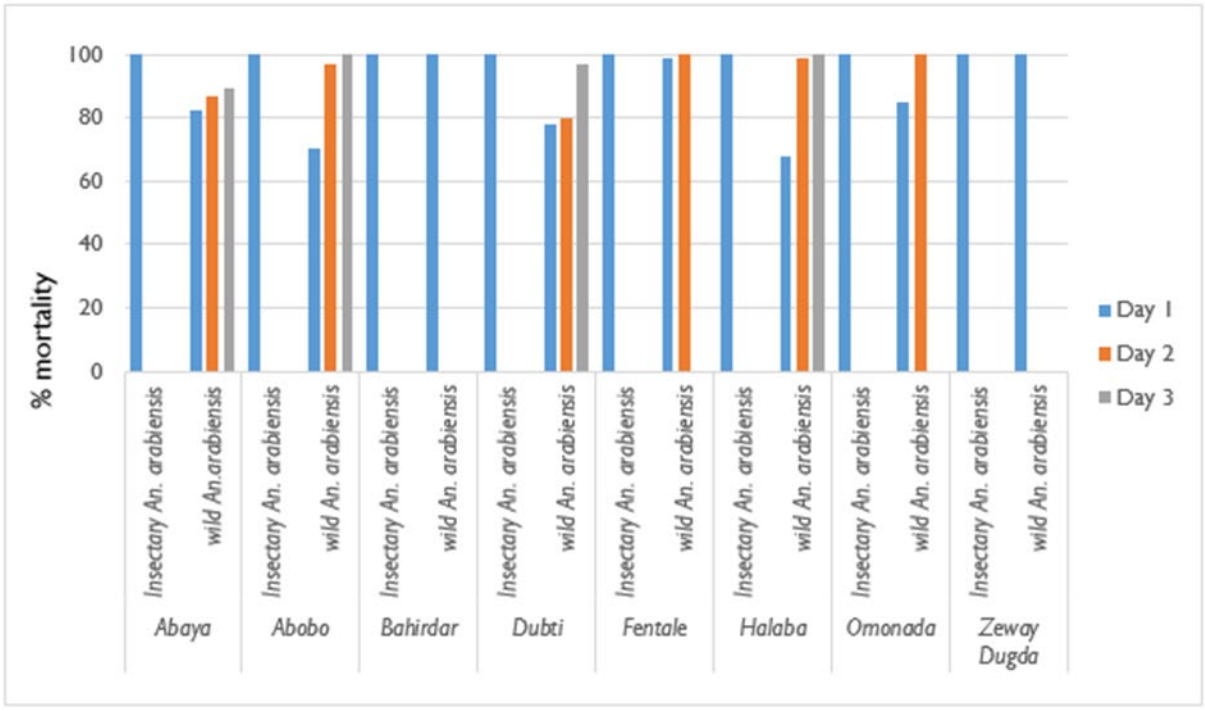
FIGURE 15. MORTALITY OF INSECTARY AND WILD *AN. ARABIENSIS* DUE TO EXPOSURE TO CLOTHIANIDIN



3.9.3 *AN. ARABIENSIS* SUSCEPTIBILITY TO CHLORFENAPYR

All of the wild *An. arabiensis* test mosquitoes from Zeway-Dugda died within 24 hours. Those from Omonada and Fentale died within 48 hours, and those from Abobo and Halaba died within 72 hours. Mortality of wild *An. arabiensis* from Abaya and Dubti after 72 hours was 89% and 97%, respectively, which puts them in resistance and possible resistance classifications. In the parallel tests, 100% mortality of the insectary colony of *An. arabiensis* was observed within 24 hours of exposure (Figure 16, Annex E).

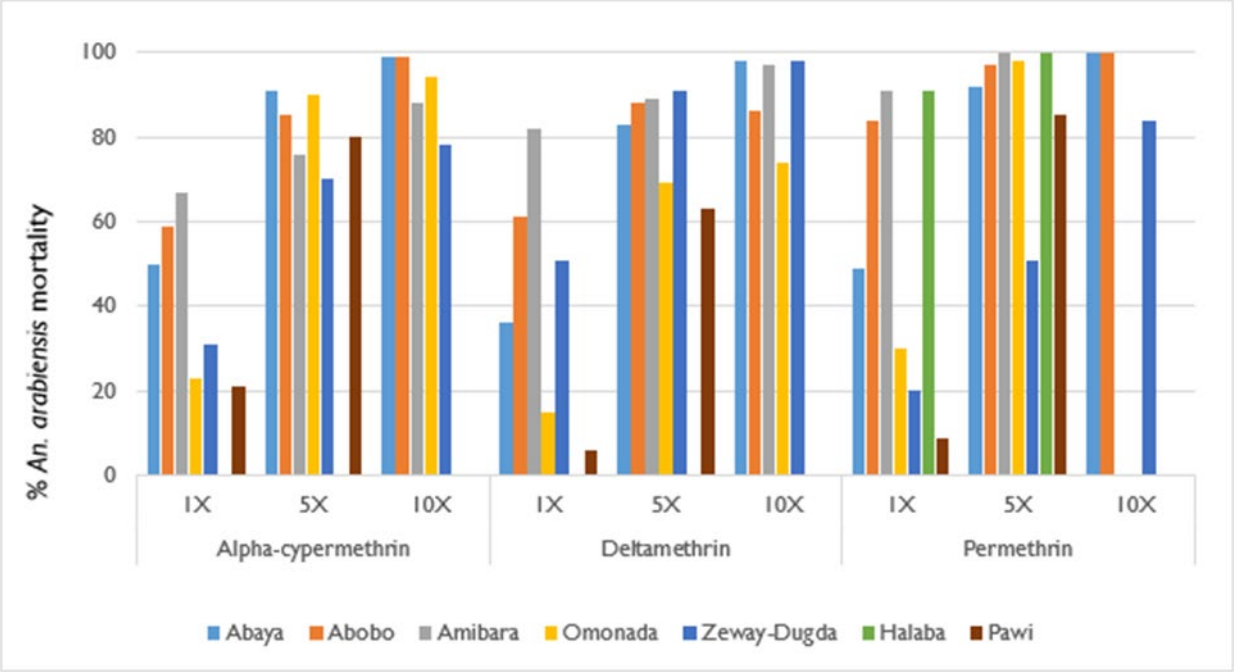
FIGURE 16. MORTALITY OF INSECTARY AND WILD *AN. ARABIENSIS* DUE TO EXPOSURE TO CHLORFENAPYR (100UG/BOTTLE)



3.9.4 RESISTANCE INTENSITY ASSAY RESULTS

High intensity resistance to alpha-cypermethrin was confirmed in Amibara, Omonada, and Zeway-Dugda, where mortality ranged from 78% to 94%. High intensity resistance to deltamethrin was recorded in *An. arabiensis* populations from Abobo, Amibara, and Omonada, where mortality ranged from 74% to 97%. High intensity resistance to permethrin was observed only in Zeway-Dugda (84% mortality at 10X). Populations of *An. arabiensis* exhibited moderate intensity resistance to alpha-cypermethrin in Abaya and Abobo (99% mortality at 10X), to deltamethrin in Abaya and Zeway-Dugda (98% mortality at 10X), and to permethrin in Abaya and Abobo (100% mortality at 10X). Low permethrin intensity resistance populations of *An. arabiensis* were recorded in Amibara, Omonada, and Halaba (98-100% mortality at 5X). Complete resistance intensity assays could not be conducted in Pawi because of a shortage of test mosquitoes. There, resistance intensity assays were conducted only at 5X concentration. *An. arabiensis* mortalities were 80%, 63%, and 85%, for alpha-cypermethrin, deltamethrin, and permethrin, respectively, suggesting moderate to high intensity resistance (Figure 17, Annex F).

FIGURE 17. MORTALITY OF *AN. ARABIENSIS* IN WHO TUBE RESISTANCE INTENSITY ASSAYS (2019)



3.9.5 *PBO* SYNERGIST ASSAY RESULTS

Pre-exposure to PBO restored susceptibility (98-100% mortality) to alpha-cypermethrin in the populations of *An. arabiensis* in seven of 11 sites (Abaya, Amibara, Dangur, Humera, Jawi, Medabay Zana, and Pawi), implicating the presence of a monoogygenase-based resistance mechanism. Partial restoration of susceptibility (83-92% mortailty) to alpha-cypermethrin was observed in four of the sites, Abobo, Omonada, Bambasi, and Zeway-Dugda (Figure 18A, Annex G).

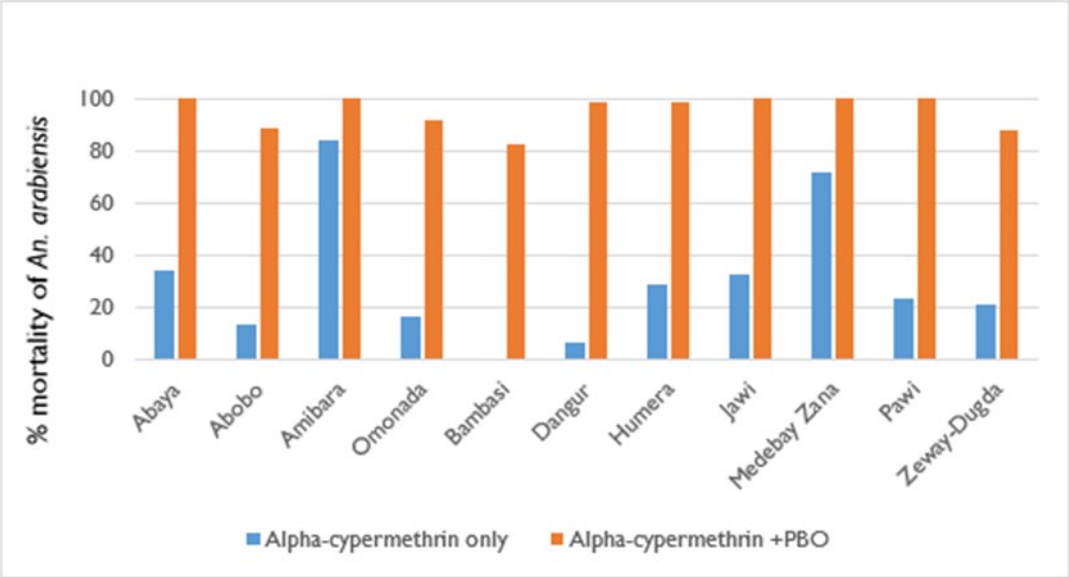
Pre-exposure to PBO restored susceptibility to deltamethrin in eight of the 11 sites (Abaya, Amibara, Omonada, Dangur, Humera, Jawi, Medabay Zana, and Pawi) and partially restored it in three of the sites (Abobo, Bambasi, and Zeway-Dugda) (Figure 18B, Annex G).

Pre-exposure to PBO restored susceptibility to permethrin in five of the 11 sites (Abobo, Abaya, Amibara, Humera, and Medabay Zana). It partially restored susceptibility to permethrin in Omonada, Bambasi, Dangur, Jawi, Pawi, and Zeway-Dugda (Figure 18C, Annex G).

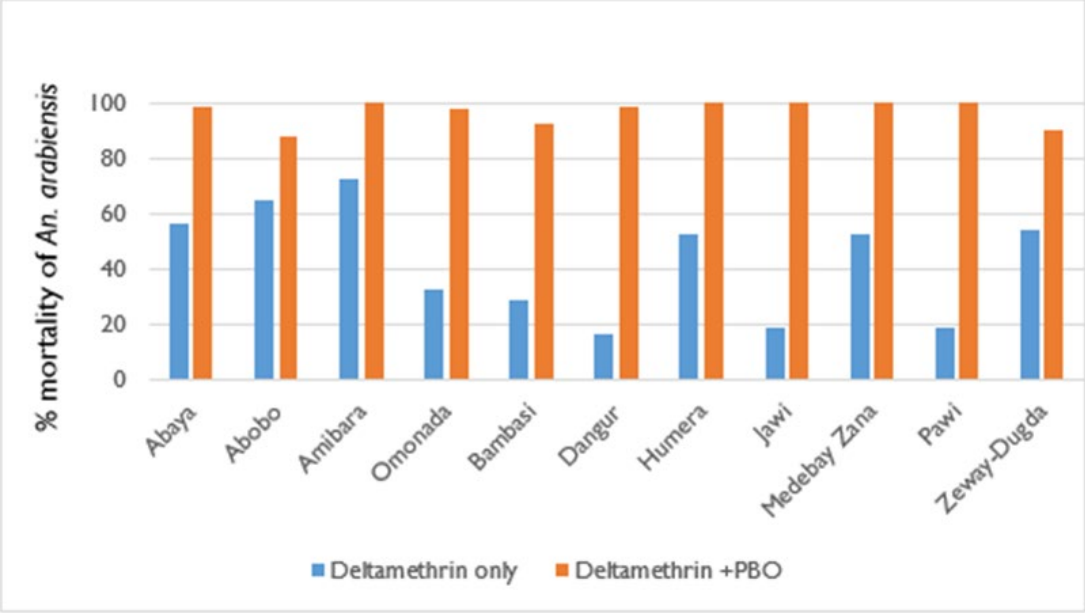
Pre-exposure to PBO did not fully restore susceptibility to alpha-cypermethrin, deltamethrin, and permethrin in some of the sites, indicating the involvement of other resistance mechanisms in those areas.

FIGURE 18. *AN. ARABIENSIS* MORTALITY IN PRE-EXPOSURE PBO SYNERGIST AND INSECTICIDE ALONE ASSAYS (2019)

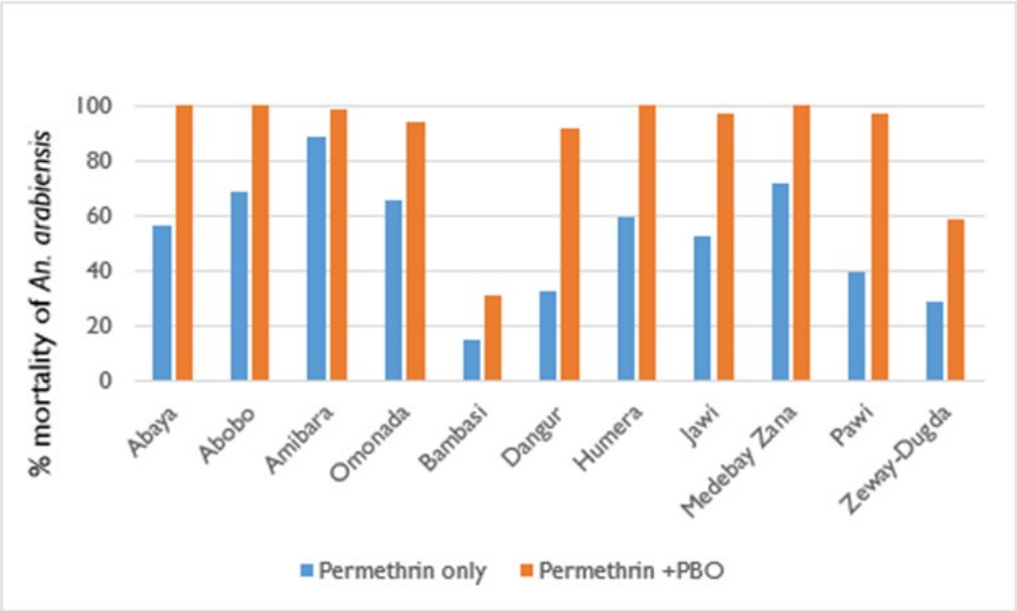
A) ALPHA-CYPERMETHRIN



B) DELTAMETHRIN



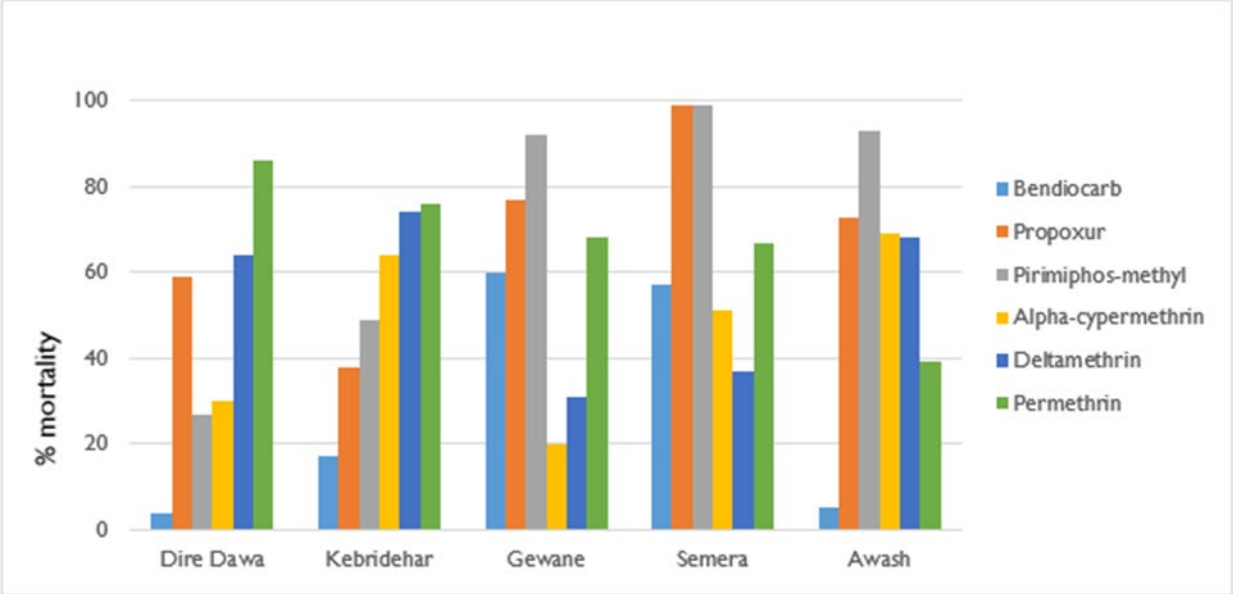
D) PERMETHRIN



3.9.6 SUSCEPTIBILITY STATUS OF *AN. STEPHENSI* TO INSECTICIDES

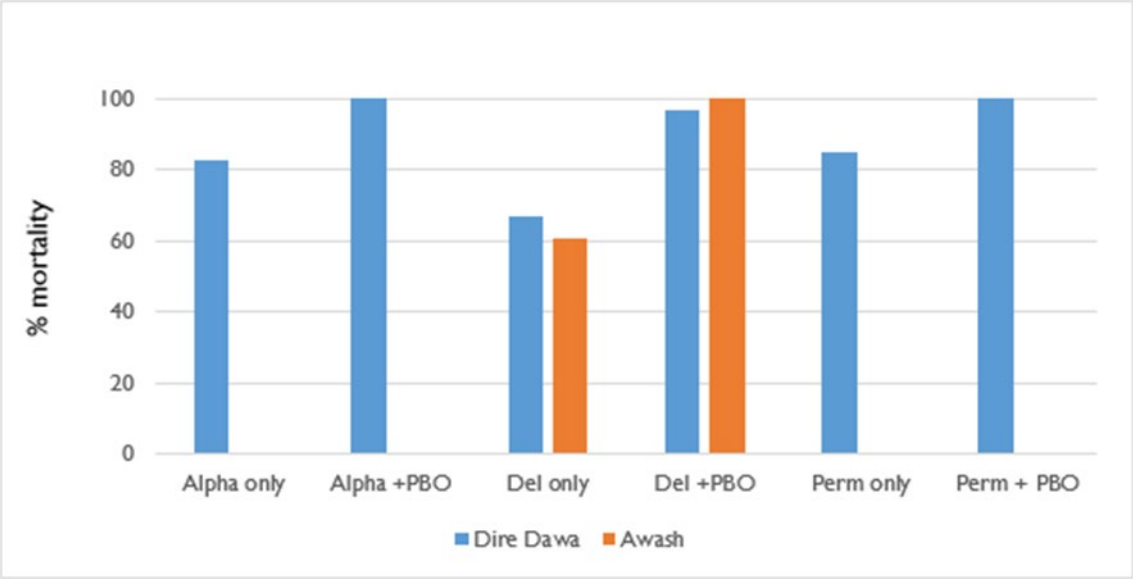
Anopheles stephensi was susceptible to propoxur and pirimiphos-methyl in only one site, Semera (99% mortality, respectively). *Anopheles stephensi* populations from two sites, Dire Dawa and Kebridehar, were resistant to pirimiphos-methyl. Possible resistance to the same insecticide was recorded in Gewane and Awash. The vector population from the five test sites were highly resistant to bendiocarb, alpha-cypermethrin, deltamethrin, and permethrin (Figure 19).

FIGURE 19. *AN. STEPHENSI* MORTALITY IN WHO TUBE TESTS (2019)



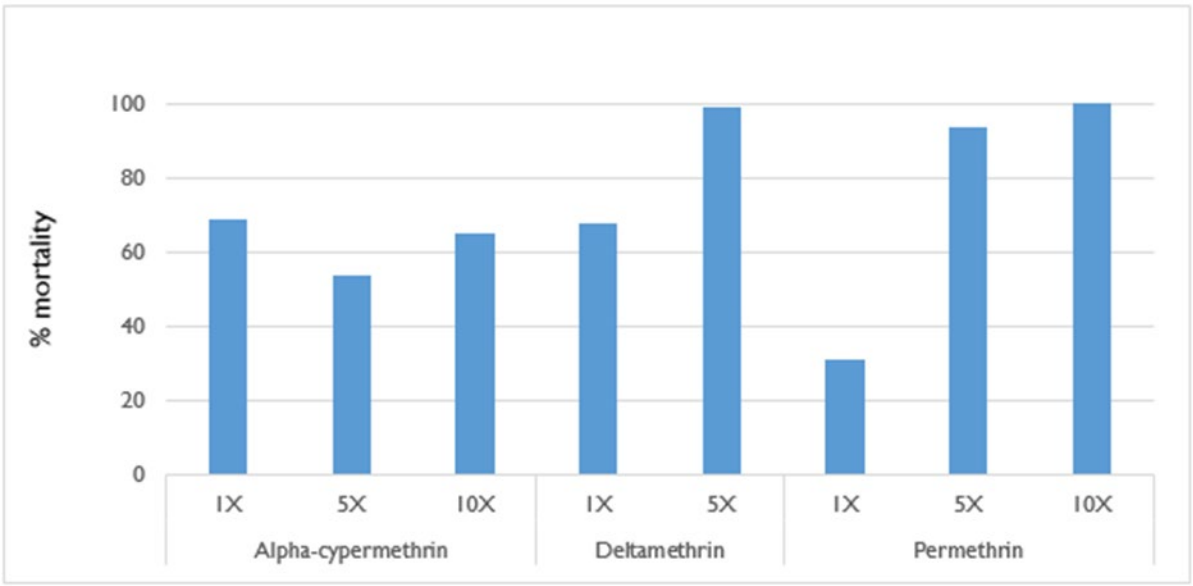
In synergists assays, pre-exposure to PBO restored susceptibility to alpha-cypermethrin and permethrin in Dire Dawa and to deltamethrin in Awash. But it only partially restored susceptibility to deltamethrin in Dire Dawa. Although further investigation is required, the present finding provides evidence that pyrethroid resistance in *An. stephensi* is mainly conferred by mixed function oxidases (Figure 20).

FIGURE 20. *AN. STEPHENSI* MORTALITY IN PRE-EXPOSURE PBO AND INSECTICIDE ALONE ASSAYS (2019)



Resistance intensity assays carried out in Awash showed that *An. stephensi* exhibited high intensity resistance to alpha-cypermethrin (65% mortality at 10X) and moderate resistance to deltamethrin (99% mortality at 5X) and permethrin (100% mortality at 10X) (Figure 21).

FIGURE 21. MORTALITY OF *AN. STEPHENSI* FROM AWASH IN RESISTANCE INTENSITY ASSAYS (2019)

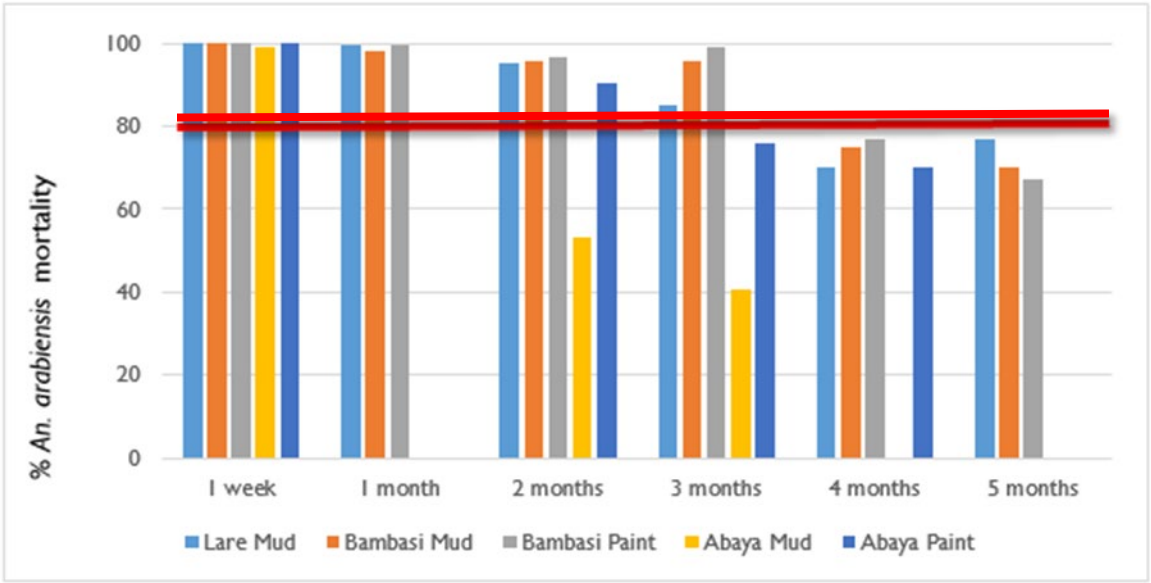


3.10 Entomological Assessment of Quality and Decay Rate of Actellic 300CS

3.10.1 Cone Bioassays

WHO cone wall bioassays conducted within a week of spraying produced 100% mortality in *An. arabiensis* on all wall surfaces tested in Lare (mud) and Bambasi (mud and painted mud), but not in Abaya, where mortality on mud surfaces was 99.3%. One month after spraying, mortality on mud surfaces was 99.5% in Lare and 98.3% in Bambasi. Painted mud surfaces in Bambasi caused 99.4% mortality of *An. arabiensis* one month after spraying. Tests were not conducted in Abaya because of security issues one month after spraying. Two months after spraying in Lare, mortality of *An. arabiensis* reared from wild collected larvae and pupae was 95.4% on mud. During the same period in Bambasi, mortality of *An. arabiensis* was 95.6% on mud and 96.7% on painted mud surfaces, and in Abaya, it was 53.3% on mud and 90.6% on painted mud surfaces. Three months after spraying, mortality of *An. arabiensis* on mud surfaces dropped to 85% in Lare; in Bambasi mortality was 95.6% on mud surfaces and 98.9% on painted mud. Mortality dropped below the cut-off value of 80% after three months for both surfaces in Abaya. In both Lare and Bambasi, mortalities were below 80% after four months on all surface types (Figure 22, Annex H).

FIGURE 22. INSECTARY *AN. ARABIENSIS* MORTALITY IN CONE BIOASSAYS (2019)



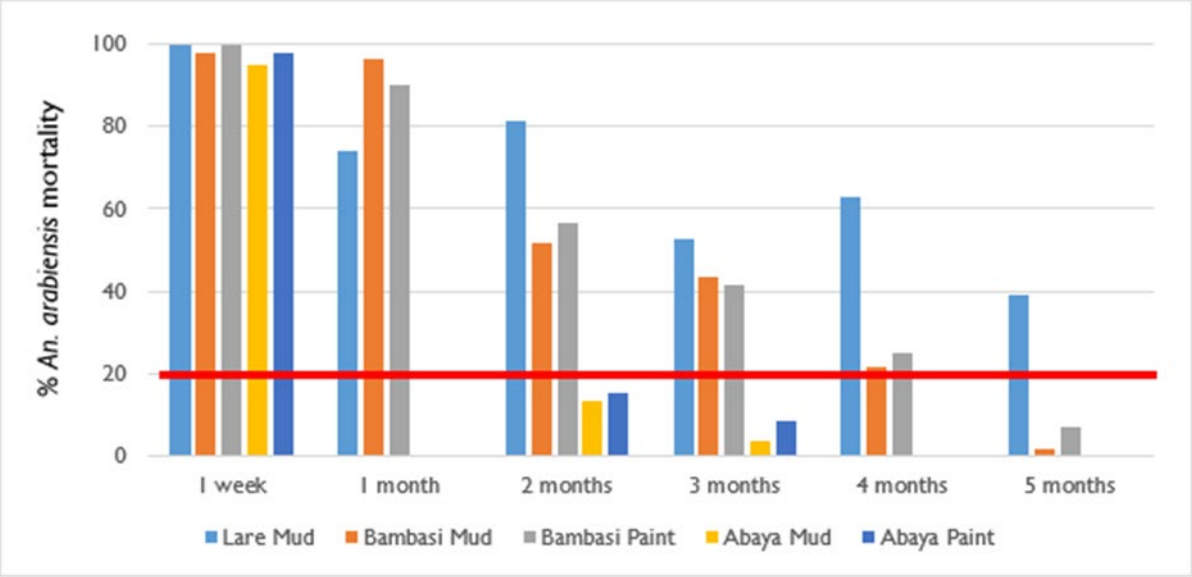
Red line shows the WHO cut of value of mosquito mortality

3.10.2 Assessment of the Fumigant Effect of Actellic 300CS

The fumigant effect of Actellic 300CS on the mortality of *An. arabiensis* was high within a week of testing after spraying in all three sites, killing 100% in Lare (mud only), 98% and 100% on mud and painted mud surfaces in Bambasi, and 95% and 98% in Abaya on mud and paint mud surfaces. After a month, two months, three months, four months and five months in Lare (mud only), mortality of *An. arabiensis* was 74.0%, 81.6%, 52.3%, 63.1%, and 39.1%, respectively all above the cut-off value of 20% mortality. After a month in Bambasi, both mud and painted mud surfaces killed more than 90%; Two months after spraying, mortality was greater than 50%, and after three, four and five months, mortality in mud houses was 43.3%, 21.6%, and 1.7%, respectively, while in painted mud houses it was 41.7%, 25.0%, and 6.7%, respectively. After two months in Abaya, Actellic 300 CS killed 6.7% of *An. arabiensis* in houses with mud surface and 28.0% in houses with painted mud surfaces; after three months, mortality dropped to below 10% for both surface types.

The fumigant effect assessment results showed that airborne Actellic 300 CS lasted much longer than anticipated, about five months on mud houses in Lare, four months on both mud and painted mud houses in Bambasi, and two months on mud and painted mud houses in Abaya (Figure 23, Annex I).

FIGURE 23. *AN. ARABIENSIS* MORTALITY IN FUMIGATION BIOASSAYS (2019)



The red line indicates the cut of value of mosquito mortality due to the fumigant effect of Actellic 300 CS.

4. ENTOMOLOGICAL CAPACITY BUILDING

As noted in the Introduction, one of PMI VectorLink's goals is to build technical capacities in the countries the project supports, so that vector control efforts are sustained. In Ethiopia, the project carried out the following entomology-related capacity-building activities.

4.1 TRAINING

In April 2019, the PMI VectorLink Ethiopia Project conducted a training workshop on morphological identification, insecticide susceptibility tests, and curation of mosquitoes. The participants were:

- Thirteen staff from nine universities: Addis Ababa University, Jimma University, ArbaMinch University, DebreMarkos University, Assosa University, Dire Dawa University, Jigjiga University, Mekelle University, and University of Gondar
- Three from the Ethiopian Public Health Institute
- Four from the Armauer Hansen Research Institute
- Two from the Oromia Public Health Research Capacity Building and Quality Assurance Laboratory
- One from the NMCP
- One from PMI VectorLink Ethiopia

4.2 SUPPORT FOR ESTABLISHMENT OF AN INSECTARY AT ASSOSA UNIVERSITY

The PMI VectorLink Ethiopia project provided training and material support to Assosa University to establish an insectary. Two technicians from Assosa University were trained at Jimma University on insectary management. Basic insectary materials were also provided. Assosa University began maintaining a colony of *An. arabiensis* last year and continuously supplies susceptible mosquito colonies for cone bioassays and for the assessment of fumigant effects in Benishangul-Gumuz.

5. CONCLUSIONS

Anopheles arabiensis remains the predominant species and the main vector of malaria transmission in Ethiopia. This species together with the secondary malaria vectors, *An. pharoensis* and *An. funestus* group tend to be early feeders indoors and outdoors and prefer to rest outdoors, which may limit the impact that IRS and ITNs would have in reducing malaria transmission in Ethiopia. Supplementary vector control interventions are required to further advance malaria control and achieve long-term malaria elimination. *Anopheles arabiensis* is susceptible to pirimiphos-methyl, propoxur, clothianidin, chlorfenapyr and bendiocarb and this might provide opportunities for pre-emptive rotation of insecticides used in IRS to mitigate resistance and preserve the tools at hand. It is, therefore, high priority to evaluate the operational efficacy of the new insecticides and facilitate their registration for use in malaria vector control in Ethiopia.

Anopheles stephensi was prevalent in Dire Dawa, Kebridehar, Awash and Metehara and was found infected with *P. vivax* in Kebridehar. The number of sites that documented the presence of this vector has increased from ten in 2019 to 13 this year with confirmation of its occurrence in Metehara, Meki and Zeway in the most recent survey. Further surveys are needed in the future in the other parts of the country to completely map its geographical distribution. The finding of *Plasmodium* infections together with its wider and more urban distribution calls for innovative vector control interventions. The populations of *An. stephensi* were resistant to pyrethroids, bendiocarb, propoxur and pirimiphos-methyl with the exception of one site, Semera, where it was susceptible to pirimiphos-methyl and propoxur. The fact that the vector is mainly detected in urban centers and resistant to most public insecticides imply conventional vector control interventions might not be effective against *An. stephensi*. The country needs to develop a control strategy based on comprehensive assessment of the vector distribution, better understanding of its breeding and biting behavior, its response to adulticides and larvicides, as well as incorporating the experiences from countries that have successfully controlled this vector.

The residual bio-efficacy of actellic 300 CS as measured by WHO cone bioassay test was about four months in Bambasi (mud and painted surfaces) and Lare (mud surface). It lasted shorter in Abaya less than two months on mud and about three months on painted surfaces. The duration of effective action of Actellic 300 CS barely fell into the lower end of WHO's estimate in Bambasi and Lare but was much shorter than expected in Abaya. Though further study is needed to figure out why that is the case, porosity of the wall surfaces, quality of the spray, and environment factors like relative humidity and temperature might be some of the factors that might have contribute to the shorter duration.

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ANNEX A. MONTHLY COLLECTIONS OF *ANOPHELES* AND CULICINES FROM SENTINEL SITES (MAY 2019-MARCH 2020)

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/cyddipis*			*An. tenebrosus*			*An. demeilloni*			Culicines		
		PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total
Abaya	May (Pre IRS)	8	44	52	0	20	20	_	_	_	0	0	0	0	3	3	_	_	_	_	_	_	_	_	_	45	304	349
	June (Post IRS)	2	63	65	0	25	25	_	_	_	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	17	694	711
	July (Post IRS)	Collection not done																										
	Aug (Post IRS)	2	37	39	0	20	20	_	_	_	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	25	448	473
	Sept (Post IRS)	0	1	1	0	3	3	_	_	_	0	4	4	0	0	0	_	_	_	_	_	_	_	_	_	100	149	249
	Oct (Post IRS)	7	22	29	0	0	0	_	_	_	0	6	6	0	0	0	_	_	_	_	_	_	_	_	_	96	237	333
	Nov (Post IRS)	1	3	4	0	0	0	_	_	_	0	8	8	1	3	4	_	_	_	_	_	_	_	_	_	70	293	363
	Dec (Post IRS)	0	7	7	0	2	2	_	_	_	0	6	6	0	0	0	_	_	_	_	_	_	_	_	_	127	426	553
	Jan (Post IRS)	4	4	8	0	0	0	_	_	_	0	2	2	0	1	1	_	_	_	_	_	_	_	_	_	62	158	220
	Feb (Post IRS)	0	1	1	0	1	1	_	_	_	0	2	2	0	0	0	_	_	_	_	_	_	_	_	_	104	8	112

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/cyddipis*			*An. tenebrosus*			*An. demeilloni*			Culicines		
		PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total
	March (Post IRS)	1	2	3	0	0	0	_	_	_	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	50	52	102
	Subtotal	25	184	209	0	71	71	_	_	_	0	28	28	1	7	8	_	_	_	_	_	_	_	_	_	696	2769	3465
Lare	May (Pre IRS)	24	27	51	0	0	0	1	0	1	0	0	0	_	_	_	0	0	0	_	_	_	_	_	_	3	2	5
	June (Post IRS)	5	61	66	8	223	231	1	6	7	0	0	0	_	_	_	0	7	7	_	_	_	_	_	_	22	54	76
	July (Post IRS)	6	257	263	15	342	357	0	0	0	0	14	14	_	_	_	0	0	0	_	_	_	_	_	_	38	286	324
	Aug (Post IRS)	6	94	100	24	255	279	0	13	13	0	107	107	_	_	_	0	0	0	_	_	_	_	_	_	18	261	279
	Sept (Post IRS)	8	83	91	5	8	13	0	0	0	0	238	238	_	_	_	0	0	0	_	_	_	_	_	_	29	200	229
	Oct (Post IRS)	20	227	247	0	12	12	0	0	0	0	16	16	_	_	_	1	2	3	_	_	_	_	_	_	16	220	236
	Nov (Post IRS)	0	36	36	0	5	5	0	0	0	0	4	4	_	_	_	0	0	0	_	_	_	_	_	_	11	104	115
	Dec (Post IRS)	9	51	60	0	0	0	0	0	0	0	6	6	_	_	_	0	0	0	_	_	_	_	_	_	14	51	65
	Jan (Post IRS)	0	7	7	0	2	2	0	10	10	0	1	1	_	_	_	0	0	0	_	_	_	_	_	_	8	27	35
	Feb (Post IRS)	0	3	3	0	0	0	0	21	21	0	0	0	_	_	_	0	0	0	_	_	_	_	_	_	0	16	16
	March (Post IRS)	0	11	11	0	0	0	0	0	0	0	0	0	_	_	_	0	0	0	_	_	_	_	_	_	0	9	9
	Subtotal	78	857	935	52	847	899	2	50	52	0	386	386	_	_	_	1	9	10	_	_	_	_	_	_	159	1230	1389
Benatsemay	May (No IRS)	77	62	139	0	0	0	0	0	0	0	0	0	_	_	_	_	_	_	0	0	0	_	_	_	25	14	39
	June (No IRS)	435	311	746	0	25	25	0	0	0	0	0	0	_	_	_	_	_	_	0	0	0	_	_	_	74	59	133
	July (No IRS)	103	104	207	0	88	88	2	0	2	0	0	0	_	_	_	_	_	_	0	36	36	_	_	_	110	455	565
	Aug	55	38	93	0	58	58	1	0	1	0	0	0	_	_	_	_	_	_	1	54	55	_	_	_	32	341	373

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/cyddipis*			*An. tenebrosus*			*An. demeilloni*			Culicines		
		PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total
	(No IRS)																											
	Sept	13	1	14	0	8	8	0	0	0	0	0	0	_	_	_	_	_	_	0	0	0	_	_	_	17	6	23
	(No IRS)																											
	Oct	1	6	7	0	0	0	3	0	3	0	0	0	_	_	_	_	_	_	0	0	0	_	_	_	17	775	792
	(No IRS)																											
	Nov	74	253	327	2	227	229	2	1	3	0	0	0	_	_	_	_	_	_	0	140	140	_	_	_	30	1164	1194
	(No IRS)																											
	Dec	20	143	163	0	417	417	3	2	5	0	307	307	_	_	_	_	_	_	0	163	163	_	_	_	58	2318	2376
	(No IRS)																											
	Jan	12	35	47	0	33	33	0	3	3	0	129	129	_	_	_	_	_	_	0	3	3	_	_	_	31	1124	1155
	(No IRS)																											
	Feb	46	16	62	0	28	28	5	4	9	0	99	99	_	_	_	_	_	_	0	14	14	_	_	_	51	757	808
	(No IRS)																											
	March	7	7	14	0	90	90	1	4	5	0	29	29	_	_	_	_	_	_	0	30	30	_	_	_	17	562	579
	(No IRS)																											
	Subtotal	843	976	1819	2	974	976	17	14	31	0	564	564	_	_	_	_	_	_	1	440	441	_	_	_	462	7575	8037
Harbu	May	13	8	21	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	0	0	0	364	285	649
	(No IRS)																											
	June	5	15	20	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	0	0	0	280	347	627
	(No IRS)																											
	July	8	16	24	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	0	0	0	180	513	693
	(No IRS)																											
	Aug	12	54	66	2	0	2	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	1	0	1	274	918	1192
	(No IRS)																											
	Sept	14	88	102	2	36	38	_	_	_	_	_	_	_	_	_	_	_	_	0	3	3	1	3	4	502	3805	4307
	(No IRS)																											
	Oct	19	160	179	2	102	104	_	_	_	_	_	_	_	_	_	_	_	_	0	10	10	0	0	0	404	4123	4527
	(No IRS)																											
	Nov	5	41	46	0	36	36	_	_	_	_	_	_	_	_	_	_	_	_	1	5	6	1	3	4	166	1436	1602
	(No IRS)																											
	Dec	4	11	15	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	7	6	13	163	842	1005
	(No IRS)																											
	Jan	5	11	16	0	1	1	_	_	_	_	_	_	_	_	_	_	_	_	1	1	2	5	1	6	402	854	1256

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/cyddipis*			*An. tenebrosus*			*An. demeilloni*			Culicines		
		PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total
	(No IRS)																											
	Feb	7	21	28	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	1	0	1	2	1	3	235	464	699
	(No IRS)																											
	March	12	8	20	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0	1	0	1	304	418	722
	(No IRS)																											
	Subtotal	104	433	537	6	175	181	_	_	_	_	_	_	_	_	_	_	_	_	3	19	22	18	14	32	3274	14005	17279
Jabitehnan	May	11	9	20	_	_	_	_	_	_	0	0	0	_	_	_	_	_	_	_	_	_	0	0	0	20	13	33
	(Pre IRS)																											
	June	5	8	13	_	_	_	_	_	_	0	0	0	_	_	_	_	_	_	_	_	_	4	0	4	30	11	41
	(Pre IRS)																											
	July	13	6	19	_	_	_	_	_	_	0	0	0	_	_	_	_	_	_	_	_	_	3	0	3	16	18	34
	(Pre IRS)																											
	Aug	90	52	142	_	_	_	_	_	_	0	0	0	_	_	_	_	_	_	_	_	_	0	0	0	26	69	95
	(Pre IRS)																											
	Sept	76	40	116	_	_	_	_	_	_	7	21	28	_	_	_	_	_	_	_	_	_	0	0	0	60	290	350
	(Post IRS)																											
	Oct	31	10	41	_	_	_	_	_	_	1	36	37	_	_	_	_	_	_	_	_	_	0	0	0	36	230	266
	(Post IRS)																											
	Nov	25	94	119	_	_	_	_	_	_	13	69	82	_	_	_	_	_	_	_	_	_	0	0	0	55	162	217
	(Post IRS)																											
	Dec	9	9	18	_	_	_	_	_	_	0	1	1	_	_	_	_	_	_	_	_	_	0	0	0	41	91	132
	(Post IRS)																											
	Jan	5	4	9	_	_	_	_	_	_	0	2	2	_	_	_	_	_	_	_	_	_	0	0	0	32	42	74
	(Post IRS)																											
	Feb	4	5	9	_	_	_	_	_	_	0	4	4	_	_	_	_	_	_	_	_	_	0	0	0	23	56	79
	(Post IRS)																											
	March	0	15	15	_	_	_	_	_	_	0	2	2	_	_	_	_	_	_	_	_	_	0	0	0	33	6	39
	(Post IRS)																											
	Subtotal	269	252	521	_	_	_	_	_	_	21	135	156	_	_	_	_	_	_	_	_	_	7	0	7	372	988	1360
Metema	May	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0
	(Pre IRS)																											
	June	2	2	4	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	1	0	1
	(Pre IRS)																											

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/cyddipis*			*An. tenebrosus*			*An. demeilloni*			Culicines		
		PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total	PSC	HLC	Total
	July (Pre IRS)	3	6	9	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0
	Aug (Pre IRS)	0	3	3	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	1	1
	Sept (Pre IRS)	7	39	46	0	1	1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0
	Oct (Post IRS)	3	15	18	0	1	1	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0
	Nov (Post IRS)	4	4	8	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0
	Dec (Post IRS)	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	12	0	12
	Jan (Post IRS)	1	0	1	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	13	0	13
	Feb (Post IRS)	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	1	0	1
	March (Post IRS)	0	0	0	0	0	0	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	0	0	0

Site	Time	*An. arabiensis*				*An. pharoensis*				*An. funestus* s.l.				*An. coustani*				*An. ziemanni*				*An. squamosus/cyddipis*				Culicines			
		PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total	PSC	CDC	HLC	Total
Bambasi	May (Pre IRS)	5	_	1	6	0	_	0	0	0	_	0	0	0	_	0	0	0	_	0	0	0	_	0	0	18	_	0	0
	June (Post IRS)	0	_	1	1	0	_	0	0	0	_	0	0	0	_	0	0	0	_	0	0	0	_	0	0	0	_	0	0
	July (Post IRS)	11	_	89	100	0	_	1	1	0	_	1	1	0	_	58	58	0	_	27	27	0	_	0	0	0	_	181	181
	Aug (Post IRS)	7	93	116	216	0	7	3	10	4	18	1	23	3	167	108	278	0	20	36	56	0	143	16	159	3	303	181	484
	Sept (Post IRS)	4	26	33	63	1	3	0	4	6	41	8	55	4	134	229	367	4	78	105	187	1	111	36	148	5	223	296	519
	Oct (Post IRS)	2	16	22	40	0	2	0	2	0	47	2	49	3	286	269	558	0	10	4	14	1	113	8	122	5	253	34	287
	Nov (Post IRS)	2	41	17	60	0	5	0	5	2	49	1	52	3	99	99	201	1	50	26	77	1	36	5	42	5	199	101	300
	Dec (Post IRS)	2	5	3	10	0	7	1	8	6	103	3	112	0	23	22	45	0	2	0	2	0	7	0	7	4	84	16	100
	Jan (Post IRS)	0	0	0	0	0	0	0	0	4	74	0	78	0	6	6	12	0	3	0	3	0	3	0	3	0	35	0	35
	Feb (Post IRS)	0	0	0	0	0	0	0	0	2	29	1	32	0	11	3	14	0	1	0	1	0	1	0	1	0	8	1	9
	March (Post IRS)	0	0	0	0	0	0	0	0	0	5	0	5	0	2	0	2	0	0	0	0	0	0	0	0	0	4	1	5
Subtotal		33	181	282	496	1	24	5	30	24	366	17	407	13	728	794	1535	5	164	198	367	3	414	65	482		1109	811	1920

ANNEX B. MONTHLY ABUNDANCE OF *AN. STEPHENSIS* FROM DIRE DAWA, KEBRIDEHAR, AWASH, AND METEHARA TOWNS (2019)

Month 2019	Dire Dawa							Kebridehar							Awash						Metehara						
	PSC	HLC	CDC	Hand Coll.	Black Box	Cattle-baited Tent Trap	Total	PSC	HLC	CDC	Hand Coll.	Black Box	Cattle-baited Tent Trap	Total	PSC	HLC	CDC	Hand Coll.	Cattle-baited Tent Trap	Total	PSC	HLC	CDC	Hand Coll.	Black Box	Cattle-baited Tent Trap	Total
June	0	0	0	ND	0	ND	0	4	1	3	ND	0	ND	8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
July	3	0	0	18	0	ND	21	1	0	0	0	0	ND	1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	
Aug	1	0	0	127	16	16	160	2	0	0	5	0	6	13	0	4	0	20	ND	24	1	4	0	2	ND	ND	7
Sept	0	0	3	24	82	9	118	4	0	0	19	0	18	41	0	2	0	36	10	48	5	7	18	19	0	4	53
Oct	0	0	1	26	56	9	92	1	4	0	79	0	29	113	2	1	0	37	10	50	2	0	2	12	0	5	21
Nov	0	0	0	5	2	6	13	3	0	0	46	0	29	78	0	0	0	11	1	12	1	0	0	3	0	3	7
Dec	0	0	0	5	3	0	8	14	0	0	63	0	37	114	1	0	0	19	0	17	0	0	3	9	0	6	18
Overall	4	0	4	205	159	40	412	29	5	3	212	0	119	368	3	7	0	123	21	154	9	11	23	45	0	18	106

ANNEX C. INSECTICIDE SUSCEPTIBILITY TEST RESULTS OF *AN. ARABIENSIS* (2019)

Region	District	% mortality											
		Bendiocarb		Propoxur		Pirimiphos-methyl		Alpha-cypermethrin		Deltamethrin		Permethrin	
		Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control
Afar	Amibara	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	67 (67/100) R	0 (0/50)	82 (82/100) R	0 (0/50)	91 (91/100) R	0 (0/50)
	Dubti	99 (99/100) S	0 (0/50)	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	81 (81/100) R	2 (1/50)	51 (51/100) R	2 (1/50)	95 (95/100) R	4 (2/50)
Amhara	Bahirdar	97 (100/100) POR	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	20 (20/100) R	0 (0/50)	22 (22/100) R	0 (0/50)	16 (16/100) R	0 (0/50)
	Jawi	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	57 (57/100) R	0 (0/50)	83 (83/100) R	0 (0/50)	83 (83/100) R	0 (0/50)
	Metema	100 (100/100) S	0 (0/50)	100 (100/100 S	0 (0/50)	100 (100/100) S	0 (0/50)	68 (68/100) R	0 (0/50)	41 (41/100) R	0 (0/50)	86 (86/100) R	0 (0/50)
Benishangul -gumuz	Bambasi	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	0 (0/100) R	0 (0/50)	29 (29/100) R	0 (0/50)	16 (16/100) R	0 (0/50)
	Dangur	98 (98/100) S	0 (0/50)	99 (99/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	5 (5/100) R	0 (0/50)	5 (5/100) R	2 (1/50)	16 (16/100) R	0 (0/50)
	Pawi	100 (100/100 S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	21 (21/100) R	0 (0/50)	6 (6/100) R	0 (0/50)	9 (9/100) R	0 (0/50)
Gambela	Abobo	100 (100/100 S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	2 (1/50)	59 (59/100) R	0 (0/50)	61 (61/100) R	0 (0/50)	84 (84/100) R	0 (0/50)

Region	District	% mortality											
		Bendiocarb		Propoxur		Pirimiphos-methyl		Alpha-cypermethrin		Deltamethrin		Permethrin	
		Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control
Oromia	Abaya	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	36 (36/100) R	0 (0/50)	50 (50/100) R	0 (0/50)	49 (49/100) R	0 (0/50)
	Metehara	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	68 (68/100) R	0 (0/50)	41 (41/100) R	0 (0/50)	86 (86/100) R	0 (0/50)
	Omonada	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	27 (27/100) R	0 (0/50)	15 (15/100) R	0 (0/50)	30 (30/100) R	0 (0/50)
	Zeway-Dugda	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	31 (31/100) R	0 (0/50)	51 (51/100) R	0 (0/50)	20 (20/100) R	0 (0/50)
SNNPR	Benatsemay	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	87 (87/100) R	0 (2/50)				
	Dilla Zuria	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	6 (6/100) R	0 (0/50)	6 (6/100) R	0 (0/50)	2 (2/100) R	0 (0/50)
	Halaba	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	58 (58/100) R	0 (0/50)	80 (80/100) R	0 (0/50)	91 (91/100) R	0 (0/50)
	Jinka	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	2 (1/50)	42 (42/100) R	0 (0/50)	35 (35/100) R	0 (0/50)	25 (25/100) R	2 (1/50)
	Misrak Badawacho	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	5 (5/100) R	0 (0/50)	5 (5/100) R	0 (0/50)	6 (6/100) R	0 (0/50)
Somali	Erer	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	64 (64/100) R	0 (0/50)	74 (74/100) R	0 (0/50)	80 (80/100) R	0 (0/50)
Tigray	Humera	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	33 (33/100) R	0 (0/50)	47 (47/100) R	2 (1/50)	75 (75/100) R	0 (0/50)
	Medabay Zana	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	78 (78/100) R	0 (0/50)	67 (67/100) R	4 (2/50)	78 (78/100) R	0 (0/50)

S= Susceptible (98-100% mortality), POR= Possible of Resistance (90-97% mortality), R- Resistnce (<90% mortality)

ANNEX D. SUSCEPTIBILITY OF INSECTARY AND WILD *AN. ARABIENSIS* TO CLOTHIANIDIN (2019)

Sentinel Site	Mosquito strain	Number tested	% mortality						
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Abobo	Insectary *An. arabiensis*	100	65	77	100				
	Wild *An. arabiensis*	100	79	92	98	100			
Amibara	Insectary *An. arabiensis*	100	50	83	89	100			
	Wild *An. arabiensis*	100	73	93	99	99			
Omonada	Insectary *An. arabiensis*	100	65	77	100				
	Wild *An. arabiensis*	100	67	88	97	100			
Bambasi	Wild *An. arabiensis*	100	68	92	100				
Halaba	Insectary *An. arabiensis*	100	91	96	99	100			
	Wild *An. arabiensis*	100	90	93	98	100			
Dubti	Insectary *An. arabiensis*	100	88	93	100				
Zeway-Dugda	Wild *An. arabiensis*	100	36	67	81	98	100		
	Insectary *An. arabiensis*	100	59	92	99	100			
	Wild *An. arabiensis*	100	29	54	77	93	94	97	98

ANNEX E. SUSCEPTIBILITY OF INSECTARY AND WILD *AN. ARABIENSIS* TO CHLORFENAPYR (2019)

Sentinel Site	Insectary and Wild *An. arabiensis*	Number tested	% Mortality		
			Day 1	Day 2	Day 3
Abaya	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	82	87	89
Abobo	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	70	97	100
Bahirdar	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	100		
Dubti	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	78	80	97
Fentale	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	99	100	
Halaba	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	68	99	100
Omonada	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	85	100	
Zeway-Dugda	Insectary *An. arabiensis*	100	100		
	Wild *An. arabiensis*	100	100		

ANNEX F. MORTALITY OF *AN. ARABIENSIS* FROM RESISTANCE INTENSITY ASSAYS (2019)

Insecticide	% mortality							
	Dose	**Abaya**	**Abobo**	**Amibara**	**Omonada**	**Zeway-Dugda**	**Halaba**	**Pawi**
Alpha-cypermethrin	1X	50 (50/100)	59 (59/100)	67 (67/100)	23 (23/100)	31 (31/100)		21 (21/100)
	5X	91 (91/100)	85 (85/100)	76 (76/100)	90 (90/100)	70 (70/100)		80 (80/100)
	10X	99 (99/100)	99 (99/100)	88 (88/100)	94 (94/100)	78 (78/100)		-
Deltamethrin	1X	36 (36/100)	61 (61/100)	82 (82/100)	15 (15/100)	51 (51/100)		6 (6/100)
	5X	83 (83/100)	88 (88/100)	89 (89/100)	69 (69/100)	91 (91/100)		63 (63/100)
	10X	98 (98/100)	86 (86/100)	97 (97/100)	74 (74/100)	98 (98/100)		-
Permethrin	1X	49 (49/100)	84 (84/100)	91 (91/100)	30 (30/100)	20 (20/100)	91 (91/100)	9 (9/100)
	5X	92 (92/100)	97 ((97/100)	100 (100/100)	98 (98/100)	51 (51/100)	100 (100/100)	85(85/100)
	10X	100 (100/100)	100 (100/100)			84 (84/100)		-

ANNEX G. *AN. ARABIENSIS* MORTALITY FROM PBO SYNERGIST TESTS (2019)

Insecticide	Abaya	Abobo	Amibara	Omonada	Bambasi	Dangur	Humera	Jawi	Medebay Zana	Pawi	Zeway-Dugda
Alpha only	34 .7 (26/75)	14 (11/75)	84 (63/75)	17 (13/75)	0 (0/75)	6.7 (5/75)	29 (22/75)	33 (25/75)	72 (54/75)	24 (18/75)	21 (16/75)
Alpha +PBO	100 (75/75)	89 (67/75)	100 (75/75)	92 (69/75)	83 (62/75)	98.7 (74/75)	98.7 (74/75)	100 (75/75)	100 (75/75)	100 (75/75)	88 (66/75)
Delta only	57 (43/75)	65 (49/75)	73 (55/75)	33 (25/75)	29 (22/75)	17 (13/75)	53 (40/75)	18.7 (14/75)	53 (40/75)	18.7 (14/75)	54.7 (41/75)
Delta +PBO	98.7 (74/75)	88 (66/75)	100 (75/75)	98 (74/75)	93 (70/75)	98.7 (74/75)	100 (75/75)	100 (75/75)	100 (75/75)	100 (75/75)	90.7 (68/75)
Perm only	57 (43/75)	69 (52/75)	89 (67/75)	66 (50/75)	15 (11/75)	33 (25/75)	60 (45/75)	53 (40/75)	72 (54/75)	40 (30/75)	29 (22/75)
Perm +PBO	100 (75/75)	100 (75/75)	98.7 (74/75)	94 (71/75)	31 (23/75)	92 (69/75)	100 (75/75)	97 (73/75)	100 (75/75)	97 (73/75)	58.7 (44/75)

ANNEX H. RESULTS OF CONE BIOASSAY TESTS (2019)

Test Site	Spray Date	Wall Surface	% Mortality of Insectary *An. arabiensis* (# tested)					
			T0	**T1**	**T2**	**T3**	**T4**	**T5**
			% 24 hrs test mortality (N)	**% 24 hrs test mortality (N)**	**% 24 hrs test mortality (N)**	**% 24 hrs test mortality (N)**	**% 24 hrs test mortality (N)**	**% 24 hrs test mortality (N)**
Lare	May-19	Mud	100 (383)	99.5 (372)	95.4* (368)	85 (371)	70 (373)	77 (369)
Bambasi	Jun-19	Mud	100 (180)	98.3 (180)	95.6 (180)	95.6 (180)	75 (180)	70 (180)
		Painted mud	100 (180)	99.4 (180)	96.7 (180)	98.9 (180)	76.7 (180)	67 (180)
Abaya	May-19	Mud	99.3 (149)	ND (security)	53.3 (180)	40.6 (180)	39.3 (150)	-
		Painted mud	100 (180)	ND (security)	90.6 (180)	76 (180)	(180)	-

*Wild *An. arabiensis* from Lare because of shortage of insectary *An. arabiensis*.

ANNEX I. RESULTS OF FUMIGANT BIOASSAY TESTS (2019)

Test Site	Spray Date	Wall Surface	% Mortality of Insectary *An. arabiensis* (#tested)					
			T0	T1	T2	T3	T4	T5
			% 24 hrs test mortality (N)	% 24 hrs test mortality (N)	% 24 hrs test mortality (N)	% 24 hrs test mortality (N)	% 24 hrs test mortality (N)	% 24 hrs test mortality (N)
Lare	May-19	Mud	100 (120)	74.2 (120)	81.6 (120)*	52.5 (120)	63.1 (120)	39.1 (123)
Bambasi	Jun-19	Mud	98 (60)	96.7 (60)	51.7 (60)	43.3 (60)	21.6 (60)	1.7 (60)
		Painted mud	100 (60)	90.0 (60)	56.7 (600	41.7 (60)	25.0 (60)	6.7 (60)
Abaya	May-19	Mud	95 (60)	ND (security)	13.3 (60)	3.3 (60)	ND	ND
		Painted mud	98 (60)	ND (security)	15 (60)	8.3 (60)	ND	ND

*Wild *An. arabiensis* from Lare because of shortage of insectary *An. arabiensis*.