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# PMI VECTORLINK PROJECT ETHIOPIA

# FINAL ENTOMOLOGY REPORT

# APRIL 2021-MARCH 2022

**Recommended Citation:** The PMI VectorLink Project. March 2022. *Ethiopia Final Entomological Report, APRIL 2021-MARCH 2022*. Rockville, MD. Abt Associates.

**Contract No.:** AID-OAA-17-00008

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

**Submitted to**: United States Agency for International Development/PMI

**Submitted on:** May 31, 2022

**Approved: November 8, 2022**

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# THE PMI VECTORLINK PROJECT ETHIOPIA FINAL ENTOMOLOGY REPORT APRIL 2021-MARCH 2022

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# ACRONYMS

| | |
|---|---|
| *An.* | *Anopheles* |
| BBI | bovine blood index |
| CBS | community-based surveillance |
| CDC | Centers for Disease Control and Prevention |
| CMC | community mosquito collector |
| ELISA | Enzyme-linked Immunosorbent Assay |
| HBI | human blood index |
| ILT | indoor light trap |
| IRS | indoor residual spraying |
| ITN | insecticide-treated net |
| ND | Not Done |
| NMEP | National Malaria Elimination Program |
| OLT | outdoor light trap |
| *Pf* | *Plasmodium falciparum* |
| *Pv* | *Plasmodium vivax* |
| PBO | piperonyl butoxide |
| PCR | polymerase chain reaction |
| PMI | President's Malaria Initiative |
| PSC | pyrethrum sheet catch |
| SNNPR | Southern Nations, Nationalities, and Peoples' Region |
| SOP | Standard Operating Procedure |
| WHO | World Health Organization |

# EXECUTIVE SUMMARY

## BACKGROUND

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are the main vector control tools to reduce the burden of malaria in Ethiopia. The President's Malaria Initiative (PMI) VectorLink Project is supporting the Federal Ministry of Health by implementing IRS operations in selected districts of Amhara, Benishangul-Gumuz, Gambela, and Oromia Regional States. Along with IRS, the project collects entomological data to inform the National Malaria Elimination Program (NMEP) on vector composition and distribution, seasonality, density, susceptibility of vectors to insecticides, residual efficacy of insecticides, sporozoite infection rates, and blood meal sources. In addition, PMI VectorLink Project planned to implement larval source management (LSM) to control the invasive malaria vector, *Anopheles stephensi*, in eight towns in eastern and central Ethiopia.

Between April 2021 and March 2022, the project conducted longitudinal entomological monitoring in seven sentinel sites using Centers for Disease Control and Prevention (CDC) light traps, pyrethrum spray catches (PSC), and Prokopack aspirator collection methods. The sites were Abaya District (Oromia Region), Bambasi District (Benishangul-Gumuz Region), Bennatsemay and Salamago Districts (Southern Nations, Nationalities, and Peoples' Regional States), Jabitehnan and Metema Districts (Amhara Region), and Lare District (Gambela Region). Community-based vector surveillance has been continued in Gelana District (Oromia Region). Larval-based routine surveillance was conducted in Meki Town (Oromia Region) in relation to *Anopheles stephensi*, the invasive species in the Horn of Africa. The presence/absence of *An. stephensi* was inspected in Amhara and Somali Regions in areas where surveys were not conducted in the previous years. Insecticide-resistance monitoring was conducted in 21 sentinel sites by carrying out World Health Organization (WHO) tube tests and CDC bottle bioassays. WHO tube tests were used to determine the susceptibility of the vectors to propoxur, pirimiphos-methyl, alpha-cypermethrin, deltamethrin, and permethrin in 15 sentinel sites. Clothianidin and chlorfenapyr tests were conducted using the CDC bottle bioassay method. The spray quality and residual efficacy of Actellic 300CS, Fludora Fusion, and SumiShield were assessed using cone bioassay tests in nine sentinel sites. Actellic 300CS was assessed in Dera, Fogera, Jawi, Metema, and Quara Districts in Amhara Region and Lare District in Gambela Region. Fludora Fusion in Abaya and Gelana Districts (Oromia Region); and SumiShield in Menge District (Benishangul-Gumuz Region). Larval source management activities have been started following the guideline prepared by the project. Based on the guideline, sensitization of health officials, community members and other stake holders, microplanning, recruiting and employing of LSM personnel and seasonal workers, training, base line entomology data collection and launching of LSM have been accomplished in six towns. Preparations are underway for the implementation of LSM in the other two towns. Standalone progress reports and final report will be submitted to PMI covering the implementation of LSM and also the baseline and post intervention entomological data collections. Results

A total of 8,210 *Anopheles* and 40,620 culicines were collected from the seven sentinel sites. At least 17 species of *Anopheles* were identified, belonging to *An. arabiensis, An. coustani, An. dancalicus, An. demeilloni, An. funestus* s.s, *An. fuscivenous, An. garnahami, An. leesoni, An. parensis, An. pharoensis, An. pretoriensis, An. rhodesiensis, An. rivulorum, An. salabi, An. squamosus/cydippis, An. tenebrosus,* and *An. ziemanni*. Polymerase chain reaction (PCR) identification confirmed *An. arabiensis* and the members of the *An. funestus* group namely *An. funestsus* s.s, *An. leesoni, An. parensis* and *An. rivulorum*. Three *Anopheles* species, namely *An. arabiensis, An. pharoensis,* and *An. ziemanni*, were

identified from 1,371 collections in Gelana District, out of which 91% were *An. arabiensis*. Gelana is the VectorLink project Community Based Surveillance (CBS) site in West-Guji.

In the seven sentinel sites, *An. arabiensis* was prevalent in all, comprising 49% of all collections. CDC light traps collected 84% of *An. arabiensis*. The remaining were from PSC and Prokopack collections. Of the CDC light trap collections of *An. arabiensis*, the indoor light trap (ILT) accounted for 44.4% and the outdoor light trap (OLT) for 55.6%.

Of all collections of *An. arabiensis* from ILT at the seven sentinel sites, 41.6%–91.4% were unfed. The *An. arabiensis* density was higher in Jabitehnan (unsprayed with IRS) than in the other sites, with a peak trap density of 10.4 indoors and 11.4 outdoors *An. arabiensis*/trap/night. PSC and Prokopack yielded more unfed *An. arabiensis* in Bambasi, Jabitehnan, and Metema. More resting feds were collected from Abaya, Bennatsemay, Lare, and Salamago. *Anopheles stephensi* surveys were conducted in 16 sites in Amhara and Somali Regions, of which it was found in eight (50%) of the sites, one in the western part of Amhara bordering Sudan and seven in Somali.

Sporozoite ELISA (Enzyme-linked Immunosorbent Assay) detected 11 infections of *Plasmodium falciparum* (n=4), *P. vivax* 247 (n=6), and *P. vivax* 210 (n=1) from 1,553 tested *An. arabiensis*. Another 23 infections of *P. vivax* 247, and *P. vivax* 210 were detected from *An. coustani*, *An. parensis,* and *An. ziemanni*. However, boiling homogenates and retesting confirmed two out of four *An. arabiensis* specimens with *P. falciparum* and two out of 20 *An. coustani* specimens with *P. vivax* 210.

Populations of *An. arabiensis* were susceptible to propoxur, pirimiphos-methyl, chlorfenapyr, and clothianidin (99–100% mortality) but resistant to alpha-cypermethrin, deltamethrin, and permethrin (mortality <90%). In most of the sites, pre-exposure to piperonyl butoxide (PBO) followed by deltamethrin and permethrin tests restored full susceptibility of *An. arabiensis*, showing the role of cytochrome P450 monooxygenase enzymes to resistance to these insecticides.

Regarding the residual efficacy of IRS in houses, Actellic 300CS persisted three to eight months, killing more than 80% of insectary *An. arabiensis*. The lowest efficacy (three months) of Actellic 300 CS was detected from mud wall houses in Lare district. Actellic 300CS performed well on all wall surface types in Dera, Fogera, Jawi, Metema and Quara districts in Amhara regional state. Fludora Fusion showed six months residual efficacy in houses with dung walls but remained active for nine months on mud walls and painted mud walls in Abaya and Gelana. SumiShield persisted for five months on mud and painted mud walls in Menge.

# CONCLUSIONS

The malaria vectors *An. arabiensis, An. funestus,* and *An. pharoensis* were prevalent in their previously known distribution areas. *Plasmodium falciparum* sporozoite infections were detected in *An. arabiensis,* which were confirmed by the boiling method. Although additional studies are required, the finding of *P. vivax* sporozoite infections in *An. coustani* might lead to considering this species as one of the secondary malaria vectors in Ethiopia. Further evidence should be generated on its feeding and resting behavior in the wild, host preference, susceptibility to insecticides and susceptibility to *Plasmodium* infections from laboratory experiments.

The presence of *An. stephensi* has been confirmed from west Amhara at the border of Sudan for the first time in the western part of Ethiopia, further confirming its spread in the country and presenting additional challenges to limiting its distribution through vector control measures. The number of sites where *An. stephensi* detected has increased to 45 from the 10 positive sites in 2018.

Both *An. arabiensis* and *An. stephensi* were highly resistant to the pyrethroid insecticides. On the other hand, widespread susceptibility to chlorfenapyr and reversion to susceptibility with pre-exposure to PBO open good opportunity for the malaria vector control program to consider implementation of PBO nets and Interceptor® G2 long-lasting insecticidal nets. That *An. arabiensis* is highly susceptible to the IRS insecticides pirimiphos-methyl and clothianidin, coupled with the long-lasting residual efficacy of Actellic 300CS, SumiShield, and Fludora Fusion, means the country can use these products in rotation as has been indicated in the draft Insecticide Resistance Management Plan in Ethiopia, 2021-2025. The strategic approach that the PMI VectorLink Project follows in applying the three products in different regions is appropriate for malaria vector control in Ethiopia.

# I. INTRODUCTION

Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are the key vector control interventions for the control of malaria vectors in Ethiopia. In this regard, the President's Malaria Initiative (PMI) VectorLink Ethiopia Project undertook IRS operations in 2021 in 37 districts of the regions of Amhara, Benishangul-Gumuz, Gambela, and Oromia, protecting a total population of 1,618,765 with 94.9% spray coverage. The insecticides sprayed were Actellic 300CS, Fludora Fusion, and SumiShield.

To make a measurable impact on human health and entomological indicators including density, longevity, and sporozoite infection of malaria vectors, IRS should be done properly at the right time and dose and achieving more than 85% coverage, the vectors should be susceptible to the insecticide and rest indoors.

To link vector control interventions with the dynamics of malaria vectors, the project collected entomological data on the species composition, seasonality, density, behavior, and susceptibility to insecticides, as well as the spray quality and residual efficacy of the IRS insecticides. From April 2021 to March 2022, the project conducted longitudinal entomological monitoring in seven sentinel sites in Amhara, Benishangul-Gumuz, Gambela, Oromia, and Southern Nations, Nationalities, and Peoples' Regional State (SNNPR). Community-based surveillance (CBS), which started in 2020, has been continued longitudinally in Gelana District in West Guji (Oromia Region). Surveys have been carried out on the presence/absence of *Anopheles stephensi* in the southeastern, central, northern, and northwestern parts of the country. Routine larval surveillance of *An. stephensi* was conducted in one town (Meki) in Oromia Region.

Insecticide-resistance monitoring targeting *Anopheles arabiensis* and *An. stephensi* was conducted in a total of 21 sentinel sites throughout the country. The insecticides screened included propoxur, pirimiphos-methyl, clothianidin, chlorfenapyr, alpha-cypermethrin, deltamethrin, and permethrin. The spray quality and residual efficacy of Actellic 300CS, Fludora Fusion, and SumiShield were assessed in nine sentinel sites.

Laboratory analysis was carried out on field-collected *Anopheles* mosquitoes to identify species and to detect sporozoite infections and the molecular markers of insecticide resistance.

After mapping the distribution of *An. stephensi* in the country and gathering useful data on the bionomics, behavior and insecticide resistance, the project planned to implement larval source management which included larviciding and larval source reduction in eight towns. The larvicide selected for LSM is VectoBac WDG, a water dispersible granule formulation of *Bacillus thuringiensis* var *israelensis*.

The project supported several stakeholders to develop capacity to conduct longitudinal monitoring, morphological species identification, and insecticide-resistance monitoring by offering short-term trainings and supplying laboratory and insectary materials.

This annual report presents entomological data gathered in the fourth year of the PMI VectorLink Project in Ethiopia.

# 2. METHODOLOGY

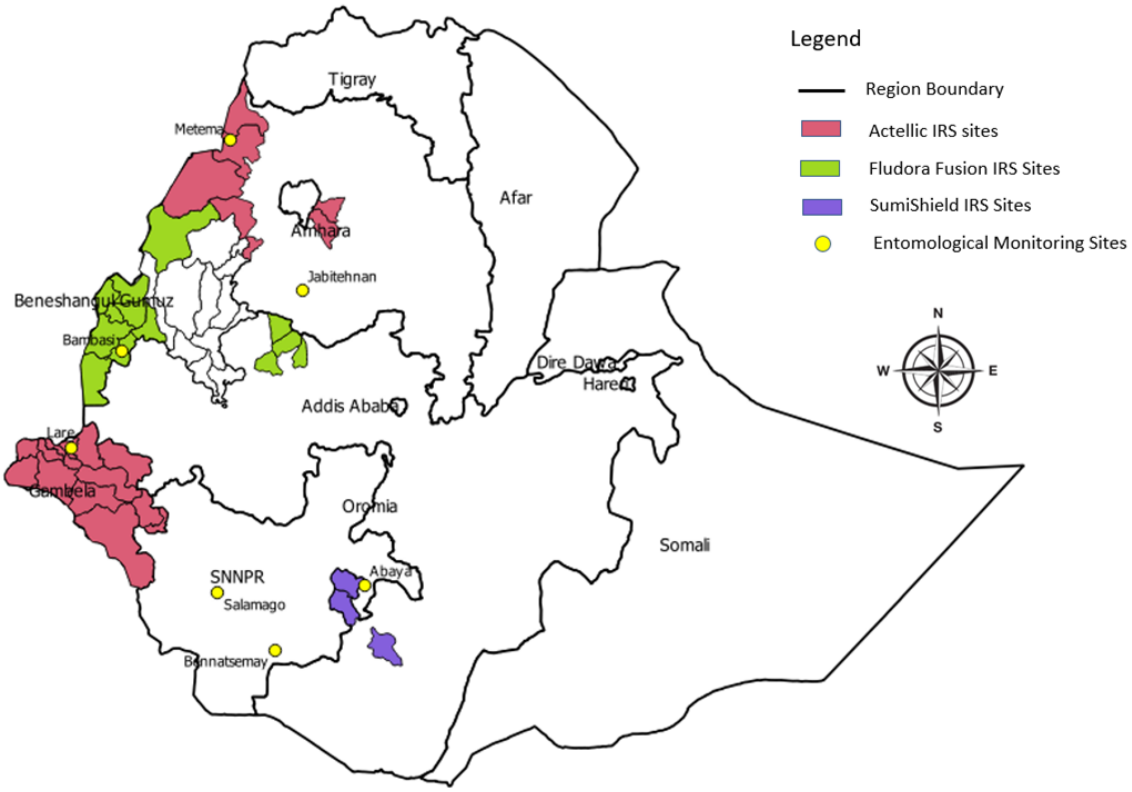
## 2.1 LONGITUDINAL ENTOMOLOGICAL MONITORING

Routine monthly entomological monitoring was conducted to generate data on *Anopheles* species composition, abundance and seasonality, vector resting and trap density, proportion of abdominal stages, and sporozoite infection rates. Entomological monitoring provides additional information on the origin of vector blood meals and determines the human blood index (HBI) and the bovine blood index (BBI).

### 2.1.1 ENTOMOLOGICAL MONITORING SITES

Routine entomological monitoring was carried out in seven sentinel sites: Abaya (Oromia), Bambasi (Benishangul-Gumuz), Bennatsemay and Salamago (SNNPR), Jabitehnan and Metema (Amhara), and Lare (Gambela). Of these, four sites (Abaya, Bambasi, Lare, and Metema) are PMI IRS Project districts and the other three (Bennatsemay, Jabitehnan, and Salamago) are non-PMI IRS Project districts. In the 2021 spray campaign, Abaya and Bambasi were sprayed with Fludora Fusion and SumiShield, respectively, whereas Lare and Metema were sprayed with Actellic 300CS. Indoor residual spraying using propoxur was conducted in Salamago by the National Malaria Elimination Program (NMEP), but not in Bennatsemay and Jabitehnan.

**FIGURE 1. ROUTINE ENTOMOLOGICAL MONITORING SITES (2021)**



### 2.1.2 MOSQUITO SAMPLING METHODS

The PMI VectorLink Standard Operating Procedures (SOPs)[1] were employed to sample mosquitoes in the seven sentinel sites using Centers for Disease Control and Prevention (CDC) light traps, pyrethrum sheet catch (PSC), and Prokopack aspirators. CDC light trap collections were made indoors (ILT) from human residential houses and outdoors (OLT) following the PMI VectorLink SOP #1. At each site, 12 houses were randomly selected, and mosquito sampling was carried out monthly in the same houses and compounds for two nights, for a total of 48 trap-nights (12 houses × 2 locations × 2 nights) per month per site.

Indoor resting *An. arabiensis*, *An. funestus* group, and *An. pharoensis* were collected from each of 10 houses using Prokopack aspirators (SOP #11) and PSCs (SOP #3) in each of the sentinel sites to estimate the indoor resting density and proportion of abdominal feeding stages (unfed, fed, half gravid, and gravid) and to detect sporozoite infection rates and origins of blood meals. Sporozoite infection rates were also determined from light trap collections. The frequency of *Anopheles* sampling is given in Table 1.

**TABLE 1. FREQUENCY OF *ANOPHELES* SAMPLING (APRIL 2021-MARCH 2022)**

| Type of collection | Time | Frequency | Sample |
|---|---|---|---|
| Pyrethrum spray catch | 6:00 am to 8:00 am | Once a month | 10 houses per site (10 houses × 7 sites = 70) per month |
| Prokopack | 6:00 am to 8:00 am | Once a month | 10 houses per site (10 houses × 7 sites = 70) per month |
| Human-baited CDC light trap indoors | 6:00 pm to 6:00 am | 12 traps × 2 nights/month per site × 7 sites | 168 trap-nights per month indoors |
| Human-baited CDC light trap outdoors (baits sleep outdoors under a temporary shelter protected by treated nets) | 6:00 pm to 6:00 am | 12 traps × 2 nights/month per site × 7 sites | 168 trap-nights per month outdoors |

## 2.2 COMMUNITY-BASED MOSQUITO SURVEILLANCE

The PMI VectorLink Ethiopia Project piloted community-based mosquito surveillance in Gelana District (Oromia Region) from August 2020 to March 2021 and then continued from April 2021 to March 2022. Monthly mosquito collections were carried out in four Kebeles by engaging the same eight female mosquito collectors and using two CDC light traps/Kebele (total = 8). Collections in each month were done indoors for five nights, giving 40 trap-nights (8 traps × 5 days).

## 2.3 MOSQUITO IDENTIFICATION, LABELLING, AND PRESERVATION

Mosquitoes collected from the routine entomological monitoring from the seven sites and from community-based surveillance in Gelana were sorted by sex and subfamilies as culicines and anophelines. Culicines and males of both groups were counted and recorded, then those specimens were discarded.

The female *Anopheles* were identified to species using Coetzee's (2020) morphological key; and categorized to abdominal feeding status as unfed, fed, half gravid, and gravid; recorded on household data forms; labelled; and then preserved in Eppendorf tubes. The tubes were sealed in zip bags with grains of silica gel. Dried specimens

[1] Complete SOPs are available at https://pmivectorlink.org/resources/tools-and-innovations/.

were submitted to Jimma and Arba Minch Universities for laboratory analysis to detect sporozoites and blood meal sources and also molecular species identification (ID)

Data collected in the forms were transferred to the VectorLink Collect database and exported to an Excel spreadsheet for analysis. Data are presented in pie charts, bar graphs, and tables in this report.

## 2.4 *ANOPHELES STEPHENSI* SURVEILLANCE

Longitudinal larval and adult surveillance of *An. stephensi* was planned in the towns of Meki (Oromia) and Semera (Afar). Larval density was determined in Meki from monthly collections between April 2021 and March 2022 from the same 15 to 18 larval habitats. In addition, attempts were made to sample adult *An. stephensi* from 20 animal shelters using Prokopack aspirators. Two female community mosquito collectors were involved in larval an adult collections, and they were supervised by the malaria focal person.

The surveillance in Semera was conducted in June, July, and September 2021, after which it was discontinued because of civil conflict.

## 2.5 ASSESSING PRESENCE/ABSENCE OF *ANOPHELES STEPHENSI* IN AMHARA AND SOMALI REGIONS

The presence/absence of *An. stephensi* was investigated in 16 localities in Amhara and Somali regions where surveys were not conducted in the previous years, out of which 14 were urban and two were rural (10-20km from an urban center). Container and natural breeding habitats were inspected for larvae and animal shelters for adults. Larvae/pupae were collected, raised to adults, and morphologically identified to species. Adult *Anopheles* were sampled using Prokopack aspirators.

## 2.6 MOLECULAR AND IMMUNOLOGICAL ASSAYS

Members of the *An. funestus* group and *An. gambiae* s.l. were identified to species using polymerase chain reaction (PCR). Enzyme-linked Immunosorbent Assays (ELISA) was used to detect sporozoite infections and origin of blood meals.

### 2.6.1 SPECIES ID PCR

The PCR method described by Scott et al. (1993) and Koekemoer et al. (2002) was employed to identify members of *An. gambiae* s.l. and *An. funestus* group, respectively.

### 2.6.2 SPOROZOITE ELISA

Specimens from sub collections of *An. arabiensis*, *An. pharoensis, An. funestus* s.l., *An. coustani,* and *An. ziemanni* were investigated for circumsporozoite proteins using the ELISA method described by Wirtz et al. (1992). Specimens found positive for sporozoites were further confirmed by the boiling method. Mosquitoes with all abdominal stages, including blood unfeds, feds, half gravids, and gravids, were tested.

### 2.6.3 BLOOD MEAL ELISA

The direct ELISA method described by Beier et al. (1988) was used to identify the blood meal sources of *An. arabiensis*. The tests were conducted to identify human, bovine, and mixed blood meals.

## 2.7 INSECTICIDE RESISTANCE MONITORING AND MECHANISM OF RESISTANCE

Insecticide resistance monitoring was undertaken targeting *An. arabiensis* and *An. stephensi*. Larvae and pupae were collected, reared to adults, and females 2-5 days old were tested for their susceptibility to insecticides used for IRS (clothianidin, pirimiphos-methyl, and propoxur) and ITN (chlorfenapyr, alpha-cypermethrin, deltamethrin, and permethrin). The tests included diagnostic concentrations (1X), intensity assays (5X and 10X), and piperonyl butoxide (PBO) assays. The World Health Organization (WHO) tube test was used, except for clothianidin and chlorfenapyr, for which the CDC bottle bioassay method was used.

Interpretation of the results from the susceptibility tests, resistance intensity assays, and PBO synergist assays was based on the criteria found in SOP #6. Abbott's formula was applied when control mortality was between 5% and 20%.

### 2.7.1 INSECTICIDE SUSCEPTIBILITY TESTS

Insecticide susceptibility tests on populations of *An. arabiensis* and *An. stephensi* were conducted using the WHO tube test method and papers impregnated with 1X concentrations of 0.1% propoxur, 0.25% pirimiphos-methyl, 0.05% alpha-cypermethrin, 0.05% deltamethrin, and 0.75% permethrin. The protocol described in PMI VectorLink Project SOP #6 was applied to conduct the susceptibility tests. All insecticide-impregnated papers were obtained from the Universiti Sains Malaysia (University of Science, Malaysia).

A new protocol based on the CDC bottle bioassay (SOP #4) was employed to investigate the susceptibility status of *An. arabiensis* and *An. stephensi* to clothianidin by coating glass bottles at a concentration of 4 µg/bottle from a solution prepared by mixing clothianidin and acetone/Mero®. The tests were conducted from September to November 2021.Mosquitoes were exposed for 60 minutes, and mortality was recorded after 24 hours.

Chlorfenapyr susceptibility tests were conducted with glass bottles coated at the concentration of 100µg/bottle following the CDC bottle bioassay method (SOP #4).

### 2.7.2 RESISTANCE INTENSITY ASSAYS

The level of resistance intensity of *An. arabiensis* and *An. stephensi* to the pyrethroid insecticides (alpha-cypermethrin, deltamethrin, and permethrin) at the concentrations of 1X, 5X, and 10X was evaluated following the method described in SOP #6.

### 2.7.3 PBO SYNERGIST ASSAYS

The response of *An. arabiensis* and *An. stephensi* to the three pyrethroid insecticides after pre-exposure to 4% PBO-impregnated papers was evaluated as described in SOP #6.

## 2.8 ENTOMOLOGICAL ASSESSMENT OF SPRAY QUALITY AND RESIDUAL EFFICACY OF IRS INSECTICIDES

IRS quality and residual efficacy of Actellic 300CS, Fludora Fusion, and SumiShield were assessed in the VectorLink Ethiopia IRS sentinel sites. The WHO wall cone bioassay tests were conducted using insectary-reared *An. arabiensis* and employing SOP #9. In addition, the fumigant effect of the insecticides on mortality of test mosquitoes was assessed.

### 2.8.1 Entomological Assessment of Spray Quality and Residual Efficacy of Actellic 300CS

IRS quality and residual efficacy of Actellic 300CS was assessed in six districts: Dera, Fogera, Jawi, Metema, and Quara in Amhara and Lare in Gambela. In the five districts in Amhara, the assessment was carried out in one Kebele each, namely Korata (Dera), Tihuaza Kana (Fogera), Mabluke (Jawi), Kokit (Metema), and Banbaho (Quara). Bioassayed houses in Lare were equally divided between Kuergeng and Bullimkun Kebeles.

From each Kebele, 10 houses were randomly selected (12 in Lare), considering the type of interior wall surfaces. Wall surfaces in Dera, Fogera, Metema, and Quara were mud, painted mud, and dung; those in Jawi and Lare were entirely mud.

In each house, three cones were fixed at heights of 0.5 meters (low), 1.0 meters (middle), and 1.5 meters (high) from the floor. At least 10 insectary-reared *An. arabiensis* were introduced into each cone. The control consisted of 10 mosquitoes introduced in insecticide-free houses or on the exterior walls of sprayed houses but placed on a cardboard covered by white paper.

The number of mosquitoes knocked down was recorded after 30 and 60 minutes and the number dead after 24 hours. Wall cone bioassay tests were conducted monthly until mortality of mosquitoes was below 80% for two consecutive months. Observed mortality was corrected using Abbott's formula when the control mortality was between 5 to 20%.

### 2.8.2 Entomological Assessment of Spray Quality and Residual Efficacy of SumiShield and Fludora Fusion

IRS quality and residual efficacy of SumiShield and Fludora Fusion were assessed in three districts: SumiShield in Menge and Abe Dongoro, and Fludora Fusion in Abaya and Gelana. The SumiShield Kebeles selected for the tests were Bane Shegole in Menge District and Mender 15 in Abe Dongoro. The Fludora Fusion Kebeles were Samaro and Guangua in Abaya and Tore Badia and Kersa in Gelana.

Wall surfaces in the houses in Menge and Abe Dongoro are mud and painted mud. Wall surfaces in the houses in Abaya were mud, painted mud, cement, and painted cement. In Gelana they were mud, painted mud, and dung. Six houses were tested in each of the Kebeles (=12/district).

The wall cone bioassay tests were conducted, and test mosquito mortality was recorded every 24 hours until the fifth day, according to SOP #9. Testing was discontinued in Abe Dongoro after three months because of security issues.

### 2.8.3 Assessment of the Fumigant Effect of Actellic 300CS, SumiShield, and Fludora Fusion

Fumigation bioassays were conducted in conjunction with cone bioassays in Abaya, Gelana, Lare, and Menge, following the PMI VectorLink Project protocol. The tests in each house were conducted by introducing 10 insectary-reared *An. arabiensis* in a small cage placed on a chair, table, or otherwise at the height of 1 meter from the floor and 10 centimeters from the walls of houses sprayed with Actellic 300CS, SumiShield, or Fludora Fusion. The same number of mosquitoes were tested in insecticide-free houses.

# 3. RESULTS

## 3.1 SPECIES COMPOSITION, SEASONALITY, AND DENSITY

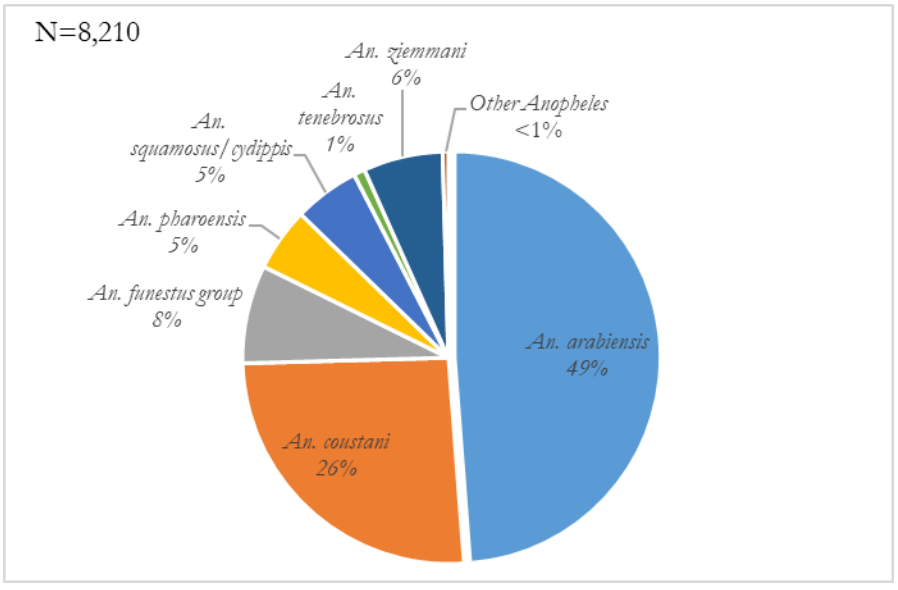
This section presents data on the species composition of *Anopheles* mosquitoes, seasonal abundance, CDC light trap density, indoor resting density, and abdominal blood feeding stages from mosquito collections using CDC light traps, PSC, and Prokopack aspirators. The discussion focuses on the malaria vectors in Ethiopia, which include *An. arabiensis*, *An. funestus,* and *An. pharoensis*. The invasive species *An. stephensi* is discussed separately in section 3.4.

### 3.1.1 SPECIES COMPOSITION, ABUNDANCE, AND DENSITY FROM ALL SEVEN SENTINEL SITES

A total of 8,210 *Anopheles* mosquitoes composed of at least 17 species were collected from the seven sentinel sites, out of which seven species were abundant and the remaining were rare. The predominant species was *An. arabiensis* (PCR confirmed), comprising 49% (N=4,006) of all collections (Figure 2). The second most abundant species was *An. coustani,* comprising 26% (N=2,116) of the collections; followed by *An. funestus* group (8%, N=634), *An. ziemanni* (6%, N=512), *An. pharoensis* (5%, N=409), *An. squamosus/cydippis* (5%, N=423), and *An. tenebrosus* (1%, N=73). The other eight rare *Anopheles* species (less than 1% of the total, N=37) were *An. dancalicus, An. demeilloni, An. fuscivenous, An. garnahami, An. pretoriensis, An. rhodesiensis, An. rivulorum,* and *An. salabi.* PCR identified four members of the *An. funestus* group: *An. funestus* s.s., *An. rivulorum*, *An. parensis,* and *An. leesoni.*

In addition, 40,620 culicines were collected from the seven sentinel sites.

**FIGURE 2. COMPOSITION OF *ANOPHELES* SPECIES FROM ALL SEVEN SITES (APRIL 2021-MARCH 2022)**



*Anopheles arabiensis* was found in all the seven sites (Table 2). Of the total 4,006 collected, 56.0% (N=2,241) were from the non-IRS site, Jabitehnan. Collections from the four PMI IRS sites were 4.8%, 7.2%, 8.0%, and 3.3% from Abaya, Bambasi, Lare, and Metema, respectively. *Anopheles funestus* group was collected from six of the seven sites. Of them, 55.2% (N=350) and 40.5% (N=257) were from Lare and Bambasi. Of 409 *An. pharoensis* collected, 72.9% (N=298) were from Lare and 22.7% (N=93) were from Bennatsemay.

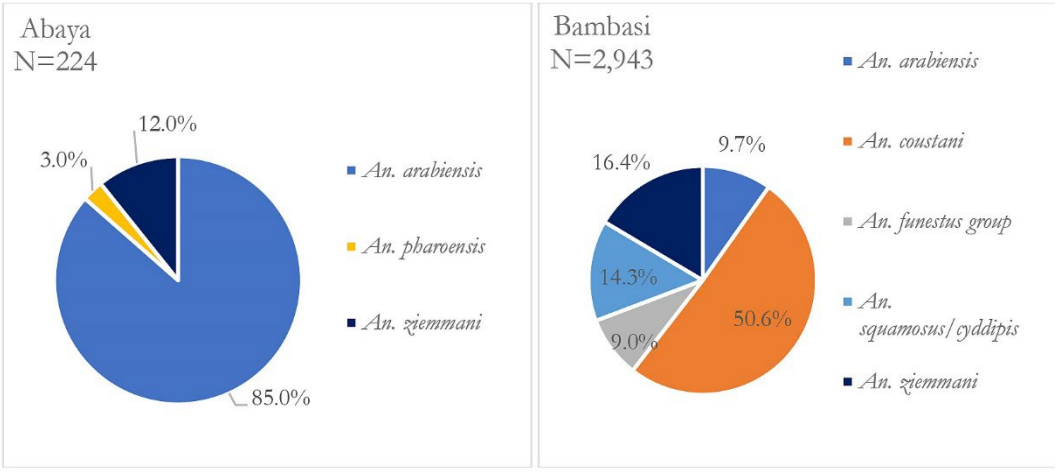
**TABLE 2. PROPORTION OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* COLLECTED IN THE SEVEN SENTINEL SITES (APRIL 2021-MARCH 2022)**

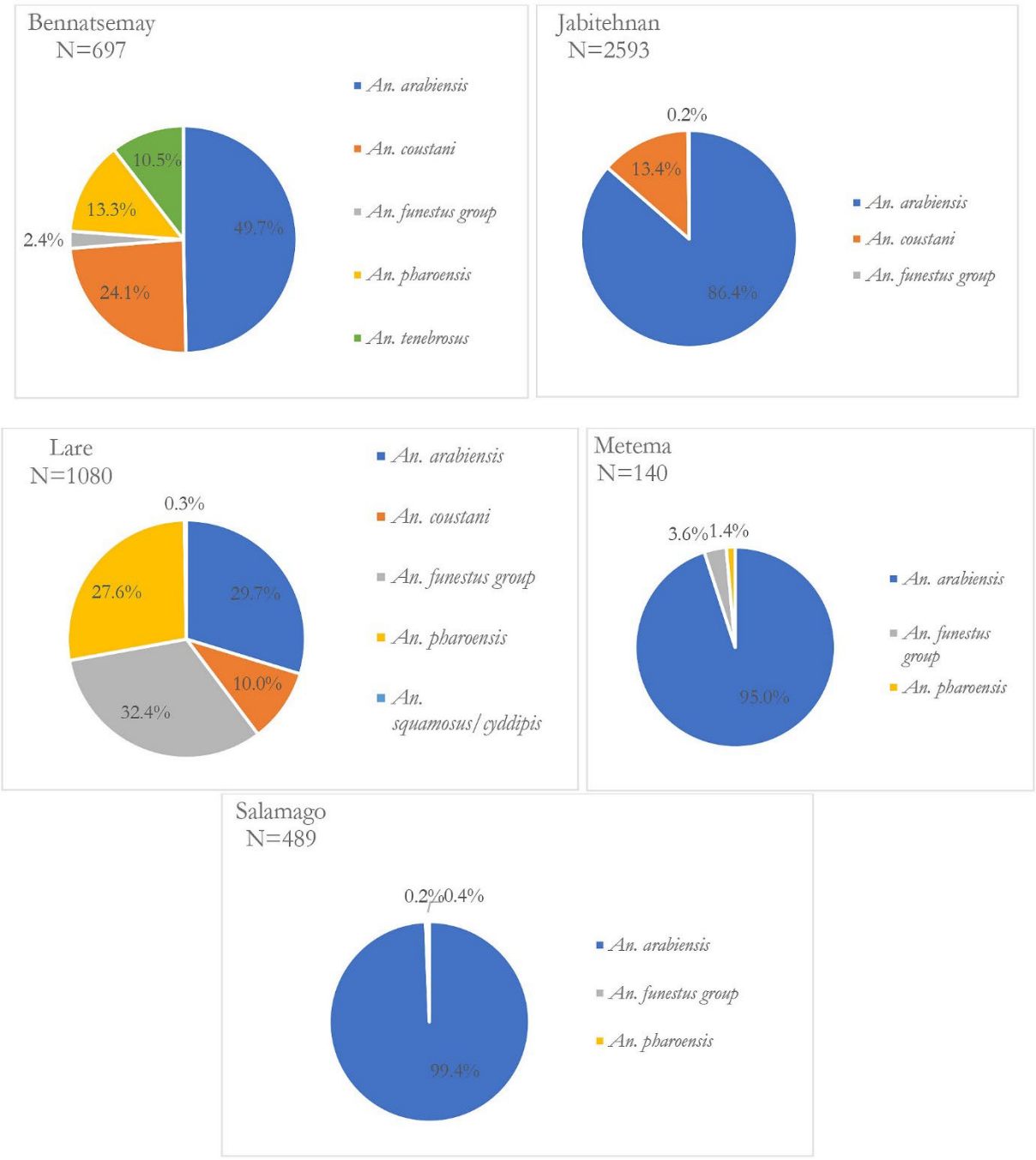
| Sentinel site | N (%) | | |
|---|---|---|---|
| | *An. arabiensis* | *An. funestus group* | *An. pharoensis* |
| Abaya | 190 (4.8) | 0 (0.0) | 7 (1.7) |
| Bambasi | 289 (7.2) | 257 (40.5) | 0 (0.0) |
| Bennatsemay | 346 (8.6) | 17 (2.7) | 93 (22.7) |
| Jabitehnan | 2241 (56.0) | 4 (0.6) | 0 (0.0) |
| Lare | 321 (8.0) | 350 (55.2) | 298 (72.9) |
| Metema | 133 (3.3) | 5 (0.8) | 9 (2.2) |
| Salamago | 486 (12.1) | 1 (0.2) | 2 (0.5) |
| **Total** | **4,006 (100)** | **634 (100)** | **409 (100)** |

### 3.1.2 SPECIES COMPOSITION AND ABUNDANCE BY SENTINEL SITE

The species composition and abundance of *Anopheles* species by sentinel site is presented in Figure 3 and Annex A. *An. arabiensis* was predominant in five of the seven sentinel sites: Abaya (85%), Bennatsemay (50%), Jabitehnan (87%), Metema (95%), and Salamago (99%). *An. arabiensis* was less abundant in Bambasi (10%) and Lare (30%). *An. funestus* group in Bambasi and Lare were 2% and 32%, respectively, of collections there. *An. pharoensis* was 13% in Bennatsemay and 28% in Lare. *An. coustani* was 51% of collections in Bambasi.

**FIGURE 3. COMPOSITION OF *ANOPHELES*, BY SENTINEL SITE (APRIL 2021-MARCH 2022)**






### 3.1.3 *Anopheles arabiensis*, *An. funestus* group, and *An. pharoensis* by Method of Collection

*Anopheles arabiensis*, *An. funestus* group, and *An. pharoensis* collections from CDC light traps accounted for 84.0% (N=3,374), 78.4% (N=507), and 93.0% (N=372), respectively, of the totals sampled from all collection methods in the seven sentinel sites. PSC collected 9.0% (N=364), 11.9% (N=77), and 3.2% (N=13), respectively. Prokopack collected 7.0% (N=268), 9.7% (N=63), and 3.8% (N=15), respectively (Table 3).

For *An. arabiensis*, CDC light traps accounted for more than 90% of collections in Bambasi and Salamago; more than 80% in Abaya, Jabitehnan, and Lare; 69.9% in Metema; and 43.1% in Bennatsemay. PSC accounted for 46.8% in Bennatsemay. Prokopack collections accounted for 12.0% or less at any site (Table 3).

For the *An. funestus* group, CDC light traps accounted for 95.3% of collections in Bambasi and 63.5% of collections in Lare. PSCs and Prokopacks contributed 3.5% and 1.2%, respectively, in Bambasi and 19.4% and 17.1%, respectively, in Lare (Table 3).

For *An. pharoensis,* CDC light traps accounted for 95.7% and 92.2% in Bennatsemay and Lare, respectively (Table 3).

**TABLE 3. PROPORTION OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* BY METHOD OF COLLECTION FROM THE SEVEN SENTINEL SITES (APRIL 2021-MARCH 2022)**

| Sentinel site | *An. arabiensis* N (%) | | | | *An. funestus group* N (%) | | | | *An. pharoensis* N (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CDC | PSC | Proko-pack | Total | CDC | PSC | Proko-pack | Total | CDC | PSC | Proko-pack | Total |
| Abaya | 169 (89.0) | 6 (3.2) | 15 (7.9) | 190 (100) | - | - | - | - | - | - | - | - |
| Bambasi | 268 (92.7) | 13 (4.5) | 8 (2.8) | 289 (100) | 245 (95.3) | 9 (3.5) | 3 (1.2) | 257 (100) | - | - | - | - |
| Bennatsemay | 149 (43.1) | 162 (46.8) | 35 (10.1) | 346 (100) | - | - | - | - | 89 (95.7) | 4 (4.3) | 0 (0.0) | 93 (100) |
| Jabitehnan | 1999 (89.2) | 95 (4.2) | 147 (6.6) | 2241 (100) | - | - | - | - | - | - | - | - |
| Lare | 257 (80.1) | 27 (8.4) | 37 (11.5) | 321 (100) | 222 (63.5) | 68 (19.4) | 60 (17.1) | 350 (100) | 283 (92.2) | 9 (2.9) | 15 (4.9) | 307 (100) |
| Metema | 93 (69.9) | 24 (18.1) | 16 (12.0) | 133 (100) | - | - | - | - | - | - | - | - |
| Salamago | 439 (90.3) | 37 (7.6) | 10 (2.1) | 486 (100) | - | - | - | - | - | - | - | - |
| **Total** | **3374 (84.0)** | **364 9.0)** | **268 (7.0)** | **4006 (100)** | **507 (78.4)** | **77 (11.9)** | **63 (9.7)** | **647 (100)** | **372 (93.0)** | **13 (3.2)** | **15 (3.8)** | **400 (100)** |

### 3.1.4 ABUNDANCE OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* BY LOCATION FROM CDC LIGHT TRAP COLLECTIONS

A total of 3,374 *An. arabiensis* were caught indoors and outdoors using CDC light traps in the seven sentinel sites, out of which 1,498 (44.4%) were from indoors and 1,876 (55.6%) were from outdoors. The indoor collections were higher than the outdoors in Abaya (58.6% versus 41.4%), Bambasi (54.5% versus 45.5%), Bennatsemay (51.7% versus 48.3%) and Salamago (54.9% versus 45.1%). More *An. arabiensis* were collected outdoors than indoors in Jabitehnan (60.6% versus 39.8%), Lare (57.2% versus 42.9%), and Metema (60.2% versus 39.8%) (Table 4).

For *An. funestus* group, the indoor and outdoor collections from Bambasi were similar (49.1% versus 50.9%). However, in Lare, the indoor collections (55.4%) were higher than the outdoor (44.6%). Overall, the indoor collections were slightly more than the outdoors (Table 4).

Most of *An. pharoensis* were collected outdoors in both Bennatsemay (56.2%) and Lare (84.8%) (Table 4).

**TABLE 4. INDOOR AND OUTDOOR LIGHT TRAP COLLECTIONS OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* FROM THREE SITES (APRIL 2021-MARCH 2022)**

| Sentinel sites | *An. arabiensis* N (%) | | | *An. funestus group* N (%) | | | *An. pharoensis* N (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | ILT | OLT | Total | ILT | OLT | Total | ILT | OLT | Total |
| Abaya | 99 (58.6) | 70 (41.4) | 169 (100) | | | | | | |
| Bambasi | 146 (54.5) | 122 (45.5) | 268 (100) | 140 (49.1) | 145 (50.9) | 285 (100) | | | |
| Bennatsemay | 77 (51.7) | 72 (48.3) | 149 (100) | | | | 39 (43.8) | 50 (56.2) | 89 (100) |
| Jabitehnan | 788 (39.4) | 1211 (60.6) | 1999 (100) | | | | | | |
| Lare | 110 (42.8) | 147 (57.2) | 257 (100) | 123 (55.4) | 99 (44.6) | 222 (100) | 43 (15.2) | 240 (84.8) | 283 (100) |
| Metema | 37 (39.8) | 56 (60.2) | 93 (100) | | | | | | |
| Salamago | 241 (54.9) | 198 (45.1) | 439 (100) | | | | | | |
| Total | 1498 (44.4) | 1876 (55.6) | 3374 (100) | 263 (51.8) | 244 (48.2) | 507 (100) | 82 (22.0) | 290 (78.0) | 372 (100) |

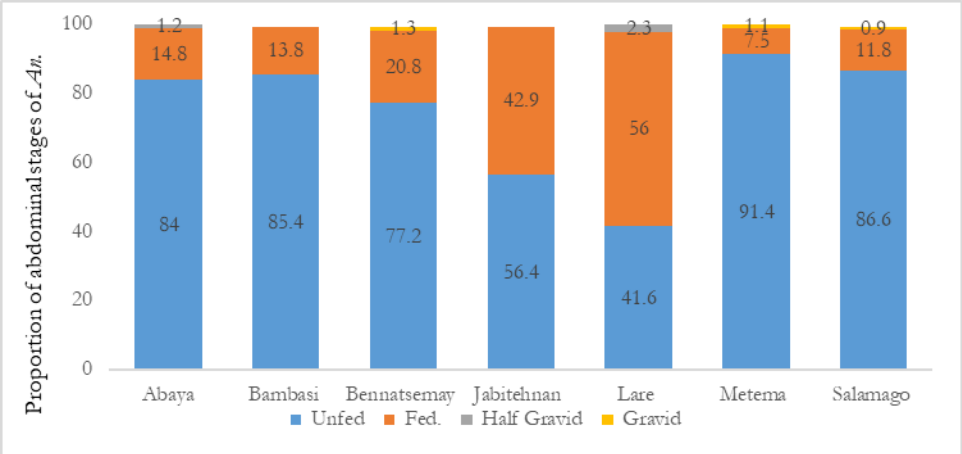
### 3.1.5 PROPORTION OF ABDOMINAL FEEDING STAGES OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* FROM CDC LIGHT TRAP COLLECTIONS

This section describes the proportion of the four abdominal stages (unfed, fed, half gravid, and gravid) for *An. arabiensis*, *An. funestus* group, and *An. pharoensis* obtained from CDC light trap collections at the seven sentinel sites.

*Anopheles arabiensis*

CDC light traps caught more unfed *An. arabiensis* than the rest of the abdominal stages in Abaya (84%), Bambasi (85.4%), Bennatsemay (77.2%), Jabitehnan (56.4%), Metema (91.4%), and Salamago (86.6%). In Lare, the unfeds were 41.6%, outnumbered by the feds, which were 56% of all collections. The second largest group in six out of the seven sentinel sites were feds, comprising 7.5% to 42.9%. The proportions of half gravids and gravids were small in all the sites (Figure 4).

**FIGURE 4. PROPORTION OF ABDOMINAL FEEDING STAGES OF *AN. ARABIENSIS* FROM CDC LIGHT TRAP COLLECTIONS (APRIL 2021-MARCH 2022)**

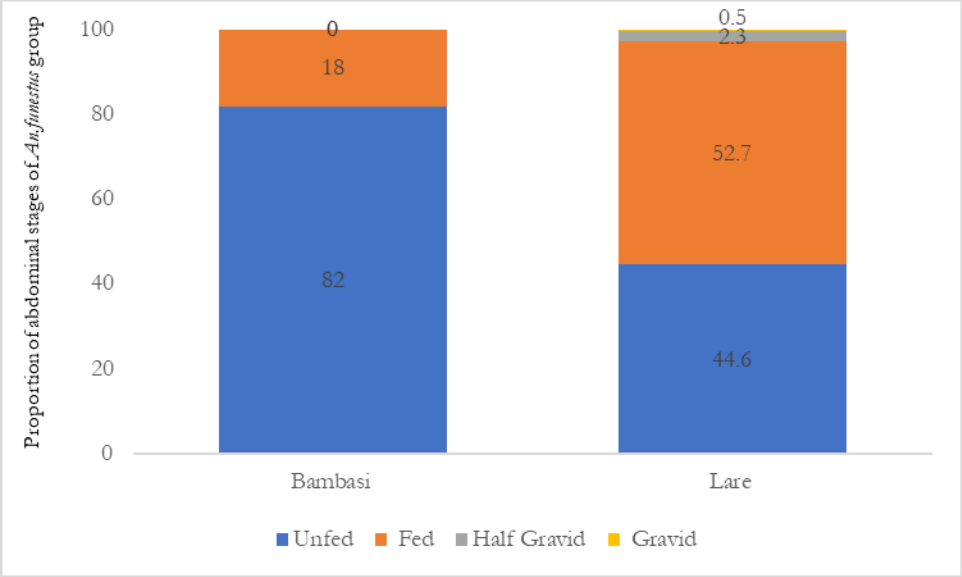


### *Anopheles funestus* group

CDC light traps in Bambasi caught more unfed *An. funestus* group (82%) than feds (18%). No half gravid or gravid were trapped there (Figure 5).

In Lare, the proportion of abdominal stages of *An. funestus* group from CDC light traps were 44.6% unfed, 52.7% fed, 2.3%, half-gravid, and 0.5% gravid (Figure 5).

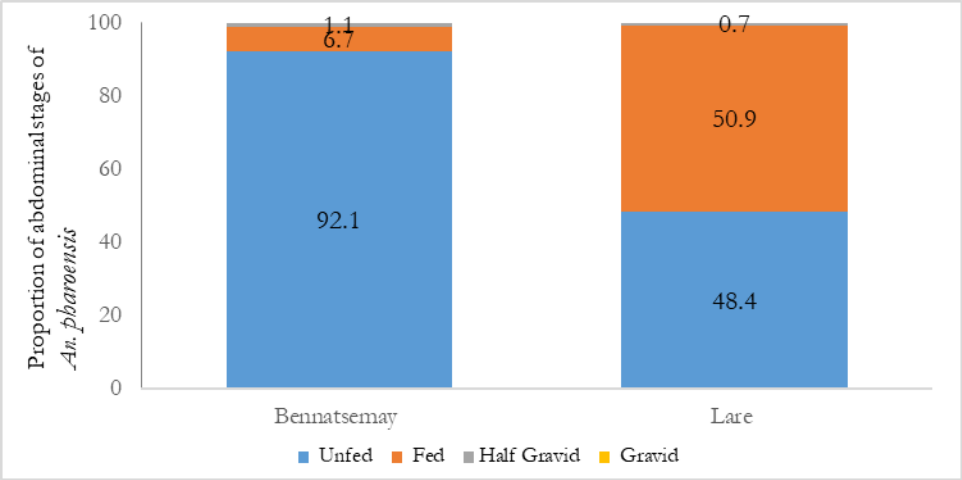
**FIGURE 5. PROPORTION OF ABDOMINAL FEEDING STAGES OF *AN. FUNESTUS* GROUP FROM CDC LIGHT TRAP COLLECTIONS (APRIL 2021-MARCH 2022)**



### *Anopheles pharoensis*

CDC light traps in Bennatsemay caught most *An. pharoensis* unfed (92.1%). At Lare, most were fed (50.9%) (Figure 6). Like *An. arabiensis* and *An. funestus* group, a small number of half gravids and no gravids were collected from these two sentinel sites (Figure 6).

**FIGURE 6. PROPORTION OF ABDOMINAL FEEDING STAGES OF *AN. PHAROENSIS* FROM CDC LIGHT TRAP COLLECTIONS (APRIL 2021-MARCH 2022)**




## 3.1.6 DENSITY AND SEASONALITY OF *AN. ARABIENSIS* FROM CDC LIGHT TRAP COLLECTIONS

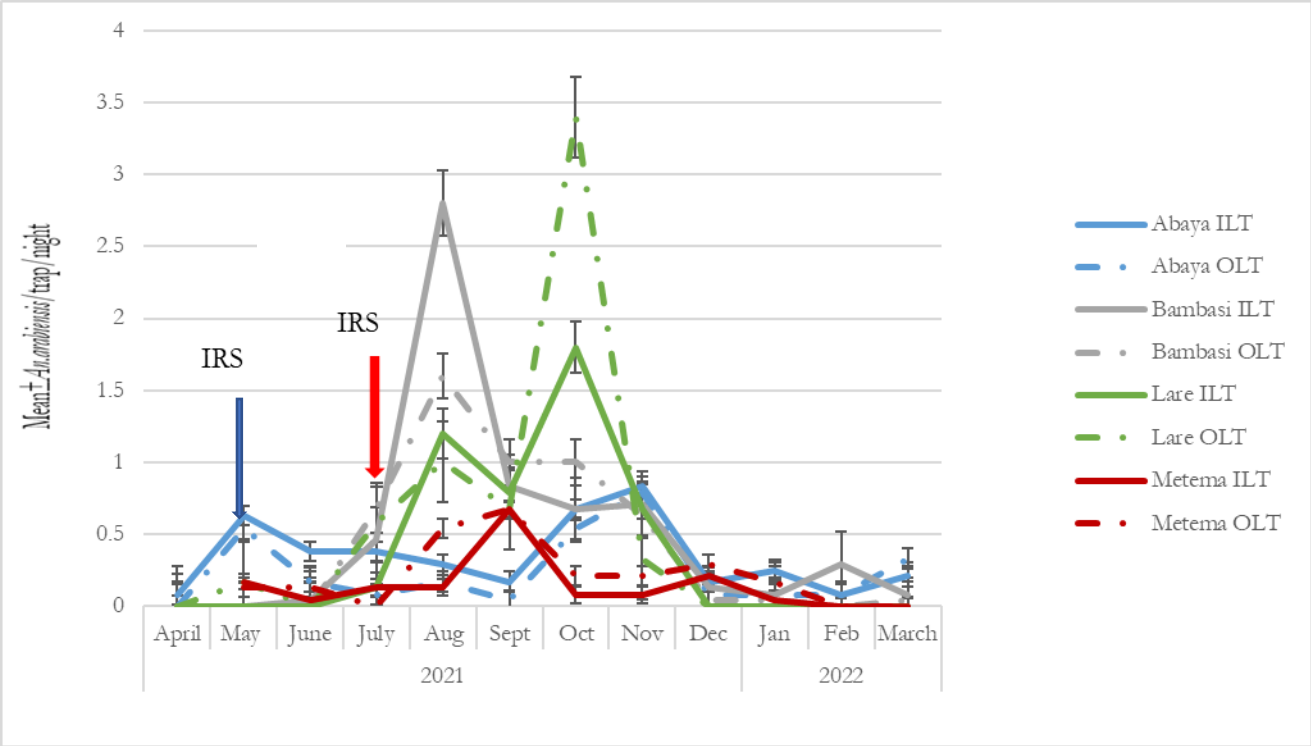
### PMI VectorLink IRS Sites

The daily mean density of *An. arabiensis* from CDC light trap collections indoors and outdoors from the PMI VectorLink IRS sites is presented in Figure 7.

The mean daily trap density of *An. arabiensis* indoors and outdoors in Abaya and Metema was less than 1.0 *An. arabiensis*/trap/night throughout the whole entomological monitoring period. The trend was also the same indoors in Bambasi, except in August when the trap density of 2.8 *An. arabiensis*/trap/night was recorded; outdoors in Bambasi, the mean daily density that month was 1.6. Furthermore, the density outdoors in September and October was 1.0 *An. arabiensis*/trap/night. In the rest of the months, density was less than 1.0. In Lare, 1.2 and 1.8 *An. arabiensis*/trap/night were recorded indoors in August and October, respectively. Outdoors, the peak was 3.4 *An. arabiensis*/trap/night in October; a smaller peak of 1.0 *An. arabiensis*/trap/night was recorded in August (Figure 7).

*Anopheles arabiensis* was entirely absent in April and May in Bambasi; in April and December-March in Lare; and in February and March in Metema (Figure 7).

## FIGURE 7. INDOOR AND OUTDOOR CDC LIGHT TRAP DENSITY OF *AN. ARABIENSIS* IN THE PMI VECTORLINK IRS SITES (APRIL 2021-MARCH 2022)




**Note:** IRS was conducted in May in Abaya, Bambasi, and Lare (blue arrow) and July in Metema (red arrow). Bars represent standard errors.

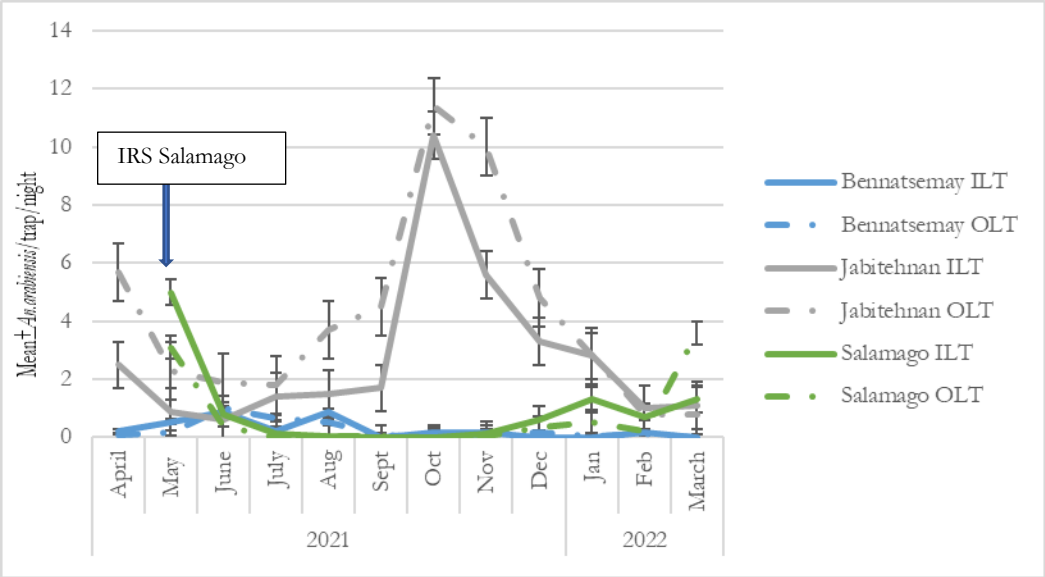
### PMI VectorLink Non-IRS Sites

The daily mean density of *Anopheles arabiensis* from CDC light trap collections indoors and outdoors from the PMI VectorLink non-IRS sites is presented in Figure 8.

In Bennatsemay, the indoor and outdoor CDC trap density of *An. arabiensis* was below 1.0, except in June, when outdoor density was 1.0 *An. arabiensis*/trap/night. In Salamago, two indoor peaks were in May, recorded as 5.0 *An. arabiensis*/trap/night; and in January and March, recorded as 1.3 *An. arabiensis*/trap/night. Outdoors, the peaks were 3.1 *An. arabiensis*/trap/night in May and 3.6 *An. arabiensis*/trap/night in March (Figure 8).

In Jabitehnan, the major peaks indoors occurred in April, October, November, December, and January, when the respective daily trap densities were 2.5, 10.4, 5.6, 3.3, and 2.8 *An. arabiensis*/trap/night. The indoor trap density was higher than 1.0 *An. arabiensis*/trap/night, but below 2.0 *An. arabiensis*/trap/night in July, August, September, February, and March. It was less than 1.0 *An. arabiensis*/trap/night in May and June. Outdoors, the major peaks were in April (5.7 *An. arabiensis*/trap/night), August (3.7 *An. arabiensis*/trap/night), September (4.5 *An. arabiensis*/trap/night), October (11.4 *An. arabiensis*/trap/night), November (10.0 *An. arabiensis*/trap/night), and December (4.8 *An. arabiensis*/trap/night). The density was also 2.3, and 2.8 *An. arabiensis*/trap/night in May and January, respectively (Figure 8).

**FIGURE 8. CDC TRAP DENSITY OF *AN. ARABIENSIS* IN PMI VECTORLINK NON-IRS SITES (APRIL 2021-MARCH 2022)**



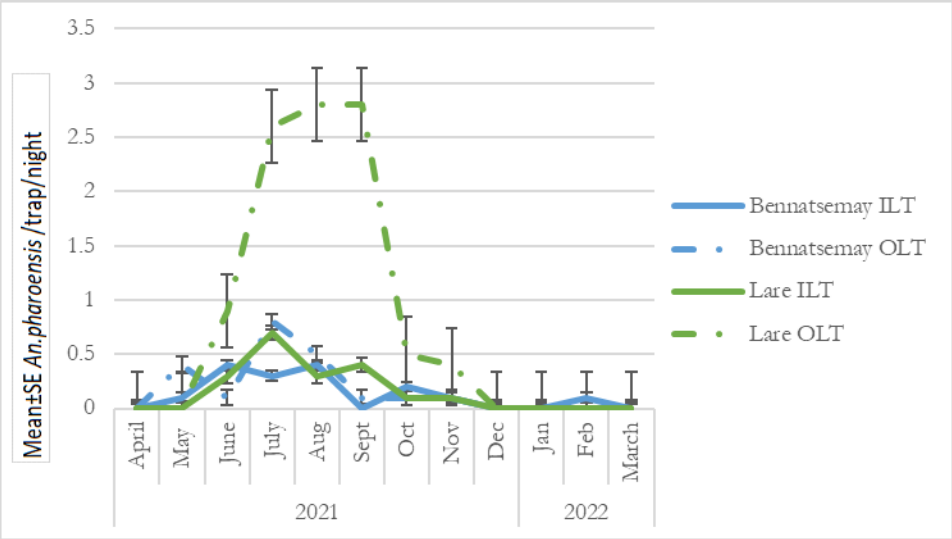

**Note:** Bars represent standard errors. There was no IRS conducted in Bennatsemay or Jabitehnan.

### 3.1.7 DENSITY OF *AN. PHAROENSIS* FROM CDC LIGHT TRAP COLLECTIONS IN BENNATSEMAY AND LARE DISTRICTS

The mean nightly CDC light trap density of *An. pharoensis* was less than 0.5 indoors and outdoors in Bennatsemay when prevalent during the months of entomological monitoring. The only exception was in July outdoors, when the density was 0.8 *An. pharoensis*/trap/night. There was no *An. pharoensis* collected from December to March in either Bennatsemay or Lare, except in February, when the density was 0.1 *An. pharoensis*/trap/night indoors at Bennatsemay (Figure 9).

In Lare, density of *An. pharoensis* indoors from June to November was 0.1 to 0.7 *An. pharoensis*/trap/night but completely absent in April-May and December-March from both indoor and outdoor collections. The highest density outdoors (2.6 to 2.8 *An. pharoensis*/trap/night) was recoded from July to September. In the rest of the months, including June, October, and November, the respective densities were 0.9, 0.5, and 0.4 *An. pharoensis*/trap/night (Figure 9).

**FIGURE 9. CDC LIGHT TRAP DENSITY OF *AN. PHAROENSIS* FROM LARE AND BENNATSEMAY (APRIL 2021-MARCH 2022)**



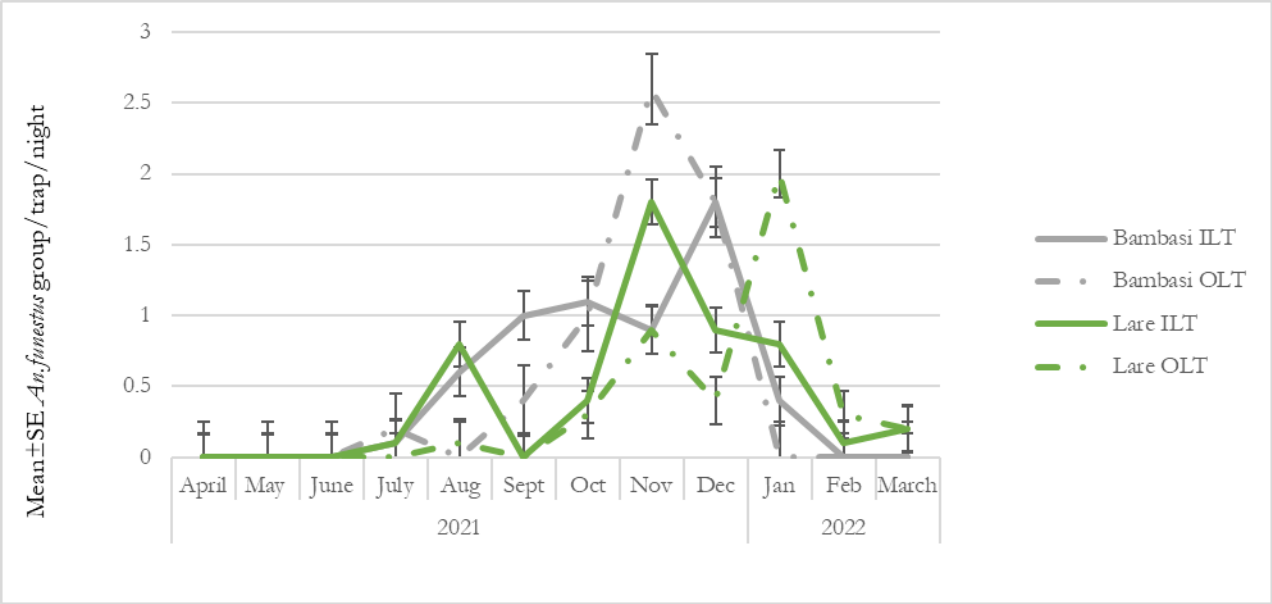

**Note:** Bars represent standard errors.

### 3.1.8 DENSITY OF *AN. FUNESTUS* GROUP FROM CDC LIGHT TRAP COLLECTIONS FROM BAMBASI TOWN AND LARE DISTRICT

In Bambasi, *Anopheles funestus* group were collected from July to January, and the nightly indoor peak CDC light trap density was 1.8 in December. The respective densities in September, October, and November were 1.0, 1.1, and 0.9 *An. funestus* group/trap/night. The outdoor peak, 2.6 *An. funestus* group/trap/night, was in November; and 1.0 *An. funestus* group/trap/night and 1.8 *An. funestus* group/trap/night were recorded in October and December (Figure 10).

In Lare, *An. funestus* group prevailed from July to March, with variable density ranging from 0.1 to 1.8 indoors and 0.1 to 2.0 outdoors, except in September, when no *An. funestus* group mosquitoes were found either indoors or outdoors. The peak density indoors and outdoors was 1.8 *An. funestus* group/trap/night in November and 2.0 in January (Figure 10).

**FIGURE 10. CDC LIGHT TRAP DENSITY OF *AN. FUNESTUS* S.L. FROM BAMBASI AND LARE (APRIL 2021-MARCH 2022)**




**Note:** Bars represent standard errors.

### 3.1.9 INDOOR RESTING DENSITY OF *AN. ARABIENSIS* DETERMINED FROM PSC

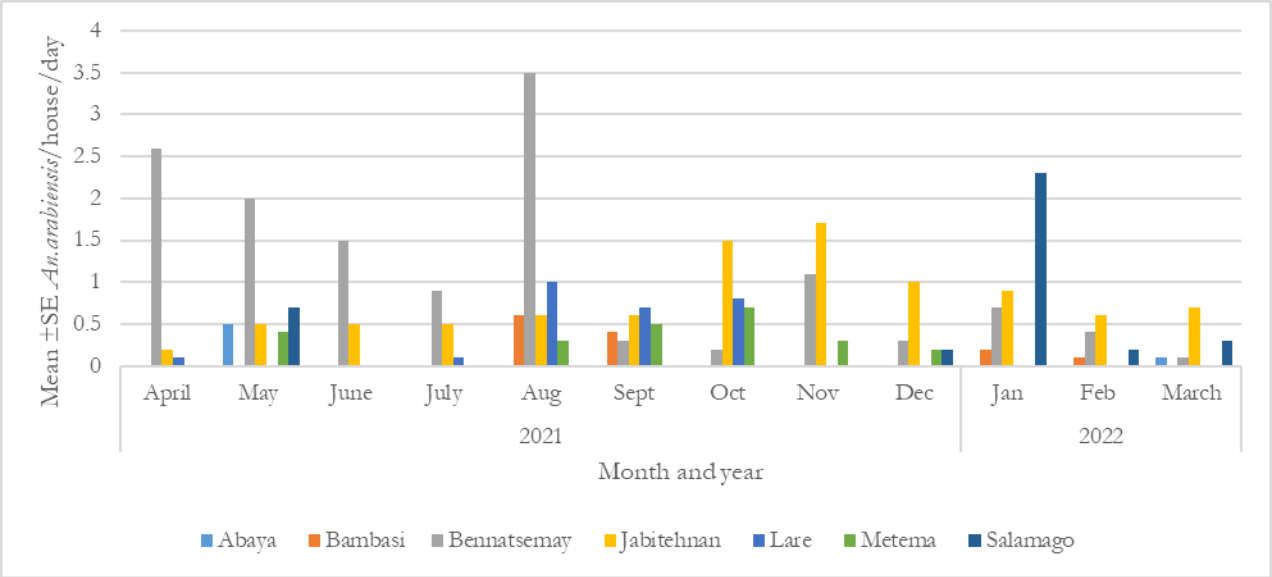
The mean daily indoor resting density of *Anopheles arabiensis* in Abaya was 0.5 and 0.1 *An. arabiensis*/house/day in May 2021 and March 2022, respectively. No indoor resting *An. arabiensis* were found in the other months (Figure 11).

In Bambasi, the indoor resting density was 0.6, 0.4, 0.2, and 0.1 *An. arabiensis*/house/day in August, September, January, and February, respectively. In the rest of the months, no mosquitoes were collected indoors. *An. arabiensis* was present in indoor collections through the year in non-IRS sentinel sites in Bennatsemay and Jabitehnan. The peak daily density in Bennatsemay was in August (3.5 *An. arabiensis*/house/day), April (2.6 *An. arabiensis*/house/day), and May (2.0 *An. arabiensis*/house/day). More than 1.0 *An. arabiensis*/house/day were observed in June (1.5 *An. arabiensis*/house/day) and November (1.1 *An. arabiensis*/house/day). In the rest of the months, it varied from 0.1 *An. arabiensis*/house/day to 0.9 *An. arabiensis*/house/day (Figure 11).

In Jabitehnan, the peak indoor resting density was in October (1.5 *An. arabiensis*/house/day) and November (1.7 *An. arabiensis*/house/day). The density was 0.2 to 0.9 *An. arabiensis*/house/day in the rest of the months; except December, which was 1.0 *An. arabiensis*/house/day (Figure 11).

In Lare, the indoor resting density of *An. arabiensis* was 1.0 in August, but less than 1.0 in the other four months when it was found at all in the mosquito collections. In Metema, the density was less than 1.0 for six months, and none were caught in the other six months. In Salamago, a daily peak density of 2.3 *An. arabiensis*/house/day was recorded in January 2022. In the other four months when any were collected, May, December, February, and March, the density was less than 1.0 *An. arabiensis*/house/day (Figure 11).

**FIGURE 11. INDOOR RESTING DENSITY OF *AN. ARABIENSIS* BASED ON PSC ACROSS SEVEN SITES (APRIL 2021-MARCH 2022)**

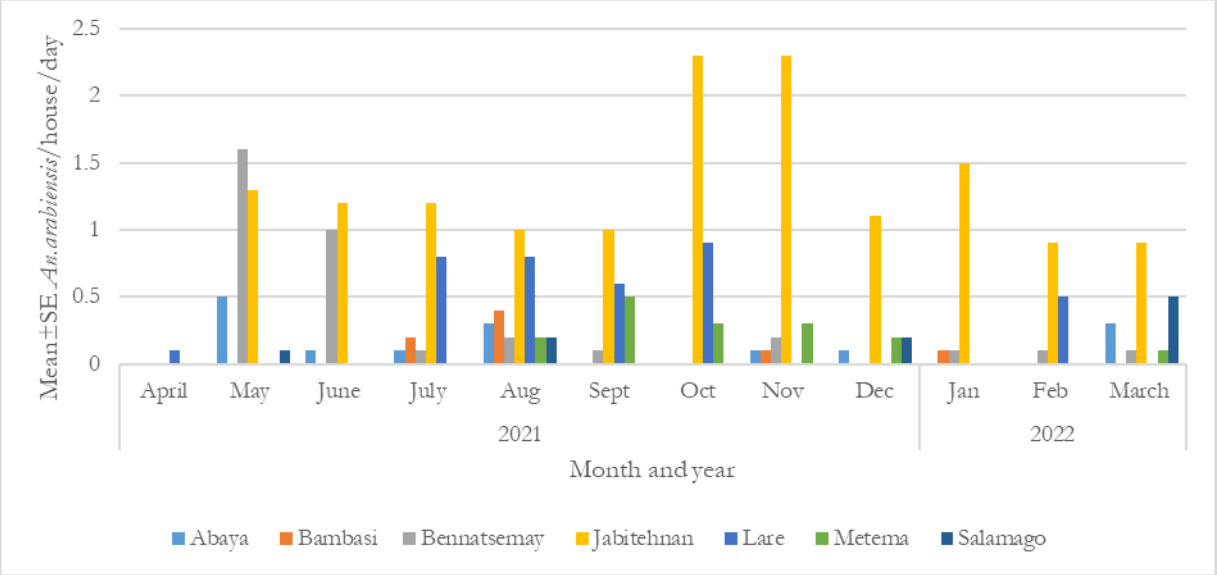



### 3.1.10 INDOOR RESTING DENSITY OF *ANOPHELES ARABIENSIS* DETERMINED FROM PROKOPACK COLLECTIONS ACROSS THE SEVEN SENTINEL SITES

In Abaya, the peak indoor resting density, 0.5 *An. arabiensis*/house/day, was recorded in May; in the rest of the months, it was 0-0.3. In Bambasi, the peak was in August, 0.4 *An. arabiensis*/house/day. In Bennatsemay, in May and June, a peak of 1.6 and 1.0 *An. arabiensis*/house/day, respectively, was recorded; in the rest of the months, density remained was 0.1-0.2. In Jabitehnan, the indoor resting density peaked at 2.3 *An. arabiensis*/house/day in October and November (Figure 12).

In contrast, in Lare, Metema, and Salamago, the indoor resting density was less than 1.0 *An. arabiensis*/house/day during the entire entomological monitoring period (Figure 12).

**FIGURE 12. INDOOR RESTING DENSITY OF *AN. ARABIENSIS* BASED ON PROKOPACK COLLECTIONS ACROSS THE SEVEN SENTINEL SITES (APRIL 2021-MARCH 2022)**




### 3.1.11 ABDOMINAL BLOOD FEEDING STAGES OF *AN. ARABIENSIS*, *AN. FUNESTUS* GROUP, AND *AN. PHAROENSIS* FROM PSC AND PROKOPACK COLLECTIONS

*Anopheles arabiensis*

In Abaya, 83.3% and 60% fed *Anopheles arabiensis* were collected from PSC and Prokopack whereas the proportions of unfeds were 16.7% and 20%, respectively. In contrast, in Bambasi, more than 75% of the collections using PSC and Prokopack were unfeds. The proportions of feds there were almost the same, 23.1% from PSC and 25.0% from Prokopack (Figure 13).

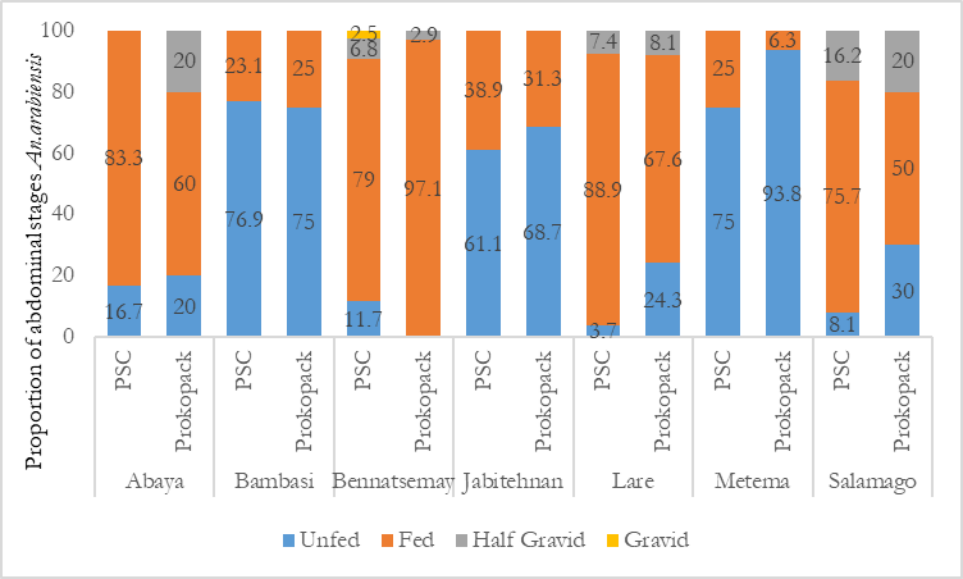
In Bennatsemay, feds were predominant in collections made using both methods: 79.0% from PSC and 97.1% from Prokopack. Of the PSC collections, 11.7% were unfeds. In Jabitehnan, unfeds accounted for 61.1% and 68.7% of PSC and Prokopack collections, respectively; feds accounted for 38.9% and 31.3%, respectively (Figure 13).

In Lare, fed *An. arabiensis* mosquitoes were dominant over the other abdominal stages from both PSC and Prokopack collections, accounting for 88.9% and 67.6%, respectively. Unfeds and half gravids accounted for the remaining 3.7% and 7.4% from PSC and 24.3% and 8.1% from Prokopack, respectively (Figure 13).

In Metema, more unfeds were collected, accounting for 75% and 93.8% from PSC and Prokopack, respectively. Feds accounted for the remaining 25% from PSC and 6.3% from Prokopack collections. In Salamago, feds were 75.7% from PSC and 50% from Prokopack; the respective unfeds were 8.1% and 30% (Figure 13).

All in all, more unfed *An. arabiensis* were sampled from three of the seven sentinel sites (Bambasi, Jabitehnan, and Metema). In the other four sites (Abaya, Bennatsemay, Lare, and Salamago), feds were predominant over the other abdominal stages.

**FIGURE 13. ABDOMINAL FEEDING STATUS OF *AN. ARABIENSIS* ACROSS THE SEVEN SENTINEL SITES (APRIL 2021-MARCH 2022)**

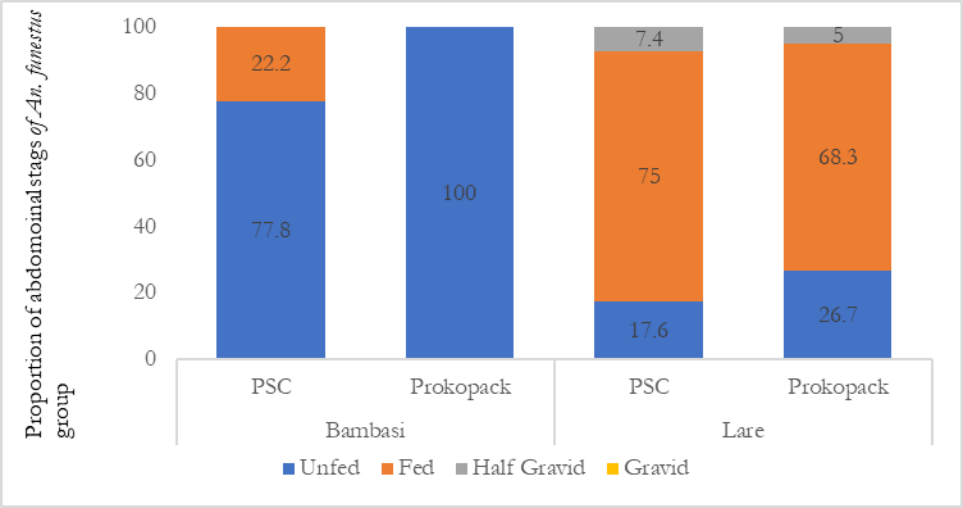



## *Anopheles funestus* group

In Bambasi, the proportion of unfed *An. funestus* group from PSC and Prokopack collections were 77.8% and 100 %, respectively; 22.2 % from PSC were feds. Half gravids and gravids were absent from the collections (Figure 14).

Unlike Bambasi, in Lare, most of the collections were fed *An. funestus* group: 75.0% from PSC and 68.3% from Prokopack; the respective unfed composition was 17.6% and 26.7%. The remaining 7.4% from PSC and 5.0% from Prokopack were half gravids (Figure 14).

**FIGURE 14. ABDOMINAL FEEDING STATUS OF *AN. FUNESTUS* GROUP (APRIL 2021-MARCH 2022)**

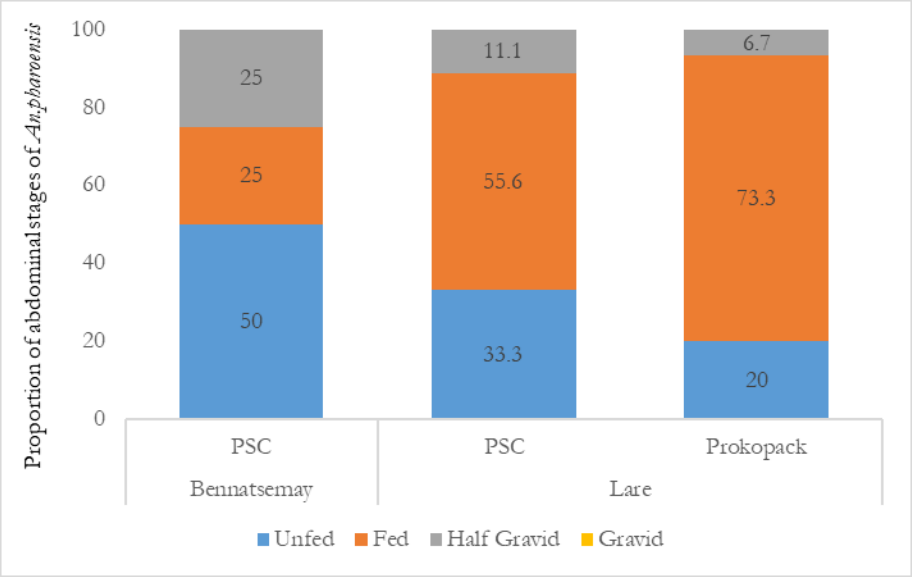


### *Anopheles pharoensis*

In Bennatsemay, *An. pharoensis* was found in PSC collections but not in Prokopack for unknown reasons. The proportion of abdominal stages of *An. pharoensis* from PSC was 50% unfed and each of 25% fed and half gravid (Figure 15).

In Lare, the proportions of feds from both PSC (55.6%) and Prokopack (73.3%) collections were more than the unfeds (33.3% and 20%, respectively) and half gravids (11.1% and 6.7%, respectively) (Figure 15).

**FIGURE 15. ABDOMINAL FEEDING STATUS OF *AN. PHAROENSIS* (APRIL 2021-MARCH 2022)**



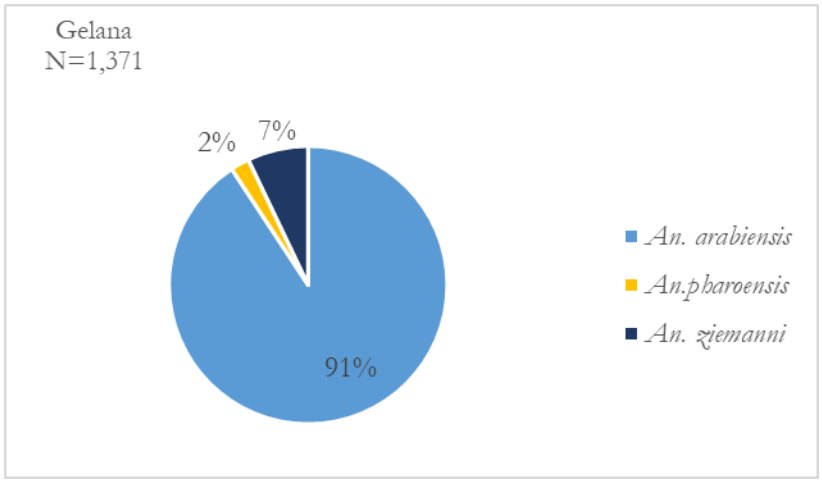

## 3.2 COMMUNITY-BASED MOSQUITO COLLECTIONS FROM GELANA DISTRICT, OROMIA REGION

This section presents data from Gelana District (Oromia Region) community-based mosquito collections on the species composition and abundance of *Anopheles* mosquitoes, the monthly CDC light trap density of *An. arabiensis,* and the proportions of abdominal stages of that species.

### 3.2.1 SPECIES COMPOSITION AND ABUNDANCE

A total of 1,371 *Anopheles* mosquitoes belonging to *An. arabiensis*, *An. pharoensis,* and *An. ziemanni* were collected from Gelana District using CDC indoor light traps. *Anopheles arabiensis* comprised 91% (N=1,244) of all collections. The rest were *An. ziemanni* (7%, N=97) and *An. pharoensis* (2%, N=30) (Figure 16).

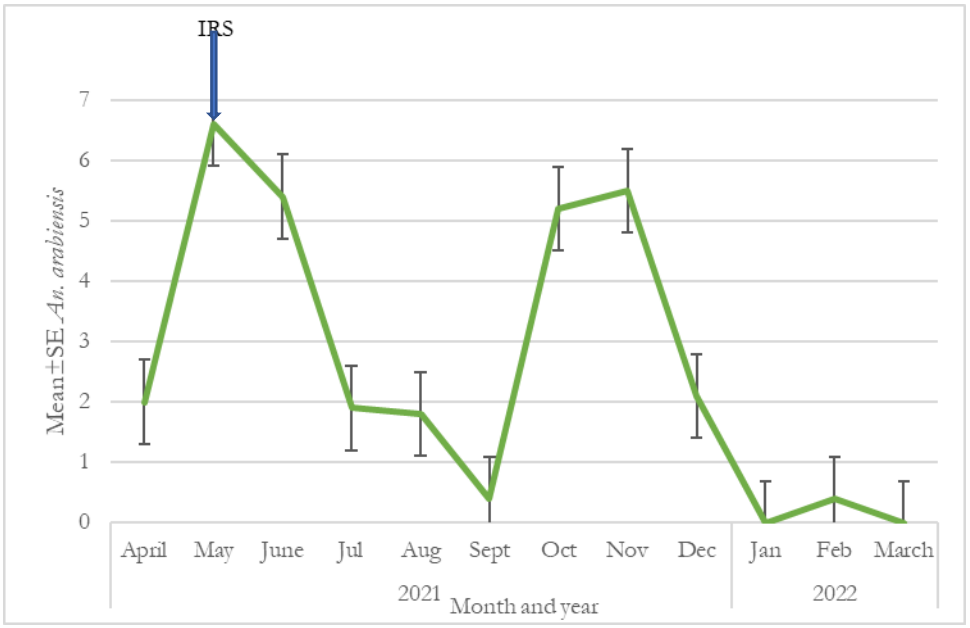
**FIGURE 16. SPECIES COMPOSITION OF *ANOPHELES* FROM GELANA DISTRICT (2020-2021)**




### 3.2.2 MONTHLY CDC LIGHT TRAP DENSITY OF *AN. ARABIENSIS*

*Anopheles arabiensis* in Gelana were collected in 10 of the 12 entomological surveillance months, and was absent from the mosquito collections in January and March. The daily mean trap densities were varied among the months. At the time of IRS in May, the mean trap density was 6.6 *An. arabiensis*/trap/night, it but sharply declined until September. Two peaks appeared, in October (5.2 *An. arabiensis*/trap/night) and November (5.5 *An. arabiensis*/trap/night). Then density sharply dropped until January.

**FIGURE 17. CDC LIGHT TRAP DENSITY OF *AN. ARABIENSIS* FROM GELANA DISTRICT (APRIL 2021-MARCH 2022)**

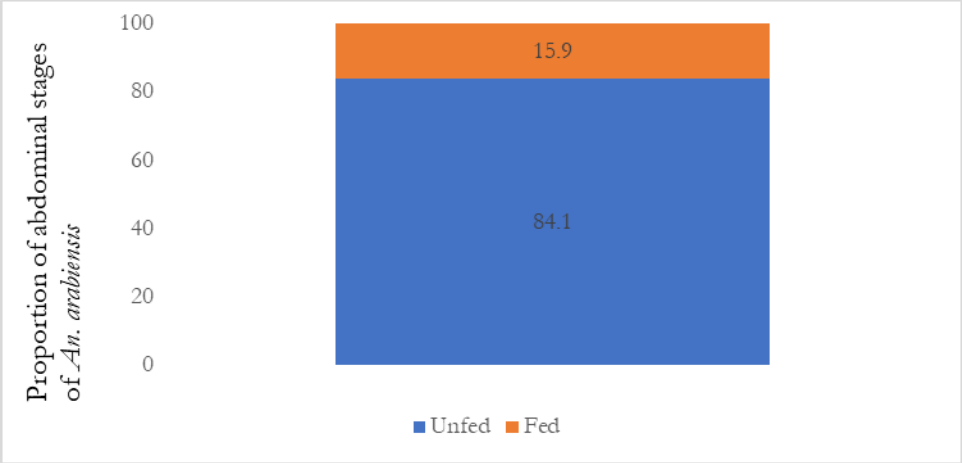



**Note:** Bars are standard errors. IRS in Gelana was conducted in May 2021 (blue arrow).

### 3.2.3 Abdominal Feeding Stages of *An. arabiensis* from CDC Light Trap Collections

The composition of abdominal stages of *An. arabiensis* from Gelana is presented in Figure 18. Unfeds were 84.1%; feds were 15.9%. There were no half gravids or gravids in the CDC light trap collections from Gelana.

**Figure 18. Proportion of Abdominal Feeding Stages of *An. arabiensis* from CDC Light Trap Collections in Gelana District (April 2021-March 2022)**




## 3.3 Larval Density of *An. stephensi* from Monthly Entomological Monitoring

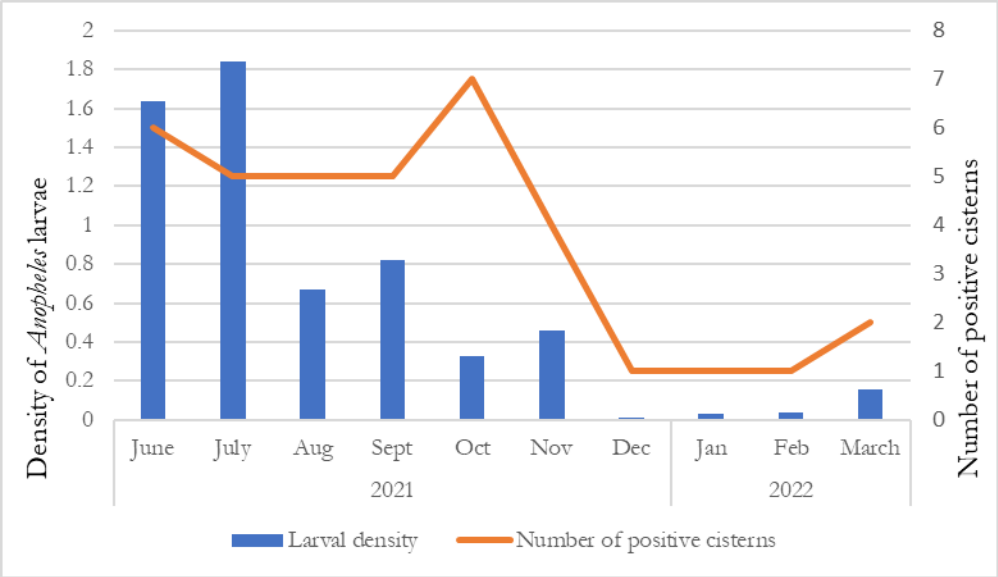
### 3.3.1 Meki Town

Surveys for larval habitats started in Meki in June 2021, and 15 permanent cisterns were found. In July, three additional permanent cisterns were found, giving the total of 18 cisterns. The 18 cisterns were inspected for larvae monthly until March 2022. The number of positive larval habitats for *Anopheles* larvae is shown on Figure 19.

A total of 2,058 *Anopheles* larvae were collected. Of them, 315 and 10 adults of *An. stephensi* and *An. arabiensis,* respectively, were raised and identified to the species. *Aede*s larvae were not encountered.

During the larval monitoring period, *An. stephensi* varied in larval density. The peak larval density, 1.84 *Anopheles*/dip/day, was observed in July. The second-largest density, 1.64 *Anopheles*/dip/day, was in June. In the rest of the months, the density ranged from 0.01 *Anopheles*/dip/day in December to 0.82 in September (Figure 19).

**FIGURE 19. DENSITY OF *ANOPHELES* LARVAE AND NUMBER OF POSITIVE CISTERNS FROM MEKI TOWN (2021-2022)**




In addition to assessment of larval density, Prokopack aspirators were surveyed by community mosquito collectors monthly from June 2021 to March 2022 in 20 cattle shelters for adult *An. stephensi*. Only nine adult *An. gambiae* s.l. were collected; no *An. stephensi* were found.

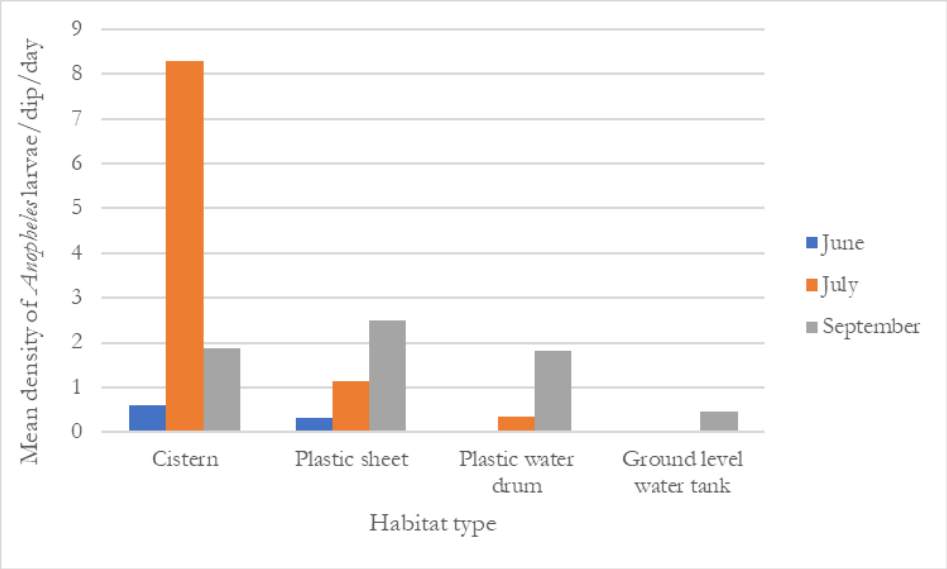
### 3.3.2 SEMERA-LOGIA TOWN

Four different types of larval habitats were identified in Semera-Logia: cisterns, plastic sheets, plastic water drums, and ground-level water tanks. In June, the number of larval habitats found positive with *Anopheles* larvae was 14 out of 35 inspected. In July, 21 out of 33 larval habitats inspected; in September, it was 22 out of 38 inspected. Water in the containers is used for hollow brick construction and residential house constructions. Therefore, availability of water filled containers vary from month to month based on the needs of business facilities and house constructing individuals. With the exception of drums, the rest containers are replenished with tap water from time to time containing water for a longer period.

A total of 5,239 *Anopheles* larvae were collected in the three months for all habitat types, and the mean daily larval density from each was estimated (Figure 20). The largest larval density, 8.3 *Anopheles* larvae/dip/day, was recorded from cisterns in July, followed by 2.51 *Anopheles* larvae/dip/day from plastic sheets in September. From cisterns, the larval density in September and June was 1.88 *Anopheles* larvae/dip/day and 0.59 *Anopheles* larvae/dip/day, respectively. From plastic sheets, larval density in June and July was 0.32 *Anopheles* larvae/dip/day and 1.15 *Anopheles* larvae/dip/day. From plastic water drums, larval density in July and September were 0.36 and 1.81 *Anopheles* larvae/dip/day, respectively. From ground-level water tanks, in September it was 0.45 *Anopheles* larvae/dip/day. Brick and new residential house constructions are undergoing in Semera. Business facilities engaged in brick constructions store water in cisterns. Storing water in plastic sheets is a common practice for house construction. Therefore, the peak density of *An. stephensi* in July and September could be associated with increased storage of water by the community.

The overall larval density estimated from the four container types was 0.43, 5.17, and 1.99 *Anopheles* larvae/dip/day in June, July, and September, respectively (Figure 20).

**FIGURE 20. DENSITY OF *ANOPHELES* BY HABITAT FROM SEMERA-LOGIA TOWN (2021-2022)**




A total of 249 *An. stephensi* were identified from larval rearing (Annex B); no other *Anopheles* were found in Semera.

## 3.4 SURVEY ON THE PRESENCE/ABSENCE OF *AN. STEPHENSI*

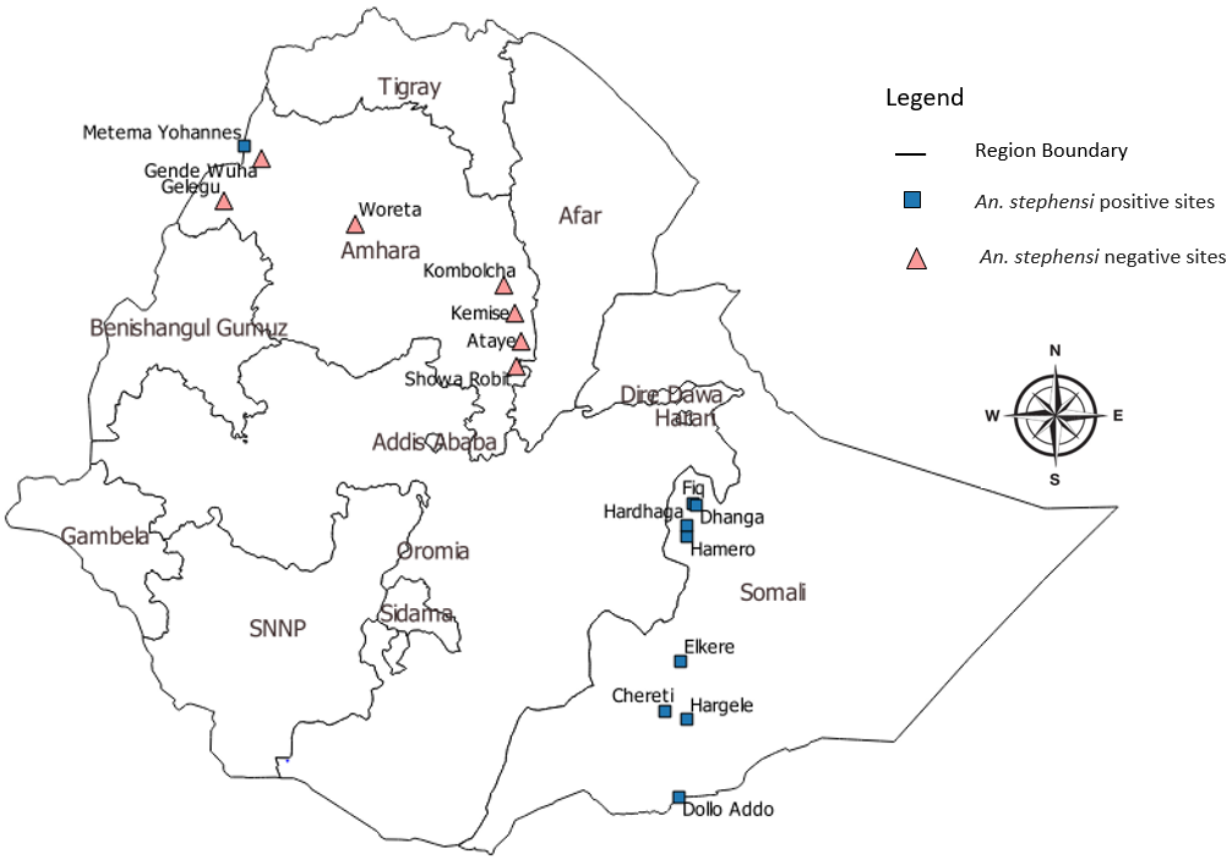
The presence of *An. stephensi* was investigated from collecting larvae and raising to adults in 16 new sites in Amhara and Somali, of which 50% of the sites were positive. The presence of *An. stephensi* was confirmed from Metema Yohannes Town in Amhara and Chereti, Dhanga, Dollo Ado, Elkere, Fik, Hamero, Hardhaga, and Hargele in Somali. From a total collection of 3,915 *Anopheles* larvae, 937 adult *An. stephensi* were identified (Table 5). The distribution of sites where *An. stephensi* was and was not detected is shown on Figure 21.

**TABLE 5. NUMBER OF *ANOPHELES* LARVAE AND ADULTS OF *AN. STEPHENSI* IDENTIFIED FROM REARED LARVAE (2021)**

| Region | Site | # *Anopheles* larvae collected | #Adult *Anopheles* raised from larvae and identified | |
|---|---|---|---|---|
| | | | *An. stephensi* | *An. gambiae* s.l. |
| Amhara | Ataye | 280 | 0 | 0 |
| | Gelegu | 66 | 0 | 22 |
| | Gendewuha | 0 | 0 | 0 |
| | Kemise | 289 | 0 | 0 |
| | Kombolcha | 443 | 0 | 0 |
| | Metema Yohannes | 19 | 4 | 0 |
| | Shoa Robit | 431 | 0 | 0 |
| | Woreta | 29 | 0 | 20 |
| Somali | Chereti | 618 | 25 | 0 |
| | Dhanga | 133 | 63 | 70 |
| | Dollo Ado | 334 | 30 | 0 |
| | Elkere | 205 | 96 | 90 |
| | Fik | 580 | 527 | 0 |

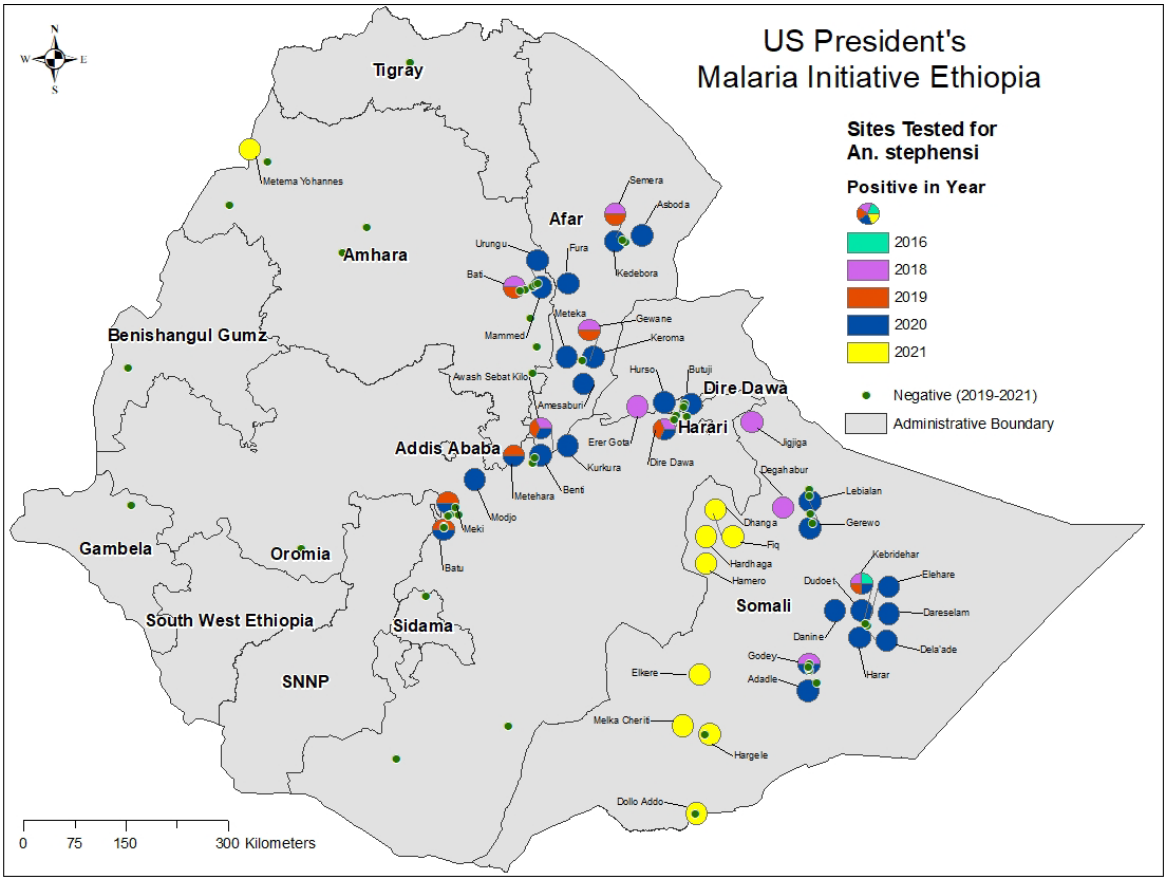
| Region | Site | # *Anopheles* larvae collected | #Adult *Anopheles* raised from larvae and identified | |
|---|---|---|---|---|
| | | | *An. stephensi* | *An. gambiae* s.l. |
| | Hamero | 209 | 168 | 0 |
| | Hardhaga | 127 | 2 | 125 |
| | Hargele | 152 | 22 | 0 |
| **Total** | | **3915** | **937** | **327** |

**FIGURE 21. SITES POSITIVE AND NEGATIVE FOR *AN. STEPHENSI* FROM THE 2021 SURVEYS IN AMHARA AND SOMALI REGIONS**




A total of 83 sites were surveyed from 2018 to 2021, out of which 45 sites were found positive for the presence of *An. stephensi* (Figure 22).

**FIGURE 22. MAP SHOWING THE NUMBER OF SITES POSITIVE AND NEGATIVE FOR THE PRESENCE OF *AN. STEPHENS* FROM ENTOMOLOGICAL SURVEYS CONDUCTED FROM 2018 TO 2021**




## 3.5 LABORATORY TEST RESULTS

This section discusses the laboratory test results on species identification of *An. gambiae* s.l. and *An. funestus* group, as well as sporozoite infections before and after boiling of mosquito homogenates and blood meal sources.

### 3.5.1 SPECIES IDENTIFICATION OF *ANOPHELES GAMBIAE* S.L.

A total of 116 specimens of *An. gambiae* s.l. collected from Bambasi and Jabitehnan were PCR analysed. Of these, 90.5% (N=105) were amplified and all of them were *An. arabiensis*. In Bambasi, 56 mosquitoes were tested, and 96.4% (N=54) of them were *An. arabiensis*. DNA of the remaining 3.6% (N=2) was unamplified. In Jabitehnan, 85% (N=51) of the 60 mosquitoes tested were *An. arabiensis*. DNA of the remaining 15.0% (N=9) was not amplified. (Table 6).

**TABLE 6. SPECIES IDENTIFICATION OF *AN. GAMBIAE* S.L.**

| Sentinel site | # Tested | *An. arabiensis* N (%) | Not amplified N (%) |
|---|---|---|---|
| Bambasi | 56 | 54 (96.4) | 2 (3.6) |
| Jabitehnan | 60 | 51 (85.0) | 9 (15.0) |
| **Total** | **116** | **105 (90.5)** | **11 (9.5)** |

### 3.5.2 SPECIES IDENTIFICATION OF MEMBERS OF *ANOPHELES FUNESTUS* GROUP

A total of 47 specimens of *An. funestus* group collected from Bambasi were PCR analyzed to identify the species. Tests detected four species—*An. funestus* s.s., *An. leesoni*, *An. parensis,* and *An. rivulorum*. Of these, the most prevalent was *An. parensis,* comprising 74.4% (N=35) of all tested specimens. Each of 4.3% were *An. funestus* s.s. and *An. leesoni*; 10.6% were *An. rivulorum*. The remaining 6.4% were not amplified (Table 7).

**TABLE 7. MOLECULAR IDENTIFICATION OF *AN. FUNESTUS* GROUP BY PCR FROM SAMPLES COLLECTED FROM BAMBASI TOWN (2021)**

| *An. funestus s.s.* N (%) | *An. leesoni* N (%) | *An. parensis* N (%) | *An. rivulorum* N (%) | Not amplified N (%) | Total N (%) |
|---|---|---|---|---|---|
| 2 (4.3) | 2 (4.3) | 35 (74.4) | 5 (10.6) | 3 (6.4) | 47 (100) |

### 3.5.3 SPOROZOITE INFECTION RATES OF *ANOPHELES* MOSQUITOES

*Anopheles arabiensis*

A total of 1,553 *An. arabiensis* collected from Abaya, Bambasi, Bennatsemay, Gelana, Jabitehnan, Metema, and Salamago using CDC light traps, PSC, and Prokopack aspirations were sporozoite ELISA tested. Of them, 11 specimens were positive for *Plasmodium falciparum* and *P. vivax* 210 and 247 sporozoites, giving overall sporozoite rates of 0.26%, 0.06%, and 0.39%, respectively (Table 8).

ELISA tests after boiling the homogenates confirmed two positive *P. falciparum* out of the 11 positives detected before boiling; the rest were negative (Table 10).

**TABLE 8. SPOROZOITE INFECTION RATES OF *AN. ARABIENSIS* (APRIL 2021-MARCH 2022)**

| Site | Method | # Tested | Pf (% +ve) | Pv-210 (% +ve) | Pv-247 (% +ve) |
|---|---|---|---|---|---|
| Abaya | CDC | 64 | 0 | 0 | 1(1.56) |
| | Prokopack | 6 | 0 | 0 | 0 |
| | Total | 70 | 0 | 0 | 1 (1.43) |
| Bambasi | CDC | 150 | 0 | 1 (0.66) | 1 (0.66) |
| | PSC | 1 | 0 | 0 | 0 |
| | Total | 151 | 0 | 1 (0.66) | 1 (0.66) |
| Bennatsemay | CDC | 6 | 0 | 0 | 0 |
| | Prokopack | 16 | 0 | 0 | 0 |
| | PSC | 44 | 0 | 0 | 0 |
| | Total | 66 | 0 | 0 | 0 |
| Gelana | CDC | 378 | 2 (0.53) | 0 | 1 (0.26) |
| Jabitehnan | CDC | 699 | 2 (0.29) | 0 | 3 (0.43) |
| | Prokopack | 18 | 0 | 0 | 0 |
| | PSC | 27 | 0 | 0 | 0 |
| | Total | 744 | 2 (0.27) | 0 | 3 (0.40) |
| Metema | CDC | 43 | 0 | 0 | 0 |
| | Prokopack | 5 | 0 | 0 | 0 |
| | PSC | 9 | 0 | 0 | 0 |
| | Total | 57 | 0 | 0 | 0 |
| Salamago | CDC | 87 | 0 | 0 | 0 |
| **Grand Total** | | **1553** | **4(0.26)** | **1 (0.06)** | **6 (0.39)** |

**Note:** No single pool of specimens was positive for both *Pv*-210 and *Pv*-247 (i.e., specimens positive for *Pv*-210 and *Pv*-247 were different). Result calculated with ELISA, without the boiling step. **Key:** *Pf*=*P. falciparum. Pv*=*P. vivax*. +ve=tested positive

## Other *Anopheles* Species

From the longitudinal entomological monitoring, 1,749 specimens representing at least seven *Anopheles* species—namely *An. coustani*, *An. demeilloni*, *An. funestus* group, *An. pharoensis*, *An. squamosus/cydippis,* and *An. ziemanni*—were ELISA tested to detect sporozoite infections. Of these, 26 were positive for *P. vivax* 247 and *P. vivax* 210. Those positive for *P. vivax* 247 were three *An. coustani* from Jabitehnan. Those positive for *P. vivax* 210 were 14 and six specimens of *An. coustani* from Bambasi and Jabitehnan, respectively; one each of *An. ziemanni* from Bambasi and Gelana; and one *An. parensis* (PCR confirmed) from Lare (Table 9).

After tests conducted by boiling mosquito homogenates, of all the 26 positives, two *An. coustani* from Bambasi were positive for *P. vivax* 210. The remaining 24 samples were negative (Table 10). This provides data that *An. coustani* might serve as a secondary vector of malaria in Ethiopia.

**TABLE 9. SPOROZOITE ELISA RESULTS FOR OTHER *ANOPHELES* SPECIES COLLECTED**

| Site | Species | Method | Total | *Pf* (% +ve) | *Pv*-210 (% +ve) | *Pv*-247 (% +ve) |
|---|---|---|---|---|---|---|
| Abaya | *An. pharoensis* | CDC | 1 | 0 | 0 | 0 |
| | *An. ziemanni* | CDC | 2 | 0 | 0 | 0 |
| | Total | | 3 | 0 | 0 | 0 |
| Bambasi | *An. coustani* | CDC | 912 | 0 | 14 (1.54) | 0 |
| | *An. coustani* | PSC | 5 | 0 | 0 | 0 |
| | *An. funestus* group | CDC | 78 | 0 | 0 | 0 |
| | *An. squamosus/cydippis* | CDC | 217 | 0 | 0 | 0 |
| | *An. ziemanni* | CDC | 164 | 0 | 1 (0.61) | 0 |
| | Total | | 1376 | 0 | 15 (1.1) | 0 |
| Gelana | *An. pharoensis* | CDC | 2 | 0 | 0 | 0 |
| | *An. ziemanni* | CDC | 31 | 0 | 1 (3.23) | 0 |
| | Total | | 33 | 0 | 1 (3.03) | 0 |
| Jabitehnan | *An. coustani* | CDC | 148 | 0 | 6 (4.05) | 3 (2.03) |
| | *An. coustani* | Prokopack | 30 | 0 | 0 | 0 |
| | *An. demeilloni* | CDC | 1 | 0 | 0 | 0 |
| | Total | | 179 | 0 | 6 (3.35) | 3 (1.68) |
| Lare | *An. coustani* | CDC | 14 | 0 | 0 | 0 |
| | *An. funestus* group | CDC | 127 | 0 | 1 (0.79) | 0 |
| | *An. funestus* group | Prokopack | 10 | 0 | 0 | 0 |
| | *An. funestus* | PSC | 3 | 0 | 0 | 0 |
| | Total | | 154 | 0 | 1 (0.65) | 0 |
| Metema | *An. funestus* | CDC | 4 | 0 | 0 | 0 |
| **Grand Total** | | | **1749** | **0 (0.0)** | **23 (1.32)** | **3 (0.17)** |

**Note:** No single pool of specimens was positive for both *Pv*-210 and *Pv*-247 (i.e., specimens positive for *Pv*-210 and *Pv*-247 were different). Result calculated with ELISA, without the boiling step. **Key:** *Pf*=*P. falciparum*, *Pv*=*P. vivax*. +ve=tested positive

## Sporozoite Infections after Boiling

Data on *Anopheles* species found positive before and after boiling of mosquito homogenates is presented in Table 10. Of the four *An. arabiensis* found positive for *P. falciparum* in Gelana and Jabitehnan, two from Gelana were positive after boiling; the two from Jabitehnan were negative. Of the 23 specimens found positive for *P. vivax* 210, ELISA confirmed two *An. coustani* from Bambasi were positive after boiling; the other 22 specimens of *An. arabiensis, An. coustani, An. ziemanni,* and *An. parensis* were negative. None of the nine *An. arabiensis* and *An. coustani* from Abaya, Bambasi, Gelana, and Jabitehnan was positive for *P. vivax* 247 after boiling.

**TABLE 10. *ANOPHELES* SPECI*ES FOUND POSITIVE/NEGATIVE FOR SPOROZOITE INFECTION IN A BOILING METHOD OF TESTING**

| Site | Species of *Anopheles* | *Pf* | | | *Pv*-210 | | | *Pv*-247 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | # +ve before boiling | after boiling | | # +ve before boiling | after boiling | | # +ve before heating | after boiling | |
| | | | # +ve | # +ve negative | | # +ve | # +ve negative | | # +ve | negative # +ve |
| Abaya | *An. arabiensis* | - | - | - | - | - | - | 1 | 0 | 1 |
| Bambasi | *An. arabiensis* | - | - | - | 1 | 0 | 1 | 1 | 0 | 1 |
| | *An. coustani* | - | - | - | 14 | 2 | 13 | - | - | - |
| | *An. ziemanni* | - | - | - | 1 | 0 | 1 | - | - | - |
| Gelana | *An. arabiensis* | 2 | 2 | 0 | - | - | - | 1 | 0 | 1 |
| | *An. ziemanni* | - | - | - | 1 | 0 | 1 | - | - | - |
| Jabitehnan | *An. arabiensis* | 2 | 0 | 2 | - | - | - | 3 | 0 | 3 |
| | *An. coustani* | - | - | - | 5 | 0 | 5 | 3 | 0 | 3 |
| Lare | *An. parensis* | - | - | - | 1 | 0 | 1 | - | - | - |
| **Total** | | **4** | **2** | **2** | **23** | **2** | **22** | **9** | **0** | **9** |

**Key:** *Pf*=*P. falciparum*. *Pv*=*P. vivax*. +ve=tested positive

### 3.5.4 BLOOD MEAL SOURCES OF *ANOPHELES*

A total of 410 specimens of *An. arabiensis* collected from Abaya, Bennatsemay, Gelana, and Jabitehnan were ELISA tested to detect the source of their blood meals as human, bovine, or mixed (human plus bovine). In two of the four sites—Abaya and Gelana—the human blood index (HBI; 0.54 and 0.83, respectively) was higher than the bovine blood index (BBI). In Bennatsemay and Jabitehnan, the opposite was observed: the HBI (0.29 and 0.19, respectively) was lower than the BBI, showing the high propensity of *An. arabiensis* to feed on cattle in those two districts. The overall HBI in the four sites was 0.30, whereas the BBI was 0.70 (Table 11).

**TABLE 11. BLOOD MEAL SOURCES AND BLOOD MEAL INDICES OF *ANOPHELES ARABIENSIS* (2021)**

| Site | # Tested | Origin of blood meal | | | | HBI | BBI |
|---|---|---|---|---|---|---|---|
| | | Human | Bovine | Mixed | Unidentified | | |
| Abaya | 13 | 6 | 1 | 1 | 5 | 0.54 | 0.15 |
| Bennatsemay | 70 | 13 | 34 | 7 | 16 | 0.29 | 0.59 |
| Gelana | 53 | 43 | 1 | 1 | 8 | 0.83 | 0.38 |
| Jabitehnan | 274 | 5 | 195 | 46 | 28 | 0.19 | 0.88 |
| **Total** | **410** | **67** | **231** | **55** | **57** | **0.30** | **0.70** |

**Key:** HBI=human blood index. BBI=bovine blood index.

## 3.6 INSECTICIDE RESISTANCE MONITORING

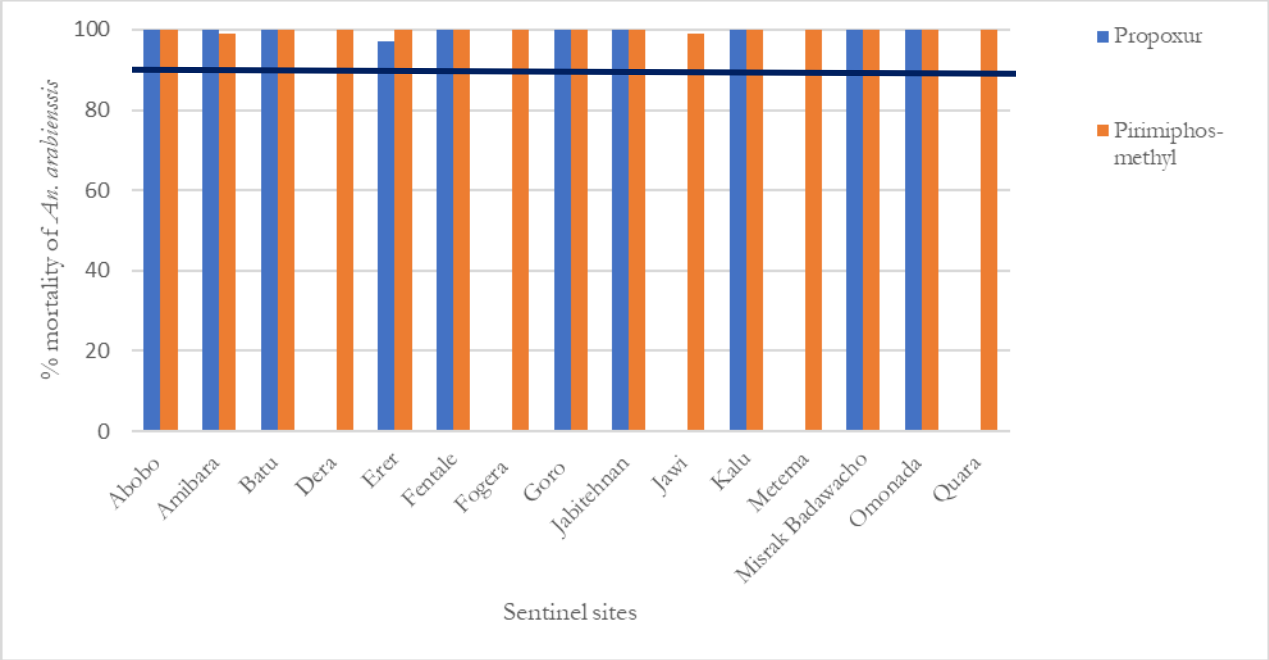
This section gives results of insecticide susceptibility tests, resistance intensity, and piperonyl butoxide (PBO) synergist assays conducted on populations of *An. arabiensis* and *An. stephensi*.

### 3.6.1 *ANOPHELES ARABIENSIS* SUSCEPTIBILITY TO INSECTICIDES

Populations of *An. arabiensis* were susceptible to propoxur in nine sentinel sites: Abobo, Amibara, Batu, Fentale, Goro, Jabitehnan, Kalu, Misrak Badawacho, and Omonada. Propoxur-impregnated papers killed 97% of *An. arabiensis* in Erer, revealing possible resistance.

*Anopheles arabiensis* was susceptible to pirimiphos-methyl in 15 sentinel sites: Abobo, Amibara, Batu, Dera, Erer, Fentale, Fogera, Goro, Jabitehnan, Jawi, Kalu, Metema, Misrak Badawacho, Omonada, and Quara (Figure 23).

**FIGURE 23. MORTALITY OF *AN. ARABIENSIS* FROM WHO TUBE TESTS CONDUCTED ON IX CONCENTRATIONS OF PROPOXUR AND PIRIMIPHOS-METHYL (2021)**

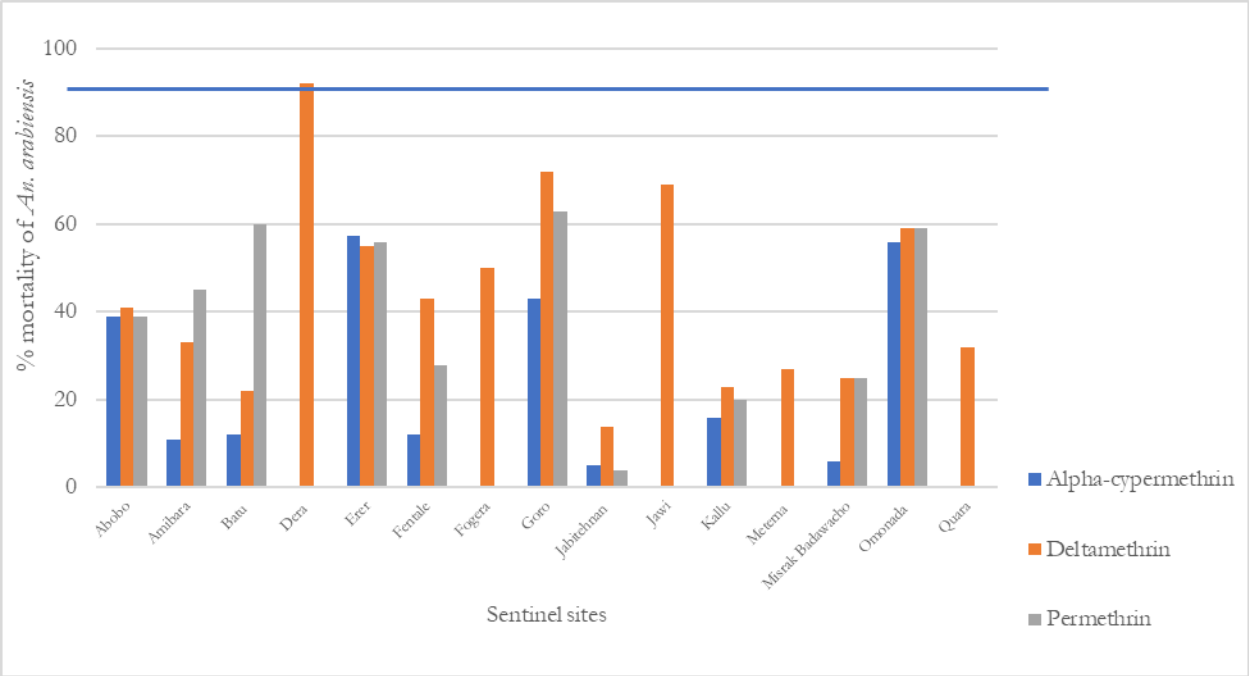



**Note:** Line indicates 90% mortality threshold for resistance.

Populations of *An. arabiensis* were resistant (4%-60% mortality) to alpha-cypermethrin and permethrin in all 10 sentinel sites where the susceptibility tests were conducted: Abobo, Amibara, Batu, Erer, Fentale, Goro, Jabitehnan, Kallu, Misrak Badawacho, and Omonada (Figure 24).

Deltamethrin susceptibility tests were conducted in 15 sentinel sites: Abobo, Amibara, Batu, Dera, Erer, Fentale, Fogera, Goro, Jabitehnan, Jawi, Kalu, Metema, Misrak Badawacho, Omonada, and Quara. Resistance of *An. arabiensis* to deltamethrin was found in 14 of 15 sites, where mortality was 14%-72%. *Anopheles arabiensis* mortality was 92% in Dera, showing possible resistance (Figure 24).

**FIGURE 24. MORTALITY OF *AN. ARABIENSIS* FROM WHO TUBE TESTS CONDUCTED ON 1X CONCENTRATIONS OF ALPHA-CYPERMETHRIN, DELTAMETHRIN, AND PERMETHRIN (2021)**




**Note:** Line indicates 90% mortality threshold for resistance.

### 3.6.2 RESULTS OF *AN. ARABIENSIS* RESISTANCE INTENSITY ASSAYS

The intensity of resistance in populations of *An. arabiensis* to alpha-cypermethrin from Batu, Fentale, Goro, Kallu and Misrak Badawacho fall under high resistance intensity of the classification of WHO since mortality was 75-94% at 10X (Figure 25A).

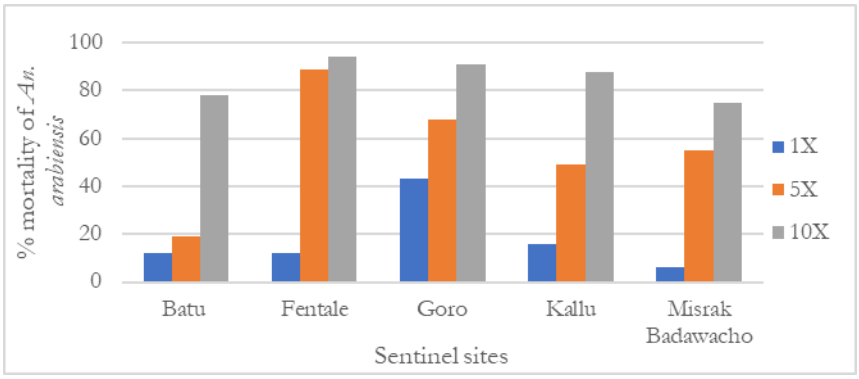
The intensity of resistance in populations of *An. arabiensis* was low to deltamethrin in Fentale and Metema, with 98% and 100% mortality at 5X, respectively. Intensity of resistance was moderate in Fogera, Goro, Jabitehnan, Jawi, and Quara, with 100% mortality at 10X. Intensity of resistance was high in Batu, Dera, and Misrak Badawacho, with 82%, 97%, and 80% mortality at 10X (Figure 25B).

The intensity of resistance in populations of *An. arabiensis* was moderate to permethrin in Goro and Kallu, with 100% mortality at 10X. Intensity of resistance was high in Batu and Misrak Badawacho, with 94% and 88% mortality at 10X (Figure 25C).

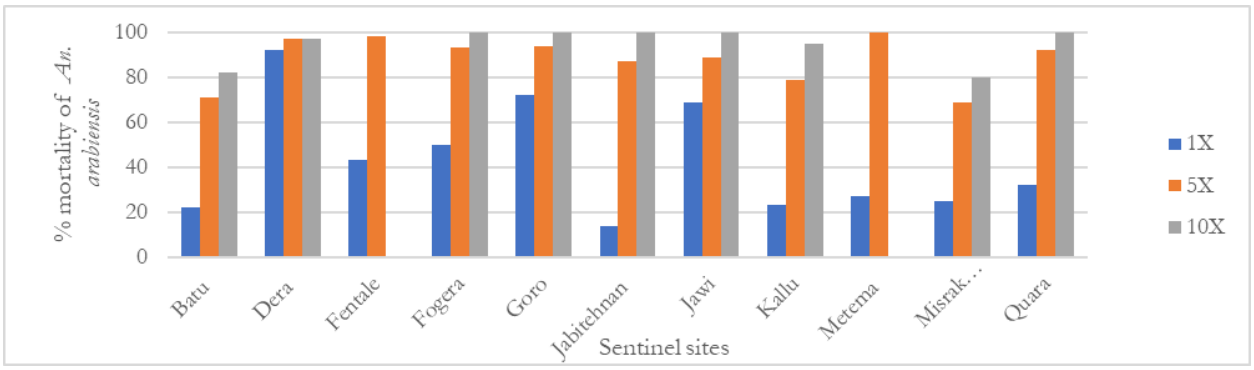
The intensity of resistance in *An. arabiensis* populations to all three pyrethroids (alpha-cypermethrin, deltamethrin, and permethrin) was high in Batu and Misrak Badawacho.

**FIGURE 25. MORTALITY OF *AN. ARABIENSIS* FROM RESISTANCE INTENSITY TESTS (2021)**

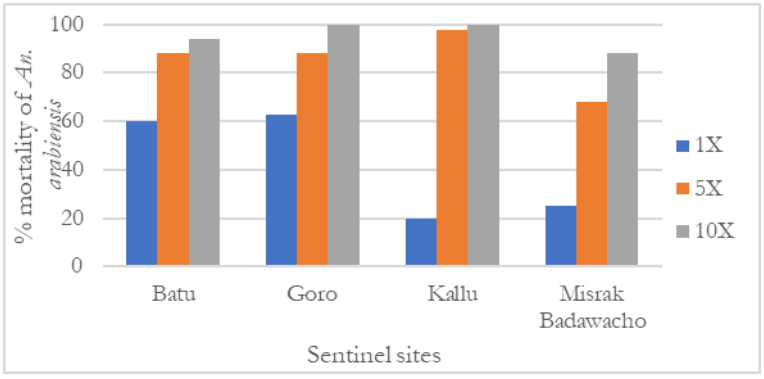
**A) ALPHA-CYPERMETHRIN**




**B) DELTAMETHRIN**




**C) PERMETHRIN**



### 3.6.3 RESULTS OF *AN. ARABIENSIS* FROM PBO SYNERGIST ASSAYS

PBO synergist assays on populations of *An. arabiensis* were conducted for alpha-cypermethrin, deltamethrin, and permethrin in nine districts: Abobo, Amibara, Batu, Fentale, Goro, Jabitehnan, Kalu, Misrak Badawacho, and Omonada. Only alpha-cypermethrin and deltamethrin were tested in Erer. Only deltamethrin was tested in Dera, Fogera, Jawi, Metema, and Quara (Figure 26 A, B, C).

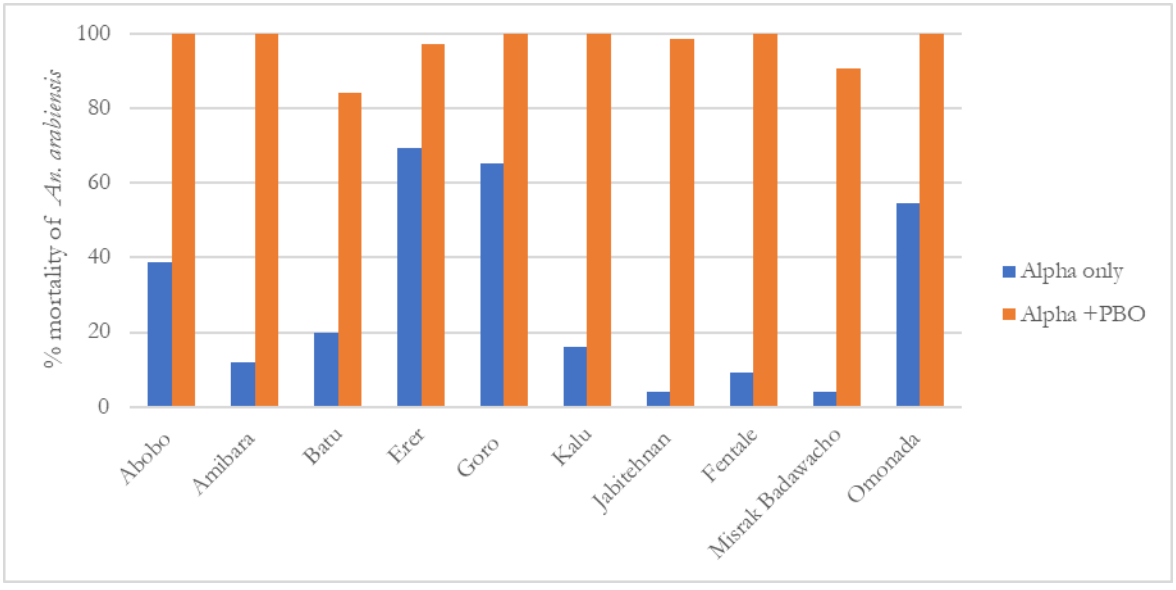
Pre-PBO exposure followed by alpha-cypermethrin caused 99-100% mortality of *An. arabiensis* in Amibara, Batu, Fentale, Goro, Kalu, and Omonada, indicating the role of cytochrome P450 monooxygenase enzymes in resistance to alpha-cypermethrin. Partial susceptibility was restored in Batu (84.0% mortality), Erer (97.3%), and Misrak Badawacho (90.7%), indicating that cytochrome P450 monooxygenase enzymes are not the only mechanisms of resistance in the areas (Figure 26 A).

PBO restored full susceptibility of *An. arabiensis* to deltamethrin with 100% mortality in 10 districts: Amibara, Dera, Fentale, Fogera, Jabitehnan, Jawi, Kalu, Metema, Omonada, and Quara. Partial susceptibility was restored in Abobo (97.3%), Batu (96.0%), Erer (94.7%), Goro (94.7%), and Misrak Badawacho (86.7%)(Figure 26 B).

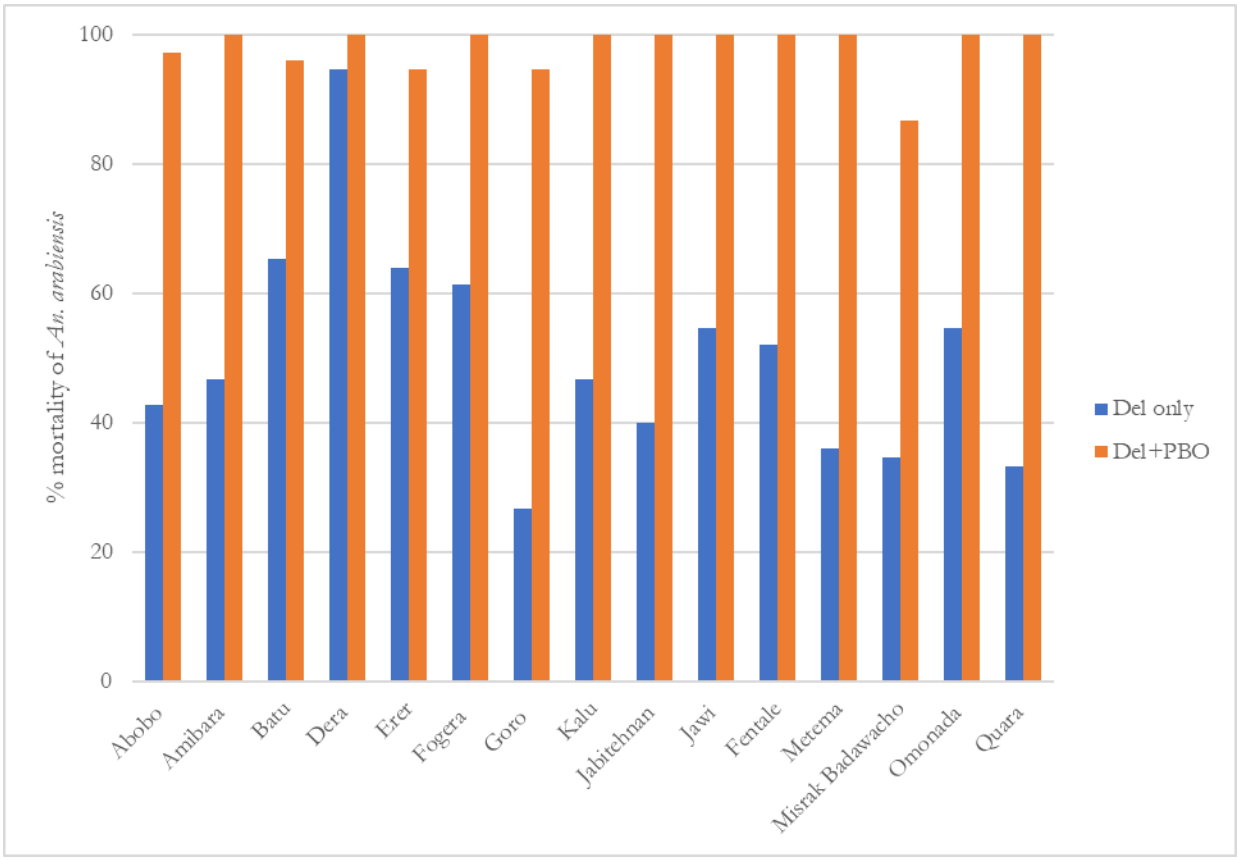
PBO resorted full susceptibility of *An. arabiensis* to permethrin in Abobo, Amibara, Batu, Fentale, Goro, Jabitehnan, Kalu, and Omonada. It restored partial susceptibility in Misrak Badawacho (65.3% mortality) (Figure 26 C).

**FIGURE 26. MORTALITY OF *AN. ARABIENSIS* FOLLOWING PBO SYNERGIST ASSAYS COMBINED WITH A) ALPHACYPERMETHRIN, B) DELTAMETHRIN, AND C) PERMETHRIN (2021)**

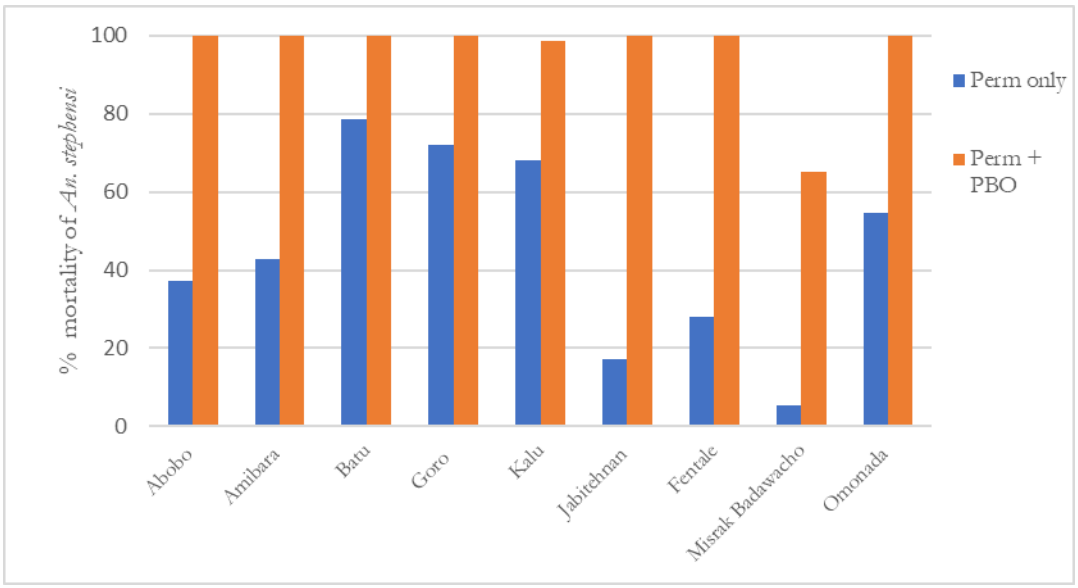
**A) ALPHACYPERMETHRIN**



## B) DELTAMETHRIN




## C) PERMETHRIN

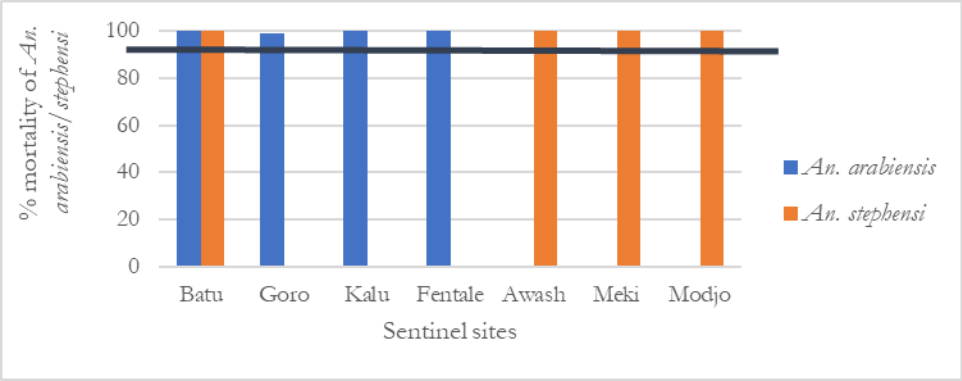



**Key:** Alpha=alpha-cypermethrin. Del=deltamethrin. PBO=piperonyl butoxide. Perm=permethrin.

### 3.6.4 *Anopheles arabiensis* and *An. stephensi* Susceptibility to Clothianidin

Populations of *An. arabiensis* were susceptible (99%-100% mortality) to clothianidin in Batu, Goro, Kalu, and Fentale. With mortality of 100%, *An. stephensi* also exhibited susceptibility to clothianidin in Batu, Awash, and Meki and Modjo Towns (Figure 27).

**Figure 27. Mortality of *An. arabiensis* and *An. stephensi* Tested against Clothianidin (2021)**

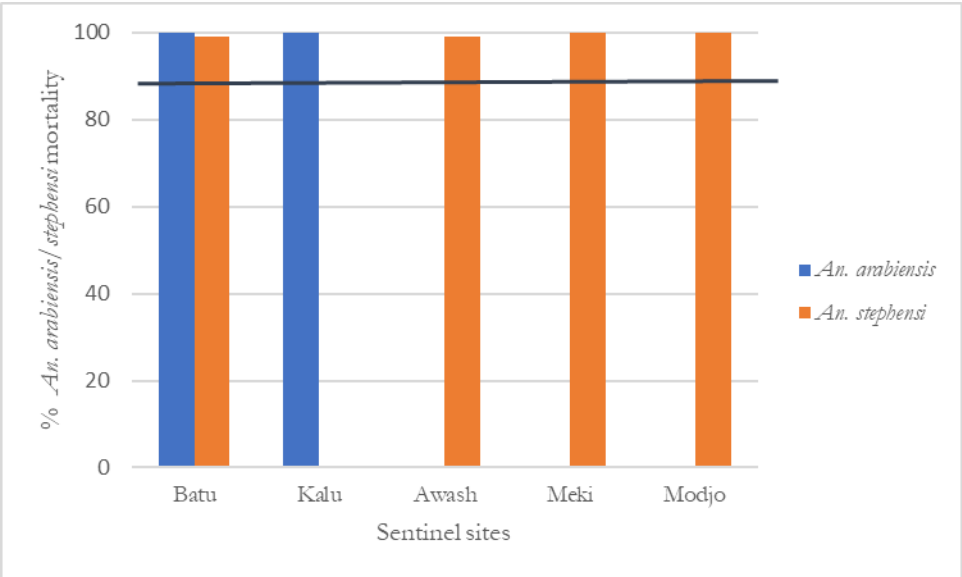



**Note:** Line indicates 90% mortality threshold for resistance.

### 3.6.5 *Anopheles arabiensis* and *An. stephensi* Susceptibility to Chlorfenapyr

Populations of *An. arabiensis* from Batu and Kalu and *An. stephensi* from Batu, Awash, and Meki and Modjo Towns were susceptible to chlorfenapyr, with mortality of 99%-100% after a 72-hour holding period (Figure 28).

**Figure 28. Mortality of *An. arabiensis* and *An. stephensi* Tested against Chlorfenapyr (2021)**



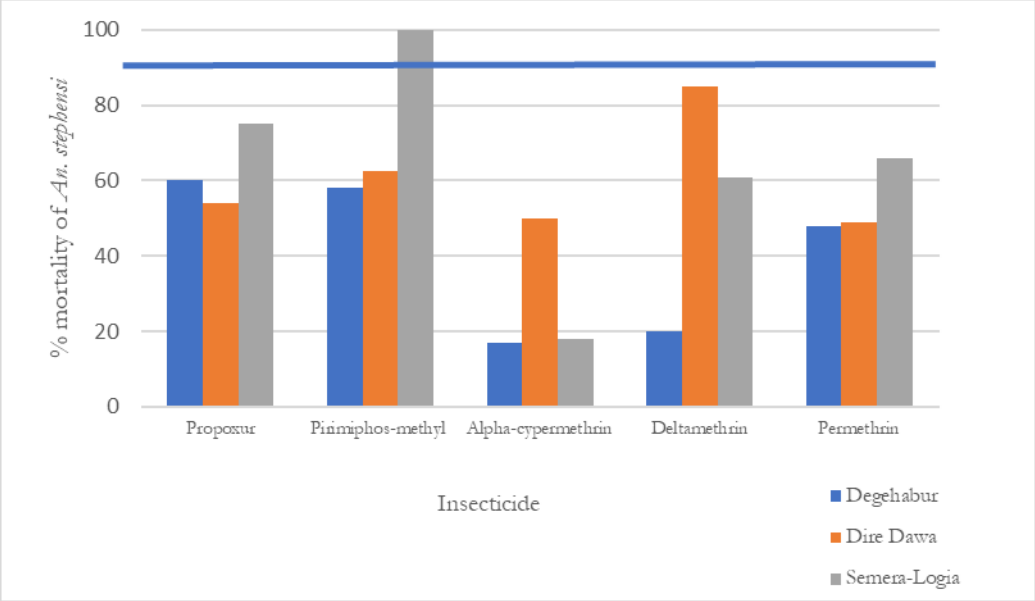

**Note:** Line indicates 90% mortality threshold for resistance.

### 3.6.6 *ANOPHELES STEPHENSI* INSECTICIDE SUSCEPTIBILITY, RESISTANCE INTENSITY, AND PBO ASSAYS

#### Susceptibility of *An. stephensi* to Insecticides

With mortality less than 90% in WHO tube tests, populations of *An. stephensi* from Degehabur, Dire Dawa, and Semara-Logia Towns were found resistant to propoxur, pirimiphos-methyl (except in Semera-Logia), alpha-cypermethrin, deltamethrin, and permethrin (Figure 29). The result of pirimiphos-methyl from Semera-Logia was consistent with that of 2020, and it is not yet clear why there is a difference in susceptibility to this insecticide among the populations of *An. stephensi* in this area.

**FIGURE 29. INSECTICIDE SUSCEPTIBILITY STATUS OF *ANOPHELES STEPHENSI* (2021)**

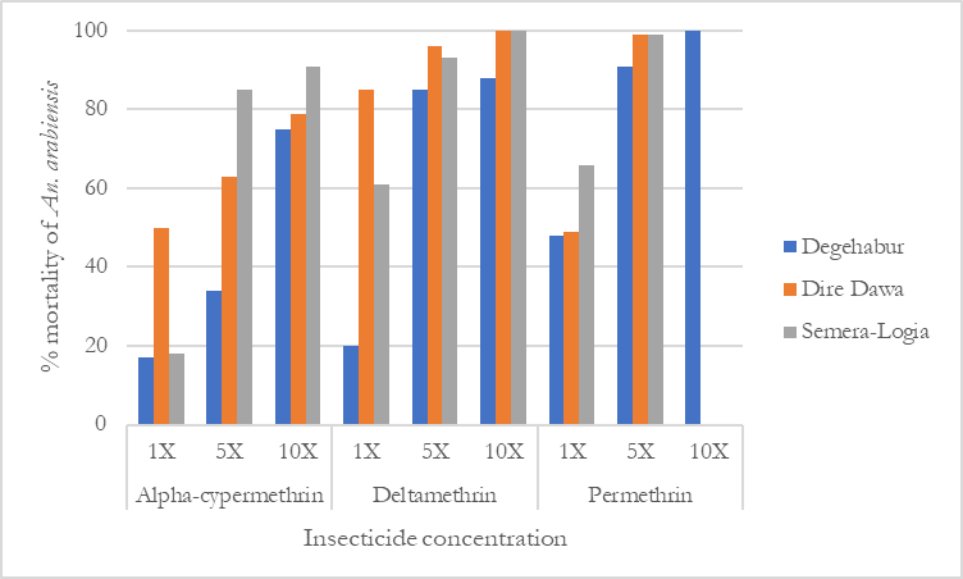



**Note:** Line indicates 90% threshold for resistance.

#### Resistance Intensity Assays on *An. stephensi*

Populations of *An. stephensi* exhibited high resistance intensity to alpha-cypermethrin in Degehabur, Dire Dawa, and Semera-Logia Towns at 75%-91% mortality at 10X and to deltamethrin in Degehabur, with 88% mortality at 10X. *An. stephensi* exhibited moderate resistance to deltamethrin in the population from Dire Dawa and Semera-Logia, with 100% mortality at 10X. Intensity of resistance was moderate to permethrin in populations of *An. stephensi* from Degehabur, with 100% mortality at 10X. However, the intensity of resistance to permethrin was low, with 99% mortality at 5X in Dire Dawa and Semera-Logia (Figure 30).

**FIGURE 30. MORTALITY OF *ANOPHELES STEPHENSI* FROM RESISTANCE INTENSITY ASSAYS (2021)**

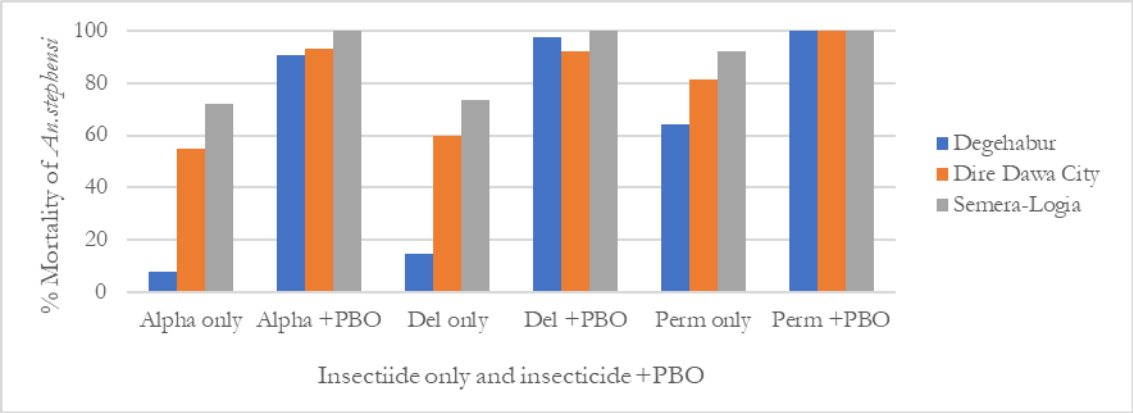



## PBO Synergist Assays on *An. stephensi*

Pre-PBO exposure followed by alpha-cypermethrin or deltamethrin caused partial restoration of susceptibility in Degehabur Town (90.7 and 97.3% mortality, respectively) and in Dire Dawa Town (93.3% and 92.0%, respectively). Susceptibility to the two insecticides was fully restored in Semera-Logia Town, where mortality was 100% (Figure 31).

PBO restored full susceptibility of *An. stephensi* to permethrin in all the three sites (Figure 30).

**FIGURE 31. MORTALITY OF *AN. STEPHENSI* FROM PBO SYNERGIST ASSAYS (2021)**




**Key:** Alpha=alpha-cypermethrin. Del=deltamethrin. PBO=piperonyl butoxide. Perm=permethrin.

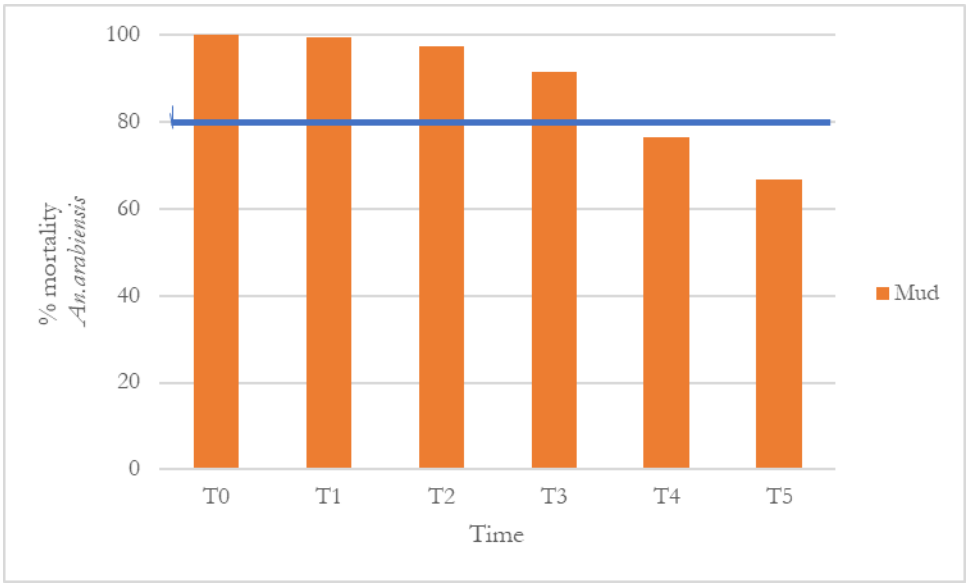
## 3.7 ENTOMOLOGICAL ASSESSMENT OF QUALITY OF SPRAYING AND DECAY RATE OF ACTELLIC 300CS, SUMISHIELD, AND FLUDORA FUSION

The spray quality assessment and residual efficacy of Actellic 300CS, SumiShield, and Fludora Fusion, which were evaluated through wall cone and fumigation bioassays, is discussed in this section.

### 3.7.1 CONE BIOASSAY TESTS: ACTELLIC 300CS IN LARE DISTRICT

The percentage mortality of *An. arabiensis* for Actellic 300CS–sprayed houses in Lare was between 91.4% and 100% from T0 to T3. Mortality was below 80% at T4 and T5, indicating three months of residual efficacy of Actellic 300CS in Lare and the need to shift to another insecticide product (Figure 32).

**FIGURE 32. MORTALITY OF *AN. ARABIENSIS* IN CONE BIOASSAYS IN LARE DISTRICT (2021)**



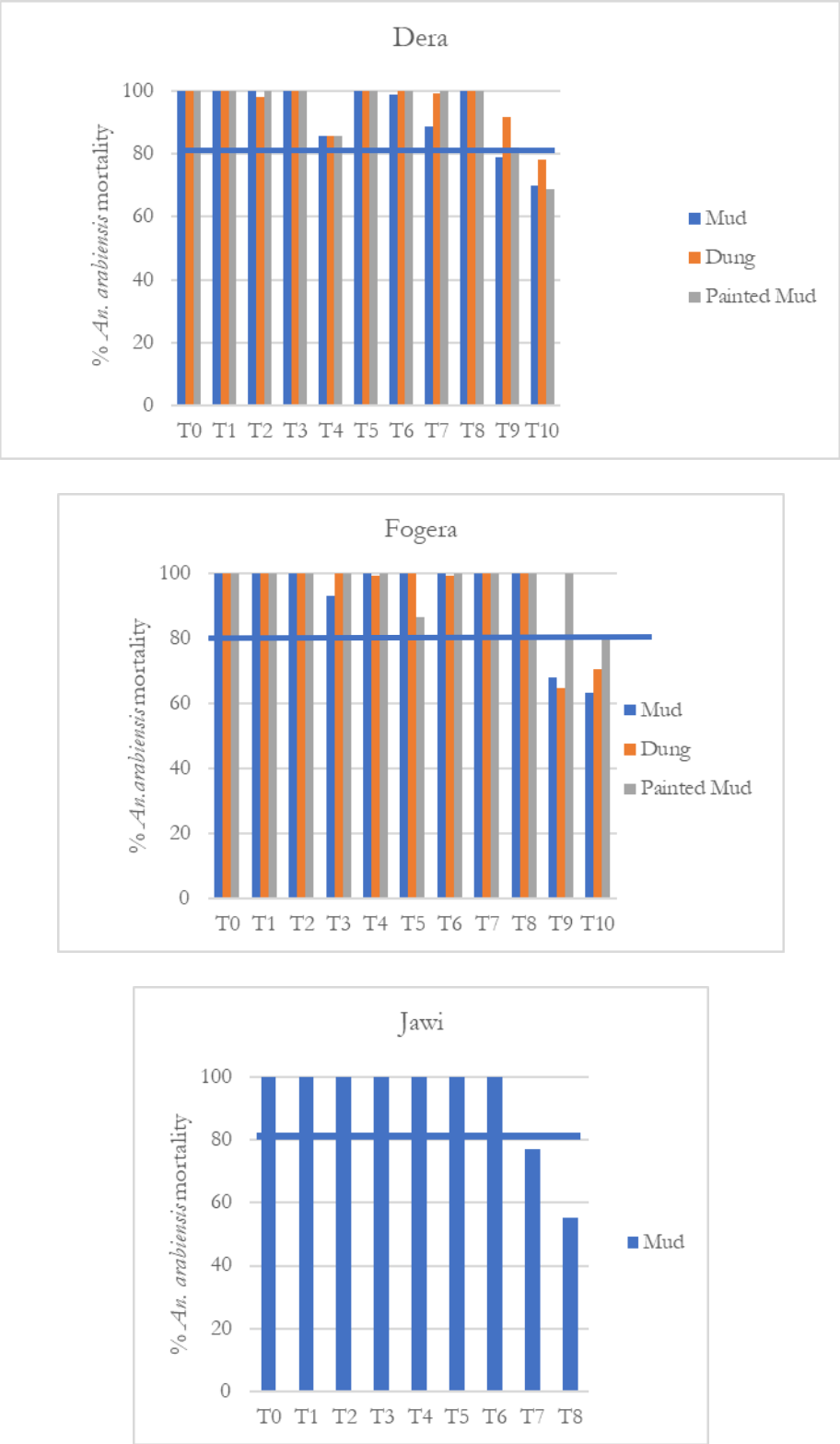

**Note:** Line indicates the WHO 80% cut-off value of mortality.

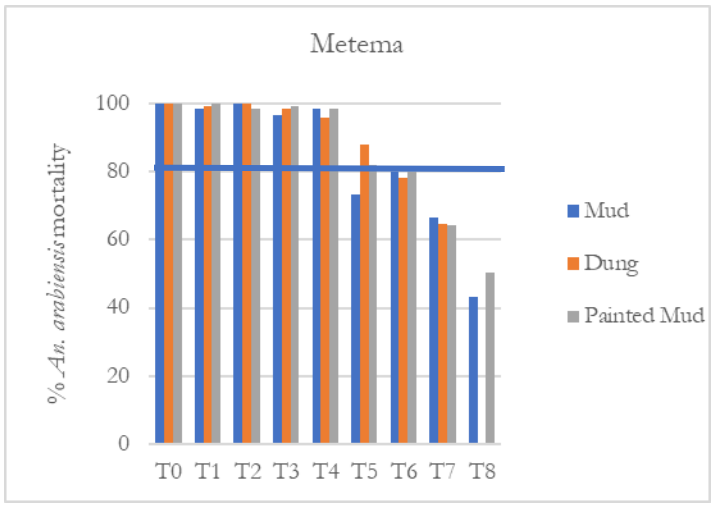
### 3.7.2 CONE BIOASSAY TESTS: ACTELLIC 300CS IN DERA, FOGERA, JAWI, METEMA, AND QUARA DISTRICTS

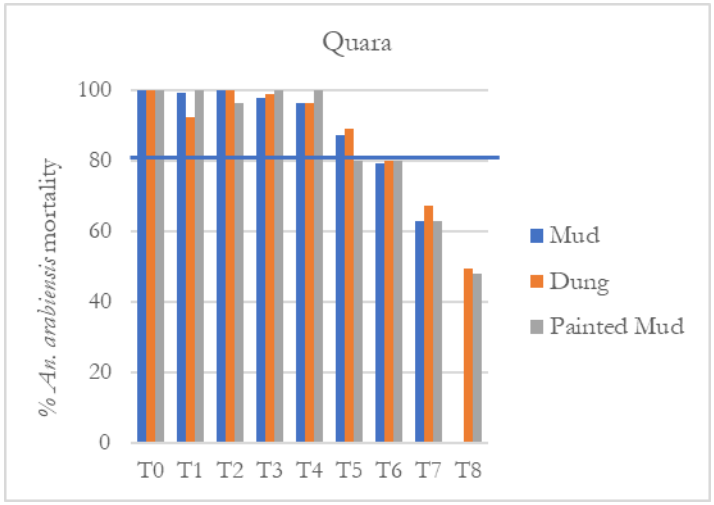
Contrary to Lare, the residual efficacy of Actellic 300CS in the five districts of Amhara Region showed long residual effects. Actellic 300CS lasted for six months in Jawi, Metema, and Quara, killing more than 80% of test mosquitoes (Figure 33). In Dera, Actellic 300CS remained efficacious in all houses for nine months, except mud houses, where mortality was 78.9% at T9. Mortality at T10 declined below the WHO 80% mortality threshold (Figure 33).

Actellic 300CS in Fogera persisted for eight months in all mud, dung, and painted mud houses, killing 100% of *An. arabiensis*. The residual efficacy in painted mud houses was up to 10 months (80% mortality at T10), whereas mortality was below 80% in houses with mud and dung wall surfaces.

**FIGURE 33. *ANOPHELES ARABIENSIS* MORTALITY IN CONE BIOASSAYS IN DERA, FOGERA, JAWI, METEMA, AND QUARA DISTRICTS**





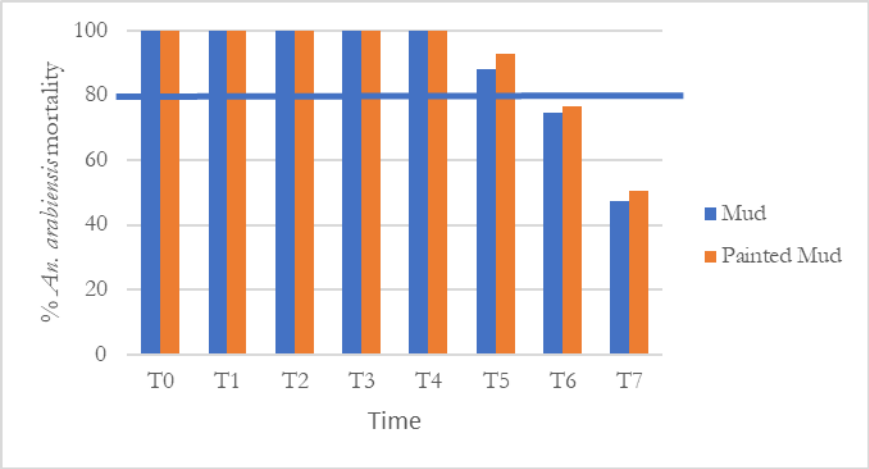




**Note:** Line indicates the WHO 80% cut-off value of mortality.

### 3.7.3 CONE BIOASSAY TESTS: SUMISHIELD IN MENGE TOWN

SumiShield in Menge persisted for five months, killing more than 80% of *An. arabiensis*. Mortality was 100% for mud and painted mud surfaces from T0 to T4, but at T5 it declined to 88.2% in mud houses and 92.9% in painted mud houses. Mortality was lower than 80% at T6 and T7 (Figure 34).

**FIGURE 34. RESIDUAL EFFICACY OF SUMISHIELD FROM CONE BIOASSAY TESTS OF INSECTARY-RAISED *AN. ARABIENSIS* IN MENGE TOWN (2021)**

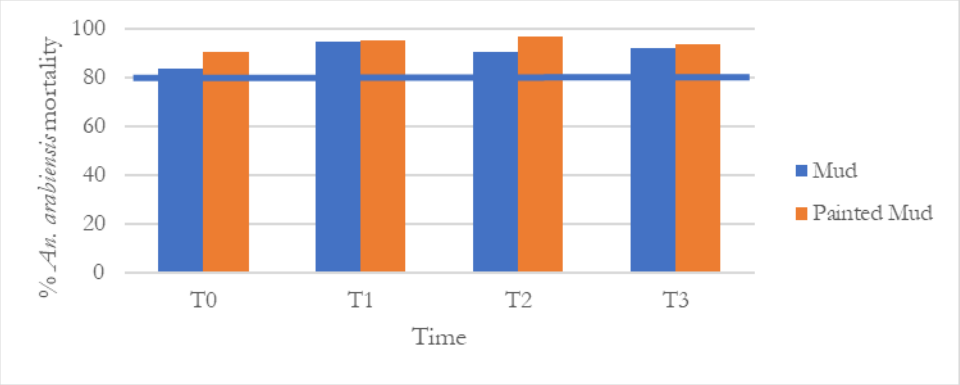



**Note:** Line indicates the WHO 80% cut-off value of mortality.

### 3.7.4 CONE BIOASSAY TESTS: SUMISHIELD IN ABE DONGORO DISTRICT

In Abe Dongoro, mortality of *An. arabiensis* from SumiShield-sprayed houses increased from T0 to T1, T2, and T3, possibly as the bioavailability of the insecticide increased. Mortality for mud houses was 83.9%, 94.7%, 90.4%, and 92.3% at T0, T1, T2, and T3, respectively. Mortality for painted mud houses over the same period was 90.7%, 95.2%, 96.7%, and 93.8%, respectively (Figure 35).

**FIGURE 35. RESIDUAL EFFICACY OF SUMISHIELD FROM CONE BIOASSAY TESTS OF INSECTARY-RAISED *AN. ARABIENSIS* IN ABE DONGORO DISTRICT (2021)**

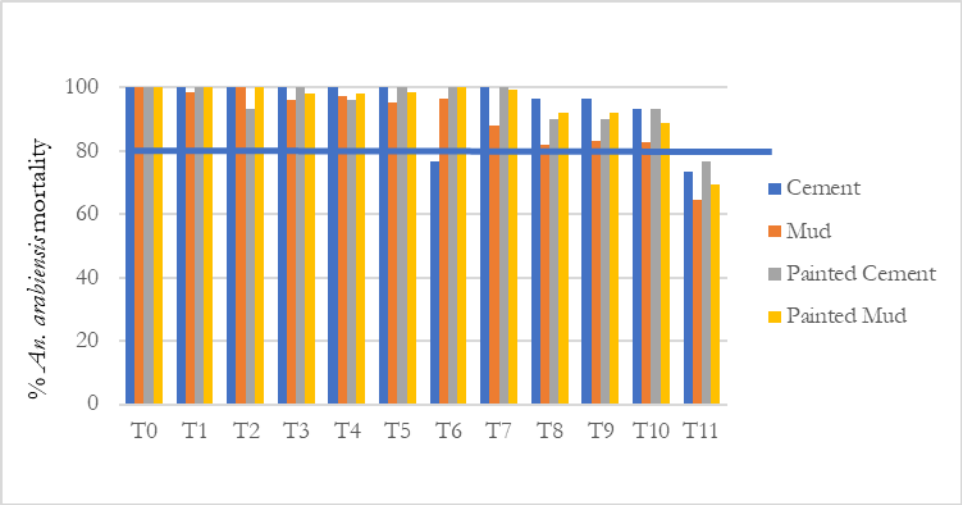



**Note:** Line indicates the WHO 80% cut-off value of mortality. Tests stopped at T3 due to security problem in the area.

### 3.7.5 CONE BIOASSAY TESTS: FLUDORA FUSION IN ABAYA DISTRICT

The residual efficacy of Fludora Fusion in Abaya was 10 months, killing 80%-100% insectary-reared *An. arabiensis* mosquitoes. However, mortality was only 76.7% at T6 for cement houses, but it increased to 100% at T7 and remained efficacious in the remaining months. Mortality of *An. arabiensis* went below 80% in all houses at T11 (Figure 36).

**FIGURE 36. RESIDUAL EFFICACY OF FLUDORA FUSION FROM CONE BIOASSAY TESTS ON INSECTARY-RAISED *AN. ARABIENSIS* IN ABAYA DISTRICT (2020-2021)**

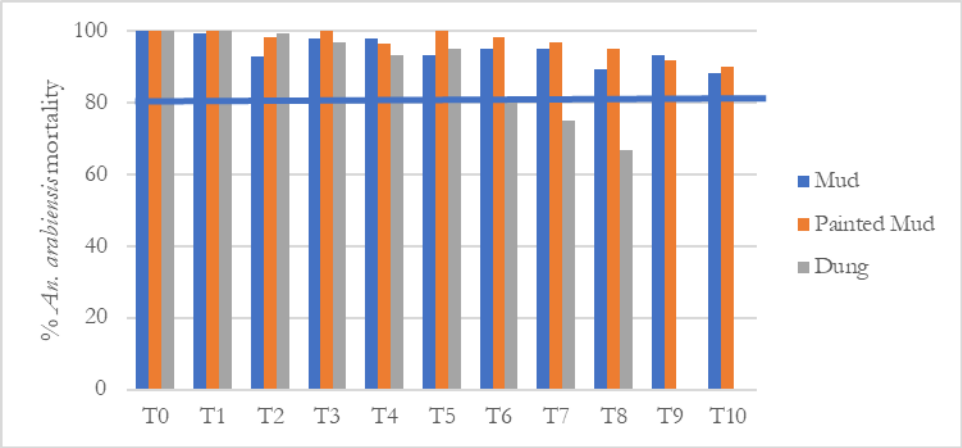



**Note:** Line indicates the WHO 80% cut-off value of mortality.

### 3.7.6 CONE BIOASSAY TESTS: FLUDORA FUSION IN GELANA DISTRICT

Fludora Fusion persisted for more than 10 months in Gelana, except for dung wall houses, in which *An. arabiensis* mortality was below 80% at T7. Mortality of *An. arabiensis* for mud and painted mud houses was more than 90% from T0 to T10 (Figure 37).

**FIGURE 37. MORTALITY OF *AN. ARABIENSIS* IN CONE BIOASSAYS CONDUCTED IN GELANA DISTRICT (2021-2022)**

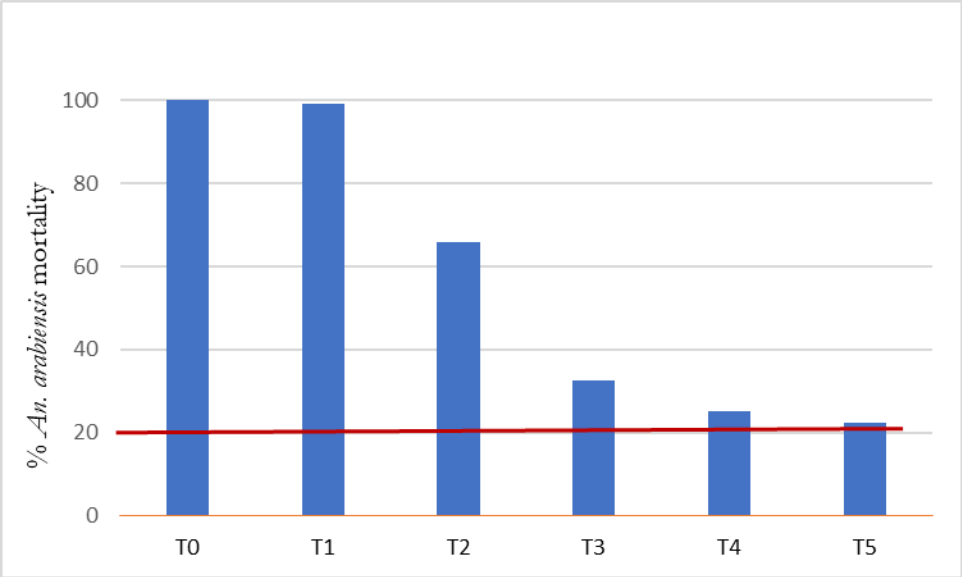



**Note:** Line indicates the WHO 80% cut-off value of mortality.

### 3.7.7 FUMIGATION BIOASSAYS: ACTELLIC 300CS IN LARE DISTRICT

Mortality of *An. arabiensis* from fumigation bioassay tests in Actellic 300CS–sprayed mud wall houses was 100%, 100%, 99.2%, 65.8%, 25.2%, and 22.2 at T0, T1, T2, T3, T4, and T5, respectively (Figure 38).

**FIGURE 38. RESIDUAL EFFICACY OF ACTELLIC 300CS FROM FUMIGATION BIOASSAYS TESTED ON INSECTARY-RAISED *AN. ARABIENSIS* IN LARE DISTRICT (2020-2021)**

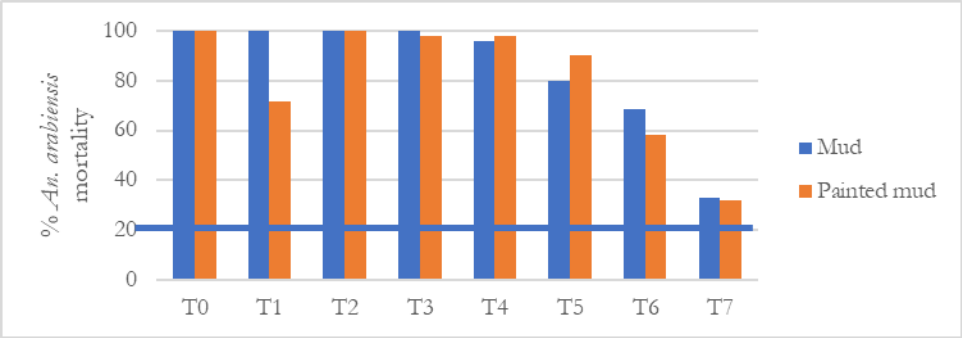



**Note:** Line indicates 20% cut-off value of mortality.

### 3.7.8 FUMIGATION BIOASSAYS: SUMISHIELD IN MENGE TOWN

The fumigant killing effect of SumiShield–sprayed houses was highly pronounced in Menge from T0 to T7 on both mud and painted mud wall houses. (Figure 39).

**FIGURE 39. RESIDUAL EFFICACY OF SUMISHIELD FROM FUMIGATION BIOASSAYS TESTED IN MENGE TOWN (2021-2022)**



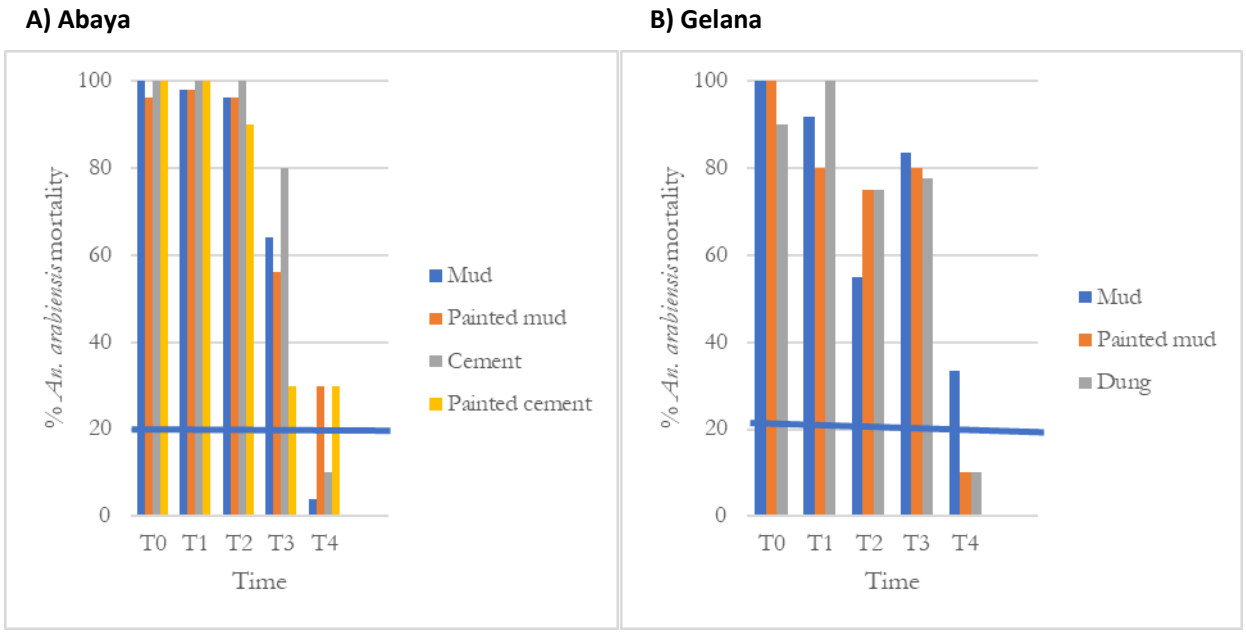

**Note:** Line indicates 20% cut-off value of mortality.

### 3.7.9 FUMIGATION BIOASSAYS: FLUDORA FUSION IN ABAYA AND GELANA DISTRICTS

In Abaya, mortality was 90%-100% of *An. arabiensis* for Fludora Fusion–sprayed mud, painted mud, cement, and painted cement houses from T0 to T2; mortality at T3 was 64%, 56%, 80%, and 30% for the respective wall surfaces. At T4, mortality declined below 20% in mud and cement wall houses; mortality was 30% for painted mud and painted cement wall houses (Figure 40A).

In Gelana, mortality for mud wall houses was 100%, 91.7%, 55%, 83.3%, and 33.3% of *An. arabiensis* at T0, T1, T2, T3 and T4, respectively. Mortality during the same time was 100%, 80.0%, 75.0%, 80%, and 10% for painted mud wall houses; mortality for dung walls was 90.0%, 100%, 75.0%, and 10.0% during the same period (Figure 40B).

**FIGURE 40. RESIDUAL EFFICACY OF FLUDORA FUSION FROM FUMIGATION BIOASSAYS TESTED ON INSECTARY-RAISED *AN. ARABIENSIS* IN (A) ABAYA AND (B) GELANA DISTRICTS (2021-2022)**




**Note:** Line indicates 20% cut-off value of mortality.

# 4. ENTOMOLOGICAL CAPACITY BUILDING

In 2021, the PMI VectorLink Ethiopia Project provided entomology training, particularly emphasizing *An. stephensi,* to 46 staff of National Malaria Elimination Program, Ethiopian Public Health Institute, Regional Health Bureaus, Regional Public Health Institutes, District Health Bureaus, and 16 Universities (Table 12). Three women, one each from Wachamo University, Ethiopian Public Health Institute, and Adama Public Health Institute, have attended the training. Two rounds of training took place in Adama Town in March and April 2021. A certificate of participation was awarded to the trainees.

Of those trained, eight (two from Oromia Health Bureau, one from Amhara Public Health Institute, and five university staff) have involved in entomological monitoring and residual efficacy assessment supported by VectorLink. The project supported three of those to establish insectaries in Dilla University, Wollega University and University of Gondar. In addition, a staff person from Adama Public Health Institute leads a team that rears insectary mosquitoes for research and training and supplies mosquitoes for various institutions and VectorLink.

**TABLE 12. LIST OF INSTITUTION AND NUMBER OF ENTOMOLOGY TRAINEES (MARCH AND APRIL 2021)**

| Institution | Number of trainees |
|---|---|
| National Malaria Elimination Program | 3 |
| Ethiopian Public Health Institute | 3 |
| Afar Public Health Institute | 1 |
| Afar Regional Health Bureau | 1 |
| Adama Public Health Institute | 2 |
| Amhara Public Health Institute | 3 |
| Three district health bureaus from Amhara | 3 |
| Benishangul-Gumuz Regional Health Bureau | 1 |
| Oromia Regional Health Bureau | 2 |
| Sidama Regional Health Bureau | 1 |
| Southern Nations, Nationalities, and Peoples' Regional Health Bureau | 2 |
| Southern Nations, Nationalities, and Peoples' Public Health Institute | 1 |
| Somali Regional Health Bureau | 1 |
| Tigray Health Research Institute | 3 |
| Universities (Ambo, Arba Minch, Assosa, Bahirdar, Debrebirhan, Dilla, Gondar, Hawassa, Jigjiga, Maddaa Wollabo, Mekelle, Mettu, Wachamo, Wolaita, Wollega, Wollo) | 19 |
| **Total** | **46** |

The project has trained 60 community mosquito collectors (CMCs) and 11 supervisors for the evaluation of PBO nets compared with IRS and standard ITNs that is ongoing in Amhara Region. Of those who took the training, 52 CMCs and one supervisor were women.

The PMI VectorLink Ethiopia Project contributed to a "train the trainer" training ("Malaria Foci Investigation and Response Standard Operating Procedures") organized by NMEP and held in four rounds in Adama Town in 2021: March 1-7, March 8-14, March 22-26, and March 29-April 2. A total of 226 staff from the malaria elimination–designated districts and regional health bureaus participated. A VectorLink staff person was engaged as a trainer; the project also provided training materials.

The project has supported Dilla University and Wollega University to train two insectary keepers each in Jimma University and Addis Ababa University, respectively.

The following supports were also provided through the project:

- Donations of office furniture, heaters, larval trays, and cages to the Adama insectary.
- Insectary support has been given to the universities of Addis Ababa, Debre Markos, Dilla, Jimma, Gondar, and Wollega.
- Various laboratory items have been donated to the Armauer Hansen Research Institute, notably a complete set of real-time PCR machines, microscopes, entomology monitoring and insectary materials, reagents, and supplies.
- Two E-imagers, laboratory furniture, microscopes, and entomology monitoring materials have been given to Amhara Public Health Institute.
- Jimma and ArbaMinch universities have received laboratory reagents, supplies and primers.

The PMI VectorLink Ethiopia Project has contributed to the preparation of an action plan document for the entomological surveillance and control of *An. stephensi*. The project played a key role in the estimation of a budget for five years.

# 5. CONCLUSIONS

The species composition of *Anopheles* revealed the presence of at least 17 species in the seven sentinel sites in Abaya District (Oromia Region), Bambasi District (Benishangul-Gumuz Region), Bennatsemay and Salamago Districts (Southern Nations, Nationalities, and Peoples' Regional States), Jabitehnan and Metema Districts (Amhara Region), and Lare District (Gambela Region). *Anopheles arabiensis* was predominantly found in Abaya, Bennatsemay, Jabitehnan, Metema and Salamago sites; *An. funestus* group in Lare; and *An. coustani* in. Bambasi.

The conventional Enzyme-linked Immunosorbent Assay detected sporozoite infections in 37 specimens of *An. arabiensis*, *An. coustani*, *An. parensis,* and *An. ziemanni*; however, only four turned positive after boiling and retesting. Two detected were *Plasmodium falciparum* from *An. arabiensis;* the other two were *P. vivax* 210 from *An. coustani*. The negatives showed false positivity. The finding of confirmed sporozoite infections from *An. coustani* implies this species might play a secondary role in the transmission of malaria in Ethiopia, as in other parts of Africa (Afrane et al., 2016; Goupeyou-Youmsi et al., 2020). Future work should look at the bloodmeal sources of the samples with false positives since that may explain why metabolites look positive

*Anopheles arabiensis* was more abundant in the unsprayed district, Jabitehnan, as compared to the rest of the sentinel sites. A drop in the density of this species was observed in Salamago after propoxur spraying by the National Malaria Elimination Program. The density was low in the PMI VectorLink Project sites, particularly in Gelana District, where density went down by more than threefold from the previous year (N=4,912 versus 1,371) after shifting from Actellic 300CS to Fludora Fusion. The latter product persisted more than nine months in mud and painted mud wall houses in Gelana as well as in Abaya District. Unlike in the past, as well as in 2021 in Lare, the residual efficacy of Actellic 300CS extended from six to nine months in Amhara.

Considering the susceptibility status of populations of *An. arabiensis* to pirimiphos-methyl, together with the long residual life of Actellic 300CS, it is recommended to continue spaying Actellic 300CS in Amhara. On the other hand, SumiShield persisted for five months in Menge, killing more than 80% of test mosquitoes. In view of the short season of *An. arabiensis* in Benishangul-Gumuz Region, SumiShield spraying can be recommended there. Populations of *An. arabiensis* are susceptible to pirimiphos-methyl, clothianidin, and chlorfenapyr. The fact that the species is susceptible to chlorfenapyr implies that NMEP could consider implementation of Interceptor G2 for impactful vector control

*Anopheles stephensi* has been detected in western Ethiopia, at the Sudan border town of Metema Yohannes, which is a transport corridor between the two countries. The species was not found in the dry port of Woreta, but repeated visits are required during the rainy and dry months to detect its presence. *Anopheles stephensi* is now known from a total of 45 sites (22 urban and 23 semiurban/rural) in Ethiopia. *Anopheles stephensi* is susceptible to clothianidin and chlorfenapyr insecticides, and the use of Interceptor G2 along with larval source management can be considered as a viable option of vector control where this species found in Ethiopia.

# 6. REFERENCES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# Annex A. Monthly Collections of *Anopheles* and Culicines from Sentinel Sites (April 2021-March 2022)

| Site | Period | *An. funestus s.l.* | | | *An. gambiae s.l.* | | | *An. coustani* | | | *An. demeilloni* | | | *An. other* | | | *An. pharoensis* | | | *An. pretoriensis* | | | *An. squamosus* | | | *An. tenebrosus* | | | *An. ziemanni* | | | *Culicines* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK |
| Abaya | April 2021 (Pre IRS) | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 137 | 311 | 21 |
| | May (Post IRS) | 0 | 0 | 0 | 5 | 28 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 234 | 995 | 123 |
| | June (Post IRS) | 0 | 0 | 0 | 0 | 13 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 167 | 826 | 162 |
| | July (Post IRS) | 0 | 0 | 0 | 0 | 11 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 239 | 1520 | 166 |
| | Aug (Post IRS) | 0 | 0 | 0 | 0 | 11 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 179 | 2142 | 130 |
| | Sept (Post IRS) | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 71 | 670 | 105 |
| | Oct (Post IRS) | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 45 | 618 | 13 |
| | Nov (Post IRS) | 0 | 0 | 0 | 0 | 39 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 10 | 638 | 11 |
| | Dec (Post IRS) | 0 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 16 | 326 | 35 |
| | Jan 2022 (Post IRS) | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 182 | 7 |
| | Feb (Post IRS) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | 187 | 20 |
| | Mar (Post IRS) | 0 | 0 | 0 | 1 | 13 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 8 | 50 | 63 |
| | Sub Total | 0 | 0 | 0 | 6 | 169 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 | 0 | 1128 | 8465 | 856 |

| Site | Period | *An. funestus s.l.* | | | *An. gambiae s.l.* | | | *An. coustani* | | | *An. demeilloni* | | | *An. other* | | | *An. pharoensis* | | | *An. pretoriensis* | | | *An. squamosus* | | | *An. tenebrosus* | | | *An. ziemanni* | | | *Culicines* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK |
| Bambasi | April 2021 (Pre IRS) | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| | May (Post IRS) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 27 | 6 |
| | June (Post IRS) | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 21 | 155 | 51 |
| | July (Post IRS) | 0 | 8 | 0 | 0 | 27 | 2 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 59 | 509 | 36 |
| | Aug (Post IRS) | 0 | 14 | 0 | 6 | 106 | 4 | 2 | 279 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 91 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 119 | 2424 | 143 |
| | Sept (Post IRS) | 1 | 33 | 0 | 4 | 45 | 0 | 3 | 427 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 105 | 1 | 0 | 0 | 0 | 1 | 98 | 0 | 162 | 2801 | 150 |
| | Oct (Post IRS) | 1 | 49 | 0 | 0 | 40 | 0 | 11 | 388 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 114 | 2 | 0 | 0 | 0 | 0 | 105 | 3 | 56 | 911 | 95 |
| | Nov (Post IRS) | 0 | 83 | 0 | 0 | 32 | 1 | 8 | 237 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 0 | 0 | 0 | 0 | 4 | 202 | 0 | 74 | 838 | 84 |
| | Dec (Post IRS) | 5 | 86 | 3 | 0 | 4 | 0 | 0 | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 35 | 306 | 45 |
| | Jan 2022 (Post IRS) | 2 | 11 | 0 | 2 | 3 | 1 | 0 | 26 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 25 | 97 | 24 |
| | Feb (Post IRS) | 0 | 0 | 0 | 1 | 7 | 0 | 0 | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 17 | 149 | 9 |
| | Mar (Post IRS) | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 22 | 148 | 17 |
| | Sub Total | 9 | 285 | 3 | 13 | 268 | 8 | 24 | 1432 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 414 | 3 | 0 | 0 | 0 | 5 | 477 | 3 | 595 | 8365 | 660 |
| Bennatsemay | April 2021 (Pre IRS) | 0 | 0 | | 52 | 7 | | 0 | 1 | | 0 | 1 | | 0 | 0 | | 0 | 1 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 5 | 9 | |
| | May (Post IRS) | 0 | 0 | 0 | 20 | 17 | 16 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 94 | 0 |
| | June (Post IRS) | 0 | 0 | 0 | 15 | 45 | 10 | 0 | 9 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 36 | 76 | 6 |
| | July (Post IRS) | 0 | 0 | 0 | 9 | 21 | 1 | 0 | 102 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 53 | 0 | 0 | 0 | 0 | 28 | 621 | 2 |
| | Aug (Post IRS) | 0 | 1 | 0 | 35 | 34 | 2 | 0 | 47 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 43 | 123 | 7 |
| | Sept (Post IRS) | 0 | 1 | 0 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 16 | 174 | 0 |
| | Oct (Post IRS) | 0 | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 36 | 295 | 0 |

| Site | Period | *An. funestus s.l.* | | | *An. gambiae s.l.* | | | *An. coustani* | | | *An. demeilloni* | | | *An. other* | | | *An. pharoensis* | | | *An. pretoriensis* | | | *An. squamosus* | | | *An. tenebrosus* | | | *An. ziemanni* | | | *Culicines* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK |
| | Nov (Post IRS) | 0 | 0 | 0 | 11 | 7 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 14 | 95 | 1 |
| | Dec (Post IRS) | 0 | 0 | 0 | 3 | 4 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 20 | 103 | 6 |
| | Jan 2022 (Post IRS) | 0 | 13 | 0 | 7 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 4 | 61 | 0 |
| | Feb (Post IRS) | 0 | 0 | 0 | 4 | 7 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 89 | 5 |
| | Mar (Post IRS) | 0 | 2 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 18 | 0 |
| | Sub Total | 0 | 17 | 0 | 162 | 149 | 35 | 0 | 168 | 0 | 0 | 3 | 0 | 0 | 1 | 0 | 4 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 0 | 0 | 0 | 0 | 258 | 1758 | 27 |
| Jabitehnan | April 2021 (Pre IRS) | 0 | 0 | | 4 | 197 | | 0 | 2 | | 0 | 3 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 0 | 0 | | 51 | 233 | 0 |
| | May (Post IRS) | 0 | 0 | 0 | 5 | 76 | 13 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 183 | 35 |
| | June (Post IRS) | 0 | 0 | 0 | 5 | 61 | 12 | 0 | 8 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 44 | 261 | 69 |
| | July (Post IRS) | 0 | 0 | 0 | 5 | 76 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 57 | 356 | 91 |
| | Aug (Post IRS) | 0 | 0 | 0 | 6 | 123 | 10 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 355 | 67 |
| | Sept (Post IRS) | 0 | 0 | 0 | 6 | 150 | 10 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 373 | 65 |
| | Oct (Post IRS) | 0 | 0 | 0 | 15 | 522 | 23 | 3 | 131 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 | 766 | 122 |
| | Nov (Post IRS) | 0 | 0 | 0 | 17 | 373 | 23 | 2 | 108 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 75 | 714 | 101 |
| | Dec (Post IRS) | 0 | 4 | 0 | 10 | 196 | 11 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 58 | 520 | 151 |
| | Jan 2022 (Post IRS) | 0 | 0 | 0 | 9 | 136 | 15 | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 117 | 650 | 108 |
| | Feb (Post IRS) | 0 | 0 | 0 | 6 | 44 | 9 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 178 | 843 | 195 |
| | Mar (Post IRS) | 0 | 0 | 0 | 7 | 45 | 9 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 162 | 879 | 177 |
| | Sub Total | 0 | 4 | 0 | 95 | 1999 | 147 | 5 | 333 | 10 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 971 | 6133 | 1181 |

| Site | Period | *An. funestus s.l.* | | | *An. gambiae s.l.* | | | *An. coustani* | | | *An. demeilloni* | | | *An. other* | | | *An. pharoensis* | | | *An. pretoriensis* | | | *An. squamosus* | | | *An. tenebrosus* | | | *An. ziemanni* | | | *Culicines* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK |
| Metema | May 2021 (Post IRS) | 0 | 0 | 0 | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 33 | 1 |
| | June (Post IRS) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 70 | 14 |
| | July (Post IRS) | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 89 | 522 | 81 |
| | Aug (Post IRS) | 0 | 0 | 0 | 3 | 16 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 99 | 615 | 118 |
| | Sept (Post IRS) | 0 | 0 | 0 | 5 | 32 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 81 | 499 | 99 |
| | Oct (Post IRS) | 0 | 0 | 0 | 7 | 7 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 276 | 75 |
| | Nov (Post IRS) | 0 | 0 | 0 | 3 | 7 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 39 | 214 | 43 |
| | Dec (Post IRS) | 0 | 0 | 0 | 2 | 12 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 171 | 31 |
| | Jan 2022 (Post IRS) | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 58 | 13 |
| | Feb (Post IRS) | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 51 | 4 |
| | Mar (Post IRS) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 44 | 4 |
| | Sub Total | 0 | 4 | 1 | 24 | 93 | 16 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 434 | 2553 | 483 |
| Lare | April 2021 (Pre IRS) | 1 | 0 | 3 | 1 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 24 | 7 |
| | May (Post IRS) | 2 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 190 | 74 |
| | June (Post IRS) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 28 | 5 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 275 | 47 |
| | July (Post IRS) | 1 | 2 | 5 | 1 | 17 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 281 | 33 |
| | Aug (Post IRS) | 14 | 23 | 9 | 10 | 53 | 8 | 1 | 32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 74 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 312 | 20 |
| | Sept (Post IRS) | 2 | 0 | 0 | 7 | 35 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 374 | 8 |
| | Oct (Post IRS) | 5 | 16 | 8 | 8 | 124 | 9 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 135 | 12 |
| | Nov (Post IRS) | 12 | 65 | 12 | 0 | 24 | 0 | 0 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 35 | 336 | 29 |

| Site | Period | *An. funestus s.l.* | | | *An. gambiae s.l.* | | | *An. coustani* | | | *An. demeilloni* | | | *An. other* | | | *An. pharoensis* | | | *An. pretoriensis* | | | *An. squamosus* | | | *An. tenebrosus* | | | *An. ziemanni* | | | *Culicines* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK | PSC | CDC | IPK |
| | Dec (Post IRS) | 11 | 32 | 9 | 0 | 0 | 0 | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 173 | 21 |
| | Jan 2022 (Post IRS) | 13 | 68 | 10 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 172 | 17 |
| | Feb (Post IRS) | 4 | 8 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 163 | 19 |
| | Mar (Post IRS) | 3 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 97 | 7 |
| | Sub Total | 68 | 222 | 60 | 27 | 257 | 37 | 2 | 106 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 283 | 15 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 232 | 2532 | 294 |
| Salamago | May (Post IRS) | 0 | 0 | 0 | 7 | 195 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 57 | 1065 | 42 |
| | June (Post IRS) | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 69 | 1271 | 39 |
| | July (Post IRS) | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | 315 | 9 |
| | Aug (Post IRS) | 0 | 1 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 159 | 6 |
| | Sept (Post IRS) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 49 | 11 |
| | Oct (Post IRS) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 28 | 0 |
| | Nov (Post IRS) | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 0 |
| | Dec (Post IRS) | 0 | 0 | 0 | 2 | 23 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 98 | 0 |
| | Jan 2022 (Post IRS) | 0 | 0 | 0 | 23 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 100 | 2 |
| | Feb (Post IRS) | 0 | 0 | 0 | 2 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 86 | 1 |
| | Mar (Post IRS) | 0 | 0 | 0 | 3 | 117 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 141 | 6 |
| | Sub Total | 0 | 1 | 0 | 37 | 439 | 10 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 232 | 3345 | 116 |

**Key:** CDC=Centers for Disease Control and Prevention. IPK=Indoor Prokopack. IRS=indoor residual spraying. PSC=pyrethrum sheet catches.

# Annex B. Results of *An. stephensi* Surveillance from Semera-Logia (2021)

| Breeding habitat type | June | | | | July | | | | September | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # Inspected | # Positives for *Anopheles* larvae | # *Anopheles* larvae (density) | # Adult *An. stephensi* identified | # Inspected | # Positives for *Anopheles* larvae | # *Anopheles* larvae (density) | # Adult *An. stephensi* identified | # Inspected | # Positives for *Anopheles* larvae | # *Anopheles* larvae (density) | # Adult *An. stephensi* identified |
| Cistern | 22 | 10 | 261 (0.59) | 19 | 19 | 13 | 3157 (8.3) | 88 | 21 | 10 | 789 (1.88) | 65 |
| Plastic sheet | 7 | 4 | 45 (0.32) | 18 | 10 | 6 | 231 (1.15) | 7 | 10 | 7 | 501 (2.51) | 50 |
| Plastic water drum | 4 | 0 | 0 | 0 | 4 | 2 | 29 (0.36) | 2 | 6 | 4 | 217 (1.81) | 0 |
| Ground-level water tank | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 9 (0.45) | 0 |
| **Total** | **35** | **14** | **306 (0.43)** | **37** | **33** | **21** | **3417 (5.17)** | **97** | **38** | **22** | **1516 (1.99)** | **115** |

# Annex C. Insecticide Susceptibility Test Results of *An. arabiensis* (2021-2022)

| Region | District | Propoxur | | Pirimiphos-methyl | | Alpha-cypermethrin | | Deltamethrin | | Permethrin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Control | Exposed | Control | Exposed | Control | Exposed | Control | Exposed | Control |
| Afar | Amibara | 100 (100/100) S | 0 (0/50) | 100 (100/100) S | 0 (0/50) | 11 (11/100) R | 0 (0/50) | 33 (33/100) R | 2 (1/50) | 45 (45/100) R | 2 (1/50) |
| Amhara | Dera | | | 100 (100/100) | 0 (0/50) | | | 92 (92/100) | 0 (0/50) | | |
| | | | | S | | | | POR | | | |
| | Fogera | | | 100 (100/100) | 0 (0/50) | | | 50 (50/100) | 0 (0/50) | | |
| | | | | S | | | | R | | | |
| | Jabitehnan | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 5 (5/100) | 0 (0/50) | 14 (14/100) | 0 (0/50) | 4 (4/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| | Jawi | | | 99 (100/100) | 0 (0/50) | | | 69 (69/100) | 0 (0/50) | | |
| | | | | S | | | | R | | | |
| | Kallu | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 16 (16/100) | 0 (0/50) | 23 (23/100) | 0 (0/50) | 20 (20/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| | Metema | | | 100 (100/100) | 0 (0/50) | | | 27 (27/100) | 0 (0/50) | | |
| | | | | S | | | | R | | | |
| | Quara | | | 100 (100/100) | 0 (0/50) | | 0 (0/50) | 32 (32/100 | 0 (0/50) | | |
| | | | | S | | | | R | | | |
| Gambela | Abobo | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 39 (39/100) | 0 (0/50) | 41 (41/100) | 2 (1/50) | 28 (28/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |

| Region | District | Propoxur | | Pirimiphos-methyl | | Alpha-cypermethrin | | Deltamethrin | | Permethrin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Control | Exposed | Control | Exposed | Control | Exposed | Control | Exposed | Control |
| Oromia | Batu | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 12 (12/100) | 0 (0/50) | 22 (22/100) | 0 (0/50) | 60 (60/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| | Fentale | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 12 (12/100) | 0 (0/50) | 43 (43/100) | 0 (0/50) | 28 (28/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| | Goro | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 43 (43/100) | 0 (0/50) | 72 (72/100) | 0 (0/50) | 63 (63/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| | Omonada | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 56 (56/100) | 0 (0/50) | 59 (59/100) | 0 (0/50) | 59 (59/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| SNNPR | Misrak Badawacho | 100 (100/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 6 (6/100) | 0 (0/50) | 25 (25/100) | 0 (0/50) | 25 (25/100) | 0 (0/50) |
| | | S | | S | | R | | R | | R | |
| Somali | Erer | 97 (97/100) | 0 (0/50) | 100 (100/100) | 0 (0/50) | 57 (43/75) | 0 (0/50) | 55 (55/100) | 0 (0/50) | 56 (56/100) | 0 (0/50) |
| | | POR | | S | | R | | R | | R | |

**Note:** S=Susceptible (98-100% mortality), POR=Possibility of Resistance (90-97% mortality), R=Resistance (<90% mortality)

# Annex D. Mortality of *An. arabiensis* from Resistance Intensity Assays (2021-2022)

| Site | Alpha-cypermethrin | | | Deltamethrin | | | Permethrin | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1X | 5X | 10X | 1X | 5X | 10X | 1X | 5X | 10X |
| Batu | 12 | 19 | 78 | 22 | 71 | 82 | 60 | 88 | 94 |
| Dera | - | - | | 92 | 97 | 97 | - | - | - |
| Fentale | 12 | 89 | 94 | 43 | 98 | | 28 | 100 | |
| Fogera | - | - | _ | 50 | 93 | 100 | | | |
| Goro | 43 | 68 | 91 | 72 | 94 | 100 | 63 | 88 | 100 |
| Jabitehnan | - | - | - | 14 | 87 | 100 | - | - | - |
| Jawi | - | - | - | 69 | 89 | 100 | - | - | - |
| Kallu | 16 | 49 | 88 | 23 | 79 | 95 | 20 | 98 | 100 |
| Metema | - | - | - | 27 | 100 | - | - | - | - |
| Misrak Badawacho | 6 | 55 | 75 | 25 | 69 | 80 | 25 | 68 | 88 |
| Quara | - | - | - | 32 | 92 | 100 | - | - | - |

# Annex E. *An. arabiensis* Mortality from PBO Synergist Tests (2021-2022)

| Insecticide | Abobo | Amibara | Batu | Dera | Erer | Fogera | Goro | Harbu | Jabitehnan | Jawi | Metehara | Metema | Misrak Badawacho | Omonada | Quara |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alpha only | 38.7 | 12 | 20 | | 69.3 | - | 65.3 | 16 | 4 | - | 9.3 | - | 4 | 54.7 | - |
| Alpha +PBO | 100 | 100 | 84 | | 97.3 | - | 100 | 100 | 98.7 | - | 100 | - | 90.7 | 100 | - |
| Del only | 42.7 | 46.7 | 65.3 | 94.7 | 64 | 61.3 | 26.7 | 46.7 | 40 | 54.7 | 52 | 36 | 34.7 | 54.7 | 33.3 |
| Del+PBO | 97.3 | 100 | 96 | 100 | 94.7 | 100 | 94.7 | 100 | 100 | 100 | 100 | 100 | 86.7 | 100 | 100 |
| Perm only | 37.3 | 42.7 | 78.7 | - | - | - | 72 | 68 | 17.3 | - | 28 | - | 5.3 | 54.7 | - |
| Perm + PBO | 100 | 100 | 100 | - | - | - | 100 | 98.7 | 100 | - | 100 | - | 65.3 | 100 | - |

# Annex F. Results of Insecticide Susceptibility and Resistance Intensity of *An. stephensi* (2021-2022)

| Insecticide/Control | % mortality (Dead/Exposed) | | |
|---|---|---|---|
| | Degehabur | Dire Dawa | Semera-Logia |
| Propoxur | 60 (60/100) | 54 (54/100) | 75 (75/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Pirimiphos-methyl | 58 (58/100) | 62.3 (63/101) | 100 (100/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Alpha-cypermethrin 1X | 17 (17/100) | 50 (50/100) | 18 (18/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Alpha-cypermethrin 5X | 34 (34/100) | 63 (63/100) | 85 (85/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Alpha-cypermethrin 10X | 75 (75/100) | 79 (79/100) | 91 (91/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Deltamethrin 1X | 20 (20/100) | 85 (85/100) | 61 (61/100) |
| Control | 2 (1/50) | 0 (0/50) | 0 (0/50) |
| Deltamethrin 5X | 85 (85/100) | 96 (96/100) | 93 (93/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Deltamethrin 10X | 88 (88/100) | 100 (100/100) | 100 (100/100) |
| Control | 0 (0/50) | 0 (0/50) | 0 (0/50) |
| Permethrin 1X | 48 (48/100) | 49 (49/100) | 66 (66/100) |
| Control | 0 (0/50) | 0 (0/50) | 1 (1/50) |

| Insecticide/Control | % mortality (Dead/Exposed) | | |
|---|---|---|---|
| | Degehabur | Dire Dawa | Semera-Logia |
| Permethrin 5X | 91 (91/100) | 99 (99/100) | 99 (99/100) |
| Control | 0 (0/50) | 0 (0/50) | 1 (1/50) |
| Permethrin 10X | 100 (100/100) | | 100 (100/100) |
| Control | 0 (0/50) | | 0 (0/50) |

# Annex G. Susceptibility of Wild *An. arabiensis* and *An. stephensi* to Chlorfenapyr (2021-2022)

| Species | Site | # tested | % mortality | | | Control | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 24 hours | 48 hours | 72 hours | # exposed | 24 hours | 48 hours | 72 hours |
| *An. arabiensis* | Batu | 100 | 100 | 100 | 100 | 25 | 0 | 0 | 0 |
| | Kallu | 100 | 85 | 91 | 100 | 25 | 0 | 0 | 0 |
| *An. stephensi* | Awash | 102 | 98 | 99 | 99 | 25 | 0 | 0 | 0 |
| | Batu | 100 | 99 | 99 | 99 | 25 | 0 | 0 | 0 |
| | Meki | 100 | 100 | 100 | 100 | 25 | 0 | 0 | 0 |
| | Modjo | 100 | 100 | 100 | 100 | 25 | 0 | 0 | 0 |

# Annex H. Results of Cone Bioassay Tests in Actellic 300CS–Sprayed Houses (2021-2022)

| Sites | Surface Type | T0 | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dera | Mud | 100 (120) | 100 (120) | 100 (120) | 100 (120) | 85.6 (120) | 100 (120) | 98.9 (120) | 88.9 (120) | 100 (120) | 78.9 (120) | 70 (120) |
| | Dung | 100 (60) | 100 (60) | 98.3 (60) | 100 (60) | 85.8 (60) | 100 (60) | 100 (60) | 99.2 (60) | 100 (60) | 91.7 (60) | 78.3 (60) |
| | Painted Mud | 100 (120) | 100 (120) | 100 (120) | 100 (120) | 85.6 (120) | 100 (120) | 100 (120) | 100 (120) | 100 (120) | 82 (120) | 68.9 (120) |
| Fogera | Mud | 100 (60) | 100(60) | 100 (60) | 93.3 (60) | 100 (60) | 100 (60) | 100 (60) | 100 (60) | 100 (60) | 68.3 (60) | 63.3 (60) |
| | Dung | 100 (210) | 100 (210) | 100 (210) | 100 (210) | 99.5(210) | 100 (210) | 99.5 (210) | 100 (210) | 100 (210) | 64.7( 210) | 70.7 (210) |
| | Painted Mud | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 86.7 (30) | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 80 (30) |
| Jawi | Mud | 100 (300) | 100 (300) | 100 (300) | 100 (300) | 100 (300) | 100 (300) | 100 (300) | 77 (300) | 55.1(300) | | |
| Lare | Mud | 100 (360) | 99.4 (360) | 97.2(3600 | 91.4(360) | 83.1 (360) | 72.9 (360) | | | | | |
| Metema | Mud | 100 (60) | 98.3 (60) | 100 (60) | 96.7 (60) | 98.3 (60) | 73.3 (60) | 80 (60) | 66.5 (60) | 43.3 (60) | | |
| | Dung | 100 (120) | 99.2 (120) | 100 (120) | 98.3 (120) | 95.6(120) | 87.8(120) | 78.3(1200 | 64.7(120) | | | |
| | Painted Mud | 100 (120) | 100 (120) | 98.3 (120) | 99.2 (120) | 98.3 (120) | 82 (120) | 80 (120) | 64.2 (120) | 50.3 (120) | | |
| Quara | Mud | 100 (150) | 99.3 (150) | 100 (150) | 98 (150) | 96.7 (150) | 87.4 (150) | 79.3 (150) | 63.1(150) | | | |
| | Dung | 100 (120) | 92.5(120) | 100 (120) | 99.2 (120) | 96.5 (120) | 89.4 (120) | 80(120) | 67.5(120) | 49.5(120) | | |
| | Painted Mud | 100 (30) | 100 (30) | 96.7 (30) | 100(30) | 100(30) | 80(30) | 80(30) | 63(30) | 48 (30) | | |

Note: Figures in brackets are number of insectary *An. arabiensis* tested.

# Annex I. Results of Cone Bioassay Tests in SumiShield–Sprayed Houses (2021-2022)

| Time of Test | Mud | | | | | Painted Mud | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| T0 | 92.4 (210) | 99.5 (210) | 100 (210) | 100 (210) | 100 (210) | 89.3 (150) | 98.7 (150) | 100 (150) | 100 (150) | 100 (150) |
| T1 | 81.6 (210) | 95.6 (210) | 99.5 (210) | 100 (210) | 100 (210) | 72.8 (150) | 90.6 (150) | 92 (150) | 100 (150) | 100 (150) |
| T2 | 60.5 (210) | 77.7 (210) | 92.3 | 97.9 (210) | 100 (210) | 64.7 (150) | 82.7 (150) | 90.8 (150) | 99.3 (150) | 100 (150) |
| T3 | 47.1 (210) | 66.7 (210) | 80.9 (210) | 96.4 (210) | 100 (210) | 53.3 (150) | 70.5 (150) | 79.9 (150) | 96.6 (150) | 100 (150) |
| T4 | 38.6 (210) | 55.2 (210) | 71 (210) | 87.3 (210) | 100 (150) | 39.3 (150) | 61.3 (150) | 74.7 (150) | 90.1 (150) | 100 (150) |
| T5 | 22.4 (210) | 42.4 (210) | 58 (210) | 74.1 (210) | 88.2 (210) | 20 (150) | 43.3 (150) | 62 (150) | 77.5 (150) | 92.9 (150) |
| T6 | 21 (210) | 35.2 (210) | 49.1 (210) | 56.8 (210) | 74.7 (210) | 20.7 (150) | 37.3 (150) | 52.7 (150) | 62.4 (150) | 76.5 (150) |
| T7 | 6.7 (210) | 15.7 (210) | 28.1 (210) | 34.6 (210) | 47.4 (210) | 5.3 (150) | 16.7 (150) | 26.7 (150) | 38 (150) | 50.7 (150) |

*Figures in brackets are number tested.

# Annex J. Results of Cone Bioassay Tests from Fludora Fusion–Sprayed Houses in Abaya Using Insectary *An. arabiensis* (2021-2022)

| Time | Cement | | | | | Mud | | | | | Painted Cement | | | | | Painted Mud | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| T0 | 80 (30) | 93.3 (30) | 100 (30) | 100 (30) | 100 (30) | 74 (150) | 83.9 (150) | 95.9 (150) | 100 (150) | 100 (150) | 67.7 (30) | 90.3 (30) | 100 (30) | 100 (30) | 100 (30) | 89.7 (150) | 96.5 (150) | 98.6 (150) | 100 (150) | 100 (150) |
| T1 | 93.3 (30) | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 62.7 (150) | 83.3 (150) | 91.3 (150) | 93.3 (150) | 98.5 (150) | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 100 (30) | 84 (150) | 94 (150) | 99.3 (150) | 100 (150) | 100 (150) |
| T2 | 66.7 (30) | 96.7 (30) | 100 (30) | 100 (30) | 100 (30) | 88.7 (150) | 96 (150) | 100 (150) | 100 (150) | 100 (50) | 83.3 (30) | 83.3 (30) | 90 (30) | 93.3 (30) | 93.3 (30) | 76.7 (150) | 92 (150) | 98 (150) | 98.7 (150) | 100 (150) |
| T3 | 30 (30) | 60 (30) | 90 (30) | 100 (30) | 100 (30) | 50 (150) | 64.7 (150) | 80.7 (150) | 92 (150) | 96 (150) | 53.3 (30) | 60 (30) | 83.3 (30) | 100 (30) | 100 (30) | 50.7 (150) | 70.7 (150) | 81.3 (150) | 91.3 (150) | 98 (150) |
| T4 | 60 (30) | 80 (30) | 100 (30) | 100 (30) | 100 (30) | 56 (150) | 68.7 (150) | 82 (150) | 92 (150) | 97.3 (150) | 30 (30) | 40 (30) | 70 (30) | 83.3 (30) | 96.3 (30) | 60 (150) | 74 (150) | 82.9 (150) | 94.6 (150) | 98 (150) |
| T5 | 53.3 (30) | 53.3 (30) | 73.3 (30) | 96.7 (30) | 100 (30) | 51.3 (150) | 60.7 (150) | 74.7 (150) | 85.3 (150) | 95.3 (150) | 60 (30) | 86.7 (30) | 90 (30) | 96.7 (30) | 100 (30) | 60 (150) | 77.3 (150) | 87.1 (150) | 98.7 (150) | 98.7 (150) |
| T6 | 33.3 (30) | 50 (30) | 50 (30) | 66.7 (30) | 76.7 (30) | 52.7 (150) | 72 (150) | 86.7 (150) | 89.3 (150) | 96.7 (150) | 70 (30) | 83.3 (30) | 100 (30) | 100 (30) | 100 (30) | 68.7 (150) | 89.3 (150) | 97.3 (150) | 99.3 (150) | 100 (150) |
| T7 | 40 (30) | 63.3 (30) | 83.3 (30) | 96.7 (30) | 100 (30) | 48 (150) | 65.3 (150) | 76.7 (150) | 84.7 (150) | 88 (150) | 60 (30) | 60 (30) | 73.3 (30) | 86.7 (30) | 100 (30) | 46.7 (150) | 70.7 (150) | 86.7 (150) | 96 (150) | 99.3 (150) |
| T8 | 20 (30) | 40 (30) | 80 (30) | 93.3 (30) | 96.7 (30) | 28 (150) | 48.7 (150) | 59.3 (150) | 72 (150) | 82 (150) | 26.7 (30) | 60 (30) | 73.3 (30) | 80 (30) | 90 (30) | 26.7 (150) | 46.7 (150) | 64 (150) | 83.3 (150) | 92 (150) |
| T9 | 23.3 (30) | 33.3 (30) | 60 (30) | 83.3 (30) | 96.7 (30) | 28 (150) | 42.7 (150) | 58 (150) | 73.3 (150) | 83.3 (150) | 30 (30) | 46.7 (30) | 60 (30) | 73.3 (30) | 90 (30) | 25.3 (150) | 44 (150) | 63.3 (150) | 83.3 (150) | 92 (150) |
| T10 | 26.7 (30) | 46.7 (30) | 63.3 (30) | 83.3 (30) | 93.3 (30) | 22.7 (150) | 32.7 (150) | 56 (150) | 74.7 (150) | 82.7 (150) | 23.3 (30) | 40 (30) | 56.7 (30) | 76.7 (30) | 93.3 (30) | 23.3 (150) | 33.3 (150) | 57.3 (150) | 81.3 (150) | 88.7 (150) |
| T11 | 20 (30) | 43.3 (30) | 56.7 (30) | 60 (30) | 73.3 (30) | 14 (150) | 24.7 (150) | 41.3 (615) | 54.7 (150) | 64.7 (150) | 23.3 (30) | 33.3 (30) | 50 (30) | 66.7 (30) | 76.7 (30) | 15.3 (150) | 32 (150) | 46.7 (150) | 63.3 (150) | 69.3 (150) |

Figures in brackets are number of tested *An. arabiensis*

# Annex K. Results of Cone Bioassay Tests in Fludora Fusion–Sprayed Houses in Gelana Using Insectary *An. arabiensis* (2021-2022)

| Time of set | Mud | | | | | Painted Mud | | | | | Dung | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| T0 | 90.6 (180) | 98.3 (180) | 98.9 (180) | 99.4 (180) | 100 (180) | 100 (60) | 100 (60) | 100 (60) | 100 (60) | 100 (60) | 90 (120) | 94.2 (120) | 94.2 (120) | 95 (120) | 100 (120) |
| T1 | 90.6 (180) | 91.1 (180) | 96.7 (180) | 99.4 (180) | 99.4 (180) | 83.3 (60) | 85 (60) | 94.8 (60) | 100 (60) | 100 (60) | 94.2 (120) | 96.7 (120) | 99.1 (120) | 100 (120) | 100 (120) |
| T2 | 56.1 (180) | 78.3 (180) | 89.4 (180) | 91.1 (180) | 92.8 (180) | 76.7 (60) | 90 (60) | 98.3 (60) | 98.3 (60) | 98.3 (60) | 67.5 (120) | 90.8 (120) | 95.8 (120) | 95.7 (120) | 99.2 (120) |
| T3 | 49.4 (180) | 69.4 (180) | 86.7 (180) | 92.8 (180) | 97.8 (180) | 55 (60) | 68.3 (60) | 91.7 (60) | 96.7 (60) | 100 (60) | 41.7 (120) | 67.5 (120) | 70.8 (120) | 95 (120) | 96.7 (120) |
| T4 | 56.1 (180) | 75 (180) | 85.6 (180) | 95.4 (180) | 97.7 (180) | 56.7 (60) | 78.3 (60) | 80 (60) | 91.7 (60) | 96.3 (60) | 53.3 (120) | 75 (120) | 79.2 (120) | 89.2 (120) | 93.3 (120) |
| T5 | 34.4 (180) | 51.1 (180) | 70.6 (180) | 86.1 (180) | 93.2 (180) | 41.7 (60) | 71.7 (60) | 96.7 (60) | 100 (60) | 100 (60) | 25 (120) | 39.2 (120) | 69.2 (120) | 85 (120) | 95 (120) |
| T6 | 62.2 (180) | 76.1 (180) | 84.4 (180) | 93.3 (180) | 95 (180) | 71.7 (60) | 83.3 (60) | 90 (60) | 98.3 (60) | 98.3 (60) | 40.8 (120) | 62.5 (120) | 62.5 (120) | 74.2 (120) | 80.8 (120) |
| T7 | 35 (180) | 68.3 (180) | 82.8 (180) | 90 (180) | 95 (180) | 33.3 (60) | 71.7 (60) | 93.3 (60) | 96.7 (60) | 96.7 (60) | 31.7 (120) | 44.2 (120) | 60.8 (120) | 71.7 (120) | 75 (120) |
| T8 | 34.4 (180) | 56.1 (180) | 70 (180) | 83.9 (180) | 89.4 (180) | 53.3 (60) | 73.3 (60) | 88.3 (60) | 93.3 (60) | 95 (60) | 19.2 (120) | 32.5 (120) | 45.8 (120) | 59.2 (120) | 66.7 (120) |
| T9 | 28.3 (180) | 43.9 (180) | 61.7 (180) | 78.9 (180) | 93.3 (180) | 23.3 (60) | 48.3 (60) | 60 (60) | 81.7 (60) | 91.7 (60) | | | | | |
| T10 | 23.3 (180) | 36.1 (180) | 61.1 (180) | 80 (180) | 88.3 (180) | 21.7 (60) | 35 (60) | 60 (60) | 73.3 (60) | 90 (60) | | | | | |

Figures in brackets are number of tested *An. arabiensis*

# Annex L. Result of SumiShield Fumigant Bioassay Tests (2021-2022)

| Surface type | Time of spraying | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours |
|---|---|---|---|---|---|---|
| Mud | T0 | 51.0 | 68.0 | 70.0 | | |
| | T1 | 57.1 | 85.7 | 97.1 | 100 | 100 |
| | T2 | 31.4 | 48.6 | 68.6 | 85.7 | 100 |
| | T3 | 24.3 | 42.9 | 61.4 | 80.0 | 100 |
| | T4 | 10.0 | 32.9 | 52.9 | 71.4 | 95.7 |
| | T5 | 1.4 | 22.9 | 40.0 | 60.0 | 80.0 |
| | T6 | 5.7 | 27.1 | 38.6 | 51.4 | 68.6 |
| | T7 | 0 | 7.1 | 11.4 | 22.9 | 32.9 |
| Painted mud | T0 | 80.0 | 98.0 | 100 | | |
| | T1 | 30 | 48.6 | 62.9 | 71.4 | 71.4 |
| | T2 | 24.0 | 52.0 | 72.0 | 88.0 | 100 |
| | T3 | 12.0 | 46.0 | 64.0 | 82.0 | 98.0 |
| | T4 | 10.0 | 38.0 | 56.0 | 78.0 | 98.0 |
| | T5 | 0 | 22.0 | 42.0 | 66.0 | 90.0 |
| | T6 | 4.0 | 22.0 | 36.0 | 52.0 | 58.0 |
| | T7 | 0 | 4.0 | 12.0 | 24.0 | 32.0 |

*Numbers are percentage mortalities after the respective holding periods*

# Annex M. Result of Fludora Fusion Fumigant Bioassay Tests from Abaya (2021-2022)

| Surface type | Time of test | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours |
|---|---|---|---|---|---|---|
| Mud | T0 | 44.0 | 74.0 | 88.0 | 100 | 100 |
| | T1 | 10.0 | 26.0 | 54.0 | 84.0 | 98.0 |
| | T2 | 22.0 | 56.0 | 88.0 | 94.0 | 96.0 |
| | T3 | 2.0 | 8.0 | 24.0 | 42.0 | 64.0 |
| | T4 | 0 | 0 | 0 | 0 | 4.0 |
| Painted mud | T0 | 48.0 | 64.0 | 86.0 | 96.0 | 96.0 |
| | T1 | 16.0 | 38.0 | 66.0 | 90.0 | 98.0 |
| | T2 | 14.0 | 52.0 | 74.0 | 86.0 | 96.0 |
| | T3 | 2.0 | 6.0 | 8.0 | 28.0 | 56.0 |
| | T4 | 0 | 4.0 | 8.0 | 20.0 | 30.0 |
| | T5 | 0 | 0 | 4.0 | 4.0 | 10.0 |
| Cement | T0 | 10.0 | 60.0 | 80.0 | 90.0 | 90.0 |
| | T1 | 20.0 | 20.0 | 60.0 | 80.0 | 100 |
| | T2 | 0 | 30.0 | 70.0 | 100 | 100 |
| | T3 | 0 | 0 | 30.0 | 50.0 | 80.0 |
| | T4 | 0 | 0 | 0 | 0 | 10.0 |
| Painted Cement | T0 | 30.0 | 60.0 | 60.0 | 100 | 100 |
| | T1 | 0 | 20.0 | 30.0 | 80.0 | 100 |
| | T2 | 20.0 | 30.0 | 80.0 | 90.0 | 90.0 |
| | T3 | 0 | 0 | 0 | 30.0 | 30.0 |
| | T4 | 0 | 0 | 30.0 | 30.0 | 30.0 |

*Numbers are percentage mortalities after the respective holding periods*

# Annex N. Result of Fludora Fusion Fumigant Bioassay Tests from Gelana (2021-2022)

| Surface type | Time of test | 24 hours | 48 hours | 72 hours | 96 hours | 120 hours |
|---|---|---|---|---|---|---|
| Mud | T0 | 73.3 | 86.6 | 93.3 | 100.0 | 100.0 |
| | T1 | 28.3 | 41.6 | 70.0 | 88.3 | 91.6 |
| | T2 | 15 | 32.5 | 57.5 | 60.0 | 75.0 |
| | T3 | 1.7 | 6.7 | 38.3 | 63.3 | 83.3 |
| | T4 | 0 | 0 | 3.3 | 8.3 | 33.3 |
| Painted mud | T0 | ND | ND | ND | ND | ND |
| | T1 | 15.0 | 30.0 | 65.0 | 80.0 | 80.0 |
| | T2 | 35.0 | 45.0 | 70.0 | 70.0 | 75.0 |
| | T3 | 0 | 6.7 | 18.3 | 18.3 | 26.7 |
| | T4 | 0 | 0 | 0 | 10.0 | 10.0 |
| Dung | T0 | 60.0 | 85.0 | 85.0 | 90.0 | 90.0 |
| | T1 | 53.3 | 60.0 | 93.3 | 100 | 100 |
| | T2 | 15.0 | 32.5 | 57.5 | 60.0 | 75.0 |
| | T3 | 0 | 26.7 | 60.0 | 70.0 | 76.7 |
| | T4 | 0 | 0 | 0 | 0 | 13.3 |

*Numbers are percentage mortalities after the respective holding periods*