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PMI VECTORLINK ETHIOPIA PROJECT
FINAL ENTOMOLOGY REPORT
MAY 2020-MARCH 2021

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Acronyms

BBI	Bovine Blood Index
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme-linked Immunosorbent Assay
GBI	Goat Blood Index
HBI	Habitat Breeding Index
ILT	Indoor Light Trap
IRS	Indoor Residual Spraying
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 Spray Catch
RBI	Relative Breeding Index
SNNPR	Southern Nations Nationalities and Peoples Region
SOP	Standard Operating Procedure
WHO	World Health Organization

Executive Summary

Background

In the 2020 reporting year, the President's Malaria Initiative (PMI) VectorLink Ethiopia Project conducted entomological investigations that included longitudinal entomological monitoring, insecticide resistance monitoring, evaluation of vector control interventions, larval indices, residual efficacies of insecticides, and cross-sectional surveys. Routine entomological monitoring was carried out from July 2020 to March 2021 in seven sentinel sites: Abaya, Bambasi, Benatsemay, Harbu, Jabitehnan, Lare, and Metema. Centers for Disease Control and Prevention (CDC) light traps placed indoors and outdoors and pyrethrum spray catches (PSCs) were used to sample *Anopheles* mosquitoes. Community mosquito collection was piloted in Gelana District. Adult *An. stephensi* surveillance was conducted monthly from July 2020 to April 2021 in Awash, Metehara, Dire Dawa, and Kebridehar towns. The sporozoite infection rates and blood meals of sampled mosquitoes were assayed using Enzyme Linked Immunosorbent Assay (ELISA). Species morphologically identified as *An. gambiae* s.l. were subjected to species-specific Polymerase Chain Reaction (PCR) tests. The presence/absence of *An. stephensi* was investigated in rural areas surrounding 11 towns: Meki, Zeway, Metehara, Awash, Gewane, Semera, Bati, Dire Dawa, Kebridehar, Degehabur, and Goday. Insecticide susceptibility tests using pirimiphos-methyl, propoxur, bendiocarb, alpha-cypermethrin, deltamethrin, permethrin, and clothianidin impregnated papers, resistance intensity, and piperonyl butoxide (PBO) synergist assays were conducted on populations of *An. arabiensis* from Amibara, Jabitehnan, Metema, Bambasi, Abobo, Abaya, Omonada, Benatsemay, Misrak Badawacho, Erer, and Humera. Tests were also conducted on *An. stephensi* from Awash, Metehara, Meki, and Goday towns. Both species were also tested against chlorfenapyr. Quality assurance and residual efficacy of Actellic 300CS was evaluated using cone bioassays in Abaya, Bambasi, Godare, Lare, and Menge. SumiShield and Fludora Fusion indoor residual spraying (IRS) was piloted in Menge District and bioassays were conducted in two kebeles to assess residual efficacy. An experimental hut trial to assess the efficacy of PBO nets and standard nets with and without Actellic 300CS IRS was carried out in Asendabo.

Results

A total of 12,359 *Anopheles* mosquitoes belonging to at least nine species (*An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. funestus* s.l., *An. pharoensis*, *An. pretoriensis*, *An. squamosus/cydippis*, *An. tenebrosus*, and *An. ziemanni*) were collected from the seven sentinel sites and Gelana District. In addition, 23,981 culicine mosquitoes were also collected from all the sites. *Anopheles arabiensis* identified through PCR assays was the predominant species in five of the seven sentinel sites and Gelana. Most of the *Anopheles* were collected from CDC light traps. Trap density of *An. arabiensis* was less than 1 per trap per night in most of the collections, particularly in the PMI VectorLink Ethiopia Project IRS sites. *Anopheles stephensi* surveillance in the four sites produced 626 adult mosquitoes. However, in the evaluation of larval indices of *An. stephensi* in Awash, Dire Dawa, and Kebridehar, 26,855 larvae were collected from November 2020 to February 2021. As in 2019, CDC light traps and PSCs were less effective; most of the collections were from Prokopack/backpack aspirations in animal shelters and cattle and goat baited tent traps. Cross-sectional surveys conducted to determine the presence/absence of *An. stephensi* in rural kebeles in eastern Ethiopia revealed the species' presence in 21 out of 48 inspected kebeles. Circumsporozoite ELISA detected *Plasmodium* (*P.*) *falciparum* infections in *An. arabiensis* in Harbu (3/1086, 0.28%) and Gelana (1/1789, 0.05%). *Anopheles arabiensis* from Gelana were also positive for *P. vivax* 247 and 210 at the infection rate of 0.11% (2/1789) and 0.05% (1/1789), respectively. The *P. falciparum* infection rate of *Anopheles funestus* s.l. from Gelana was 8.33% (1/12). *Anopheles pharoensis* from Benatsemay was positive for *P.*

falciparum (1/88, 2.60%), and from Lare for *P. vivax* 210 (1/554, 0.18%). Moreover, *An. coustani* and *An. tenebrosus* were positive for *P. vivax* 210 in Bambasi and Harbu, at infection rates of. 0.10% (1/962) and 4.90% (2/41), respectively. *Anopheles stephensi* from Kebridehar was positive for *P. vivax* 247 and *P. vivax* 210 at the same infection rate of 1.33% (1/175). *Anopheles arabiensis* exhibited high susceptibility to bendiocarb, propoxur, pirimiphos-methyl and clothianidin (100% mortality) but resistance to alpha-cypermethrin, deltamethrin, and permethrin (<90% mortality).. In contrast, *An. stephensi* was highly resistant to all insecticides except for chlorfenapyr. Larvae of *An. stephensi* were susceptible to temephos. Although the presence of high resistance intensity was observed in *An. arabiensis* in a few sites, most of the sites showed moderate resistance. Except in a few cases, pre-exposure to PBO returned susceptibility to populations of *An. arabiensis* (alpha-cypermethrin and deltamethrin in 7/10, permethrin in 5/10 sites) and *An. stephensi* (alpha-cypermethrin in 2/4, deltamethrin in 3/4, permethrin in 4/4 sites) despite intense resistance manifested in the latter species. Actellic 300CS persisted for 2–3 months, while SumiShield and Fludora Fusion did so for at least seven months. A separate report on a PBO net/IRS codeployment hut trial in Asendabo has been submitted to PMI.

Conclusions

The findings of *Plasmodium* infections in *An. arabiensis*, *An. funestus* s.l., and *An. pharoensis* support the significance of these species in the transmission of malaria irrespective of the absence of infected mosquitoes in most of the sentinel sites. Although found with *P. vivax* infections, the vectorial role of *An. stephensi*, *An. coustani,* and *An. tenebrosus* requires further investigation. The cross-sectional studies on *An. stephensi* since 2018 provided evidence that this species is already established in Ethiopia, as it has been found in 35 urban (14) and rural (21) localities. Given the high resistance of populations of *An. arabiensis* and *An. stephensi* to the pyrethroid insecticides and reversion to susceptibility following pre-exposure to PBO, PBO nets might be an appropriate vector control intervention for *An. arabiensis* and *An. stephensi.* Populations of both species were also found susceptible to chlorfenapyr, indicating the need to evaluate the efficacy and effectiveness of nets impregnated with this insecticide (Interceptor G2). The greater susceptibility of *An. arabiensis* to clothianidin and persistence of SumiShield and Fludora Fusion for more than six months as well as the shorter life of Actellic 300CS are evidence for replacing the latter product with the former ones. However, further evaluation of the residual efficacy of SumiShield and Fludora Fusion under different geographical settings as part of regular monitoring is important for malaria vector control in the country.

1. INTRODUCTION

The President's Malaria Initiative (PMI) VectorLink Ethiopia Project has been generating entomological data since 2017 so that it can make evidence-based decisions when selecting vector control interventions and assessing the entomological impact of indoor residual spraying (IRS) and insecticide-treated nets. The project has also been conducting operational research to evaluate vector control tools. Cross-sectional surveys in the last two years in eastern and central Ethiopia enabled the project to map the distribution of *An. stephensi.* As part of entomological capacity development, the project provided entomological materials to nine universities.

From May 2020 to March 2021, VectorLink Ethiopia in collaboration with universities and a research institute conducted longitudinal entomological monitoring in 11 sentinel sites, seven of which were for the established vectors while the rest were for *An. stephensi.* The longitudinal survey in the four sites were conducted for two consecutive years. A community mosquito collection was piloted in West Guji Zone of Oromia Region to evaluate the performance of mosquito collectors drawn from the community and scale up this activity to other districts as part of the effort to expand entomological monitoring in a cost-effective manner. Insecticide resistance monitoring targeting the main vector, *An. arabiensis,* was conducted in 11 sites and *An. stephensi* in four sites. Cross-sectional surveys on *An. stephensi* were undertaken in rural areas in eastern Ethiopia to determine the species' presence/absence from adult identifications reared from larvae and pupae. The project also evaluated the residual efficacy of Actellic 300CS in five sentinel sites and SumiShield and Fludora Fusion in two sites. Finally, laboratory analysis was conducted for species identification, detection of sporozoite infections, and source of blood meals.

2. METHODOLOGY

2.1 ROUTINE ENTOMOLOGICAL MONITORING

Data on entomological indices including species composition, indoor and outdoor biting density, indoor resting density, sporozoite infection rates, and blood meal sources were collected through longitudinal monitoring from July 2020 to March 2021 from seven sentinel sites using Centers for Disease Control and Prevention (CDC) light trap and pyrethrum spray catch (PSC) collections. The PMI VectorLink Ethiopia Project does not conduct human landing catches, which are not allowed by the National Malaria Elimination Program (NMEP). Mosquitoes were sorted to *Anopheles* and culicines. Those identified as the latter were counted, number recorded and specimens discarded. *Anopheles* collections were identified to species using morphological key of Coetzee (2020), labelled, and preserved dry on silica gel. *Anopheles* data were recorded in the field on the standard PMI VectorLink Mosquito Collection, Identification and Dissection Record Form (Household Form) and entered directly into the VectorLink Collect database.

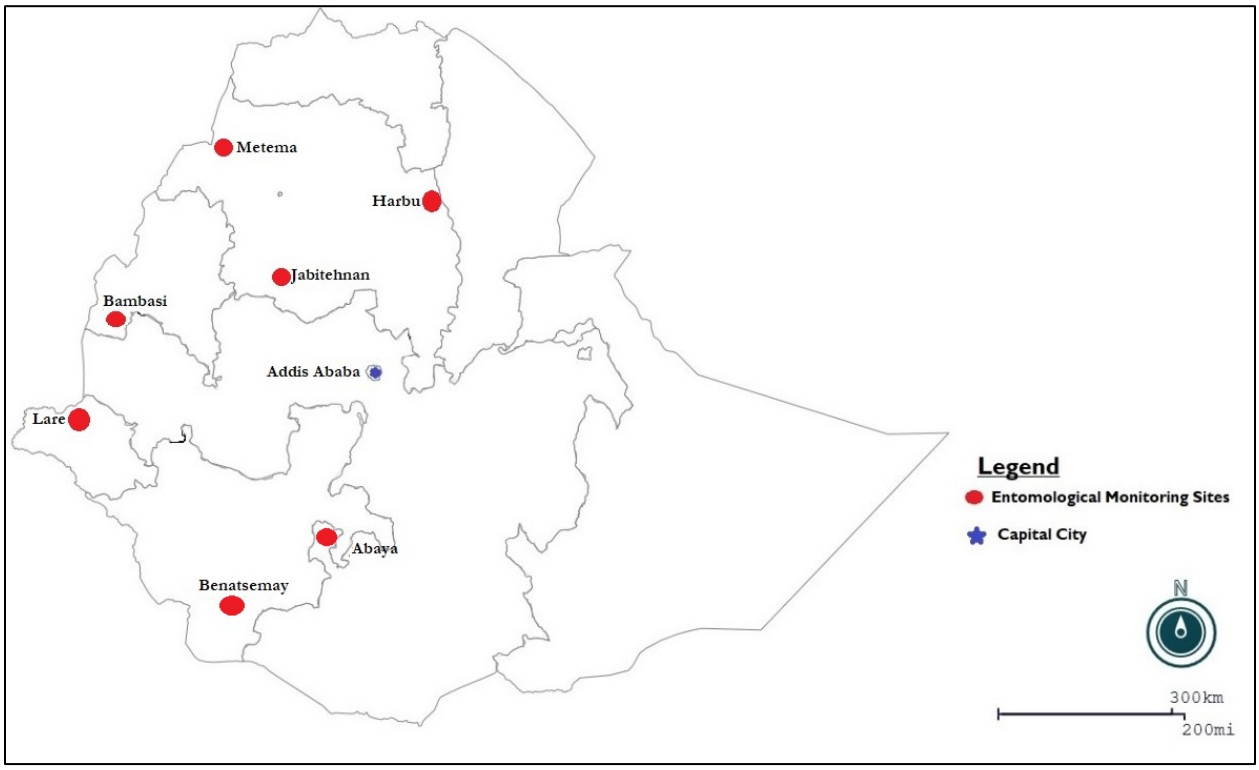
2.1.1 ENTOMOLOGICAL MONITORING SITES

The routine entomological monitoring conducted in 2020-2021 were in the same sentinel sites as in 2019-2020 work plan year: three PMI-supported IRS sites – Abaya (Oromia), Lare (Gambela), and Bambasi (Benishangul-Gumuz) – and four sites that did not receive IRS support from PMI –- Benatsemay (SNNPR), Jabitehnan (Amhara), Harbu (Amhara), and Metema (Amhara) (Figure 1). Among the four sites that did not receive PMI-supported IRS, Metema received government-supported IRS with propoxur in 2020, and Benatsemay, Harbu, and parts of Jabitehnan did not receive any IRS in 2020. Standard insecticide-treated nets are used in all seven sites, and PBO nets (PermaNet 3.0) are used in Lare.

For security reasons, entomological monitoring was not conducted in November 2020 in Benatsemay and in March 2021 in Harbu.

For each sampling, investigators recorded the following information: roof type, wall surface type, presence/absence of eaves, type of ceiling, whether the house was sprayed or not, presence/absence of domestic animals in the house, number of nets in the house, number of occupants who slept in the house during the collection night and who didn't, and brand of insecticide-treated nets.

FIGURE 1. ROUTINE ENTOMOLOGICAL MONITORING SENTINEL SITES, 2020



2.1.2 MOSQUITO SAMPLING METHODS

Mosquito collections in all seven sentinel sites were done using CDC light trap and PSC methods following the procedures described in the PMI VectorLink Standard Operating Procedures (SOPs).[1] The frequency of *Anopheles* sampling is given in Table 1.

Mosquito collections using CDC light traps indoors and outdoors were conducted following the PMI VectorLink SOP #1. In each site, 12 houses were randomly selected, and mosquito sampling was carried out monthly in the same houses for two nights indoors and outdoors giving a total of 48 trap-nights per month indoors and outdoors.

As in the past several years, 20 houses from each sentinel site were sampled to estimate the daily indoor resting density and sporozoite infection rates of the principal malaria vector using PSC, by following the protocol in SOP #3. From these collections, the proportion of mosquitoes with abdomens that were empty, engorged with a blood meal, half gravid, and gravid were recorded. The blood meal sources were identified from the blood-fed mosquitoes.

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

TABLE 1. FREQUENCY OF ANOPHELES SAMPLING (JULY 2020-MARCH 2021)

Type of collection	Time	Frequency	Sample
PSC	*6:00 am to 8:00 am*	Once a month	20 houses per site (total 140) per month
CDC light trap baited with human sleeping under net indoors	*6:00 pm to 6:00 am*	12 traps x 2 nights/month per site x 7 sites	168 trap nights per month indoors
CDC light trap baited with human sleeping under net outdoors	*6:00 pm to 6:00 am*	12 traps x 2 nights/month per site x 7 sites	168 trap nights per month outdoors

2.2 PILOTING COMMUNITY MOSQUITO COLLECTIONS

The current approach of mobilizing a handful of trained entomologists to collect entomological data from limited sites, does not provide the high-resolution entomological data needed to inform tailored sub-national deployment of vector control interventions. When properly implemented, community-based entomological surveillance enables parallel collection of longitudinal entomological data from multiple sites in a cost-effective manner. This data can inform the selection of vector control activities and assess the impact of vector control interventions. In August 2020, PMI VectorLink project selected eight community mosquito collectors (all females) in Gelana District in West Guji Zone of the Oromia Region to conduct monthly entomological monitoring from four kebeles namely Metere, Oda Negelle, Tore Budiya, and Wacho. The selection was carried out in consultation with the Chairmen of the kebeles, the Gelana district malaria focal person and health extension workers. The mosquito collectors have varied levels of education, including some graduates of technical colleges. They are not employed either by government or non-governmental organization.

The project trained the collectors on mosquito collection using CDC light traps, identification to the genus level labelling, and preservation. VectorLink Ethiopia staff identified mosquitoes to the species level using the morphological key of Coetzee (2020). The collectors conducted mosquito collections for eight months, from August 2020 to March 2021. Each month, mosquito sampling in each of the kebeles was conducted for five consecutive nights using two traps in two houses (10 trap-night per kebele per month indoors). Specimens were sent to VectorLink Ethiopia monthly. Based on the collection, the species composition, diversity and seasonal occurrence, trap density of the principal vector, and abdominal blood feeding status were documented. The indoor biting density was determined as the mean number of *Anopheles* species per trap per night.

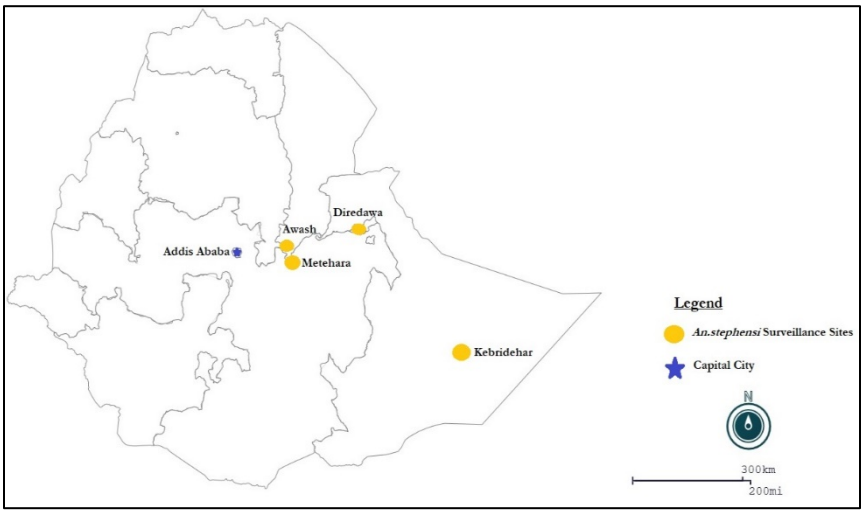
2.3 MOSQUITO IDENTIFICATION, LABELLING, AND PRESERVATION

Mosquito collections from both longitudinal entomological monitoring and community mosquito collections were identified to the species, labelled, and preserved on silica gel and shipped monthly to Jimma University and ArbaMinch University for further analysis to identify species using Polymerase Chain Reaction (PCR), sporozoite infection rates, and origin of blood meals with Enzyme-Linked Immunosorbent Assay (ELISA).

2.4 *ANOPHELES STEPHENSI* SURVEILLANCE

The same four sites as in 2019-2020 (Awash, Dire Dawa, Kebridehar, and Metehara) were used to conduct monthly *An. stephensi* surveillance for ten months, from July 2020 to April 2021 (Figure 2).

FIGURE 2. *ANOPHELES STEPHENSI* SURVEILLANCE SITES (2020-2021)



Monthly *An. stephensi* surveillance was carried out from July 2020 to April 2021 in Awash, Metehara, and Kebridehar towns and Dire Dawa City, except for Awash in August 2020 when monitoring was interrupted due to a confirmed case of COVID-19 on the team. The mosquito sampling methods were PSC, CDC light traps, black boxes, cattle-baited tent traps, clay pots, and hand collections with Prokopack and backpack aspirators (Table 2). Adult mosquitoes sampled from Dire Dawa and Kebridehar were preserved and submitted to Jimma University for sporozoite and blood meal analysis. VectorLink has received monthly entomological data from the Armauer Hansen Research Institute, which were collected from Metehara and Awash, keeping the specimens for laboratory analysis by the same methods.

TABLE 2. FREQUENCY OF *AN. STEPHENSI* SAMPLING (JULY 2020-APRIL 2021)

Type of collection	Time	Frequency	Sample
PSC	*6:00 am to 8:00 am*	Once a month per house	20 houses per site per month in four sites
CDC light trap	*6:00 pm to 6:00 am*	6 traps x 2 nights/month	48 indoors and 48 outdoors collections per night per month
Black box	*6:00 am to 8:00 am*	6 days per month per site	24 boxes per month in four sites
Cattle and goat baited net trap	*6:00 pm to 6:00 am*	3 days per month per site	12 nights per month in four sites
Clay pots	*6:00 am to 8:00 am*	6 days per month per site	24 clay pots per month in four sites
Prokopack and backpack aspirators from animal sheds	*6:00 am to 8:00 am*	Depends on the number of available sheds	Variable

2.5 ASSESSING PRESENCE/ABSENCE OF *AN. STEPHENSI* IN RURAL SITES

The presence/absence of *An. stephensi* was investigated in the rural sites located up to 20 kms from 11 towns where the vector was previously detected: Awash, Bati, Degehabour, Dire Dawa, Gewane, Goday, Kebridehar, Meki, Metehra, Semera and Zeway. Natural and artificial breeding habitats were investigated, and geographical coordinates, number of positive and negative larval breeding habitats, and habitat characteristics were recorded.

Also recorded were habitats containing *Anopheles* and *Aedes aegypti*. Larvae and pupae of *Anopheles* were collected, reared to adults, and identified to species following the morphological key of Coetzee (2020).

2.6 Assessing Larval Indices of *An. stephensi*

The density, relative breeding index (RBI), and container index (CI) of *An. stephensi* larvae from Awash, Dire Dawa, Kebridehar and Semera were assessed monthly from November 2020 to February 2021. Monthly data were recorded in a standard Excel sheet format that contained breeding habitat type, number of *Anopheles* larvae, and number of adult *An. stephensi* identified. Larvae were collected from 20 dips of a positive habitat and 20 dips from negative habitats using a 350 ml capacity dipper. Larvae in small containers were transferred into larval pans and counted.

Monthly mean larval density of *An. stephensi* was estimated as the number of larvae per dip per day. The RBI was measured by dividing the number of habitats positive for *An. stephensi* by the total number of habitats positive for any mosquito breeding. The container index of larvae was computed as the total number of containers found with *An. stephensi* larvae/pupae divided by the total number of surveyed containers multiplied by 100.

2.7 Evaluating the Performance of In2Care Traps

To evaluate attraction of modified In2Care traps (without insecticide) to *An. stephensi* for oviposition, surveillance was conducted monthly from November 2020 to April 2021 in Dire Dawa City and Kebridehar town. In each site, 20 traps were tested; 10 contained yeast treated tap water and 10 contained untreated tap water. Traps also contained a gauze strip to allow mosquitoes to easily land in the trap. Five of the yeast-treated traps were placed at a distance of five meters from a known larval breeding sites and the other five were placed at a distance of 100 meters away from the known breeding sites. The untreated water traps were placed in the same manner – five near a known breeding site, and five away from a known breeding site. From February to April 2021, gauze strips in the trap were replaced by sticky tape.

Larvae of *Anopheles* and *Ae. aegypti* were collected and counted. Adult *An. stephensi* were identified from reared larvae. In addition, the number of adult *An. stephensi* and *Ae. aegypti* collected from sticky tapes were recorded.

Trap efficiency was estimated by dividing the positive traps by the total number of traps and multiplied by 100.

2.8 Molecular and Immunological Assays

PCR was used to identify species of *An. gambiae* s.l., sporozoite infection and origin of blood meals were detected through ELISA. *Anopheles funestus* ID PCR results will be added to this report in the form of an addendum.

2.8.1 Species ID PCR

The PCR method described in Scott et al. (1993) was employed to identify members of *An. gambiae* s.l.

2.8.2 Sporozoite ELISA

The ELISA method described by Wirtz et al. (1992) was used to examine specimens of *An. arabiensis*, *An. pharoensis, An. funestus* s.l, *An. coustani*, *An. tenebrosus* and *An. stephensi* for circumsporozite proteins. Mosquitoes with all abdominal stages including blood unfeds, feds, half-gravids, and gravids were tested.

2.8.3 Blood meal ELISA

Blood meal sources of *An. arabiensis*, *An. stephensi,* and *An. funestus* group were investigated by conducting blood meal direct ELISA as described in Beier et al. (1988). The tests were conducted to identify human, bovine, goat, dog and mixed blood meals.

2.9 Insecticide Resistance Monitoring and Mechanism of Resistance

Insecticide susceptibility tests using 1X concentration, resistance intensity assays using 5X and 10X concentrations, and piperonyl butoxide (PBO) synergist assays were conducted in populations of *An. arabiensis* located in 11 sentinel sites in eight regions: Amibara (Afar), Jabitehnan (Amhara), Metema (Amhara), Bambasi (Benishangul-Gumuz), Abobo (Gambela), Abaya (Oromia), Omonada (Oromia), Benatsemay (SNNPR), Misrak Badawacho (SNNPR), Erer (Somali), and Humera (Tigray).

The susceptibility status of *An. stephensi* to the insecticides listed below (Section 2.9.1) from Awash, Metehara, Meki, and Goday were investigated. In addition, chlorfenapyr was tested in the population of *An. stephensi* from Semera Town.

2.9.1 Insecticide Susceptibility Tests

The response of populations of *An. arabiensis* and *An. stephensi* to 0.1% bendiocarb, 0.1% propoxur, 0.25% pirimiphos-methyl, 0.05% alpha-cypermethrin, 0.05% deltamethrin, and 0.75% permethrin was assessed using the World Health Organization (WHO) tube test described in PMI VectorLink Project SOP #6. All insecticide-impregnated papers were obtained from the Universiti Sains Malaysia (Science University of Malaysia). The WHO tube test method was also used to test 2% clothianidin impregnated papers received from Sumitomo Chemicals. Clothianidin tests against *An. arabiensis* were conducted in six sentinel sites, Abobo, Amibara, Omonada, Bambasi, Jabitehnan, and Benatsemay.

Chlorfenapyr susceptibility tests were conducted by impregnating bottles at the concentration of 100µg/bottle following the CDC bottle bioassay method (SOP #4). Bottles were impregnated by VectorLink staff. Populations of *An. arabiensis* from Abaya, Amibara, Bambasi, and Metema as well as *An. stephensi* from Awash and Semera towns were tested against chlorfenapyr.

Larvae and pupae were collected from breeding habitats and raised to adults. All tests including susceptibility to diagnostic concentration, resistance intensity, and PBO synergist were conducted on 2–5-day-old *An. arabiensis* and *An. stephensi.*

The criteria in SOP #6 were used to interpret results obtained from the susceptibility tests, resistance intensity assays, and PBO synergist assays. Abbott's formula was applied to any instances of control mortality between 5 and 20%.

2.9.2 Resistance Intensity Assays

Resistance intensity to the pyrethroid insecticides (alpha-cypermethrin, deltamethrin, and permethrin) at the concentrations of 1X, 5X, and 10X was assessed on seven populations of *An. arabiensis* from Abaya, Abobo, Amibara, Omonada, Bambasi, Jabitehnan, and Benatsemay following the method described in PMI VectorLink SOP #6. The intensity of resistance in *An. stephensi* was measured from Awash, Meki, Metehara, and Goday.

2.9.3 PBO Synergist Assays

The response of *An. arabiensis* to the pyrethroid insecticides (alpha-cypermethrin, deltamethrin, and permethrin) after pre-exposure to 4% PBO impregnated papers was evaluated from ten sentinel sites, Amibara, Bambasi, Abobo, Abaya, Omonada, Benatsemay, Erer, Humera, Metema, and Misrak Badawacho. PBO synergist assays to the same pyrethroids were also conducted on *An. stephensi* from Awash, Meki, Metehara, and Goday.

2.9.4 Temephos Susceptibility Tests

The susceptibility status of the larval stage of *An. stephensi* to the larvicide temephos was assessed from Awash, Semera, Dire Dawa, and Kebridehar using the WHO bioassay kit supplied by Universiti Sains Malaysia and following the methods of WHO (1981, 2005). The kit contains four concentrations of temephos in alcohol and a control with alcohol only. The four concentrations are 1.25mg/L, 6.25mg/L, 31.25mg/L, and 156.25mg/L. Preliminary tests on each of the concentrations was conducted in two to four replicates using third and early fourth instars. The concentration that was found to kill 100% of larvae was further subjected to serial dilution to compute the LD_{50} (the dose that kills 50% of test larvae) and LD_{95} (the dose that kills 95% of larvae). Larvae were tested by transferring 249ml of water in a 500ml capacity beaker and by adding 1ml of temephos. Larvae were also tested at the WHO discriminating concentration of 0.25mg/L.

2.10 Entomological Assessment of Quality and Residual Efficacy of IRS Insecticides

The quality assurance and residual efficacy of Actellic 300CS was evaluated in the VectorLink Ethiopia sentinel sites. A pilot assessment on the residual efficacy of SumiShield and Fludora Fusion was undertaken to generate data for future use of these products for IRS both by the project and NMEP. The WHO cone bioassay tests were conducted using insectary *An. arabiensis* and employing the protocol of PMI VectorLink SOP #9. In addition, the fumigant effect of the insecticides on mortality of test mosquitoes was assessed.

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

The spray quality assessment and residual efficacy of Actellic 300CS was assessed in Abaya, Bambasi, Menge, and Lare. In each sentinel site, 12 houses were equally divided between two kebeles and were randomly selected based on the available surface types. In Abaya, the two kebeles were Samaro and Guangua; the wall surfaces of the houses were dung, mud, cemented mud, painted mud, and painted cement. In Bambasi, the bioassays were conducted in the kebeles of Keshmando 1 and Keshmando 2, where the surface types were mud and painted mud. Kuayu kebele from Menge was selected and the wall surface types were mud, painted mud, and cemented mud. All the houses in Lare (Bullimkun and Kuregegn kebeles) were made of mud.

In each house, three cones were fixed at heights of 0.5 m (low), 1.0 m (middle), and 1.5 m (high) from the floor and at least ten *An. arabiensis* were introduced into each cone. The control consisted of ten mosquitos and was exposed in insecticide-free houses or on the exterior wall of sprayed houses on a board covered by white paper. Mortality was recorded after 24 hours and tests were conducted monthly until mortality of mosquitoes went down to 80% for two consecutive months.

2.10.2 Entomological Assessment of Quality and Residual Efficacy of SumiShield and Fludora Fusion

The persistence of SumiShield was evaluated in Bane Shegole kebele and of Fludora Fusion in Belmuga kebele, both in Menge District, in the same number of houses and on the same wall surface types as those conducted

with Actellic 300CS. Insectary *An. arabiensis* were used for the bioassays. In addition, Fludora Fusion-sprayed houses were tested after the third month of spraying using wild *An. gambiae* s.l. raised from larvae. Mosquito mortality was recorded every 24 hours until the fifth day according to the protocol of SOP #9. When necessary, observed mortality was corrected using Abbott's formula. This pilot study was conducted from June 2020 to February 2021.

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

Fumigation bioassays were conducted side by side with cone bioassays following project protocol. Ten insectary *An. arabiensis* in a small cage were suspended in each of Actellic 300CS-, SumiShield-, and Fludora Fusion-sprayed houses. The same number of mosquitoes were tested in insecticide-free houses. Cages were placed either on a chair, table, or other available surface at the height of 1m from the floor and 10cm from the wall. Wild *An. gambiae* s.l. reared from larvae were also used to test Fludora Fusion-sprayed houses after three months of spraying to observe the impact of deltamethrin. Mortality was recorded every 24 hours for up to five days.

3. Results

3.1 Species Composition, Abundance, and Density

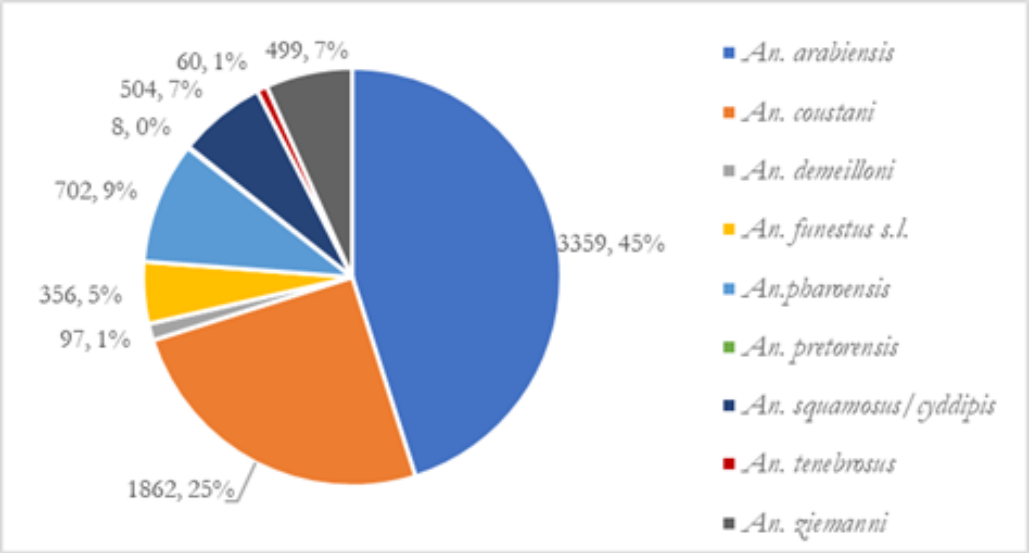
This section presents findings on species composition, abundance, and density from the seven sentinel sites together as well from each site. It also treats species by method of collection, trap density, indoor density, abdominal blood feeding stages from CDC light trap collections and PSCs.

3.1.1 Species Composition, Abundance, and Density from All Seven Sentinel Sites

A total of 7,447 *An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. funestus* s.l., *An. pharoensis*, *An. pretoriensis*, *An. squamosus/cydippis*, *An. tenebrosus*, and *An. ziemanni* were collected from all seven sites using CDC light traps and PSCs. *Anopheles arabiensis* was the predominant species, comprising 45% (n=3,359) of all collections. It was followed by *An. coustani* at 25% (n=1,862); all remaining *Anopheles* comprised 30% (n=2,226) (Figure 3). *Anopheles arabiensis* was identified from molecular analysis of specimens morphologically found to be *An. gambiae* s.l. (Section 3.7.1).

Anopheles arabiensis is the principal malaria vector in Ethiopia, while *An. pharoensis* and *An. funestus* s.s. are secondary vectors. The role of the remining *Anopheles* remains unclear, particularly *An. coustani*, *An. tenebrosus,* a *An. ziemanni* and *An. stephensi. An. coustani* is the main vector of malaria in one village in Madagascar (Goupeyou-Youmsi et al. 2020), and in a review on the secondary malaria vectors in sub-Saharan Africa, Afrane et al. (2016) presented the significance of *An. coustani* and *An. ziemanni* as secondary vectors of malaria in several countries. *An. coustani* is suspected to transmit malaria in Ethiopia but more data is needed on its anthropophagic behavior, sporozoite infection rates, and role in malaria transmission supported by epidemiological studies as has been shown in the Madagascar study (Goupeyou-Youmsi et al. 2020).

FIGURE 3. COMPOSITION OF *ANOPHELES* SPECIES FROM ALL SEVEN SITES (JULY 2020-MARCH 2021)



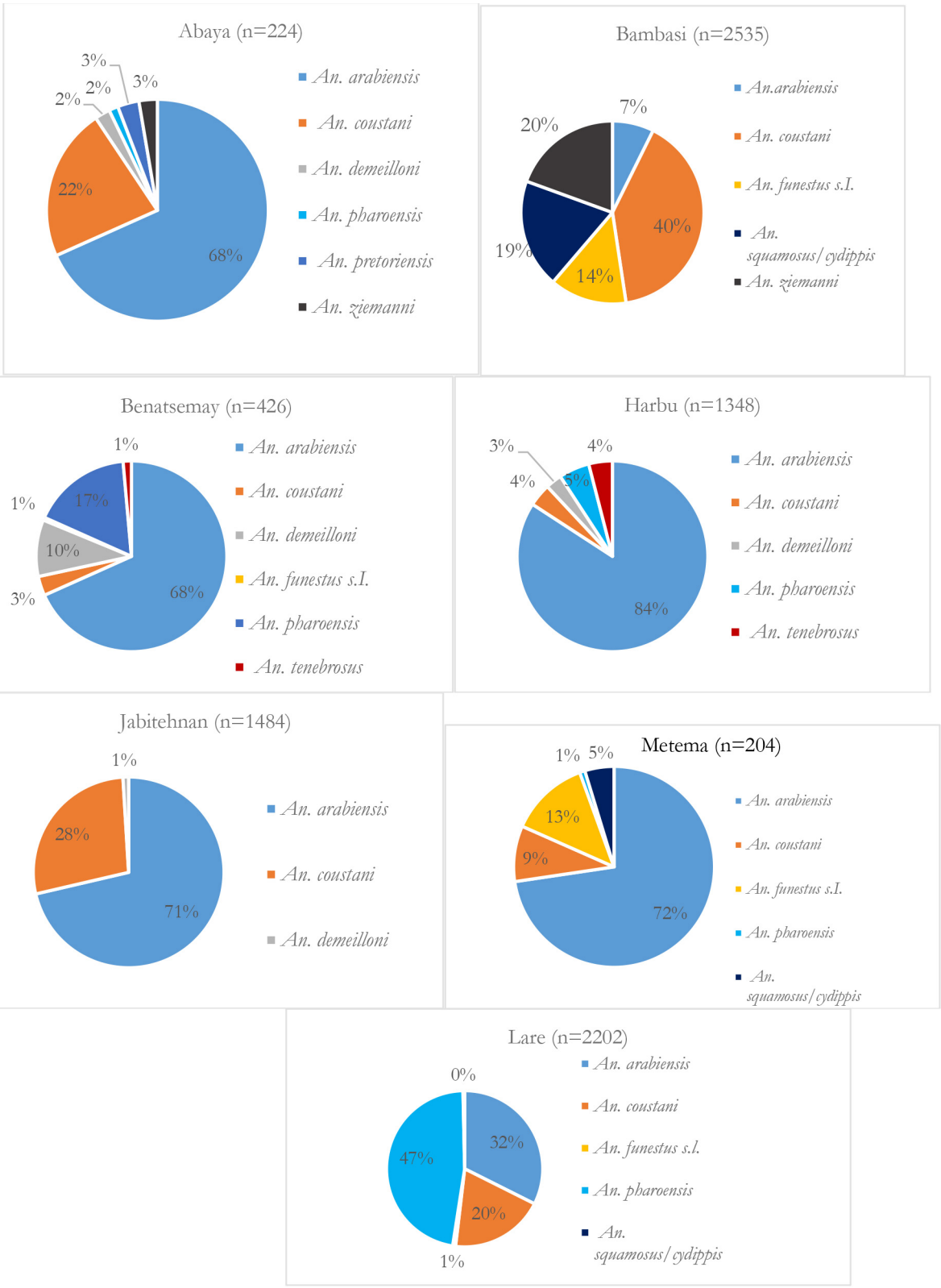
3.1.2 SPECIES COMPOSITION AND ABUNDANCE BY SENTINEL SITE

Anopheles arabiensis is dominant in five of the seven sentinel sites: Abaya (n=153, 68%), Benatsemay (n=291, 68%), Harbu (n=1134, 84%), Jabitehnan (n=1044; 71%), and Metema (n=170, 72%). The most abundant species in Bambasi and Lare were *An. coustani* (n=1021, 40%) and *An. pharoensis* (n=555, 47%), respectively. The number of *An. arabiensis* caught in Bambasi was 186, which was 7% of all mosquitoes collected there. This implies that, in Bambasi, CDC light traps were less efficient at catching *An. arabiensis* as compared to other *Anopheles* such as *An. ziemanni* (n=493, 20%), *An. squamosus/cydippis* (n=489, 19%), and the *An. funestus* group (n=346, 14%). *Anopheles pharoensis* (n=555, 47%) and *An. arabiensis* (n=381, 32%) were the first and second most abundant species in Lare. (Figure 4).

The diversity of *Anopheles* varies from site to site. The second most common species in all the sites together is *An. coustani*. *An. demeilloni* was found in Abaya, Benatsemay, Harbu, and Jabitehnan, and *An. funestus* group in Bambasi, Benatsemay, Metema, and Lare. The distribution of *An. pharoensis* was limited to Abaya, Benatsemay, Harbu, Metema, and Lare. *An. squamosus/cydippis* was found in Bambasi, Metema, and Lare; *An. tenebrosus* in Harbu and Benatsemay; and *An. ziemanni* in Abaya and Bambasi (Figure 4).

The monthly species composition and abundance is presented in Annex A.

FIGURE 4. COMPOSITION OF *ANOPHELES*, BY SENTINEL SITE (JULY 2020-MARCH 2021)



3.1.3 *Anopheles arabiensis* by Method of Collection

Anopheles arabiensis was captured from CDC light traps indoors and outdoors as well as PSCs, but the number and proportion differ by sentinel site. The indoor CDC light trap (ILT) collection was greater than the outdoor (OLT) in Abaya (n=90 vs. 46) and Bambasi (n=101 vs 70) whereas the OLT collection was greater than the ILT in Harbu (n=583 vs 477), Jabitehnan (n=384 vs 560), Metema (n=113 vs 50), and Lare (n=218 vs 128). The only site where PSC collections were more than the CDC light traps was Benatsemay, where PSCs yielded 68% (n=198) of mosquitoes collected (Table 3).

Overall, 49%, 38%, and 13% of all *An. arabiensis* were collected from OLT, ILT, and PSC collections, respectively. The ratio of *An. arabiensis* collected outdoors to indoors was 1.3:1 (Table 3).

Table 3. Proportion of *An. arabiensis* by Method of Collection (July 2020-March 2021)

Site	Number (%)			
	ILT	OLT	PSC	Total
Abaya	90 (59)	46 (30)	17 (11)	153 (100)
Bambasi	101 (54)	70 (38)	15 (8)	186 (100)
Benatsemay	40 (14)	53 (18)	198 (68)	291 (100)
Harbu	477 (42)	583 (51)	74 (7)	1134 (100)
Jabitehnan	384 (36.8)	560 (53.6)	100 (9.6)	1044 (100)
Metema	50 (29)	113 (67)	7 (4)	170 (100)
Lare	128 (34)	218 (57)	35 (9)	381 (100)
Overall	1270 (38)	1643 (49)	446 (13)	3359 (100)
Ratio of OLT to ILT is 1.3:1 (1643/1270)				

3.1.4 Proportion of Abdominal Feeding Stages of *An. arabiensis* from CDC Light Trap Collections

The number and percentage composition of the four abdominal stages of *An. arabiensis* from CDC light trap collections indoors and outdoors from all the seven sentinel sites is presented in Table 4. The total number of unfed mosquitoes was more than the total collection of fed mosquitoes, accounting for 76.6 % (n=969) indoors and 72.1% (n=942) outdoors. The second highest number was of blood feds, accounting for 18.7% (n=237) indoors and 22.2% (n=290) outdoors. The number of the half gravids and gravids in all the sentinel sites was small, and some sites had none. In six of the seven sites, the number and proportion of the unfed group indoors was similar to that of the outdoors, but in Harbu the indoor unfed group (n=310, 65%) was more than the outdoor (n=91, 39.2%).

Table 4. Proportion of Abdominal Feeding Stages of *An. arabiensis* from CDC Light Trap Collections (July 2020-March 2021)

Location	Abdominal feeding stage	Abaya	Bambasi	Benatsemay	Harbu	Jabitehnan	Metema	Lare	Overall
Indoor	UF (%)	75 (84.3)	100 (100)	26 (65)	310 (65.0)	306 (79.7)	28 (54.9)	124 (100)	969 (76.6)
	F (%)	10 (11.2)	0 (0)	14 (35)	123 (25.8)	77 (20.1)	13 (25.5)	0 (0)	237 (18.7)
	HG (%)	0 (0)	0 (0)	0 (0)	32 (6.7)	0 (0)	5 (9.8)	0 (0)	37 (2.9)
	G (%)	4 (4.5)	0 (0)	0 (0)	12 (2.5)	1 (0.2)	5 (9.8)	0 (0)	22 (1.8)
	Total	**89 (100)**	**100 (100)**	**40 (100)**	**477 (100)**	**384 (100)**	**51 (100)**	**124 (100)**	**1265 (100)**
Outdoor	UF (%)	39 (83.0)	69 (98.6)	30 (63.8)	91 (39.2)	435 (75)	66 (58.4)	212 (97.7)	942 (72.1)

F (%)	0 (0)	1 (1.4)	17 (36.2)	113 (48.7)	144 (24.8)	10 (8.8)	5 (2.3)	290 (22.2)
HG (%)	0 (0)	0 (0)	0 (0)	15 (6.5)	0 (0)	15 (13.3)	0 (0)	30 (2.3)
G (%)	8 (17.0)	0 (0)	0 (0)	13 (5.6)	1 (0.2)	22 (19.5)	0 (0)	44 (3.4)
Total	**47 (100)**	**70 (100)**	**47 (100)**	**232 (100)**	**580 (100)**	**113 (100)**	**217 (100)**	**1306 (100)**

3.1.5 Density of *An. arabiensis* from CDC Light Traps

This section discusses the trap density of *An. arabiensis* in the PMI VectorLink Project sites (Abaya, Bambasi, and Lare) and non-project sites (Benatsemay, Harbu, Jabitehnan, and Metema).

PMI VectorLink IRS Sites

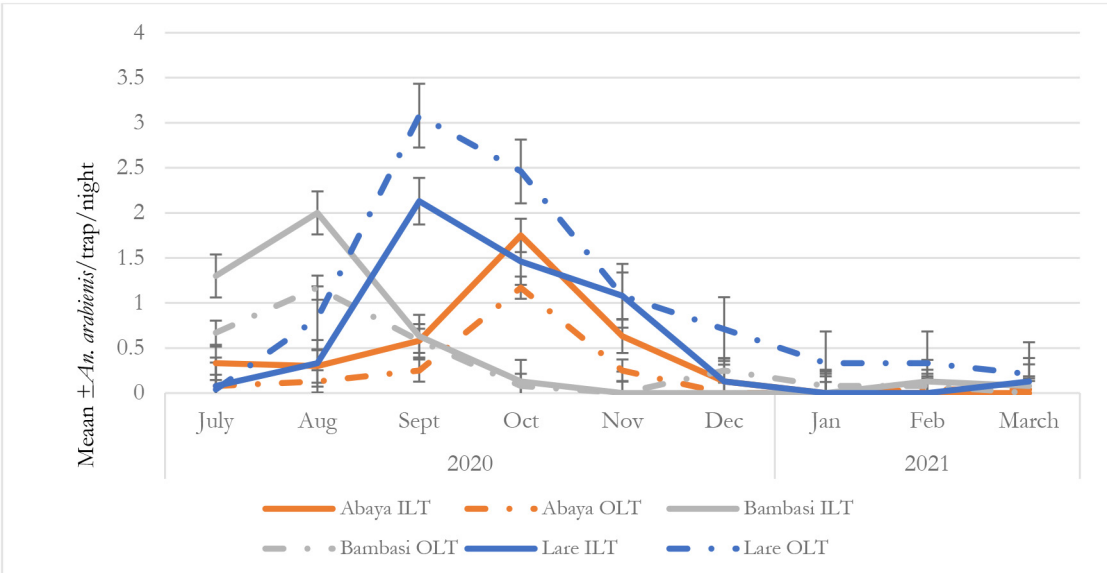
The trap density of *An. arabiensis* in Abaya was less than 1.0 *An. arabiensis*/trap/night in all months except October, when it was close to 1.8 *An. arabiensis*/trap/night indoors and 1.2 *An. arabiensis*/trap/night outdoors (Figure 5).

In Bambasi, density was less than 1.0 *An. arabiensis*/trap/night except indoors in July (1.3 *An. arabiensis*/trap/night) and August (2.0 *An. arabiensis*/trap/night), and outdoors in August (1.2 *An. arabiensis*/trap/night) (Figure 5).

In Lare, the peak density was in September, with 2.3 *An. arabiensis*/trap/night indoors and 3.0 *An. arabiensis*/trap/night outdoors. Another peak was in October when the indoor density was 1.5 *An. arabiensis*/trap/night and the outdoor 2.5 *An. arabiensis*/trap/night (Figure 5).

In general, the trap density was 0-3 *An. arabiensis*/trap/night, mostly falling below 1.0 *An. arabiensis*/trap/night, in these three sites, all of which were sprayed with Actellic 300CS during the IRS campaign.

Figure 5. Indoor and Outdoor CDC Light Trap Density of *An. arabiensis* in the PMI VectorLink IRS Sites (July 2020-March 2021)



PMI VectorLink non-IRS sites

Among the four sites that did not receive PMI-supported IRS, Metema received government-supported IRS with propoxur in 2020, and Benatsemay, Harbu, and parts of Jabitehnan did not receive any IRS in 2020.

The trap density of *An. arabiensis* in Benatsemay, both indoors and outdoors, was very low (less than 1.0), the only exceptions being in July and August when the indoor density was 0.7 *An. arabiensis*/trap/night. The outdoor density in July was the same as the indoor density, but the outdoor density in August was close to 0.9 *An. arabiensis*/trap/night (Figure 6).

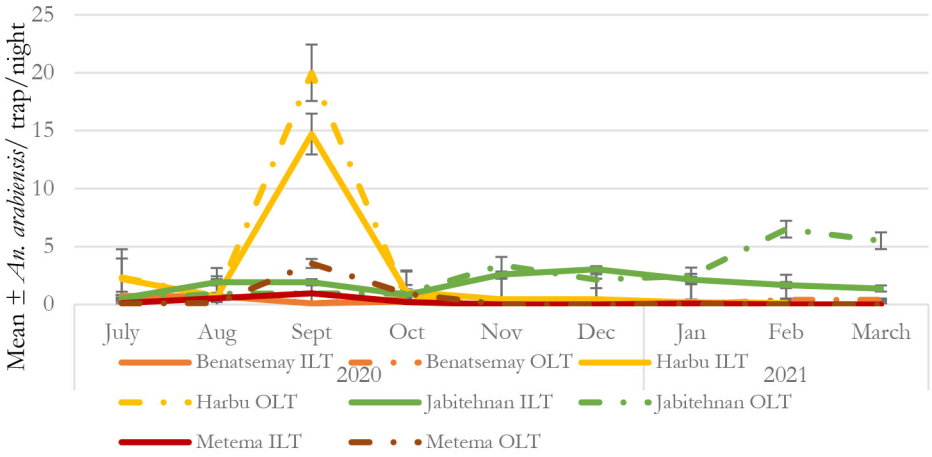
The highest trap density in Harbu, 14.7 *An. arabiensis*/trap/night indoors and 20.0 *An. arabiensis*/trap/night outdoors, was in September. The second highest was in July, when 2.2 *An. arabiensis*/trap/night indoors and 2.3 *An. arabiensis*/trap/night outdoors were recorded. In the other months, less than 1.0 *An. arabiensis*/trap/night was recorded (Figure 6).

In Jabitehnan, the largest outdoor density was 6.5 and 5.5 *An. arabiensis*/trap/night in February and March 2021, respectively. The other peak outdoor density was in November, at 3.4 *An. arabiensis*/trap/night. In the same location in January and December, the respective densities were 2.5 and 2.1 *An. arabiensis*/trap/night. On the other hand, nearly 2.0 *An. arabiensis*/trap/night density were caught indoors in August and September, and more than or equal to 3.0 *An. arabiensis*/trap/night in November and January (Figure 6).

In Metema, the density was less than 1.0 *An. arabiensis*/trap/night indoors and outdoors in most months with the one exception in September, at 3.5 *An. arabiensis*/trap/night outdoors (Figure 6).

In summary, trap density was highest in Harbu and Jabitehnan, and this could be related to absence of IRS. There was no IRS in Benatsemay, and yet the density was low; the reason for this remains to be determined. Is there a strain difference or could it be the interruption of sampling in November because of security? This needs further investigation.

FIGURE 6. CDC TRAP DENSITY OF *AN. ARABIENSIS* IN PMI VECTORLINK NON-IRS SITES (JULY 2020-MARCH 2021)



Note: Bars represent standard errors. There was no IRS in Benatsemay, Harbu, and Jabitehnan; IRS was conducted in Metema in September.

3.1.6 Density of *An. pharoensis* from CDC Light Traps in Lare

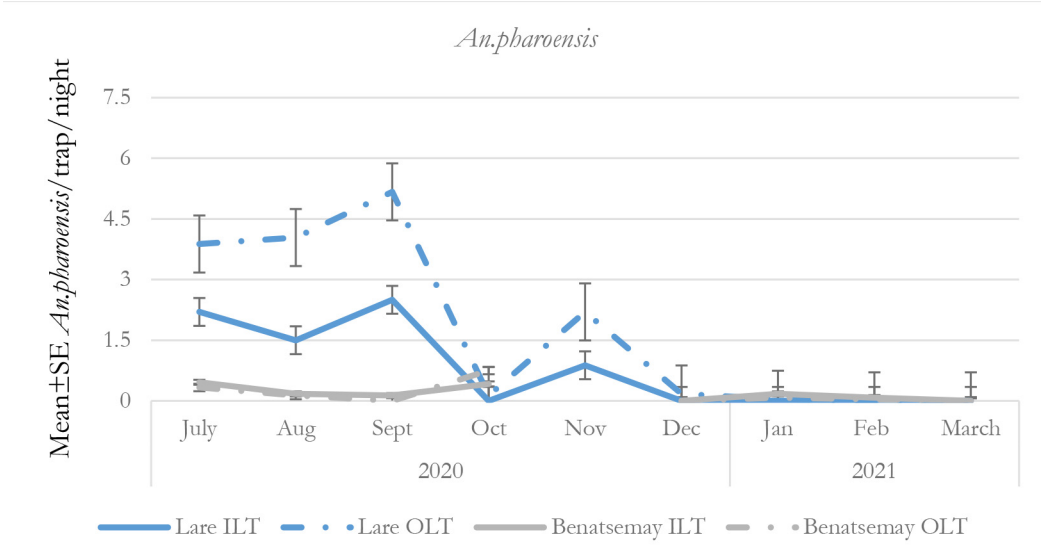
An. pharoensis was prevalent in Lare from July 2020 to January 2021, but it was absent in the indoor collections from December 2020 to March 2021. The ILTs and OLTs caught 170 (31.2%) and 375 (68.8%) *An. pharoensis,* respectively (Figure 7).

ILT collection peaked in July (2.2 *An. pharoensis*/trap/night) and September (2.5 *An. pharoensis*/trap/night), and outdoor peaks were in July (3.9 *An. pharoensis*/trap/night), August (4 *An. pharoensis*/trap/night), and September (5.2 *An. pharoensis*/trap/night).

The ratio of outdoor to indoor collection of *An. pharoensis* was 2.2:1 (375/170), suggesting a tendency to feed more outdoors than indoors.

Almost equal number of *An. pharoensis* were trapped indoors (n=34) and outdoors (n=32) in Benatsemay. This species was not found in traps in December and March, either indoors or outdoors, or in September outdoors. The indoor peak was in July with 0.5 *An. pharoensis*/trap/night, and the outdoor peak was in October with 0.8 *An. pharoensis* /trap/night (Figure 7).

Figure 7. CDC Light Trap Density of *An. pharoensis* from Lare and Benatsemay (July 2020-March 2021)

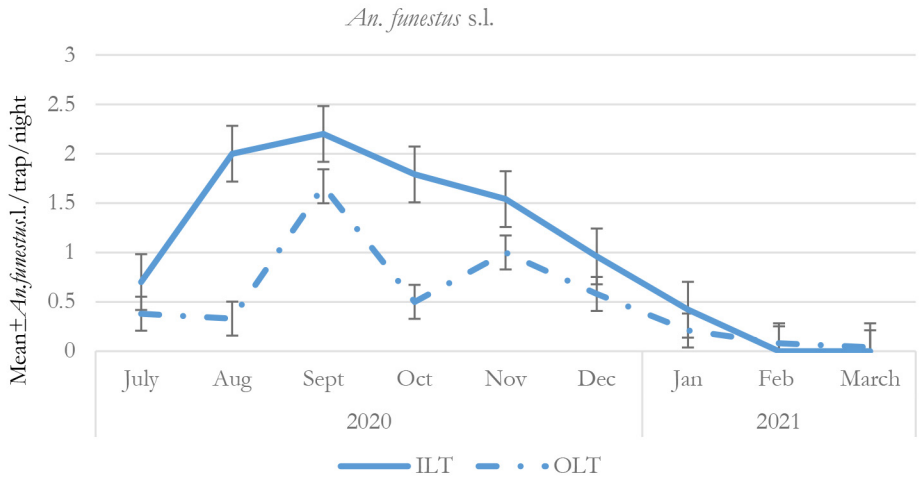


Note: Bars represent standard errors.

3.1.7 Density of *An. funestus* Group from CDC Light Traps from Bambasi

The total number of *An. funestus* s.l. collected from CDC light traps in Bambasi was 231 (69.2%) indoors and 103 (30.8%) outdoors. This species was found in traps in all months except in February and March 2021, when none were caught indoors but caught outdoors. The trap density indoors was higher in August (2.0 *An. funestus* s.l./trap/night) and September (2.2 *An. funestus* s.l./trap/night) than in the other months. Outdoors, two peaks were observed, in September (1.7 *An. funestus* s.l./trap/night) and November (1.0 *An. funestus* s.l./trap/night) (Figure 8).

FIGURE 8. CDC LIGHT TRAP DENSITY OF *AN. FUNESTUS* S.L. FROM BAMBASI (JULY 2020-MARCH 2021)



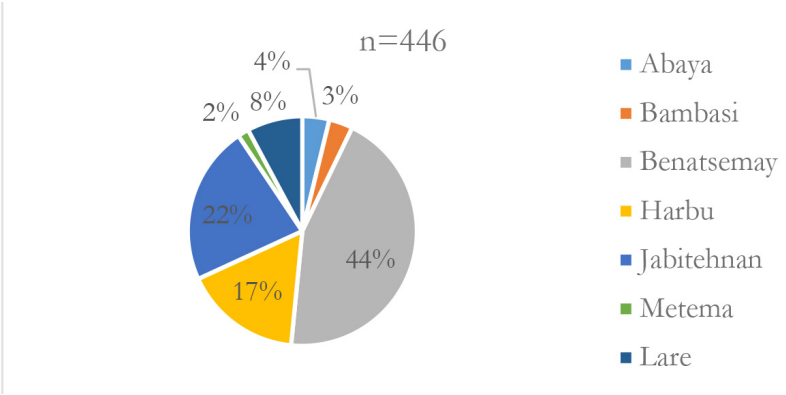
Note: Bars represent standard errors.

3.1.8 INDOOR RESTING PROPORTION OF *AN. ARABIENSIS* DETERMINED FROM PSC

A total of 446 *An. arabiensis* were sampled from PSC houses; 44% of the *An. arabiensis* were from Benatsemay, the highest indoor proportion of all the sentinel sites. The second and the third largest proportions were from Jabitehnan (22%) and Harbu (17%). The remaining sites had 2–8% of *An. arabiensis* (Figure 9).

In general, the indoor resting proportion of *An. arabiensis* was very low in the PMI VectorLink IRS sites (Abaya, Bambasi, and Lare). There was no IRS in Benatsemay, Harbu, and Jabitehnan, and the high proportion of *An. arabiensis* might be associated with this.

FIGURE 9.DISTRIBUTION OF *AN. ARABIENSIS* COLLECTED BY PSC ACROSS SEVEN SITES.



3.1.9 ABDOMINAL BLOOD FEEDING STAGES OF *AN. ARABIENSIS* FROM PSC

Of all four blood feeding stages (unfed, fed, half gravid, and gravid), most mosquitoes were in the unfed and fed groups. The proportion of unfed *An. arabiensis* collected by PSC was greater than the rest of the blood feeding stages combined in Bambasi (n=15, 100%), Jabitehnan (n=65, 66.3%), and Lare (n=24, 68.6%). In contrast, the blood feds were dominant in Benatsemay (n=146, 74.2%) and Harbu (n=55, 74.3%). Overall, the unfeds accounted for 35% (n=159) and the feds for 57.5% (n=57.5) of all collections (Table 5).

TABLE 5. ABDOMINAL FEEDING STATUS OF *AN. ARABIENSIS* (MAY 2020-MARCH 2021

Site	Unfed N (%)	Fed N (%)	Half Gravid N (%)	Gravid N (%)	Total
Abaya	6 (33.3)	8 (44.4)	3 (16.7)	1 (5.6)	18 (100)
Bambasi	15 (100)	0 (0)	0 (0)	0 (0)	15 (100)
Benatsemay	45 (22.8)	146 (74.2)	3 (1.5)	3 (1.5)	197 (100)
Harbu	1 (1.4)	55 (74.3)	15 (20.3)	3 (4.0)	74 (100)
Jabitehnan	65 (66.3)	33 (33.7)	0 (0)	0 (0)	98 (100)
Lare	24 (68.6)	10 (28.6)	1(2.8)	0 (0)	35 (100)
Metema	3 (42.9)	3 (42.9)	1 (14.2)	0 (0)	7 (100)
Overall	159 (35.8)	255 (57.5)	23 (5.2)	7 (1.6)	444 (100)

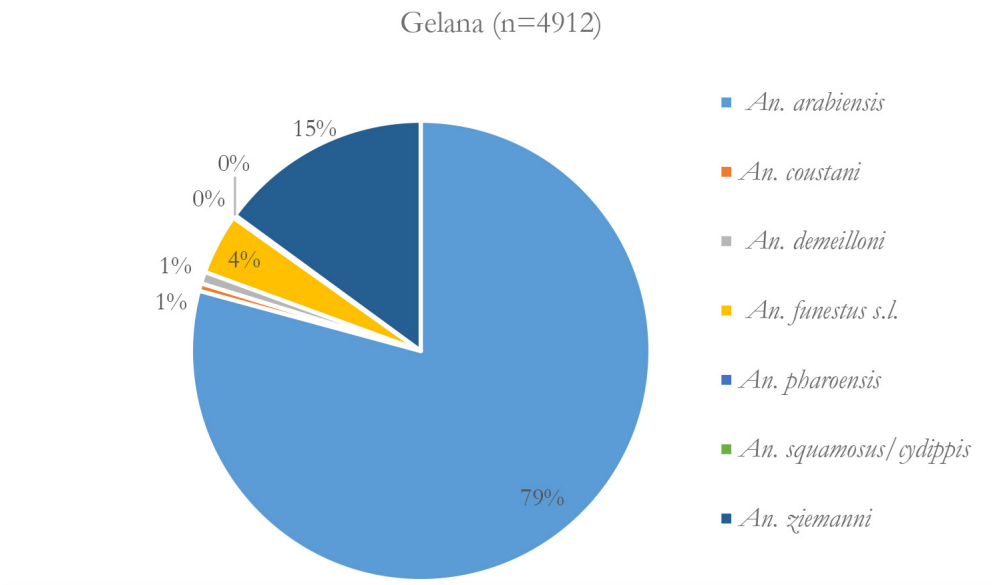
3.2 COMMUNITY MOSQUITO COLLECTIONS FROM GELANA DISTRICT

This section presents data from Gelana District collections on the species composition and abundance of *Anopheles* mosquitoes, the monthly CDC light trap density of *An. arabiensis,* and the proportion of abdominal stages of the same species.

3.2.1 SPECIES COMPOSITION AND ABUNDANCE

A total of 4,912 *Anopheles* mosquitoes belonging to at least seven species including *An. arabiensis*, *An. coustani*, *An. demeilloni*, *An. funestus* group, *An. pharoensis*, *An. squamosus/cydippis,* and *An. ziemanni* were collected from four kebeles in Gelana. *An. arabiensis* was the predominant species, accounting for 79% (n=3,891) of mosquitoes collected. The second and third largest numbers were of *An. ziemanni* (735, 15%) and *An. funestus* group (209, 4%). The remaining species were 6% of all collections (Figure 10).

FIGURE 10. SPECIES COMPOSITION OF *ANOPHELES* SPECIES FROM GELANA DISTRICT (2020-21)

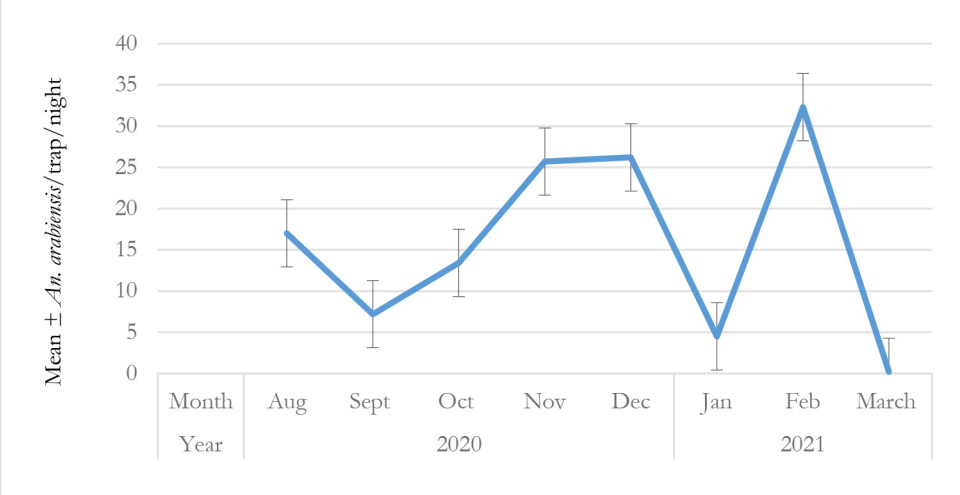


3.2.2 Monthly CDC Light Trap Density of *An. arabiensis*

Anopheles arabiensis was collected throughout the entomological monitoring period from August 2020 to March 2021 with variable monthly CDC light trap density expressed as mean number of *An. arabiensis*/trap/night from 40 trap-nights per month. The mean trap density peaked in August (17.0 *An. arabiensis*/trap/night), November (25.7 *An. arabiensis*/trap/night), December (26.2 *An. arabiensis*/trap/night), and February (32.3 *An. arabiensis*/trap/night). The lowest density was in September (7.2 *An. arabiensis*/trap/night) followed by January (4.5 *An. arabiensis*/trap/night) (Figure 11).

Gelana is one of the districts known to have perennial malaria transmission, and this might be linked to the availability of abundant potential vectors, adequate breeding habitats, and poor house construction, as well as the tendency among residents to sleep outdoors. A thorough entomological and epidemiological study is required to comprehend the contribution of each factor in the transmission of malaria.

Figure 11. CDC Trap Density of *An. arabiensis* from Gelana District (August 2020-March 2021)



Note: Bars are error bars. IRS in Gelana was conducted in June 2020.

3.2.3 Abdominal Feeding Stages of *An. arabiensis* and *An. funestus* s.l. from CDC Light Trap Collections

The majority of *An. arabiensis* (89.7%) trapped in CDC light traps in Gelana District were unfed, while 10.2% were blood fed. Close to 94% of *An. funestus* s. l. were unfed and 6.0% were blood fed (Table 6).

Table 6. Proportion of Abdominal Feeding Stages of *An. arabiensis* and *An. funestus* s.l. from CDC Light Trap Collections in Gelana District (August 2020-March 2021)

Species	Unfed N (%)	Fed N (%)	Half Gravid N (%)	Gravid N (%)	Total
An. arabiensis	3487 (89.7)	400 (10.2)	1 (0.05)	1 (0.05)	3889 (100)
An. funestus s.l.	196 (93.8)	13 (6.2)	0	0	209 (100)

3.3 Abundance of *An. stephensi* from Monthly Entomological Monitoring

A total of 626 adult *An. stephensi* were collected from Awash (n=89, 14.2%), Metehara (n=13, 2.1%), Dire Dawa (n=149, 23.8%), and Kebridehar (n=375, 59.9%) (Table 7). The monthly abundance by site and method of collection is presented in Annex B.

The highest proportion of collections were from Prokopack/backpack aspirations from animal shelters (73.0%) followed by animal-baited tent traps (17.6%). PSC and CDC light trap collections were 8.0% and 1.4%, respectively, of all collections. No *An. stephensi* were collected from black box or clay pot methods (Table 7).

TABLE 7. ABUNDANCE OF *AN. STEPHENSI* BY COLLECTION METHOD (JULY 2020-APRIL 2021)

Site	# *An. stephensi* collected (%)						Total	Proportion from total collected (%)
	PSC	CDC	Prokopack/ Backpack	Black box	Clay pot	Cattle baited tent trap		
Awash	0	0	64 (71.9)	0	0	25 (28.1)	89 (100)	89 (14.2)
Metehara	2 (15.4)	1 (7.6)	5 (38.5)	0	0	5 (38.5)	13 (100)	13 (2.1)
Dire Dawa	8 (5.4)	0	97 (65.1)	0	0	44 (29.5)	149 (100)	149 (23.8)
Kebridehar	40 (10.7)	8 (2.1)	291 (77.6)	0	0	36 (9.6)	375 (100)	375 (59.9)
Overall	50 (8.0)	9 (1.4)	457 (73.0)	0	0	110 (17.6)	626 (100)	626 (100)

3.4 PRESENCE/ABSENCE OF *AN. STEPHENSI* IN RURAL PARTS OF EASTERN ETHIOPIA

A total of 48 rural kebeles within a 20 km radius of the nearest town were visited. Out of the 48 rural kebeles surveyed, 21 kebeles were positive for *An. stephensi,* increasing the total number sites positive for *An. stephensi* to 35 (14 urban and 21 rural sites). A total of 589 larval breeding habitats were inspected, out of which 44 were positive for *An. stephensi* larvae(Table 8).

TABLE 8. RESULTS OF *AN. STEPHENSI* CROSS-SECTIONAL SURVEYS IN EASTERN ETHIOPIA (2020)

Nearest town	Number of rural kebeles visited	Number of larval breeding sites inspected	Number of breeding sites positive for *An. stephensi* larvae	Number of positive kebeles
Semera	5	136	3	2
Gewane	4	127	10	3
Bati	7	165	6	3
Metehara	3	12	1	1
Kebridehar	8	40	13	6
Meki	1	17	0	0
Zeway	1	16	0	0
Dire Dawa	6	17	2	2
Awash	1	3	1	1
Goday	6	24	1	1
Degehabur	6	32	7	2
Total	**48**	**589**	**44**	**21**

As in the urban sites, *An. stephensi* in rural areas were found to breed in artificial containers such as concrete cisterns, *birkas*, plastic sheets, tires, carwashes, and small/broken discarded materials. Puddles in two sites near Dire Dawa City were positive, but few adults emerged from the larvae (Table 9).

In total, 3,158 *Anopheles* larvae were sampled from *An. stephensi* breeding habitats, and 684 of the resulting emerged adults were identified as *An. stephensi*. No *An. stephensi* were collected from the rural villages close to Meki and Zeway towns (Table 9).

TABLE 9. NUMBER OF *ANOPHELES* LARVAE AND ADULTS OF *AN. STEPHENSI* IDENTIFIED FROM REARED LARVAE (2020)

Nearest town	Number of *Anopheles* larvae	Number of adult *An. stephensi* identified from reared larvae
Semera	285	20
Gewane	657	94
Bati	296	53
Metehara	50	17
Kebridehar	751	227
Meki	0	0
Zeway	0	0
Dire Dawa	53	2
Awash	125	5
Goday	200	60
Degehabur	741	206
Total	3158	684

The diverse data generated from the larval investigation including type and number of breeding habitats for larvae of *Anopheles*, *Aedes*, and *Culex*, larval breeding habitats shared by *An. stephensi* and other mosquito species has been provided as a supplementary to the recently published article by Balkew and colleagues (2021). The supplementary data in the manuscript also gives GPS coordinates of the larval breeding habitats and contains a distribution map of the rural areas newly found to be positive for *An. stephensi*.

3.5 EVALUATION OF *AN. STEPHENSI* LARVAL INDICES

In this section, results on the types of breeding habitats, larval abundance, number of *An. stephensi* identified from rearing of larvae, monthly RBI, HBI, and larval density are presented and discussed.

3.5.1 TYPES OF LARVAL BREEDING HABITATS AND ABUNDANCE OF *AN. STEPHENSI*

The four months (November 2020 to February 2021) of larval indices evaluation in Awash, Semera, Dire Dawa, and Kebridehar found ten types of breeding habitats of *An. stephensi* larvae: cemented cisterns (in all sites), *birkas* (Kebridehar), water tank (Awash and Semera), water drums (all sites), plastic sheets (Semera and Dire Dawa), tires (Awash and Kebridehar), discarded plastic bottles (Semera), open pipe tubes (Semera), carwashes (Dire Dawa), and stream banks (Dire Dawa). The most numerous habitats were cisterns, although the number varied from month to month. The number of positive cisterns ranged from 13 to 19 per month over the study period in Awash, from 9 to 18 in Semera, from 14 to 22 in Dire Dawa, and from 34 to 58 in Kebridehar (Table 10).

TABLE 10. COMMON BREEDING HABITATS OF *AN. STEPHENSI* LARVAE AND ABUNDANCE (2020)

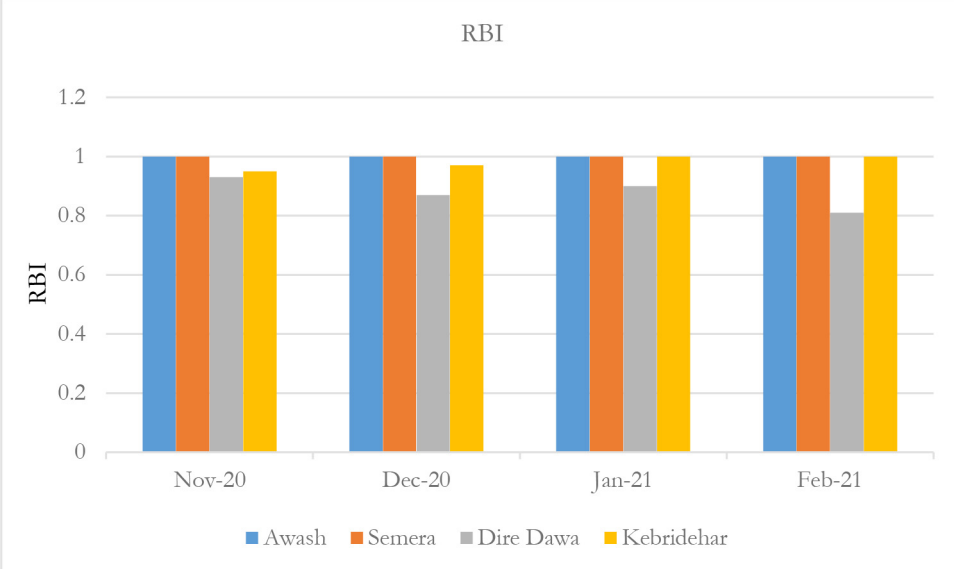
District	Type of breeding habitat	# of breeding habitats positive for *Anopheles* larvae	# of *Anopheles* larvae collected	# of adult *An. stephensi* emerged from larvae and identified
Awash	Cemented cistern	13-19	5515	418
	Water tank	1-3	543	216
	Water drum	1	73	NI
	Tire	1	87	NI
	Total		**6218**	**634**
Semera	Cemented cistern	9-18	3769	233
	Water tank	2-4	348	28
	Plastic sheet	2-6	1708	99
	Plastic bottle	1	44	10
	Water drum	1-6	578	53
	Open pipe tubes	1-2	51	4
	Total		**6498**	**427**
Dire Dawa	Cemented cistern	14-22	4572	739
	Plastic sheet	1-4	1188	105
	Water drum	1	13	4
	Car wash	1	708	81
	Cemented drum	1-2	302	47
	Stream edge	1	162	2
	Total		**6945**	**978**
Kebridehar	Cisterns (Birka)	34-58	11460	10645
	Tire	1-11	267	248
	Plastic containers	1-2	1684	1604
	Water drum	3-4	281	277
	Total		**13692**	**12774**

NI=No adult identification, since larvae were not transformed to pupae.

3.5.2 MONTHLY RBI OF *AN. STEPHENSI* LARVAE

The RBI of larvae of *An. stephensi* in Awash and Semera in all the four months was 1.0, showing that larvae were present in all inspected larval breeding habitats. Unlike in the two towns, the RBI of larvae of *An. stephensi* in Dire Dawa was less than 1.0, ranging from 0.8 to 0.9. This indicates that *An. stephensi* larvae were not found breeding in any of the few available breeding habitats. In Kebridehar, the RBI was 1.0 or nearly 1.0 in each of the four months (Figure 12).

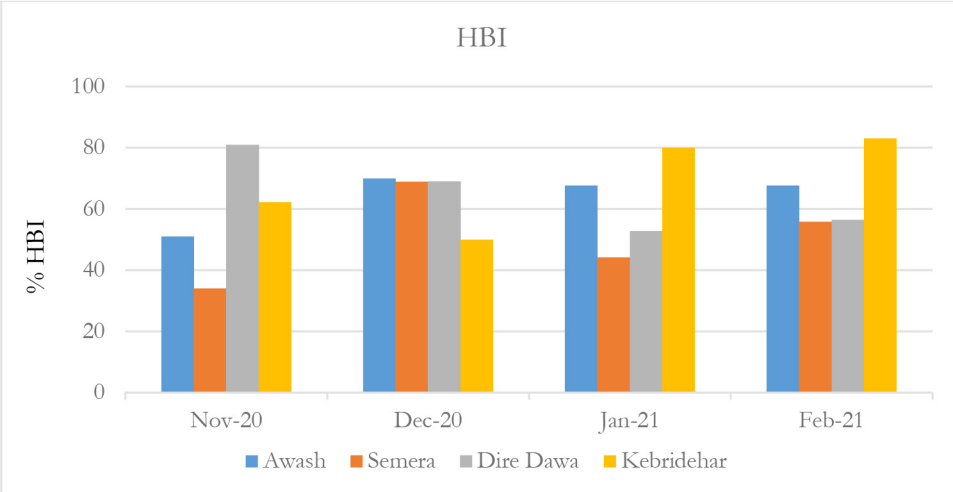
FIGURE 12. RBI OF LARVAE OF *AN. STEPHENSI* (2020-21)



3.5.3 HBI OF *AN. STEPHENSI* LARVAE

Despite the presence of manmade containers that retained water, *An. stephensi* larvae were not found in any of the breeding habitats, as shown by the HBI in all four sites. In Awash, the HBI was 51%, 70.0%, 67.7%, and 67.7% in November, December, January, and February, respectively. In the same months in Semera, the HBI was 34.0%, 68.9%, 44.2%, and 55.8%; in Dire Dawa 81.0%, 69.0%, 52.8%, and 56.4; and in Kebridehar 62.2%, 50.0%, 80.0%, and 83.0% (Figure 13).

FIGURE 13. HBI OF LARVAE OF *AN. STEPHENSI* (2020-21)

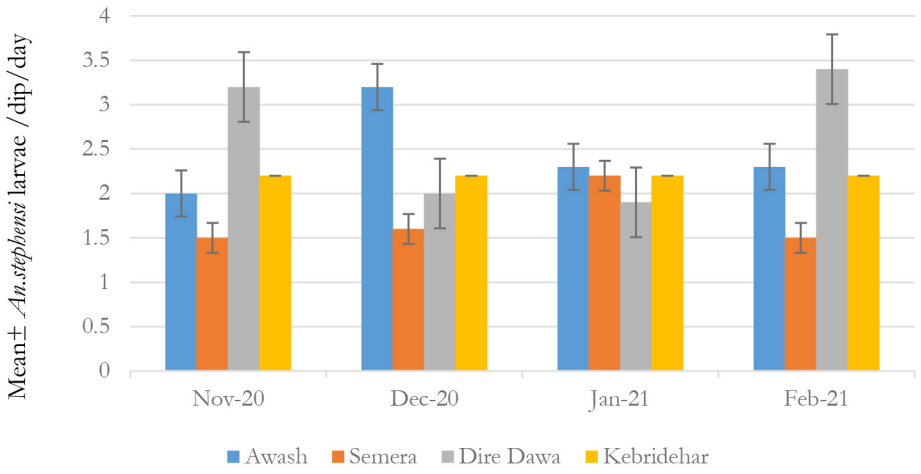


3.5.4 MONTHLY LARVAL DENSITY OF *AN. STEPHENSI*

The highest larval density of *An. stephensi* in Awash, 3.2 *An. stephensi*/dip/day, was in December, whereas it was 2.0 in November and 2.3 in January and February. The highest larval density in Semera was 2.2 *An. stephensi*/dip/day in January, 1.6 in December, and 1.5 in both November and February. Dire Dawa had the highest larval density, 3.2 and 3.4 *An. stephensi*/dip/day, in November and February, respectively; and 2.0 and

1.9 in December and January, respectively. The density in Kebridehar was the same in all four months, 2.0 *An. stephensi*/dip/day.

FIGURE 14. MONTHLY LARVAL DENSITY OF *AN. STEPHENSI* (2020-21)



Note: Bars represent standard errors.

3.6 EVALUATION OF IN2CARE TRAP PERFORMANCE

This section presents results of the evaluation of modified In2Care traps (without insecticide) in Kebridehar and Dire Dawa. For Kebridehar, the efficiency of traps with gauze strips versus traps with sticky tapes is discussed separately. In Dire Dawa, all traps but one were positive for eggs of *Ae. aegypti;* the remaining trap was positive for *An. stephensi.*

3.6.1 KEBRIDEHAR TOWN: TRAPS WITH GAUZE STRIP, NOVEMBER 2020-JANUARY 2021

Six untreated gauze-strip traps placed near a known breeding habitat in Kebridehar were found positive for 233 *Anopheles* larvae and reared to adults. *Anopheles stephensi* was identified from 209 (89.7%) of the adult specimens. The trap efficiency was 40% (6/15). No larvae were trapped in the untreated traps placed away from breeding habitats. Eight traps treated with yeast water were set near breeding habitats and three yeast-water treated traps were placed away from the habitats; the numbers of larvae recovered from the trap locations were 311 and 63, respectively, and the numbers of adult *An. stephensi* identified from the specimens were 294 and 63. The respective trap efficiency was 53.3% and 20%. The overall trap efficiency considering the positives and total traps placed was 28.3% (17/60) (Table 11).

Table 11. In2Care Traps with Gauze Strips Positive for *An. stephensi* from Kebridehar and Trap Efficiency (November 2020-January 2021)

Water used in trap	Location of traps	# In2care traps positive for larvae of *Anopheles* (out of 15)	# of *Anopheles* larvae	# adult *An. stephensi* reared from larvae and identified	Trap efficiency
Untreated	Near breeding habitats	6	233	209	40 % (6/15)
	Away from breeding habitats	0	0	0	0 % (0/15)
Yeast - treated	Near breeding habitats	8	311	294	53.3% (8/15)
	Away from breeding habitats	3	63	63	20% (3/15)
Total		**17**	**607**	**566**	**28.3 % (17/60)**

3.6.2 Kebridehar: Traps with Sticky Tape, February-April 2021

Four treated sticky-tape traps near breeding habitats were positive for 185 *An. stephensi* larvae; all the remaining traps were negative (Table 12). Sticky tapes caught no adult *An. stephensi*. *Ae. aegypti* larvae (n=114) and adults (n=23) were recovered from both treated and untreated traps. Two traps out of 120 were positive for larvae of both *An. stephensi* and *Ae. aegypti*.

Table 12. In2Care Traps with Sticky Tapes Positive for *An. stephensi* and *Ae. aegypti* from Kebridehar (February-April 2021)

		An. stephensi			*Ae. Aegypti*			
Water	Location of traps	# In2care traps positive for larvae (out of 60)	# larvae recovered from traps	# adult recovered from traps	# In2Care traps positive for larvae (out of 15)	# *larvae* recovered from traps	# In2Care traps positive for adults (out of 60)	# adults recovered from traps
Untreated	Near breeding habitats	0	0	0	0	0	4	6
	Away from breeding habitats	0	0	0	0	0	0	0
Yeast - treated	Near breeding habitats	4	185	0	4	114	10	14
	Away from breeding habitats	0	0	0	0	0	2	3
Total		**4**	**185**	**0**	**4**	114	16	23

3.6.3 Dire Dawa: Traps with Gauze Strip and Sticky Tape, November 2020-April 2021

Twenty-three traps were positive for 1,418 *Ae. aegypti* larvae and the overall trap efficiency was 19.2% (23/120) in the six-month evaluation. Adults were not found attached to the sticky traps. One trap with gauze strip and untreated water was positive for 59 *An. stephensi* eggs in November 2020; after that, no *An. stephensi* larvae/adult

were recovered. No difference was detected between traps with gauze strips and with sticky tape; therefore, unlike for Kebridehar, data obtained from both types of traps over the six months are aggregated in Table 13.

TABLE 13. IN2CARE TRAPS POSITIVE FOR *AN. STEPHENSI AE. AEGYPTI* FROM DIRE DAWA (NOVEMBER 2020-APRIL 2021)

Water	Location of traps	# In2care traps positive for eggs of *An. stephensi* (out of 30)	# In2care traps positive for eggs of *Ae. Aegypti* (out of 30)	# of *Aedes* eggs	# adult *Ae. aegypti* reared from eggs and identified	Trap efficiency
Untreated	Near breeding habitats	59	0	0	0	3.3% (1/30)
	Away from breeding habitats	0	12	713	130	40.0% (12/30)
Yeast-treated	Near breeding habitats	0	1	28	Not reared	3.3% (1/30)
	Away from breeding habitats	0	10	677	129	33.3% (10/30)
Total		**59**	**23**	**1418**	**259**	**20 (24/120)**

3.7 LABORATORY TEST RESULTS

3.7.1 SPECIES IDENTIFICATION

A total of 611 morphologically identified as *An. gambiae* s.l. specimens from ten sentinel sites were analyzed by PCR; the DNA of 602 specimens was amplified. *An. arabiensis* was identified from 601 (98.4%) specimens and *An. amharicus* from one (Table 14).

TABLE 14. *ANOPHELES ARABIENSIS* IDENTIFIED FROM PCR ASSAYS (2020)

Site	# analyzed	# amplified	*#An. arabiensis* (%)	*#An. amharicus* (%)
Abaya	60	59	58 (98.3)	1 (1.7)
Abobo	60	60	60 (100.0)	0
Amibara	60	60	60 (100.0)	0
Omonada	60	59	59 (98.3)	0
Bambasi	59	59	59 (100.0)	0
Erer	60	59	59 (98.3)	0
Jabitehnan	60	59	59 (98.3)	0
Metema	60	60	60 (100.0)	0
Misrak Badawacho	51	51	51 (100.0)	0
Benatsemay	81	76	76 (93.8)	0
Total	**611**	**602**	**601 (98.4)**	**1 (0.2)**

3.7.2 Sporozoite Infection Rates of *Anopheles* Mosquitoes

Out of 1,086 *An. arabiensis* from Harbu, three specimens were positive for circumsporozoite proteins of *Plasmodium (P.) falciparum*, and two specimens each for *P. vivax* 210 and 247. The infection rate of *P. falciparum* was 0.28%, and it was 0.18% for both *P. vivax* 210 and *P. vivax* 247. *Anopheles arabiensis* was negative for *P. falciparum* infections in Abaya (n=61), Bambasi (n=172), Lare (n=308), Benatsemay (n=88), Jabitehnan (n=419), and Metema (n=168) (Table 15).

A single specimen from 554 *An. pharoensis* from Lare was positive for *P. vivax* 210, an infection rate of 0.18%. Of 39 specimens of *An. pharoensis* from Benatsemay, one was positive for *P. falciparum,* an infection rate of 2.6%.

A single specimen out of 962 *An. coustani* from Bambasi and two specimens out of 41 *An. tenebrosus* from Harbu were positive for *P. vivax* 210, with infection rates of 0.1% and 4.9%, respectively (Table 15).

TABLE 15. SPOROZOITE INFECTION RATES OF *AN. ARABIENSIS*, *AN.PHAROENSIS*, *AN. COUSTANI*, AND *AN. TENEBROSUS* (2020)

Site	*Anopheles* species																			
	An. arabiensis				*An. pharoensis*				*An. funestus* group				*An. coustani*				*An. tenebrosus*			
	Tested	Pf +ve (%)	Pv247 +ve (%)	Pv 210+ve (%)	Tested	Pf +ve (%)	Pv247 +ve (%)	Pv 210+ve (%)	Tested	Pf +ve (%)	Pv247 +ve (%)	Pv 210+ve (%)	Tested	Pf +ve (%)	Pv247 +ve (%)	Pv 210+ve (%)	Tested	Pf +ve (%)	Pv247 +ve (%)	Pv 210+ve (%)
Abaya	61	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bambasi	172	0	0	0	—	—	—	—	263	0	0	0	962	0	0	1 (0.10)	—	—	—	—
Lare	308	0	0	0	554	0	0	1 (0.18)	5	0	0	0	4	0	0	0	—	—	—	—
Benatsemay	88	0	0	0	39	1 (2.60)	0	0	2	0	0	0	—	—	—	—	—	—	—	—
Harbu	1086	3 (0.28)	2 (0.18)	2 (0.18)	44	0	0	0	—	—	—	—	1	—	—	—	41	0	0	2 (4.90)
Jabitehnan	419	0	0	0	—	—	—	-	—	—	—	—	82	0	0	0	—	—	—	—
Metema	168	0	0	0	—	—	—	-	—	—	—	—	—	—	—	—	—	—	—	—

Note: PF=*P. falciparum*, PV=*P. vivax*

3.7.3 *Anopheles arabiensis* and *An. funestus* Sporozoite Infection Rates from Gelana

Community mosquito collectors from Gelana collected *Anopheles arabiensis* and *An. funestus* s.l. using CDC light traps. A total of 1,789 *An. arabiensis* were ELISA tested, out of which one specimen was positive for *P. falciparum* and one for *P. vivax* 210, giving a sporozote infection rate of 0.05%. Two specimens were found with *P. vivax* 247, for a sporozote infection rate of 0.11%. Of 12 *An. funestus* group, one was positive for *P. falciparum* (8.3% infection rate) (Table 16).

Table 16. Sporozoite Infection Rates of *An. arabiensis* and *An. funestus* Group from Gelana (2020)

Species	# tested	# positives (%)		
		Pf	*Pv* 210	*Pv* 247
An. arabiensis	1789	1 (0.05)	1 (0.05)	2 (0.11)
An. funestus group	12	1 (8.33)	0	0

Note: PF=*P. falciparum*, PV=*P. vivax*

3.7.4 *Anopheles stephensi* Infection Rates

Sporozoite ELISA tests were conducted on 118 and 175 *An. stephensi* collected from Dire Dawa and Kebridehar, respectively. All of those sampled from Dire Dawa were negative for *P. falciparum* and *P. vivax* and two positives were samples from Kebridehar, one each of *P. vivax* 210 and *P. vivax* 247 for an infection rate of 1.33% for each variant (Table 17).

Table 17. *Anopheles stephensi* Infection Rates (2020)

Site	# tested	# positive (%)		
		Pf	*Pv*210	*Pv*247
Dire Dawa	118	0	0	0
Kebridehar	175	0	1 (1.33)	1 (1.33)

Note: *PF=P. falciparum*, *PV=P. vivax*

3.7.5 Blood Meal Sources of *Anopheles*

A total of 693 blood-fed *An. arabiensis, An. funestus* group, *An. pharoensis,* and *An. stephensi* were tested for blood meal sources by direct ELISA using human, bovine, and goat antibodies. Overall, the human blood index (HBI), bovine blood index (BBI) and goat blood index (GBI) of *An. arabiensis* was 21.5%, 40.6%, and 56.2%, respectively. *An. stephensi* had an HBI, BBI, and GBI of 4.2%, 19.5%, and 71.6%, respectively (Table 18).

Detailed information on the number and species of *Anopheles* tested by site is provided in Annex C.

Table 18. Blood Meal Sources and Blood Meal Indices of *Anopheles* (2020)

Species	# tested	Number of blood meal sources								% BM indices		
		H	B	G	H+B	H+G	B+G	H+B+G	UN	HBI	BBI	GBI
An. arabiensis	404	20	12	27	3	51	136	13	142	21.5	40.6	56.2
An. funestus s.l.	12	1	0	7	0	0	4	0	0	8.3	33.3	91.7
An. pharoensis	16	1	0	0	0	0	5	0	10	6.3	31.3	31.3
An. stephensi	261	7	3	137	0	2	46	2	64	4.2	19.5	71.6

Note: H=human, B=bovine, G=goat, UN=unidentified, BM=blood meal

3.8 Insecticide Resistance Monitoring

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

3.8.1 *Anopheles arabiensis* Susceptibility to Insecticides

An. arabiensis in ten sentinel sites (Abaya, Abobo, Amibara, Bambasi, Benatsemay, Erer, Jabitehnan, Humera, Misrak Badawacho, and Omonada) was susceptible to pirimiphos-methyl, bendiocarb, and propoxur. Mosquito mortality was 100%. As in the last decade, resistance to the pyrethroids is not only widely present but it is also intense (Figures 15A and 15B).

FIGURE 15A. MORTALITY OF *AN. ARABIENSIS* FROM WHO TUBE TESTS CONDUCTED ON 1X CONCENTRATIONS OF BENDIOCARB, PROPOXUR AND PIRIMIPHOS-METHYL (2020)

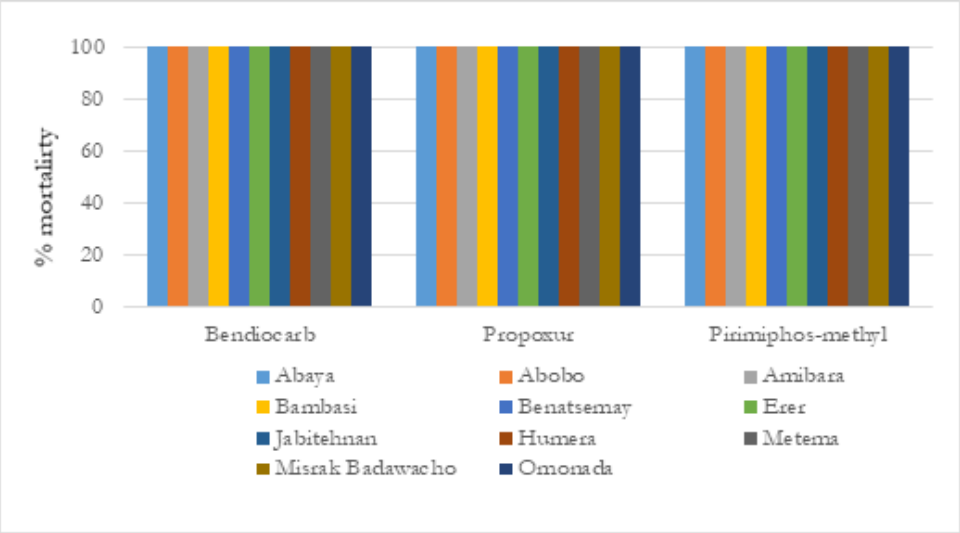
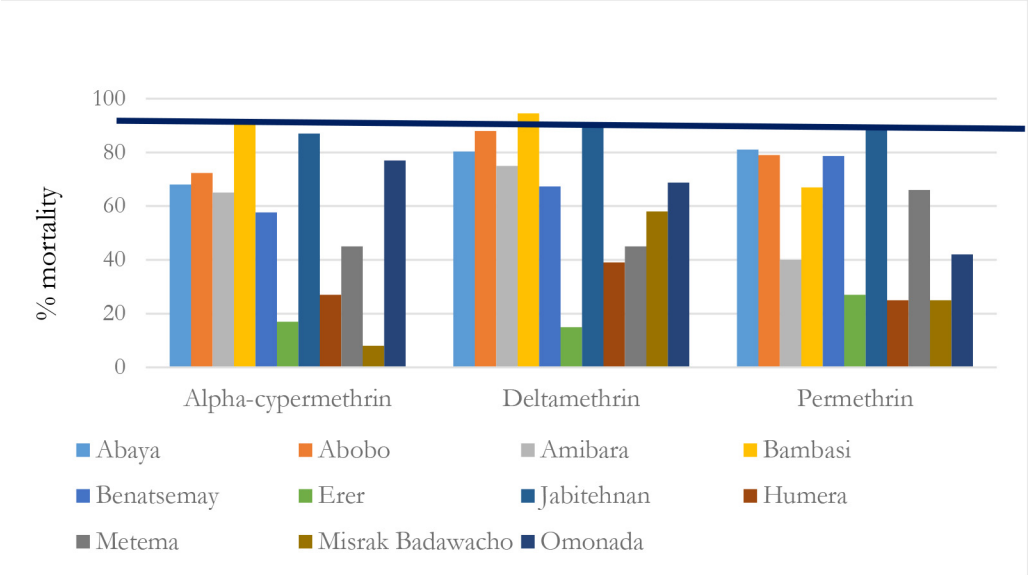


FIGURE 16B. MORTALITY OF *AN. ARABIENSIS* FROM WHO TUBE TESTS CONDUCTED ON 1X CONCENTRATIONS OF ALPHA-CYPERMETHRIN, DELTAMETHRIN AND PERMETHRIN (2020)

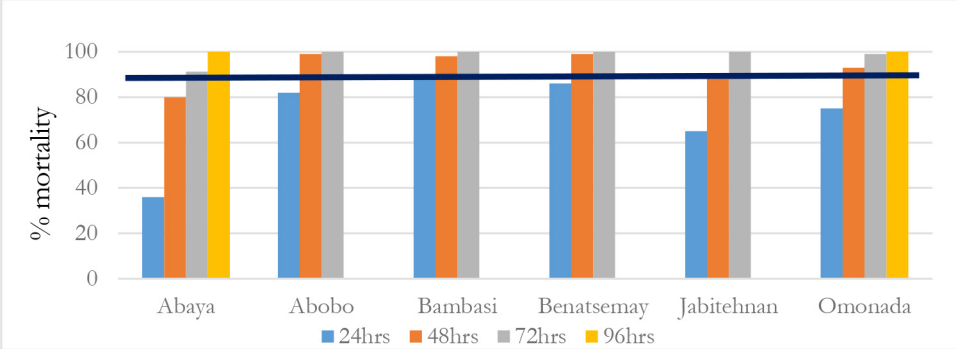


Note: Line indicates 90% mortality threshold for resistance.

3.8.2 *ANOPHELES ARABIENSIS* SUSCEPTIBILITY TO CLOTHIANIDIN

Clothianidin killed 100% of wild *An. arabiensis* from Abobo, Bambasi, Benatsemay, and Jabitehnan within 72 hours and from Abaya and Omonada within 96 hours, showing susceptibility of *An. arabiensis* to clothianidin in all sites tested (Figure 16).

FIGURE 17. MORTALITY OF *AN. ARABIENSIS* TESTED AGAINST CLOTHIANIDIN



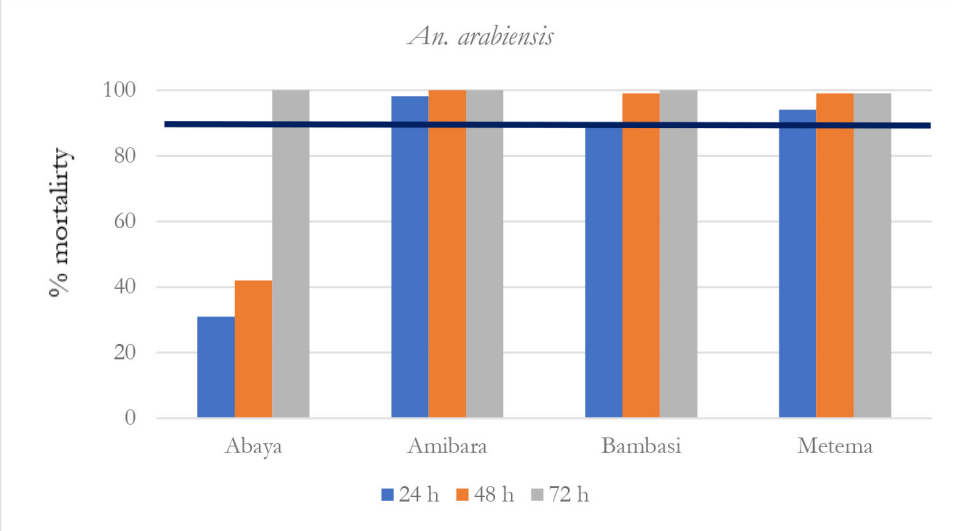
Note: Line indicates 90% mortality threshold for resistance.

3.8.3 *ANOPHELES ARABIENSIS* AND *AN. STEPHENSI* SUSCEPTIBILITY TO CHLORFENAPYR

ANOPHELES ARABIENSIS

Chlorfenapyr caused 99-100% mortality to four populations of *An. arabiensis* within 72 hours showing susceptibility of the vector to the insecticide (Figure 17).

FIGURE 18. MORTALITY OF *AN. ARABIENSIS* TESTED AGAINST CHLORFENAPYR (2020)

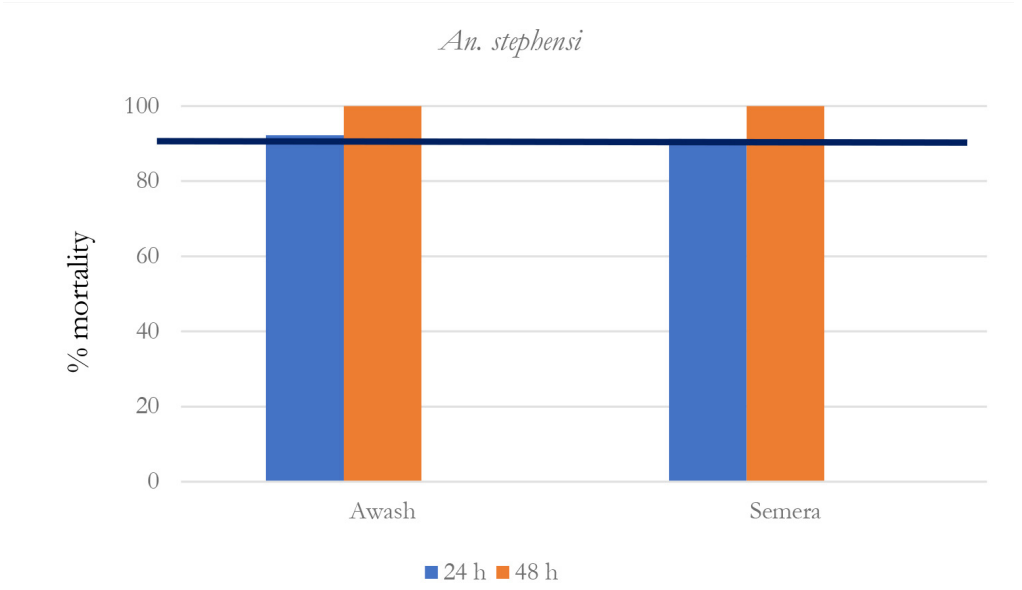


Note: Line indicates 90% mortality threshold for resistance.

ANOPHELES STEPHENSI

Anopheles stephensi from Awash and Semera were susceptible to chlorfenapyr. All test mosquitoes died within 48 hours (Figure 18).

FIGURE 19. MORTALITY OF *AN. STEPHENSI* TESTED AGAINST CHLORFENAPYR (2020)

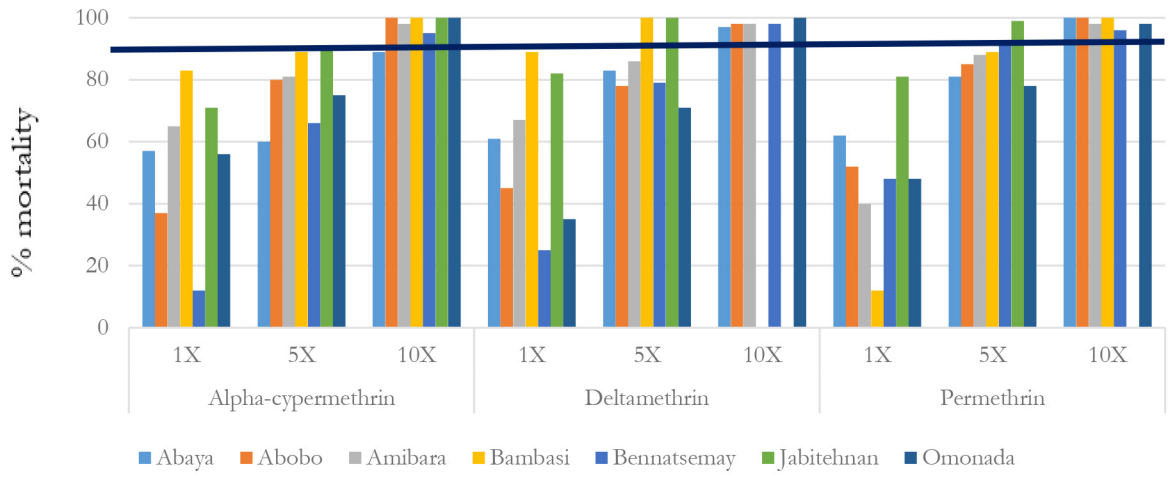


Note: Line indicates 90% mortality threshold mortality for resistance.

3.8.4 RESULTS OF *AN. ARABIENSIS* RESISTANCE INTENSITY ASSAYS

Anopheles arabiensis exhibited high resistance intensity to alpha-cypermethrin from Abaya with 89% mortality at 10X and Benatsemay at 95% mortality at the same concentration, moderate resistance intensity from Abobo, Amibara, Bambasi, Jabitehnan, and Omonada (98-100% mortality at 10X). At 100% mortality at 5X deltamethrin concentration, low resistance intensity prevailed in the population of *An. arabiensis* from Bambasi and moderate resistance in those from Abobo, Amibara, Benatsemay, and Omonada (98-100% at 10X). High resistance was noted from Abaya, with 97% mortality at 10X. The population manifested low permethrin resistance in Jabitehnan (99% mortality at 5X), moderate resistance in Abaya, Abobo, Amibara, Bambasi, and Omonada (98-100% mortality at 10X), and high resistance in Benatsemay (96% mortality at 10X) (Figure 19).

FIGURE 20. MORTALITY OF *AN. ARABIENSIS* FROM RESISTANCE INTENSITY TESTS (2020)



3.8.5 RESULTS OF *AN. ARABIENSIS* PBO SYNERGIST ASSAYS

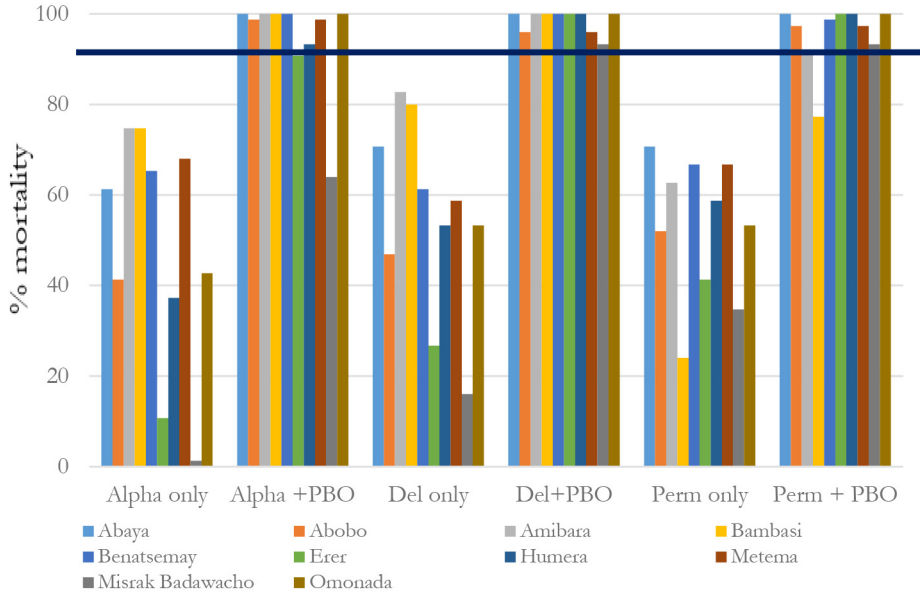
Pre-exposure to PBO followed by exposure to alpha-cypermethrin returned the populations of *An. arabiensis* to full susceptibility in Abaya, Abobo, Amibara, Bambasi, Benatsemay, Metema, and Omonada (98-100%) and partial susceptibility in Erer (90.7%), Misrak Badawacho and Humera (93.3%) (Figure 20).

Pre-exposure to PBO followed by exposure to deltamethrin resulted in restoration of susceptibility in most of the sites except in Abobo (93.3% morality), Metema (96%), and Misrak Badawacho (93.3%) (Figure 20).

Pre-exposure to PBO partially restored susceptibility of *An. arabiensis* to permethrin in three sites, namely, Amibara (92% mortality), Bambasi (77.3%), and Misrak Badawacho (93.3%), while in the rest of the sites, the population reverted to full susceptibility (Figure 20) after pre-exposure to PBO.

The role of mixed function oxidases is profound in those sites which returned to full susceptibility. Other resistance mechanisms might also be involved in those vector populations, demonstrating partial restoration of susceptibility.

FIGURE 21. MORTALITY OF *AN. ARABIENSIS* FOLLOWING PBO SYNERGIST ASSAYS (2020)



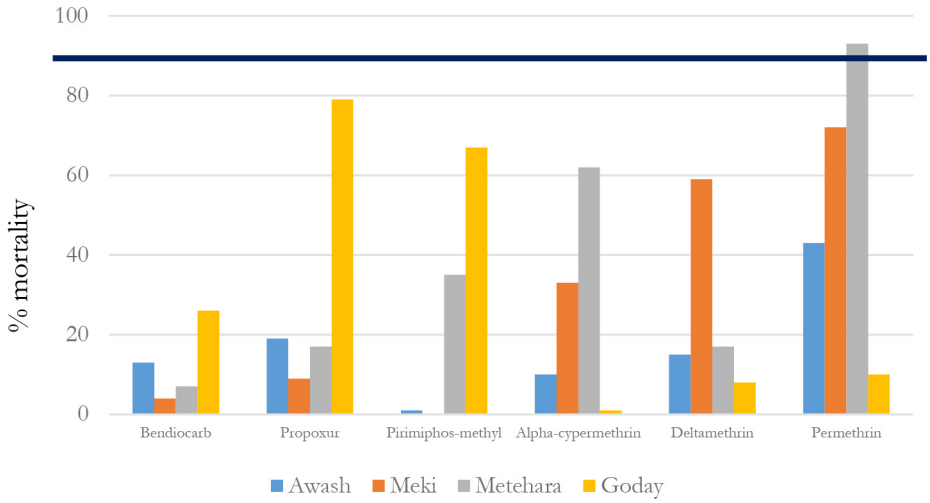
Note: Alpha= Alpha-cypermethrin, Del= Deltamethrin, Perm=permethrin

3.8.6 *ANOPHELES STEPHENSI* INSECTICIDE SUSCEPTIBILITY, RESISTANCE INTENSITY, AND PBO ASSAYS

SUSCEPTIBILITY OF AN. STEPHENSI TO INSECTICIDES

Anopheles stephensi was highly resistant to bendiocarb, propoxur, pirimiphos-methyl, alpha-cypermethrin, deltamethrin, and permethrin (mortality less than 90%) in Awash, Meki, Metehara, and Goday. The exception was permethrin, in which mortality was 93% in the population from Metehara. According to the WHO criteria, this puts the resistance status of *An. stephensi* in the category of possible resistance (Figure 21).

FIGURE 22. MORTALITY OF *AN. STEPHENSI* TESTED AGAINST SIX INSECTICIDES AT A CONCENTRATION OF 1X (2020)

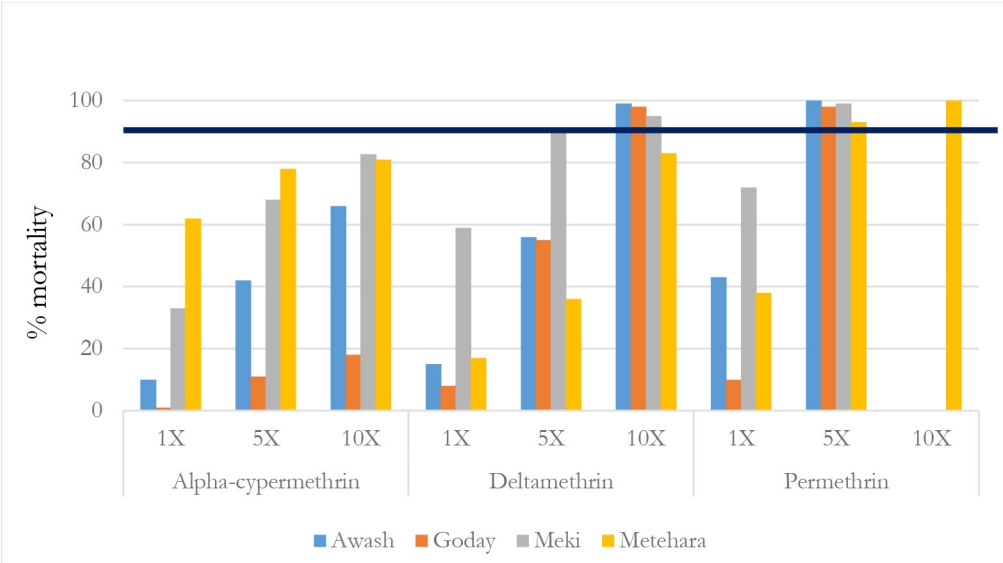


Note: Line showing 90% threshold value of resistance.

RESISTANCE INTENSITY ASSAYS ON *AN. STEPHENSI*

Anopheles stephensi exhibited high intensity resistance to alpha-cypermethrin in Awash, Goday, Meki, and Metehara with mortalities of 66%, 18%, 83%, and 81% at 10X concentration, respectively. Moderate resistance to deltamethrin was scored in Awash (99% mortality at 10X) and Goday (98% mortality at 10X), and high resistance intensity in Meki (95% mortality at 10X) and Metehara (83% mortality at 10X). Low resistance to permethrin was observed in Awash (100% mortality at 5X), Goday (98% mortality at 5X), and Meki (99% mortality at 5X), while moderate resistance was observed in Metehara (100% mortality at 10X) (Figure 22).

FIGURE 23. MORTALITY OF *AN. STEPHENSI* FROM RESISTANCE INTENSITY ASSAYS (2020)

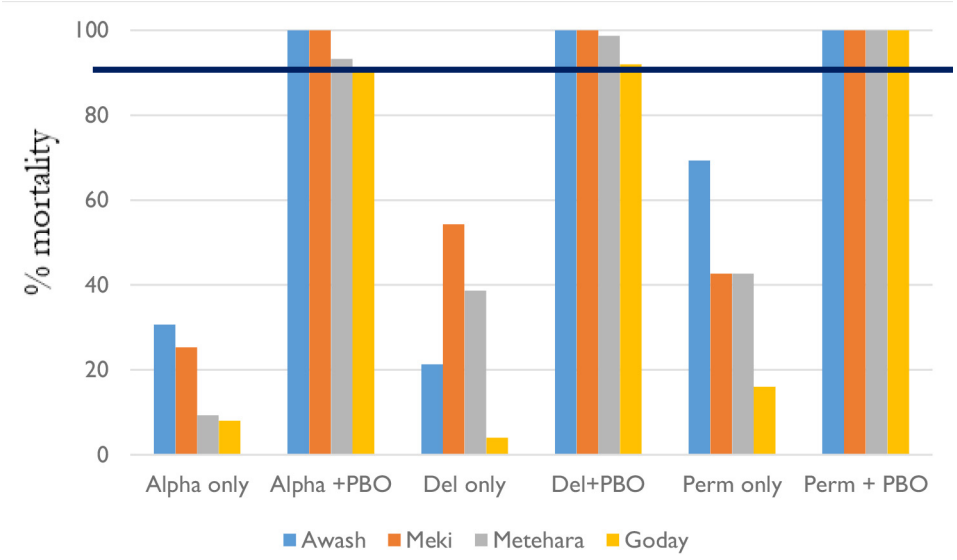


PBO Synergist Assays on An. stephensi

In Awash and Meki, pre-exposure to PBO fully restored *An. stephensi* susceptibility (100% mortality) to alpha-cypermethrin and deltamethrin. This also happened with deltamethrin in Metehara (98.7% mortality) and to permethrin in all four towns (100% mortality). Pre-exposure to PBO restored partial resistance to alpha-cypermethrin in Metehara (93.3% mortality) and Goday (91% mortality) as well as to deltamethrin in Goday (92% mortality) (Figure 23).

In conclusion, mixed function oxidases play an important role in the population of *An. stephensi* where 100% mortality occurred. Partial restoration of susceptibility indicates the involvement of other resistance mechanisms in addition to the oxidases.

Figure 24. Mortality of *An.stephensi* from PBO Synerist Assays (2020)



Note: Alpha= Alpha-cypermethrin, Del= Deltamethrin, Perm=permethrin

Temephos Susceptibility of An. stephensi Larvae

Temephos at a concentration of 31.25 mg/L resulted in 100% mortality of *An. stephensi* larvae from Awash, Semera, and Kebridehar after one hour of exposure and a 24-hour holding period (Table 19). The population in Dire Dawa required a higher dose, 156.25 mg/L, to observe the same effect. The concentration of 31.25 mg/L in Awash, Kebridehar, and Meki and of 156.25 mg/L in Dire Dawa was used for serial dilutions to determine LD_{50} and LD_{95}. In addition, serial dilutions were made from the 31.25 mg/L to obtain the WHO discriminating dose of 0.25 mg/L concentration and test larvae of *An. stephensi* from Awash, Kebridehar, and Meki. On the other hand, serial dilutions made from the 156.25 mg/L were used to test larvae in Dire Dawa.

Table 19. Mortality of Larvae of *An. stephensi* to Different Concentrations of Temephos

Concentration	% mortality of larvae of *An. stephensi*				
	Awash	Semera	Dire Dawa	Kebridehar	Meki
1.25 mg/L	0	0	0	0	0
6.25 mg/L	92	94	0	78	71
31.25 mg/L	100	100	90	100	100
156.25 mg/L	100	100	100	100	ND

In Dire Dawa, the LD_{50} was 0.105 mg/L and the LD_{95} was 0.118 mg/L (Table 20). The values for Kebridehar and Meki were much lower than those for Dire Dawa. Despite repeated tests, the lethal doses could not be determined in Semera because of variable results.

At the WHO discriminating dose, mortality of larvae was 100%, showing susceptibility to temephos (Table 20).

TABLE 20. MORTALITY OF LARVAE OF *AN. ARABIENSI* AT TWO LETHAL DOSES AND WHO DISCRIMINATING DOSE OF TEMEPHOS

Site	LD_{50} (95%CI) mg/L	LD_{95} (95%CI) mg/L	% Mortality at WHO discriminating dose of 0.25 gm/L
Dire Dawa	0.105 (0.099-0.109)	0.118 (0.114-0.113)	100
Kebridehar	0.019 (0.015-0.027)	0.031 (0.024-0.122)	100
Meki	0.012 (0.011-0.013)	0.025 (0.021-0.032)	100
Semera	_	_	100

3.9 ENTOMOLOGICAL ASSESSMENT OF QUALITY AND DECAY RATE OF ACTELLIC 300CS, SUMISHIELD, AND FLUDORA FUSION

The residual efficacy of Actellic 300CS, SumiShield, and Fludora Fusion, which was evaluated by cone and fumigation bioassays, is discussed in this section.

3.9.1 CONE BIOASSAY TESTS

ACTELLIC 300CS

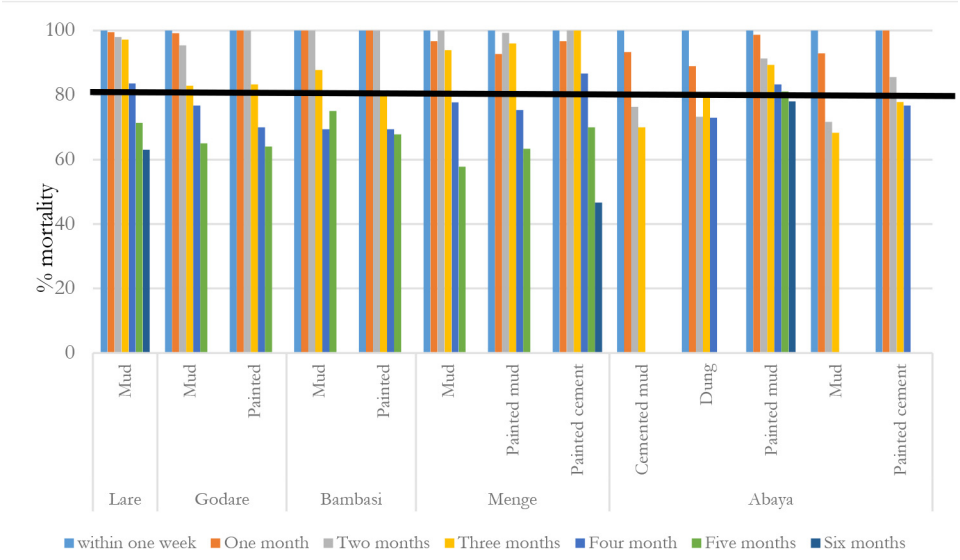
The Actellic 300CS sprayed in mud houses in Lare was efficacious for four months, causing mortality of 100% of insectary *An. arabiensis* within a week of spraying and 83% after the fourth month. Mortality declined to 71.4% and 63% in the fifth and six months (Figure 24).

In Godare, mortality of mosquitoes tested on mud and painted houses was more than the 80% WHO threshold value from within a week of spraying (100%) to the end of the third month (82.9% in mud houses and 87.8% in painted houses); after the third month, mortality went down to 64% and 67% on the respective surfaces. Results were almost identical in Bambasi in both types of houses (Figure 24).

Although mortality was over 90% on the third month in Menge, the persistence of Actellic was similar to Godare and Bambasi except from painted cement houses in which the insecticide persisted for four months. Mortality on the fourth month was 86.7% (Figure).

In Abaya, mortality after a month of spraying on mud, dung, and cement houses was 92.9%, 88.9%, and 93.3%, respectively, after which mortality fell to less than 80% for two consecutive months. The persistence of Actellic 300CS was for two, three, and five months on painted cement, dung, and painted mud, respectively (Figure 24).

FIGURE 24. RESIDUAL EFFICACY OF ACTELLIC 300CS FROM CONE BIOASSAY TESTS OF INSECTARY *AN. ARABIENSIS* (2020)

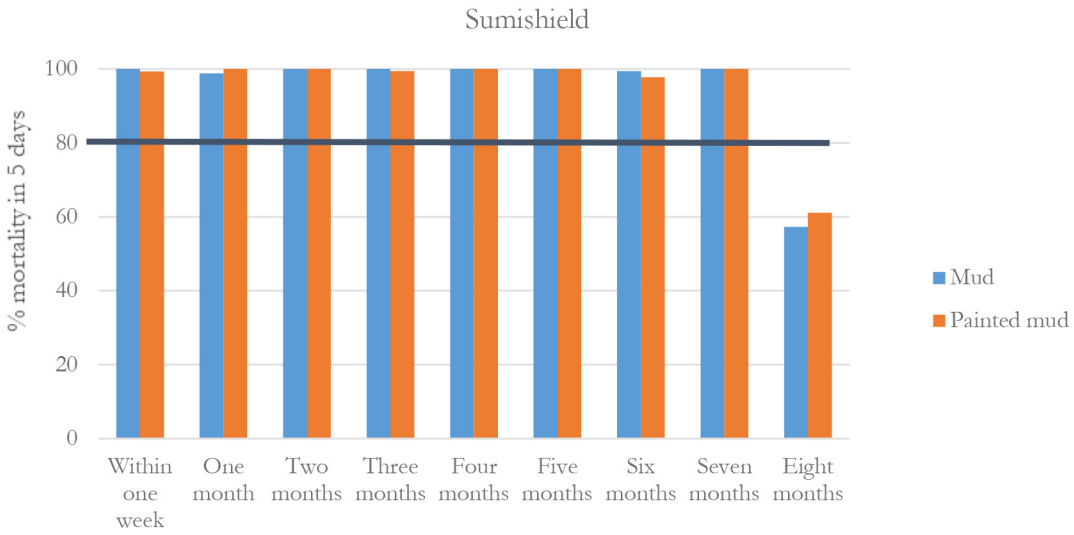


Note: Line indicates the WHO 80% cut-off value of mortality.

SUMISHIELD: BANESHEGOL KEBELE IN MENGE

SumiShield was efficacious killing 99-100% of insectary *An. arabiensis* on both mud and painted mud houses for seven months. Figure 25 illustrates mortality data after five days of cone bioassay tests. The daily mortality rate is presented in Annex K.

FIGURE 25. RESIDUAL EFFICACY OF SUMISHIELD FROM CONE BIOASSAY TESTS OF INSECTARY *AN. ARABIENSIS* (2020-21)



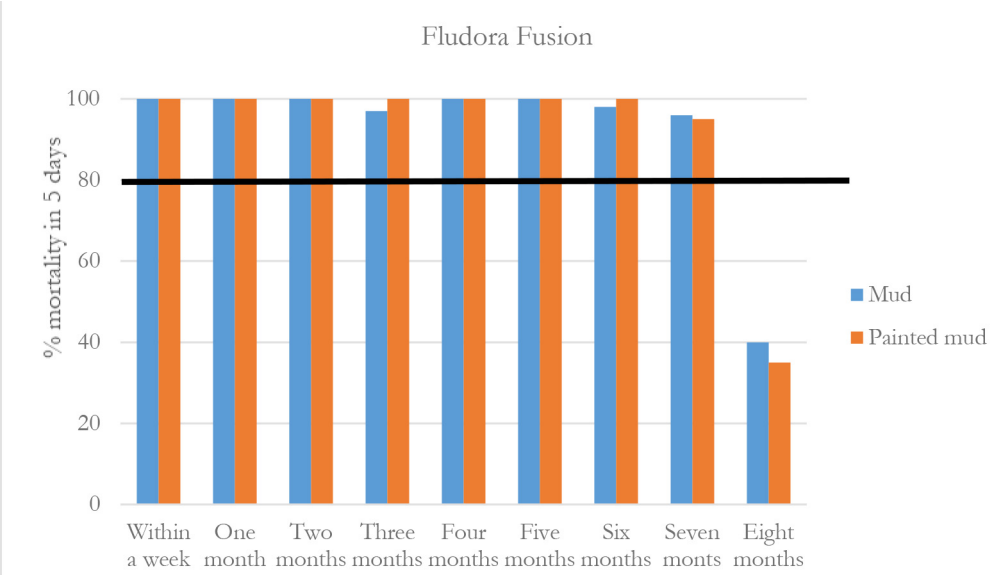
Note: Line indicates the WHO 80% cut-off value of mortality.

Fludora Fusion: Belmuga Kebele in Menge, *Insectary An. arabiensis*

As in SumiShield houses, Fludora Fusion sprayed on mud and painted mud wall surfaces killed 98-100% of insectary *An. arabiensis* at day 5 for seven consecutive months. Mortality in the eighth month dropped to 54% in mud houses and 46% in mud painted houses (Figure 26).

Results of daily mortality rates of *An. arabiensis* due to Fludora Fusion is depicted in Annex L.

Figure 26. Residual Efficacy of Fludora Fusion from Cone Bioassay Tests on Insectary *An. arabiensis* (2020-21)

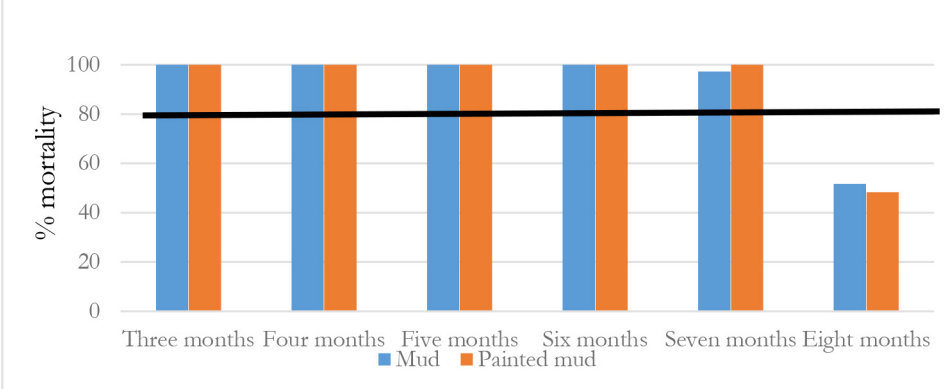


Note: Line indicates the WHO 80% cut-off value of mortality.

Fludora Fusion: Belmuga Kebele in Menge, *Wild An. arabiensis*

Like the insectary *An. arabiensis*, Fludora Fusion was efficacious with wild *An. arabiensis* in the sprayed houses, killing 97-100% of tested mosquitoes from the third to the seventh month. In the eighth month, mortality was 51.7% in mud houses and 48.7% in painted houses (Figure 27 and Annex M).

Figure 27. Residual Efficacy of Fludora Fusion from Cone Bioassay Tests on Wild *An. arabiensis* (2020-21)



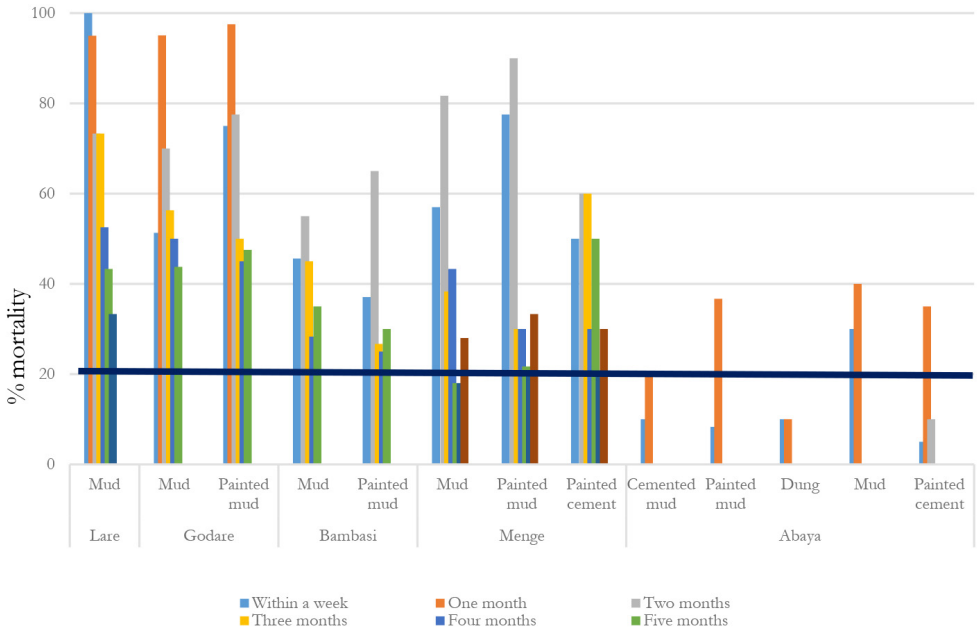
Note: Line indicates the WHO 80% cut-off value of mortality.

3.9.2 Fumigation Bioassays

Actellic 300CS

The fumigant effect of Actellic 300CS on mortality of insectary *An. arabiensis* was remarkably high in mud houses in Lare, Godare, and Bambasi and in painted mud houses in Menge, with mortality greater than 30% for more than six months. In Abaya, all test mosquitoes survived in the second month in all house types except in painted cement houses, with 10% mortality (Figure 28 and Annex N).

Figure 28. Residual Efficacy of Actellic 300CS from Fumigation Bioassays Tested on Insecary *An. arabiensis* (2020-21)

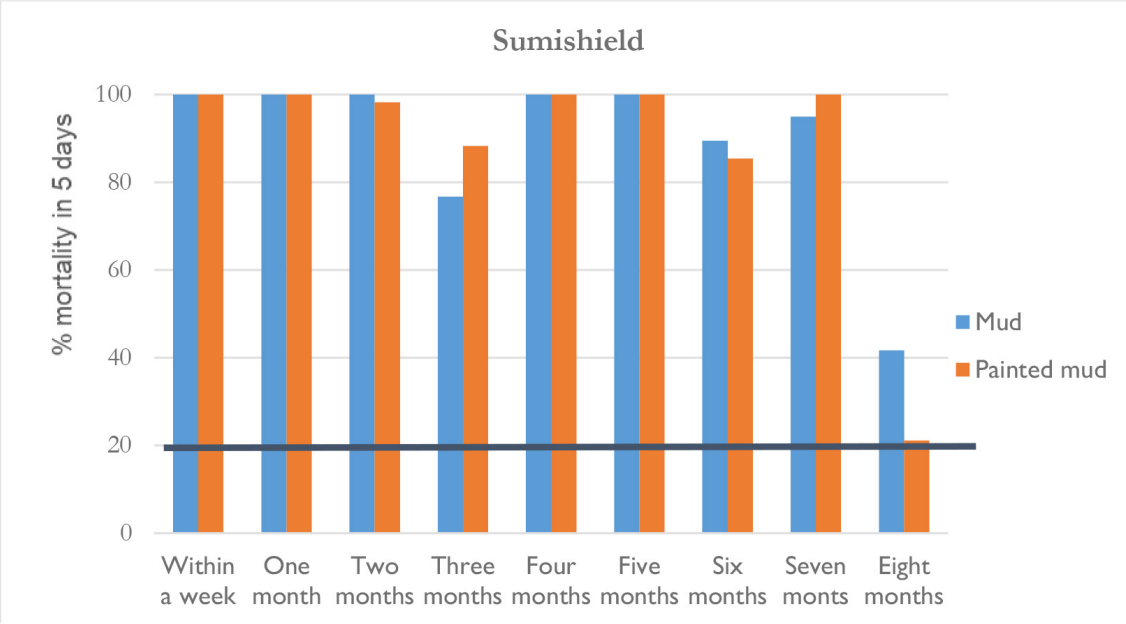


Note: Line indicates 20% cut-off value of mortality.

SumiShield: Baneshegol Kebele in Menge

The fumigant effect of SumiShield-sprayed houses on mortality of insectary *An. arabiensis* was significantly high, killing 76.7-100% in mud and 88.3-100% in painted mud houses for seven months, based on mortality records on day 5 (Figure 29). In addition, mortality was more than 20% in the eighth month. Mortality records after 24, 48, 72, 96, and 120 hours are in Annex O.

FIGURE 29. RESIDUAL EFFICACY OF SUMISHIELD FROM FUMIGATION BIOASSAYS TESTED ON INSECTARY *AN. ARABIENSIS* (2020-21)



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

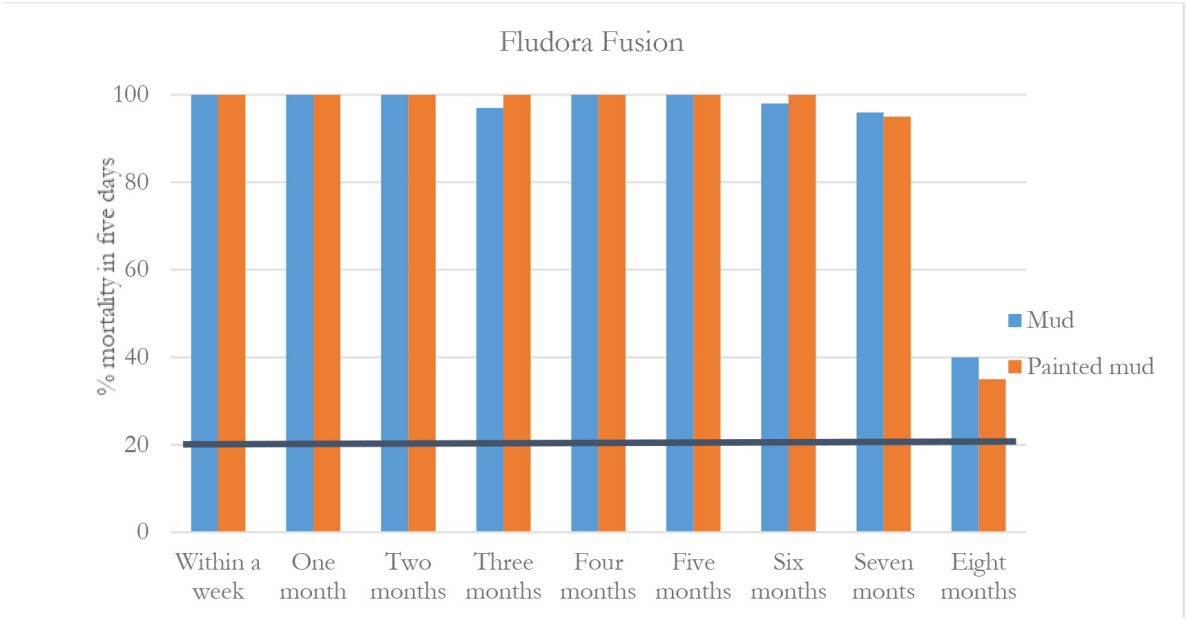
FLUDORA FUSION: BELMUGA KEBELE IN MENGE

Fludora Fusion-sprayed houses remained highly efficacious (95-100% mortality on both mud and painted mud wall surfaces) with insectary *An. arabiensis* for seven months as determined from fumigation bioassay tests. In the eighth month, mortality was far above 20% threshold mortality: 40% in mud and 35% in painted mud houses.

The fumigant effect of Actellic 300CS on wild *An. arabiensis* was similar.

Figure 30 and Annex P show percent mortality after five days of bioassays and 24 hrs-120 hrs, respectively.

FIGURE 30. RESIDUAL EFFICACY OF FLUDORA FUSION FROM FUMIGATION BIOASSAYS TESTED ON INSECTARY *AN. ARABIENSIS* (2020-21)



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

4. Entomological Capacity Building

4.1 Material and Training Support to Universities and Public Health Institutes

The PMI VectorLink Project in Ethiopia has provided insectary and entomological monitoring materials to Gondar University, Debre Markos University, Tigray Public Health Institute, the Amhara Public Health Institute, and the Oromia Public Health Research Capacity Building and Quality Assurance Laboratory. The project also supported Debre Markos University by having two staff trained on insectary management at Jimma University. Entomological and laboratory materials were procured and distributed to Jimma University (PCR machine, ELISA machine and washer), ArbaMinch University (freezer, safety cabinets, and water distillers), the Armauer Hansen Research Institute (microscopes and centrifuges), and the Ethiopian Public Health Institute (microscopes workshop on *An. stephensi*).

A virtual meeting of stakeholders on the control of *An. stephensi* took place in May 2020. Upon the recommendation of the NMEP, the project staff have contributed to the development of a policy brief document and the Ministry of Health Special Bulletin article on *An. stephensi.*

4.2 Technical Support to NMEP

The project has provided technical support to the NMEP in the development of the National Strategic Plan document for 2021-2025, and the Malaria Performance Review for 2017-2020. The project subcontracted the Malaria Consortium to prepare a strategic document on Insecticide Resistance Monitoring and Management. The Consortium reviewed the existing data on vector distribution and the insecticide resistance profile of the main vector, and recommended activities to be performed in the future; at the time this annual entomology report was being prepared, the strategic document is being revised.

4.3 Piloting Community Mosquito Collection

The results from the community mosquito collection in Gelana encourage expansion of entomological monitoring at a lower cost and also opens opportunities to train and involve mosquito collectors in insecticide resistance monitoring.

5. CONCLUSIONS

Anopheles arabiensis remains the main malaria vector in Ethiopia based on evidence of its high abundance, trap density and findings of *P. falciparum* and *P. vivax* sporozoite infections in the sentinel site of Harbu and the community mosquito collection site in Gelana District. The secondary vectors, *An. pharoensis and An. funestus,* were found infected with *P. falciparum* in Benatsemay and Gelana, respectively. Although the roles of *An. coustani* and *An. tenebrosus* remain to be clarified by additional entomological and epidemiological findings, *P. vivax* sporozoite infections in the two species were detected in Bambasi and Harbu, respectively. *P. vivax* infections were also detected in *An. stephensi* from Kebridehar.

The majority of *An. arabiensis* was collected from CDC light traps both indoors and outdoors. Outdoor density was slightly greater than indoor density. Although it varied from site to site, monthly trap density was mainly less than one *An. arabiensis*/trap/night. The density was less in the PMI VectorLink Ethiopia IRS project sites than in the non-IRS sites. The highest monthly trap density was from Harbu and Jabitehnan, where IRS was not implemented. The proportion of unfed mosquitoes was larger in the CDC light trap collections, whereas the proportion of fed mosquitoes was larger in the PSC. An appreciable number of *An. arabiensis* was collected from Gelana throughout the sampling months showing the perennial nature of this species; therefore, Gelana needs additional vector control interventions including nets .

An. stephensi was found in 21 rural sites in eastern Ethiopia, breeding in containers resembling those found in urban sites, and this raised the total number of positive sites to 35 based on cross-sectional surveys that VectorLink Ethiopia has conducted since 2018. The pilot survey investigating larval indices in Awash, Semera, Dire Dawa, and Kebridehar showed that cisterns are the main breeding habitats since they store water for a longer period replenished with tap water periodically.

The majority of adult *An. stephensi* from Awash, Dire Dawa, Kebridehar and Metehara were collected from animal shelters using Prokopack aspirators and cattle and goat baited tent traps. The conventional methods such as CDC light traps and PSC are less efficient for *An. stephensi*. Modified In2Care traps, primarily designed to collect adult *Ae. aegypti* also showed ineffective at collecting adult *An. stephensi* even in locations with large populations. Therefore, future adult surveillance of *An. stephensi* should depend on Prokopack aspirators until such time another method is identified, with the best current method of collection targeting immature stages.

Anopheles arabiensis was susceptible to bendiocarb, propoxur, pirimiphos-methyl, clothianidin, and chlorfenapyr but still highly resistant to the pyrethroids alpha-cypermethrin, deltamethrin, and permethrin. *An. stephensi* was resistant to all insecticides tested except for chlorfenapyr. Return to susceptibility after pre-exposure to PBO and susceptibility to chlorfenapyr of both *An. arabiensis* and *An. stephensi* suggests considering PBO-treated nets and Interceptor G2 nets for vector control in the country. *Anopheles stephensi* was found to be susceptible to temephos.

The residual efficacy of Actellic 300CS from the cone bioassays was short, from two to five months. A thorough study is needed as to how to estimate this effect on the epidemiology of malaria. Looking at the seven-month residual efficacy of SumiShield and Fludora Fusion, these products should be considered for IRS in the country.

6. References

Afrane A Yaw, Bonnizoni M, and Yan G. 2016. Secondary Malaria Vectors of Sub-Sahara Africa: Threat to Malaria Elimination on the Continent? Chapter in *Current Topics in Malaria*. http://dx.doi.org/10.5772/65359.

Balkew M, Mumba P, Dengela D, Yohannes G, Getachew D, Yared S, Chibsa S, Murphy M, Georgr K, Lopez K, Janies D, Choi SH, Spear J, Irish RS, and Carter ET. 2020. Geographical distribution of *Anopheles stephensi* in eastern Ethiopia. *Parasites & Vectors*, 13:35.

Balkew M, Mumba P, Yohannes G et al. 2021. An update on the distribution, bionomics, and insecticides susceptibility of *Anopheles stephensi* in Ethiopia, 2018-2020. *Malar J* , 20:263.

Beier JC, Perkings PV, Wirth RA, Koros J, Diggs D, Garganii TP, and Koech DK. 1988. Blood meal identification by direct enzyme linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera Culicidae) in Kenya. *J Med Entomol* 25: 9-16.

Coetzee M. 2020. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malar J*. 19:70.

Goupeyou-Youmsi, J, Rakotondranaivo, T, Puchot, N et al. 2020. Differential contribution of *Anopheles coustani* and *Anopheles arabiensis* to the transmission of *Plasmodium falciparum* and *Plasmodium vivax* in two neighboring villages of Madagascar. *Parasites & Vectors* 13:430. https://doi.org/10.1186/s13071-020-04282-0

Scott JA, Brogdon WG, and Collins FH. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 49(4): 520-9.

World Health Organization. 1981. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807.

———. 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides, WHO Pesticides Evaluation Scheme. Geneva: WHO/CDS/WHOPES/GCDPP/2005:13.

Wirtz RA, Sattabongkot J, Hall T, Burkot TR, and Rosenberg R. 1992. Development and Evaluation of an Enzyme-Linked Immunosorbent Assay for *Plasmodium vivax*-VK247 Sporozoites. *J Med Entomol* 29: 854-7.

ANNEX A. MONTHLY COLLECTIONS OF *ANOPHELES* AND CULICINES FROM SENTINEL SITES (JULY 2020-MARCH 2021)

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/ cydippis*			*An. tenebrosus*			*An. demeilloni*			*An. pretoriensis*			Culicines		
		CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total
Abaya	July (Post IRS)	10	1	11	2	0	2	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	798		798
	Aug (Post IRS)	10	0	10	1	0	1	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	568		568
	Sept (Post IRS)	20	0	20	0	0	0	0	0	0	9	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	544		544
	Oct (Post IRS)	70	14	84	0	0	0	0	0	0	25	0	25	3	0	3	0	0	0	0	0	0	2	1	3	3	0	3	753		753
	Nov (Post IRS)	21	2	23	0	0	0	0	0	0	11	0	11	2	0	2	0	0	0	0	0	0	2	0	2	4	0	4	507		507
	Dec (Post IRS)	3	0	3	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	331		331
	Jan (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	158		158
	Feb (Post IRS)	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	259		259
	March	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	95		95
	Subtotal	**136**	**17**	**153**	**3**	**0**	**3**	**0**	**0**	**0**	**50**	**0**	**50**	**6**	**6**	**6**	**0**	**0**	**0**	**0**	**0**	**0**	**4**	**1**	**5**	**7**	**0**	**7**	4013	0	4013
Bambasi	July (Post IRS)	47	13	60	0	0	0	25	0	25	141	4	145	55	1	56	75	0	75	0	0	0	0	0	0	0	0	0	112		112
	Aug (Post IRS)	75	0	75	0	0	0	57	2	59	213	2	215	146	1	147	171	5	176	0	0	0	0	0	0	0	0	0	358		358
	Sept (Post IRS)	29	1	30	0	0	0	81	0	81	300	8	308	223	4	227	171	1	172	0	0	0	0	0	0	0	0	0	948		948
	Oct (Post IRS)	5	0	5	0	0	0	55	1	56	201	6	207	43	1	44	45	0	45	0	0	0	0	0	0	0	0	0	834		834

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/ cydippis*			*An. tenebrosus*			*An. demeilloni*			*An. pretoriensis*			Culicines		
		CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total
	Nov (Post IRS)	2	1	3	0	0	0	61	2	63	64	8	72	6	0	6	19	1	20	0	0	0	0	0	0	0	0	0	650		650
	Dec (Post IRS)	6	0	6	0	0	0	37	6	43	55	0	55	10	0	10	1	0	1	0	0	0	0	0	0	0	0	0	532		532
	Jan (Post IRS)	0	0	0	0	0	0	15	0	15	11	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87		87
	Feb (Post IRS)	5	0	5	0	0	0	2	1	3	4	0	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	38		38
	March (Post IRS)	2	0	2	0	0	0	1	0	1	4	0	4	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	11		11
	Subtotal	**171**	**15**	**186**	0	0	0	**334**	**12**	**346**	**993**	**28**	**1021**	**486**	**7**	**493**	**482**	**7**	**489**	0	0	0	0	0	0	0	0	0	**3570**	**0**	**3570**
Lare	July (Post IRS)	38	0	38	146	1	147	0	0	0	38	0	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Aug (Post IRS)	28	0	28	133	2	135	0	0	0	48	0	48	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0		
	Sept (Post IRS)	125	17	142	184	2	186	0	0	0	53	0	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Oct (Post IRS)	94	5	99	3	0	3	0	0	0	8	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Nov (Post IRS)	52	11	63	74	4	78	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Dec (Post IRS)	20	0	20	4	0	4	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Jan (Post IRS)	8	0	8	1	1	2	2	1	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	Feb (Post IRS)	8	1	9	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		
	March (Post IRS)	8	1	9	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		
	Subtotal	381	35	416	545	10	555	6	1	7	149	0	149	**0**	**0**	**0**	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Benatsemay	July (No IRS)	34	29	63	19	5	24	0	0	0	5	0	0	0	0	0	0	0	0	5	1	6	0	0	0	0	0	0	393		393
	Aug (No IRS)	38	109	147	7	0	7	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	237		237
	Sept (No IRS)	4	16	20	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	515		515
	Oct (No IRS)	14	33	47	28	0	28	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	5	6	0	0	0	1		1
	Nov (No IRS)	No entomological monitoring has been done because of security problem.																													
	Dec (No IRS)	0	2	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	10	1	11	0	0	0	114		114

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/ cydippis*			*An. tenebrosus*			*An. demeilloni*			*An. pretoriensis*			Culicines		
		CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total
	Jan (No IRS)	1	4	5	6	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	4	9	0	0	0	155		155
	Feb (No IRS)	1	3	4	3	0	3	1	0	1	6	0	0	0	0	0	0	0	0	0	0	0	8	5	13	0	0	0	165		165
	March (No IRS)	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	38		38
	Subtotal		**198**	**291**	**66**	**6**	**72**	**1**	**1**	**2**	**14**	**0**	0	0	0	0	0	0	**0**	**5**	**1**	**6**	**24**	**17**	**41**				**1618**	**0**	**1618**
Harbu	July (No IRS)	109	15	124	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	4	0	4	4	3	7	0	0		931		931
	Aug (No IRS)	33	12	45	2	0	2	0	0	0	0	0	00	0	0	0	0 0	00	0	0	0	0	0	2	2	0	0		1721		1721
	Sept (No IRS)	833	35	868	54	0	54	0	0	0	52	0	52 0	0	0	0	0 0	00	0	38	0	38	0	2	2	0	0	0	4344		4344
	Oct (No IRS)	38	6	44	8	0	8	0	0	0	0	0	0 0	0	0	0	0 0	00	0	10	0	10	12	3	15	1	0	0	650		650
	Nov (No IRS)	17	2	19	0	0	0	0	0	0	0	0	0 **0**	**0**	**0**	**0**	0 **0**	0**0**	0	0	0	0	3	0	3	0	0	0	650		650
	Dec (No IRS)	15	3	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	5	0	5	0	0	0	634		634
	Jan (No IRS)	9	1	10	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	629		629
	Feb (No IRS)	6		6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	363		363
	Subtotal	**1060**	**74**	**1134**	**70**	**0**	**70**	**0**	**0**	**0**	**52**	**0**	**52**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**54**	**27**	**10**	**37**	**1**	**0**	**0**	**9922**	**0**	**9922**
Jabitehnan	July (No IRS)	22	1	23	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	481		481
	Aug (No IRS)	72	8	80	0	0	0	0	0	0	17	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	710		710
	Sept (No IRS)	69	14	83	0	0	0	0	0	0	43	0	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	686		686
	Oct (No IRS)	43	52	95	0	0	0	0	0	0	73	5	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	833		833
	Nov (No IRS)	143	12	155	0	0	0	0	0	0	65	5	70	0	0	0	0	0	0	0	0	0	3	0	3	0	0	0	389		389
	Dec (No IRS)	124	6	130	0	0	0	1	0	1	93	3	96	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	416		416
	Jan (No IRS)	110	2	112	0	0	0	0	0	0	57	0	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	584		584
	Feb (No IRS)	196	1	197	0	0	0	0	0	0	26	1	27	0	0	0	0	0	0	0	0	0	5	0	5	0	0	0	424		424
	March (No IRS)	165	4	169	0	0	0	0	0	0	16	0	16	0	0	0	0	0	0	0	0	0	4	1	5	0	0	0	335		335

Site	Time	*An. arabiensis*			*An. pharoensis*			*An. funestus* group			*An. coustani*			*An. ziemanni*			*An. squamosus/ cydippis*			*An. tenebrosus*			*An. demeilloni*			*An. pretoriensis*			Culicines		
		CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total	CDC	PSCC	Total
Subtotal		**944**	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**391**	**14**	**405**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**13**	**1**	**14**	**0**	**0**	**0**	**0**	**0**	**4858**
Metema	July (Pre 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	
	Aug (Pre IRS)	16	1	17	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	
	Sept (Post IRS)	112	2	114	1	0	0	11	0	0	12	0	12	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Oct (Post IRS)	27	3	30	0	0	0	15	0	0	9	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	Nov (Post IRS)	0	0	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	
	Dec (Post IRS)	0	1	1	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	
	Jan (Post IRS)	3	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	0	
	Feb (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	
	March (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	
	Subtotal	**158**	**7**	**165**	**1**	**0**	**0**	**29**	**1**	**0**	**21**	**0**	**21**	**0**	**0**	**0**	**11**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Annex B. Monthly Abundance of *An. stephensi* from Dire Dawa, Kebridehar, Awash, and Metehara Towns (July 2020-April 2021)

Month (2020-2021)	Dire Dawa							Kebridehar							Awash							Metehara						
	PSC (200 trap-days)	CDC (240 trap-nights)	Prokopack (56 trap-days)	Clay Pot (90 trap-night)	Black Box (90 trap-nights)	Cattle-baited Tent Trap (30trap nights)	Total	PSC(200 trap-days)	CDC(240 trap-nights)	Prokopack (90 trap-days	Clay Pot(90 trap-night)	Black Box (90 trap-nights)	Cattle-baited Tent Trap 30trap nights)	Total	PSC (180 trap-days)	CDC (216 trap-nights)	Backpack (36 trap-das)	Clay Pot (81 trap nights)	Black Box(81 trap nights)	Cattle-baited Tent Trap(27 trap nights)	Total	PSC (200 trap-days)	CDC (240 trap-nights	Backpack (60 trap-days)	Clay Pot (90 trap-nights)	Black Box (30 trap-nights)	Cattle-baited Tent Trap (10 trap nights)	Total
July	0	0	0	0	0	0	0	4	0	2	0	0	2	8	0	0	26	0	0	0	26	0	0	1	0	0	1	2
Aug	1	0	29	0	0	10	40	8	1	16	0	0	9	34	There was no surveillance							1	0	3	0	0	0	4
Sept	5	0	45	0	0	13	63	0	0	14	0	0	4	18	0	0	13	0	0	0	13	1	1	0	0	0	0	1
Oct	0	0	3	0	0	4	7	2	3	13	0	0	0	18	0	0	16	0	0	0	16	0	0	1	0	0	0	1
Nov	0	0	5	0	0	1	6	13	0	81	0	0	10	104	0	0	3	0	0	5	8	0	0	0	0	0	0	0
Dec	0	0	1	0	0	4	5	1	0	72	0	0	6	79	0	0	1	0	0	8	9	0	0	0	0	0	2	2
January	1	0	3	0	0	0	4	2	0	33	0	0	3	38	0	0	2	0	0	6	8	0	0	0	0	0	0	0
February	0	0	2	0	0	0	2	0	2	19	0	0	0	21	0	0	1	0	0	2	3	0	0	0	0	0	0	0
March	0	0	2	0	0	8	10	4	0	30	0	0	2	36	0	0	1	0	0	2	3	0	0	0	0	0	1	1
April	1	0	7	0	0	4	12	6	2	11	0	0	0	19	0	0	1	0	0	2	3	0	0	0	1	0	1	2

Month (2020-2021)	Dire Dawa							Kebridehar							Awash							Metehara						
	PSC (200 trap-days)	CDC (240 trap-nights)	Prokopack (56 trap-days)	Clay Pot (90 trap-night)	Black Box (90 trap-nights)	Cattle-baited Tent Trap (30trap nights)	Total	PSC(200 trap-days)	CDC(240 trap-nights)	Prokopack (90 trap-days	Clay Pot(90 trap-night)	Black Box (90 trap-nights)	Cattle-baited Tent Trap 30trap nights)	Total	PSC (180 trap-days)	CDC (216 trap-nights)	Backpack (36 trap-das)	Clay Pot (81 trap nights)	Black Box(81 trap nights)	Cattle-baited Tent Trap(27 trap nights)	Total	PSC (200 trap-days)	CDC (240 trap-nights	Backpack (60 trap-days)	Clay Pot (90 trap-nights)	Black Box (30 trap-nights)	Cattle-baited Tent Trap (10 trap nights)	Total
Overall	8	0	97	0	0	44	149	40	8	291	0	0	36	375	0	0	64	0	0	25	89	2	1	5	0	0	5	13

ANNEX C. RESULTS OF *AN. STEPHENSI* SURVEYS (2020)

Site	Breeding habitat type		Nov-20			Dec-20			Jan-21			Feb-21		
		# of sites sampled	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified
Awash	Cemented cistern	19	13	1008	302	17	1697	70	17	1380	21	19	1430	25
	Water tanker	5	3	317	203	2	163	5	2	52	5	1	11	3
	Water drum	6	0	0	0	1	73	NE	0	0	0	0	0	0
	Tire	1	0	0	0	1	16	NE	1	63	NE	1	8	NE
Total		31	16	1325	505	21	1949	75	20	1495	26	21	1449	28
Semera	Cemented cistern	18	9	1127	125	14	945	41	11	755	18	18	942	49
	Water tanker	3	2	70	0	4	49	25	2	8	0	3	221	3
	Plastic sheet	5	6	225	60	5	111	0	5	1089	25	2	283	14
	Tire	0	0	0	0	0	0	0	0	0	0	0	0	0
	Plastic bottle	2	0	0	0	1	18	10	1	26	0	0	0	0
	Water drum (barrel)	6	1	108	30	6	317	12	2	60	1	2	93	10
	Pipe open tube	6	1	10	0	2	6	4	2	9	0	1	26	0

Site	Breeding habitat type	# of sites sampled	Nov-20			Dec-20			Jan-21			Feb-21		
			# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified	# of breeding habitats positive for *Anopheles* larvae	# larvae sampled	Adult *An. stephensi* identified
Total		40	19	1540	215	32	1446	92	23	1947	44	26	1565	76
Dire Dawa	Cemented cistern	22	22	1644	149	14	798	115	14	813	210	15	1317	265
	Plastic sheet	4	1	401	11	3	347	41	3	85	18	4	355	35
	Water drum	1	1	13	4	0	0	0	0	0	0	0	0	0
	Car wash	1	1	73	1	1	71	16	1	235	42	1	316	18
	Cemented drum	2	2	136	28	2	86	3	1	25	5	1	55	11
	Stream edge	1	0	0	0	0	0	0	0	0	0	1	162	2
Total		31	27	2267	193	20	1302	175	19	1158	275	22	2205	331
Kebridehar	Cisterns (*Birka*)	68	43	3116	2918	34	3115	2693	52	2241	2086	58	2988	2948
	Tire	12	11	267	248	0	0	0	0	0	0	0	0	0
	Plastic containers	2	2	287	319	2	72	72	2	1012	900	2	313	313
	Water drum	6	3	98	94	3	45	45	3	44	44	4	94	94
Total		88	59	3768	3579	39	3232	2810	57	3297	3030	64	3395	3355

Annex D. Blood Meal Sources and Indices of *Anopheles* Mosquitoes by Sites of Collection (2020)

Site (# mosquitoes)	Species	No tested	H	B	G	H+B	H+G	B+G	H+B+G	Unknown	HBI	BBI	GBI
Abaya (n = 1)	*An. arabiensis*	1	0	0	0	0	1	0	0	0	100.0	0.0	100.0
Bambasi (n = 28)	*An. arabiensis*	1	0	0	0	0	0	1	0	0	0.0	100.0	100.0
	An. funestus	1	0	0	0	0	0	1	0	0	0.0	100.0	100.0
	An. coustani	18	1	0	2	0	3	3	0	9	22.2	16.7	44.4
	An. ziemanni	4	1	0	0	0	1	0	0	2	50.0	0.0	25.0
	An. squamosus	4	0	0	0	0	1	3	0	0	25.0	75.0	100.0
Gelana (n = 38)	*An. arabiensis*	24	1	0	2	0	19	1	0	1	83.3	4.2	91.7
	An. funestus	11	1	0	7	0	0	3	0	0	9.1	27.3	90.9
	An. ziemanni	2	0	0	0	0	1	0	1	0	100.0	50.0	100.0
	An. squamosus	1	0	0	0	0	0	0	1	0	100.0	100.0	100.0
Harbu (n = 282)	*An. arabiensis*	251	10	6	18	2	21	95	5	94	15.1	43.0	55.4
	An. pharoensis	11	1	0	0	0	0	5	0	5	9.1	45.5	45.5
	An. coustani	1	0	0	0	0	0	0	0	1	0.0	0.0	0.0
	An. demeilloni	15	0	0	2	0	0	11	0	2	0.0	73.3	86.7
	An. tenebrosus	4	0	0	0	0	0	4	0	0	0.0	100.0	100.0
Jabitehnan (n = 84)	*An. arabiensis*	76	4	6	2	1	6	34	7	16	23.7	63.2	64.5
	An. coustani	7	1	0	0	0	0	5	0	1	14.3	71.4	71.4
	An. demeilloni	1	0	0	0	0	0	1	0	0	0.0	100.0	100.0
Lare (n = 35)	*An. arabiensis*	30	4	0	0	0	2	0	0	24	20.0	0.0	6.7
	An. pharoensis	5	0	0	0	0	0	0	0	5	0.0	0.0	0.0
Metema (n = 18)	*An. arabiensis*	18	1	0	3	0	2	4	1	7	22.2	27.8	55.6
Dire Dawa (n = 109)	*An. arabiensis*	3	0	0	2	0	0	1	0	0	0.0	33.3	100.0
	An. stephensi	106	1	1	50	0	0	16	0	38	0.9	16.0	62.3

Site (# mosquitoes)	Species	No tested	H	B	G	H+B	H+G	B+G	H+B+G	Unknown	HBI	BBI	GBI
Kebri Dehar (n = 155)	*An. stephensi*	155	6	2	87	0	2	30	2	26	6.5	21.9	78.1
Total (n = 750)		**750**	**32**	**15**	**175**	**3**	**59**	**218**	**17**	**231**	**14.8**	**33.7**	**62.5**

Note: H=human, B=bovine, G=goat

ANNEX E. INSECTICIDE SUSCEPTIBILITY TEST RESULTS OF *AN. ARABIENSIS* (2020)

Region	District	% mortality											
		Bendiocarb		Propoxur		Pirimiphos-methyl		Alpha-cypermethrin		Deltamethrin		Permethrin	
		Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control
Afar	Amibara	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	65 (65/100) R	0 (0/50)	67 (67/100) R	2 (1/50)	40 (40/100) R	0 (0/50)
Amhara	Jabitehnan	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	71 (71/100) R	0 (0/50)	82 (82/100) R	0 (0/50)	82 (82/100) R	0 (0/50)
	Metema	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	45 (45/100) R	0 (0/50)	45 (45/100) R	0 (0/50)	66 (66/100)	0(0/50)
Benishangul-Gumuz	Bambasi	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	83 (83/100) R	0 (0/50)	89 (89/100) R	0 (0/50)	12 (12/100) R	0 (0/50)
Gambela	Abobo	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	2 (1/50)	37 (37/100) R	0 (0/50)	45 (45/100) R	2 (1/50)	52 (52/100) R	0 (0/50)
Oromia	Abaya	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	57 (57/100) R	0 (0/50)	61 (61/100) R	0 (0/50)	62 (62/100) R	0 (0/50)
	Omonada	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	56 (56/100) R	0 (0/50)	35 (35/100) R	0 (0/50)	48 (48/100) R	0 (0/50)
SNNPR	Benatsemay	100 (100/100)	0	100 (100/100)	0	100 (100/100)	0 (0/50)	12	0	25	0	48	0

Region	District	% mortality											
		Bendiocarb		Propoxur		Pirimiphos-methyl		Alpha-cypermethrin		Deltamethrin		Permethrin	
		Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control
		S	(0/50)	S	(0/50)	S		(12/100) R	(0/50)	(25/100) R	(0/50)	(48/100) R	(0/50)
	Misrak Badawacho	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	8 (8/100) R	0 (0/50)	48 (48/100) R	0 (0/50)	25 (25/100) R	0 (0/50)
Somali	Erer	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	17 (17/100) R	0 (0/50)	15 (15/100) R	0 (0/50)	27 (27/100) R	0 (0/50)
Tigray	Humera	100 (100/100) S	2 (1/50)	100 (100/100) S	0 (0/50)	100 (100/100) S	0 (0/50)	27 (27/100) R	4 (2/50)	39 (39/100) R	2 (1/50)	25 (25/100) R	0 (0/50)

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

Annex F. Results of Insecticide Susceptibility and Resistance Intensity of *An. stephensi* (2020)

Insecticide/Control	% mortality (Dead/Exposed)			
	Awash	Meki	Metehara	Goday
Bendiocarb	13 (13/100)	4 (4/100)	7 (7/100)	26 (26/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Propoxur	19 (19/100)	9 (9/100)	17 (17/100)	79 (79/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Pirimiphos-methyl	1 (1/100)	0 (0/100)	35 (35/100)	67 (67/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Alpha-cypermethrin 1X	10 (10/100)	33 (33/100)	62 (62/100)	1 (1/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Alpha-cypermethrin 5X	42 (42/100)	68 (68/100)	78 (78/100)	11 (11/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Alpha-cypermethrin 10X	66 (66/100)	83 (83/100)	81 (81/100)	18 (18/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Deltamethrin 1X	15 (15/100)	59 (59/100)	17 (17/100)	8 (8/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Deltamethrin 5X	56 (56/100)	91 (91/100)	36 (36/100)	55 (55/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Deltamethrin 10X	99 (99/100)	95 (95/100)	83 (83/100)	98 (98/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Permethrin 1X	43 (43/100)	72 (72/100)	93 (93/100)	10 (10/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)
Permethrin 5X	98 (98/100)	99 (99/100)	100 (100/100)	98 (98/100)
Control	0 (0/50)	0 (0/50)	0 (0/50)	0 (0/50)

Annex G. Susceptibility of Wild *An. arabiensis* and *An. stephensi* to Chlorfenapyr (2020)

Species	Site	# tested	% mortality*			Control			
			24 h	48 h	72 h	# exposed	24 h	48 h	72 h
An. arabiensis	Abaya	113	31.0	55.8	100.0	43	2.3	7.0	7.0
	Amibara	104	98.1	100.0		20	0	0	0
	Bambasi	100	90.0	99.0	100.0	20	0	0	0
	Metema	100	94.0	99.0	100.0	25	4.0	4.0	4.0
An. stephensi	Awash	103	92.2	100.0		20	0	0	0
	Semera	101	92.1	100.0		23	0	0	0

*Number of *An. arabiensis* and *An. stephensi* tested in each WHO tube test was 100.

ANNEX H. MORTALITY OF *AN. ARABIENSIS* FROM RESISTANCE INTENSITY ASSAYS (2020)

Sentinel site	% mortality*								
	Alpha-cypermethrin			Deltamethrin			Permethrin		
	1X	5X	10X	1X	5X	10X	1X	5X	10X
Abaya	57	60	89	61	83	97	62	81	100
Abobo	37	80	100	45	78	98	52	85	100
Amibara	65	81	98	67	86	98	40	88	98
Bambasi	83	89	100	89	100		12	89	100
Benatsemay	12	66	95	25	79	98	48	92	96
Jabitehnan	71	90	100	82	100		81	99	
Omonada	56	75	100	35	71	100	48	78	98

*Number of *An. arabiensis* tested was in each WHO tube test 100.

ANNEX I. *AN. ARABIENSIS* MORTALITY FROM PBO SYNERGIST TESTS (2020)

Insecticide*	Abaya	Abobo	Amibara	Bambasi	Benatsemay	Erer	Humera	Metema	Misrak Badawacho	Omonada
Alpha only	61.3	41.3	74.7	74.7	65.3	10.7	37.3	68	1.3	42.7
Alpha +PBO	100	98.7	100	100	100	90.7	93.3	98.7	64	100
Del only	70.7	46.9	82.7	80	61.3	26.7	53.3	58.7	16	53.3
Del+PBO	100	96	100	100	100	100	100	96	93.3	100
Perm only	70.7	52	62.7	24	66.7	41.3	58.7	66.7	34.7	53.3
Perm + PBO	100	97.3	92	77.3	98.7	100	100	97.3	93.3	100

*Number of *An. arabiensis* used for each test was 100.

Annex J. Results of Cone Bioassay Tests (2020)

Test site	Spray date	Wall surface	Mosquito origin	Within a week	One month	Two months	Three months	Four months	Five months	Six months
Lare (Gambela)	**Jun-20**	Mud	*An. arabiensis* (Susceptible colony)	100.0	99.5	98.0	97.2	83.6	71.4	63.0
Godare (Gambela)	**Jun-20**	Mud	*An. arabiensis* (Susceptible colony)	100.0	99.2	95.4	82.9	76.7	65.0	Dropped
		Painted mud		100.0	100.0	100.0	83.3	70	64.0	Dropped
Abaya (Oromia)	**Jun-20**	Cemented mud	*An. arabiensis* (Susceptible colony)	100.0	93.3	76.3	70.0	Dropped	Dropped	Dropped
		Dung		100.0	88.9	73.3	80.0	73	Dropped	Dropped
		Painted mud		100.0	98.7	91.3	89.3	83.3	81.1	78.0
		Mud		100.0	92.9	71.7	68.3	Dropped	Dropped	Dropped
		Painted cement		100.0	100.0	85.6	77.8	76.7	Dropped	Dropped
Bambasi (Benishan gul-Gumuz)	**Jun-20**	Mud	*An. arabiensis* (Susceptible colony)	100.0	NA	100.0	87.8	69.4	75.0	Dropped
		Painted mud		100.0	NA	100.0	81.0	69.4	67.8	Dropped
		Mud	*An. arabiensis* (wild)	NA	100.0	NA	NA	NA	NA	NA
		Painted mud		NA	100.0	NA	NA	NA	NA	NA
Menge (Benishan gul-Gumuz)	**Jun-20**	Mud	*An. arabiensis* (Susceptible colony)	100.0	NA	100.0	93.9	77.7	57.8	Dropped
		Painted mud		100.0	NA	99.3	96.0	75.3	63.3	Dropped
		Painted cement		100.0	NA	100.0	100.0	86.7	70.0	46.7
		Mud	*An. arabiensis* (wild)	NA	96.7	NA	NA	NA	NA	NA
		Painted mud		NA	92.7	NA	NA	NA	NA	NA
		Painted cement		NA	96.7	NA	NA	NA	NA	NA

NA=Not Applicable

Annex K. Results of Cone Bioassays of SumiShield (2020-21)

Time of test	Mud*					Painted mud*				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Within a week	61.7% (180)	83.3% (180)	94.4 % (180)	98.1% (180)	100 % (180)	70.6 % (180)	90.6% (180)	98.3% (180)	99.4 % (180)	100% (180)
One month	82.% (180)	91.7 % (180)	95% (180)	98.3% (180)	98.9% (180)	86% (180)	91.7% (180)	94.4% (180)	98.3 % (180)	100 % (180)
Two months	90.6% (180)	97.2% (180)	100% (180)			96.7% (180)	98.9% (180)	100% (180)		
Three months	74.3% (180)	84.8% (180)	94.5% (180)	98.8% (180)		86.7% (180)	95.6% (100)	99.4% (180)	100% (180)	
Four months	67.8% (180)	80.6 % (180)	89.4% (180)	98.3% (180)	100% (180)	62.2% (180)	80% (180)	90.6% (180)	98.3% (180)	100 % (180)
Five months	57 (180)	71.7 (180)	80.6 (180)	97.8 (180)	100 (180)	60(180)	68 (180)	75 (180)	96 (180)	100 (180)
Six months	53.9 (180)	72.0 (180)	81.7 (180)	90.6 (180)	98.9 (180)	48.3 (180)	61.7 (180)	81 .0 (180)	88.9 (180)	97.8 (180)
Seven months	49.4 (180)	56.7 (180)	72.2 (180)	86.1 (180)	98.9 (180)	50.6 (180)	58.3 (180)	77.2 (180)	91.0 (180)	100 (180)
Eight months	17 (180)	26 (180)	35.6 (180)	46 (180)	58.9 (180)	20.6 (180)	32 (180)	41 (180)	48.9 (180)	60.6 (180)

*Figures in brackets are number tested.

Annex L. Results of Cone Bioassays of Fludora Fusion Using Insectary *An. arabiensis* (2020-21)

Time of test	Mud*					Painted mud				
	Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day5
Within a week	98 % (300)	100 % (300)				85 % (60)	90 % (60)	100 % (60)		
One month	93.7 % (300)	98.3% (300)	99.7% (300)	99.7 % (300)	100 % (300)	83% (60)	100 % (60)			
Two months	99% (300)	99.7% (300)	100% (300)			100% (60)				
Three months	69.3% (300)	81.3% (300)	99% (300)	100% (300)		63.3% (60)	81.7% (60)	98.3% (60)	100% (60)	
Four months	63% (300)	74.3% (300)	85.7% (300)	96% (300)	100% (300)	70% (60)	83.3% (60)	95% (60)	98.3% (60)	100% (60)
Five months	77.3 (300)	87.7 (300)	97 (300)	100 (300)		55 (60)	68.3 (60)	88.3 (60)	95(60)	100 (60)
Six months	55.0 (300)	65.0 (300)	75.7 (300)	88.7 (300)	100 (300)	53.3 (60)	60.0 (60)	76.7 (60)	91.7 (60)	100 (60)
Seven months	34.3 (300)	60.7 (300)	74.3 (300)	84.7 (300)	99 (300)	33.3 (60)	63.3 (60)	73.3 (60)	83.3 (60)	96.7 (60)
Eight months	20.7 (300)	32.7 (300)	41 (300)	52 (300)	61.3 (300)	16.7 (60)	19.3* (60)	29.8** (60)	40.3** (60)	49** (60)

**Figures in brackets are number tested.

Annex M. Results of Cone Bioassays of Fludora Fusion Using Wild *An. arabiensis* (2020-21)

Surface type	Time of bioassay test	% mortality						
		24	48	72	96	120	144	168
Mud	Three months	68.0	80.0	93.0	99.0	100.0		
	Four months	53.3	69	78.7	90.7	100.0		
	Five months	51.3	68.0	80.3	92.7	100.0		
	Six months	51.7	68.7	81.0	91.3	100.0		
	Seven months	30.0	50.3	67.7	84.3	97.3	99.7	
	Eight months	15.0	23.0	28.7	40.7	51.7	56.7	61.3
Painted mud	Three months	65.0	81.7	91.7	95.0	100.0		
	Four months	43.3	65.0	80.0	90.0	100.0		
	Five months	46.7	63.3	80	85.0	100.0		
	Six months	50.0	63.3	71.7	93.3	100		
	Seven months	35.0	55.0	70.0	83.3	100.0		
	Eight months	10.0	20.0	30.0	38.3	48.3	55.0	61.7

Annex N. Results of Actellic 300cs Fumigant Bioassay Tests (2020)

	Lare	Godare		Bambasi		Menge			Abaya				
Time	Mud	Mud	Painted mud	Mud	Painted mud	Mud	Painted mud	Painted cement	Cemented mud	Painted mud	Dung	Mud	Painted cement
Within a week	100	51.3	75	45.6	37.1	57	77.5	50	10	8.3	10	30	5
One month	95	95.1	97.5						20	36.7	10	40	35
Two months	73.3	70	77.5	55	65	81.7	90	60	0	0	0	0	10
Three months	73.3	56.3	50	45	26.7	38.3	30	60					
Four months	52.5	50	45	28.3	25	43.3	30	30					
Five months	43.3	43.8	47.5	35	30	18	21.7	50					
Six months	33.3					18	20	20					
Seven months						28	33.3	30					
Eight months						0	0	0					

ANNEX O. RESULT OF SUMISHIELD FUMIGANT BIOASSAY TESTS (2020-2021)

Surface type	Time of test	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
Mud	Within a week	50.0	83.0	94.3	100.0			
	One month	65.0	75.0	81.7	86.7	93.3	95.0	
	Two months	53.3	70.00	95.0	100.0			
	Three months	20.0	41.7	48.3	61.7	76.7	86.7	90.0
	Four months	38.3	56.7	75.0	91.7	100		
	Five months	31.7	43.3	55	78.3	100		
	Six months	26.7	46.7	58.3	76.7	88.3		
	Seven months	15	33.3	55	73.3	95	96.7	100
	Eight months	6.7	13.3	21.7	28.3	41.7	46.7	55
Painted mud	Within a week	37.3	75.0	90.9	94.5	98.2	100.0	
	One month	50.0	76.7	80.0	83.3	90.0	90.0	
	Two months	35.0	71.7	76.7	100.0			
	Three months	30.0	48.3	71.7	81.7	88.3	95.0	100.0
	Four months	28.3	50.0	71.7	80.0	100.0		
	Five months	33.3	35	53.3	76.7	100		
	Six months	20	35	55	76.7	90		
	Seven months	15	31.7	56.7	78.3	100		
	Eight months	5	16.7	25	28.3	36.7	50	56.7

Annex P. Result of Fludora Fusion Fumigant Bioassay Tests (2020-2021)

Surface type	Time of spraying	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
Mud	Within a week	78.8	87.9	92.9				
	One month	77.0	89.0	92.0	92.0	93.0		
	Two months	88.0	95.0	96.0	100.0			
	Three months	32.0	61.0	84.0	92.0	97.0		
	Four months	36.0	53.0	70.0	83.0	97.0	100.0	
	Five months	19.0	49.0	64.0	86.0	100.0		
	Six months	29.0	39.0	59.0	80.0	98.0		
	Seven months	13.0	42.0	54.0	71.0	96.0	99.0	100.0
	Eight months	6.0	14.0	24.0	32.0	41.0	56.0	67.0
Painted mud	Within a week	65.0	80.0	100.0				
	One month	55.0	85.0	90.0	95.0	95.0		
	Two months	85.0	85.0	100.0				
	Three months	20.0	65.0	90.0	90.0	100.0		
	Four months	30.0	50.0	70.0	70.0	95.0	100.0	
	Five months	15.0	50.0	70.0	90.0	100.0		
	Six months	35.0	40.0	60.0	75.0	10		
	Seven months	15.0	45.0	55.0	70.0	95.0	100.0	
	Eight months	5.0	15.0	20.0	25.0	35.0	50.0	65.0