Laboratory-based efficacy evaluation of Bacillus thuringiensis subspecies israelensis and temephos larvicides against larvae of Anopheles stephensi in Ethiopia

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Abstract

Background: Malaria, transmitted by the bite of infected female *Anopheles* mosquitoes, remains a global public health problem. The presence of an invasive *Anopheles stephensi,* capable of transmitting *Plasmodium vivax* and *P. falciparum* parasites was first reported in Ethiopia in 2016. The ecology of *An. stephensi* is different from that of *An. arabiensis*, the primary Ethiopian malaria vector, and this suggests that alternative control strategies may be necessary. Larviciding may be an effective alternative strategy, but there is limited information on the susceptibility of Ethiopian *An. stephensi* to common larvicides. This study aimed to evaluate the efficacy of temephos and *Bacillus thuringiensis var. israelensis* (*Bti*) larvicides against larvae of invasive *An. stephensi*.

Methods: The diagnostic doses of two larvicides, temephos (0.25ml/l) and *Bti* (0.05mg/l) were tested in the laboratory against the immature stages (late third to early fourth stages larvae) of *An. stephensi* collected from the field and reared in a bio-secure insectary. Larvae were collected from two sites (Haro Adi and Awash Subuh Kilo). For each site, three hundred larvae were tested against each insecticide (as well as an untreated control), in batches of 25. The data from all replicates were pooled and descriptive statistics prepared.

Results: The mortality of larvae exposed to temephos was 100% for both sites. Mortality to *Bti* was 99.7% at Awash and100% at Haro Adi site.

Conclusions: Larvae of *An. stephensi*are susceptible to temephos and *Bti*larvicides suggesting that larviciding with these insecticides as vector control program may be effective against *An. stephensi* in these localities.

Introduction

Malaria is a global public health problem that mainly affects tropical countries (WHO, 2019b). It is transmitted by the bite of infective female *Anopheles* mosquito species. Globally, there are some 3,530 species of mosquitoes under 43 genera in the family Culicidae, which further divide into the subfamilies of *Culicinae, Anophelinae* and *Toxorhynchitinae* (Bockarie, 2005). Of these, malaria vectors belong to the genus *Anopheles* (Bockarie, 2005).

In Ethiopia, *Anopheles arabiensis* is the main malaria vector while *An. pharoensis, An. funestus* and *An. nili* are secondary vectors (MoH, 2018). The recently reported invasive species, *An. stephensi* in the country has exhibited the potential of transmitting *Plasmodium falciparum* and *P. vivax* (Ashine et al., 2020; Balkew et al. 2021). The species has also been reported from different Horn of African countries including Djibouti (2012), Sudan (2016), and Somalia (2019) raising concern about appropriate vector control strategies to target this invasive species in those countries (Balkew et al., 2020; WHO, 2019a).

Unlike the other malaria vectors, *An. stephensi* is also considered an urban and peri-urban adapted malaria vector, which breeds in man-made habitats such as overhead tanks, ditches and canals (Balkew

et al., 2020, 2021; Thomas et al., 2016). *Anopheles stephensi* feeds on both humans and animals, with a preference for the latter, and it exhibits more outdoor feeding (Ashine et al., 2020). *Anopheles stephensi* in Ethiopia is resistant to most insecticides used for bed-nets and indoor residual spraying (IRS) (Yared et al., 2020; Balkew et al., 2021) so larviciding might be an effective control method (Balkew et al., 2021).

Larval source management (LSM) is one of the oldest and primary strategies used throughout the world to control malaria targeting the immature stages of the mosquito vectors in their aquatic habitats; however it has been less commonly used in African countries following the introduction of indoor residual spraying (IRS) in the 1950s and long-lasting insecticide impregnated nets (LLINs) in the 1990s (Thwing et al., 2013; WHO, 2014). Organophosphates such as temephos and pirimiphos-methyl interfere with the nervous system of the immature larval stages, whereas microbes such as *Bacillus thuringiensis israeliensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) kill the larvae with their toxins when ingested (Thwing et al., 2013; WHO, 2014).

In Ethiopia, concerted efforts have been made in the fight against malaria since the 1950s. The intervention strategies include early diagnosis and prompt treatment of cases, IRS, prevention and control of epidemics, and recently, scale-up of LLINs and LSM through larviciding and environmental management at small scale, where breeding sites are few, findable and manageable (MoH, 2018; WHO, 2014). However, resistance to insecticides used in vector control by *An. stephensi* has been reported from within the country (Yared et al., 2020) and other places (WHO, 2019a). Therefore, in order to tailor the local strategies to the types of resistance present in the vectors (WHO, 2012; Balkew et al. 2021), it is crucial to investigate the efficacy of selected malaria vector control interventions towards the control of *An. stephensi*, especially in areas where research has not yet covered in a holistic manner.

Materials And Methods

Anopheles stephensi **collection, rearing and identification**

Anopheles stephensi larval and pupal collection sites

Larvae and pupae of *An. stephensi* were collected from Awash Subah Kilo Town (also spelled as Awash Sebat Kilo in other publications) and Haro Adi around Metehara from January 2021 to June 2021. Awash Subah Kilo Town is located in Administrative Zone 3 of the Afar Region, just above a gorge of the Awash River, after which it is named. The town lies on the Addis Ababa–Djibouti Railway line at about 217 km from Addis Ababa. This town is the largest settlement in Awash Fentale district, lying at a longitude of 08°59'N 40°10'E at an elevation of 986 meters, with favourable climate and altitude for malaria vector breeding and optimal to parasite sporogonic cycle completion. Metehara is also a town in central Ethiopia; located in the East Shewa Zone of the Oromia Region, on a longitude of 08°54'N 39°55'E, at an elevation of 947 meters above sea level. Haro Adi village, from where the larvae and pupae of *An. stephensi* were collected, is a village to the south of Metehara Town along Lake Beseka located about two kilometers away from Metehara Town (Table 1 and Fig. 1).

A total of 45 breeding sites/habitats, in and around the towns of Awash Subah Kilo and Metehara and Haro Adi village areas were visited for larval and pupal surveys. Of these, 31 breeding habitats were from Awash Subah Kilo Town, 7 from Metehara Town, and 7 from Haro Adi village. The survey of *An. stephensi* larvae and pupae was carried out in three sites, namely; *Awash* Subah Kilo Town, Metehara Town and Haro Adi around Metehara Town (Fig. 1). The survey for *An. stephensi* larvae and pupae was conducted in metal tanks near houses under construction, in jerry cans where water is reserved for daily household consumption, on cemented water banks for daily household consumption, on water reservoirs with geo-membrane plastic (near *Metehara health center*), overhead water tanks, and in cemented burrows of water reserved for production of cement blocks. These sites were selected based on the previous reports of the presence of *An. stephensi* (Ashine et al., 2020; Balkew et al., 2020). The sampling of breeding sites was managed based on the WHO guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005). All natural and man-made breeding sites around the study areas were assessed for the presence or absence of *An. stephensi* larvae. All larval instars and pupae were collected and taken to a bio-contained facility in the insectary of Aklilu Lemma Institute of Pathobiology (ALIPB).

Larvae and pupae were collected using a WHO standard dipper and transferred into a plastic jar of five-litre capacity with a handle and a cover with plenty of holes to allow air circulation. The jar was used for handling and transporting the larvae and pupae. The scooped larvae and pupae were filtered using clean cheesecloth prepared for this purpose and transferred to a plastic jar. Then approximately 1-1.5 litres of water along with debris of plants, from their natural breeding sites was added for larvae to feed on until they reached the insectary.

Rearing Anopheles stephensi mosquitoes

The larvae and pupae collected from the field were transported and reared to adults in the insectary. During mosquito rearing all of the lab conditions, such as maintaining the temperature at 27 ± 2°C and 75 ± 10% relative humidity, were met and monitored. Upon arrival in the insectary, larvae were transferred into a white enamel plastic tray. Once larvae were removed from their natural water source in the plastic container using plastic micropipettes, a diet of baker's yeast was added to the larval tray. After 5 minutes the tray was swirled to distribute the powder and prevent suffocation from undiluted/accumulated powder (EPHI, 2017). Larvae were provided food twice per day, and trays were checked to see if food remained unconsumed, and if larvae food remained unconsumed, no food was added.

Sorting of pupae from larvae was undertaken on a daily basis. Pupae were picked with plastic pipettes and transferred into a beaker with fresh deionized water and then transferred to adult holding cages. Adults in the cage were provided with sugar solution using soaked/wetted cotton ball placed on the top of the meshed cage. The cotton was maintained wet so that mosquitoes could feed on the sugar. The cotton balls were changed every 5 or 6 days, in order to avoid the growth of mold spores and/or fungus on the pad exposed to sugar (EPHI, 2017). Concurrent with sugar feeding, 3–7 days old female mosquitoes were fed on rabbit blood meals twice per week (ethical approval was obtained from Addis Ababa University-Aklilu Lemma Institute of Pathobiology (AAU-ALIPB) Ethical Review Board)). Water filled petri-dish and/or wet filter paper supported with cotton and placed on a petri-dish were provided for

mosquitoes to lay eggs on. Breeding of wild-collected *An. stephensi* colonies continued until the end of the study. The tests were done on F_1 and F_2 generations of the field-collected larvae and pupae.

Anopheles stephensi species identification

Mosquito species identification was undertaken morphologically under a dissecting microscope. Before commencing any efficacy test of the selected larvicides against *An. stephensi*, 30 adult female mosquitoes were randomly aspirated from cages. Then these mosquitoes were transferred into a glass tube and exposed to chloroform by cotton ball wetted at tip. Each of these mosquitoes was laid under a stereomicroscope at 40X for morphological identification using the updated key to the females of Afro-tropical *Anopheles* mosquitoes, which includes *An. stephensi* (Coetzee, 2020). All were confirmed to be *An. stephensi* mosquito species. There were fewer *Culex* and *Aedes* species larvae as compared to *An. stephensi*, collected from the same habitats. Though there were a few *Culex* and *Aedes* species larvae collected with *An. stephensi*, all those aspirated from the cage were *An. stephensi.* The typical features with *An. stephensi* mosquito's morphology are (i) the appearance with 3 pale bands in the palpus and the two apical pale bands are very broad with speckling on palpus segment 3 and (ii) in the 2nd main dark area on the vein 1of its wing, there are 2 pale interruptions (Coetzee, 2020). The specimens were not stored for further molecular confirmation because of financial limitations and the inability to preserve the specimens for a longer time. However, rearing of the colony in the insectary has continued.

Efficacy of Bacillus thuringiensis var. subspecies israelensis **and temephos against** An. stephensi **larvae**

Bacillus thuringiensis var. species israelensis (Bti); VectoBac WDG (FourStar®Briquets) of a solid form; produced by *DBA FourStar Microbials* LLC. 1501 East Woodfield Road, #200W (https://www.centralmosquetocontrol.com/all-products/fourstar/fourstar-briquet-180) in January 2019 and with expiry date of December 2023, were acquired from ICIPE/ILRI. The powder form of this bacterial larvicide was weighed on digital weighing scale and prepared in increasing doses of 0.05g, 0.1g and 0.2g, in such a way that it was to be applied in a container of 2000cm^2 with one litre water volume until the dose mortality response was reached. Based on this design, the lowest prepared concentration of *Bti* (0.05g/l), was added to the water and remained for 48 hours by covering the container to prevent insects from landing or laying egg in it (Demissew et al., 2016). In order to assure no insects entered into the larvicide-treated water, the tray remained closed. The insectary had two secured doors, a double door at the entrance and each unit of the insectary had its own door and closed glass windows. The subsequent tests were done following the same procedure.

In preparation to expose the larvae to larvicides, late third to early fourth instar larvae were sorted in disposable cups with water using pipettes. Larvae were filtered first through cheesecloth on a separate container for this purpose. The filtered larvae were immediately transferred into plastic containers having an area of 2000cm^2 and containing one litre of water treated with *Bti* of 0.05g, as per the application recommended for spot spray (BASF SE, n.d.). Batches of 25 larvae were exposed per testing container. Simultaneously an equal number of larvae (negative controls) were tested using untreated deionized water and with same number of larvae per container. The tests were done in four replicates. The

experiment was repeated three times on different days. This test was repeated for larvae collected from each site. The two higher doses (0.1g and 0.2g) were not tested because larvae had already responded to the lowest dose of *Bti* (0.05g).

Temephos, an emulsifiable liquid concentrate containing 500g of active ingredient per liter, brand name BASF-Abate®500E, developed in Malaysia in 2018 (https://www.mkhardware.com.my/pages/pages_id/13613/) with no stated expiry date, was acquired from Ethiopian public health institute (EPHI), and tested against *An. stephensi* larvae. Following the same procedure used for *Bti* testing, temephos of 0.25ml/l, 0.5ml/l and 1ml/l was prepared to be tested in increasing concentrations, until the dose response was saturated. Temephos (0.25ml) was added to a container of 2000cm^2 with one litre water volume using 1000ml capacity micropipette. Four replicates were set up for each concentration and each was run three times on different days. An equal number of negative controls were set up simultaneously with deionized water. The late third and early fourth stage larvae, collected from the field and from reared adults (F0, F1 and F2), were used for the larvicidal test. Larvae were first collected from the tray using pipettes into disposable plastic cups containing water. Then 25 larvae were filtered on and immediately transferred into the container of 1000ml water treated with 0.25ml of temephos. Larvae were confirmed susceptible to the lowest concentration of temephos (0.25ml/l) and the higher prepared concentrations were left not tested.

While conducting the efficacy tests of both larvicides, larval mortality was recorded after 24 hrs. Larvae that sank down to the bottom of water, in the case of temephos, and appeared floating on the water with swollen and blackened bodies, in the case of *Bti*, were considered dead. The WHO guidelines for laboratory and field testing of larvicides, states that the test should be rejected if the control mortality is > 20% or pupation is > 10% (WHO, 2005).

Data Analysis

The data were recorded using the WHO larvicide efficacy evaluation result recording form (WHO, 2005). The data from all replicates was pooled and entered into an excel spreadsheet for analysis using STATA version 14.0. Statistical analysis was not done because of the high susceptibility to the larvicides, with the exception of one larva exposed to *Bti*.

If the control mortality was between 5% and 20%, the mortalities of treated groups were corrected according to Abbott's formula. Tests with control mortality greater than 20% or pupation greater than 10% were discarded.

The mortality of the test sample was calculated by summing the number of dead larvae across all exposure replicates and then expressing this as a percentage of the total number of exposed larvae.

Data Quality Assurance

The work to generate quality data started from strictly implementing the control of other factors, such as temperature, humidity, dose of larvicides, and conducting the test as per the laboratory procedures to gather all the important information from the study. In addition, data were rechecked for proper capturing at recording, organizing, cleaning, and analysis steps.

Ethical Consideration

This study involved no human subjects and it was implemented after obtaining ethical clearance (Ref. No.: ALIPB IRB/40/2013/21, dated: Feb 10, 2021) from the IRB of Aklilu Lemma Institute of Pathobiology, Addis Ababa University.

Results

Larvae and pupae were found only in two of the surveyed sites (Awash Subah Kilo Town and Haro Adi village around Metehara), and only in water reserved for production of cement blocks with small manual factories for construction purposes. Larvae and pupae of *Anopheles* species were found in cemented water reservoirs and cisterns, but the plastic tankers, overhead metal tankers, barrels, jerry cans, geo-membrane sheets, burrow and some cisterns were observed, mainly, with larvae of *Culex/Aedes*. From the total of 45 surveyed habitats, 5 out of 31 (16.2%) in Awash Subah Kilo Town, 3 out of 7 (42.9%) in Haro Adi, and none out of 7 in Metehara Town were found positive for *An. stephensi*.

For the detailed descriptions of timeliness on visit of habitats for larval and pupae collections in the study sites refer to Supplement 1. Habitats represented as 17–26 in **Supplement 1** were visited during March 28–30/2021 and all were found negative for *An. stephensi* larvae, but *Culex/Aedes* species were found. Habitat 1 in Awash Subah Kilo Town and habitats 1 and 2 in Haro Adi village of Table 1 were found positive for larvae of *An. stephensi*, and all surveyed habitats in Metehara Town and habitats represented as 1–16 in Awash Subah Kilo Town were negative for larvae of *An. stephensi* during 2–3 February 2021. During 1–3 March of 2021, in Awash Subah Kilo Town habitat 1 in Table 1 and all surveyed habitats in Metehara Town and in Haro Adi village habitats 1–4 in **Supplement 1** were negative for larvae of *An. stephensi*, whereas habitats 4 and 5 in Awash Subah Kilo Town and habitat 3 in Haro Adi *village*, as presented in Table 1 were positive for larvae of *An. stephensi.* During 8–10 of April 2021, habitats represented as 1–3 both in Awash Subah Kilo Town and in Haro Adi village, Table 1, were found positive for larvae of *An. stephensi.* On June 3, 2021, three sites (all cisterns) were found positive for *An. stephensi* in Awash Subah Kilo, and three sites (all cisterns) were found positive in Haro Adi.

Table 1
Description of larvae and pupae positive habitats visited from Feb.2021 to June 2021

Site	**Geographic Location**				**Region**	**Climate Zone**	***Anopheles* species**
Awash Subah Kilo	**Habitat type**	**Latitude**	**Longitude**	**Altitude**	**Afar**	**Semi-arid**	An. stephensi
Habitat 1	Cistern	8.9833	40.1609	944 m	"	"	"
Habitat 2	"	9.00148	40.1674	807 m	"	"	"
Habitat 3	"	8.99812	40.1684	934 m	"	"	"
Habitat 4	"	8.97848	40.151	940 m	"	"	"
Habitat 5	"	8.9809	40.1598	938 m	"	"	"
Haro Adi (Metehara)	**Habitat type**	**Latitude**	**Longitude**	**Altitude**	**Region**	**Climatic zone**	Anopheles species
Habitat1	Cistern	8.8723	39.9199	964 m	Oromia	Semi-arid to dry sub-humid	*An. stephensi*
Habitat2	"	8.8721	39.9199	968 m	"	"	"
Habitat3	"	8.8719	39.9199	967 m	"	"	"

Efficacy of Bacillus thuringiensis subspecies israelensis against Anopheles stephensi larvae

Out of the total of 600 exposed larvae, only one survived this bacterial larvicide after 24 hours. All other larvae exposed in each replicate died within 24 hours, and all larvae appeared floating on the water with swollen and blackened bodies. All 600 larvae under negative control conditions survived during the course of the experiment (24hrs). Higher concentrations of *Bti* and temephos were not tested because the larvae were confirmed to be susceptible to the lowest concentrations.

Table 2
Efficacy of *Bacillus thuringiensis subspecies israelensis* against the larvae of *Anopheles stephensi* collected from Awash Subah Kilo and Haro Adi around Metehara Towns, January–March, 2021

Site	**Larvicide**	**Concentration**	**No. Exposed Larvae**	**Mortality (%)**
Awash	*Bti*	0.05g/l	300	99.7
	Control(water)	Deoxygenated water	300	0
Metehara	*Bti*	0.05g/l	300	100
	Control(water)	Deoxygenated water	300	0

Generally, *Bti* caused mortality of 100% and 99.7% in larvae from Awash Subah Kilo and Haro Adi around Metehara Town, respectively.

Efficacy of temephos against Anopheles stephensi larvae

All exposed larvae from both sites were susceptible to temephos and sank down to the bottom of the water within a short period of time (starting at 2 hrs post exposure). All of the 600 larvae exposed to 0.25ml/l concentration of temephos were found dead within 24 hours (Table 3).

Table 3
Efficacy of temephos against the larvae of *Anopheles stephensi* collected from Awash Subah Kilo and Haro Adi around Metehara Towns, January–March, 2021

Site	Larvicide	Concentration	No. Exposed Larvae	Mortality (%)
Awash	temephos	0.25ml/l	300	100
	Control(water)	Deoxygenated water	300	0
Metehara	temephos	0.25ml/l	300	100
	Control(water)	Deoxygenated water	300	0.33

Only descriptive statistics were used because nearly all control larvae survived and nearly all treated larvae died. From the total of 600 larvae in the control group, only 1 (0.2%) died and the test was accepted, without correction.

Discussions

In the present study, larvicide bioassays revealed that larvae of *Anopheles stephensi* from both study localities *(*Awash Subah Kilo and Haro Adi around Metehara Towns) were susceptible to both *Bti* granules and temephos liquid formulation at the lowest prepared doses. The findings also suggested that there is no difference in susceptibility status to the tested larvicides between larvae collected from the two sites.

The bacterial larvicide, *Bti*, was efficient in killing 99.7% of exposed *An. stephensi* larvae, at the concentration of 0.05gm/l water. This finding is inline with studies conducted in Iran, though tetsted with different concentration units of 512 and 4096 ppm for Bio- flash® granules and powder formulation after 24 hrs post-treatment, that *An. stephensi* larvae were seen 100% susceptible (Gezelbash *et al*., 2014). The finding of this study, aligns with the laboratory test findings on 0.046 mg/L and 0.149 mg/L, and 0.05, 0.1, 0.2, 0.5, and 1g/m^2 dosages of *Bti* VectoBac WDG against *An. stephensi* in Pakistan (Naz *et al*., 2014) and in India (Tiwari *et al*., 2011), respectively, that have shown high efficacy against *An. stephensi* larvae within 24 hours of post treatment. Future work could consider these lower concentrations for testing against larvae. The residual efficacy of the larvicides was not included in this study.

In this study, temephos showed 100% efficacy in killing all exposed *An. stephensi* larvae. This finding is similar to findings from other countries and other studies within Ethiopia (Balkew *et al.* 2021). Laboratory based studies in India and southern Iran revealed that larval bioassays on *An. stephensi* collected from the field were susceptible to a temephos larvicide diagnostic dose of (0.25 mg/l) (Abai *et al*., 2016; Tikar *et al*., 2011). The only larval habitats found to be positive for *An. stephensi* were permanent water containers filled with water for the purpose of cement block production during January through March 2021 larval collections. Unlike findings by others (Balkew *et al*., 2020; Yared *et al*., 2020; Thomas *et al*., 2016; Balkew *et al*. 2021), barrels, geometric plastic water reservoirs, overhead water containers, and Jerry Cans were negative for larvae and pupae of *An. stephensi* in Awash Subah Kilo, Metehara and Haro Adi, around Metehara Town. This absence of *An. stephensi* larvae and pupae in those breeding sites may be due to difference in the sampling season, among other reasons.

In conclusion, the present study revealed that *An. stephensi* larvae from two locations, Haro Adi around Metehara and Awash Subah Kilo Town, are susceptible to Vectobac *Bti* and temephos larvicides, unlike their adult stage, which has shown resistance to adulticides used in insecticide treated nets and indoor residual spraying (Balkew *et al*. 2021). Both *Bacillus thuringiensis subspecies israelenesis* (Bti) and temephos were found to be 99.7% and 100% effective in killing *An. stephensi* larvae, respectively. The vector's preference for breeding in artificial habitats suggests possible control of this vector through the application of larvicides to these fixed habitats. Further laboratory and field-based studies are necessary to determine efficacy of larvicides against *An. stephensi* and other malaria vectors at different localities and presumably under field settings. Exhaustive assessment of breeding sites and identifying the cohabitants of this vector can also help in identifying effective tool(s) to control these vectors in an integrated approach.

Abbreviations

AAU: Addis Ababa University; ALIPB: Aklilu Lemma institute of pathobiology; BS: *Bacillus sphaericus*; BASF: Baden Aniline and Soda Factory; *Bti*: *Bacillus thuringiensis subspecies israelensis*; CG: Corn granule; EPHI: Ethiopian public health institute; GPS: Global positioning system: ICIPE: International center of insect physiology and ecology; ILRI: International livestock research institute; IRB: Institutional review board; IRS: Indoor residual spraying; ITU: International toxic units; IVM: Integrated vector management; LLINs: long lasting insecticide impregnated Nets; LSM: Larval source management; MOH: Ministry of health; RH: Relative humidity; WDG: Water dispersible granule; WHO: World health organization.

Declarations

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Authors' contributions

AT: Conceived and designed the study, collected all the necessary materials, including the larvae and pupae from the field, conducted the experiment, conducted mosquito species identification, analyzed the data, and involved in the interpretation and manuscript writing as well compiling all the comments provided from all co-authors throughout the work; SD: participated in study design, facilitated the readiness of laboratory facility, supervised the study, participated in reading and commenting the manuscript; LG: participated in critically reviewing and enriching the manuscript; BE: participated in designing the study, in critically reading word for word of the manuscript and enriching the manuscript. GY: assisted in the mosquito morphological identification and cooperated in providing guidance on identifying major sites potential for the presence of *An. stephensi* larvae during larvae collection. He also had contributed in reading, commenting and enriching this manuscript. SZ and SI have contributed in reviewing, commenting and restructuring this article. All authors read and approved the final manuscript.

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Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the U.S. President's Malaria Initiative.

Availability of data and materials

All datasets on which the conclusions of this study relied on are presented in this paper.

Ethical Consideration

-Not applicable

Consent for publication

-Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

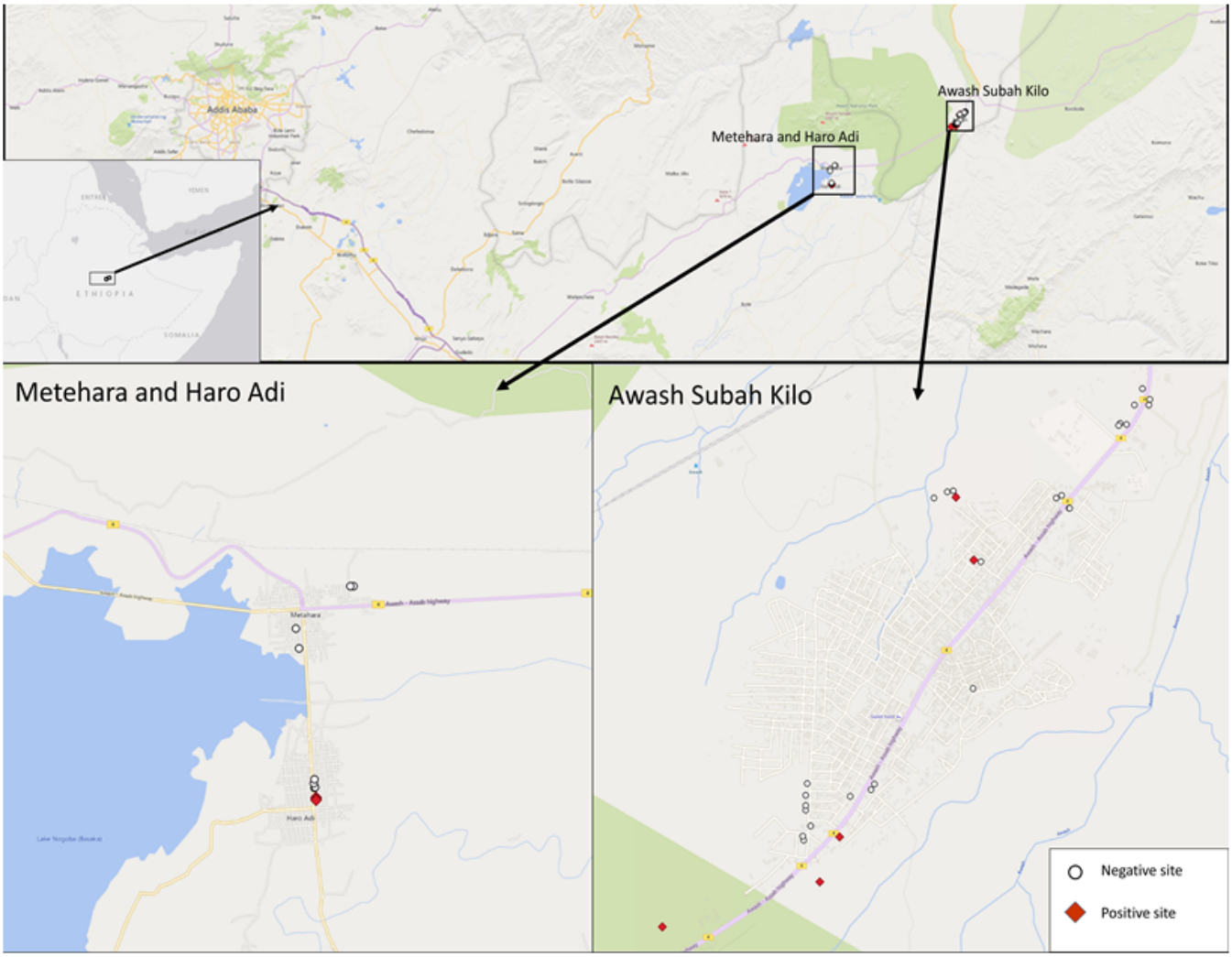

Figure 1

Map of Awash Subah Kilo and Haro Adi around Metehara area showing *Anopheles stephensi* larvae and pupae collection habitats

Supplementary Files

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