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Outdoor and early hour human biting activities of malaria mosquitoes and the suitability of clay pot for outdoor resting mosquito collection in malaria endemic villages of southern Rift Valley, Ethiopia

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ARTICLE INFO

Keywords: Blood meal index Clay pot Human landing catch Pit shelter

ABSTRACT

Background: Sampling adult *Anopheles* mosquitoes is important for assessing vector density, estimating the sporozoite infection rate, and quantifying the impact of vector control interventions. The objective of this study was to assess the *Anopheles* mosquito species composition, and their outdoor and indoor biting activities, and to evaluate the suitability of clay pots for indoor and outdoor resting mosquito collections.

Methods: Two malaria-endemic villages in the Gamo zone were purposely selected. Forty clay pots were deployed for outdoor resting mosquitoes sampling and another forty for indoor resting sampling. Twenty pit shelters were constructed for outdoor resting mosquito collection. The human landing catch (HLC) technique was employed to collect indoor and outdoor host-seeking mosquitoes in two households in each village. Morphological identification of the *Anopheles* mosquito was done using an identification key. Enzyme-linked immunosorbent assay technique was used for blood meal origin and circumsporozoite proteins (CSP) test. Speciation of *An. gambiae* complex was done using polymerase chain reaction. A Chi-square test was used to compare the effectiveness of clay pot and pit shelters for outdoor resting sampling.

Results: A total of 904 female *Anopheles* mosquitoes comprising *An. gambiae* complex, *An. pharoensis*, *An. tenebrosus*, *An. dencalicus* and *An. demelloni* were sampled. The majority (64%) of them were sampled by the HLC technique. There was a slight difference between the outdoor clay pot (19%) and pit shelter (17%) collection. No *Anopheles* mosquitoes were collected indoor using clay pots. All mosquitoes were tested for CSPs, but none of them were found to be positive. *Anopheles* mosquitoes were tending to bite humans outdoor than indoors, and their peak biting hours was 10–11 pm. The human blood meal index of *Anopheles* mosquitoes was 0.07 from pit shelters and it was 0.04 from clay pots. The bovine blood meal index was 0.45 for mosquitoes from both pit shelters and clay pot collections.

Conclusion: Anopheles arabiensis was the predominant species and it was tending to bite cattle more than humans. Clay pot could be suitable for outdoor resting mosquito collection, but not for indoor resting species.

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https://doi.org/10.1016/j.parepi.2022.e00278

Received 22 August 2021; Received in revised form 21 August 2022; Accepted 26 October 2022

Available online 30 October 2022

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1. Introduction

1.1. Background

Malaria is one of the serious public health problems worldwide, with estimated millions of cases and thousands of deaths (WHO, 2021). Africa contributed to most of the cases and deaths. Despite this high burden, in the past few decades, there was a remarkable reduction in malaria infection across African countries (Bhatt et al., 2015). The widespread distribution and extensive utilization of the vector control interventions might have contributed to the decline of malaria. Long-lasting insecticidal nets (LLINs) and indoor residual spray (IRS) were the two major players (Bhatt et al., 2015). In Ethiopia, millions of people are at risk of malaria infection. The highest proportion of malaria infection is due to *Plasmodium falciparum* compared to *P. vivax*, but the proportion varies based on the topography and altitude (Taffese et al., 2018). The dominant malaria vector is *Anopheles arabiensis* (Massebo et al., 2013a, 2013b; Abrahama et al., 2017).

Entomological sampling is an important process to estimate the vector density, measure the circumsporozoite protein (CSPs) and entomological inoculation rates (EIR), and quantify the effect of interventions directed against the vector population (Kelly-Hope and McKenzie, 2009). The success of the vector control method can be confirmed by assessing the reduction in the number of infectious mosquito bites on people in a particular time and place which is known as the EIR (Kelly-Hope and McKenzie, 2009). Thus, assessing the man-biting rate and the proportion of malaria parasite-infected mosquitoes are important components to evaluate the impact of existing malaria control tools.

Many techniques have been used in sampling mosquitoes to estimate EIR (Drakeley et al., 2003). For example, human landing catch (HLC) is the gold standard method in estimating the human vector contact both indoors and outdoors (Service, 2008). This method has ethical concerns and is labor intensive and expensive. Pyrethrum spray catch (PSC) is a suitable technique to collect indoor resting malaria mosquitoes (Sikaala et al., 2013). The challenges related with PSC are that some mosquitoes might leave the houses before and after spraying with aerosols. It is also labor intensive, and challenging for the collectors as it is conducted early in the morning. The occupants are also unhappy to leave their houses early in the morning. Another indoor resting mosquito sampling method is prokopack aspirator. This method is easy to operate and efficient in collecting indoor resting malaria mosquitoes (Maia et al., 2011). Prokopack,



Fig. 1. The map of Ethiopia, the region, the study districts and villages in Gamo Zone, southwest Ethiopia (GIS version 2.8).

Backpack and mouth aspirators can be used to collect outdoor resting malaria mosquitoes (Service, 2008). These collections can be done in tree holes, caves, animal burrows, and vegetation. In addition, the diverse mosquito species can be collected outdoors from those resting sites using the above mentioned entomological techniques. Though the prokopack, and backpack aspirators methods are efficient in resting mosquito collections, we didn't use them due to the lack of the equipment. Pit shelter is another technique suitable for outdoor resting mosquito collection (Service, 2008). There are several challenges in preparing pit shelters. It is very difficult to dig many pit shelters. The durability of pit shelters is low especially in rainy season. Most importantly, it is hard to collect mosquitoes inside pit shelters during rainy seasons due to waterlogging. On the other hand, there are little evidences about the effectiveness of clay pot for resting malaria mosquitoes collection (Knols and Farenhorst, 2009; van de Straat et al., 2021).

In current study, we used HLCs to sample the host seeking malaria mosquitoes and to determine the indoor and outdoor human biting activities of malaria mosquitos. The other two techniques such as clay pots and pit shelters were used to sample indoor and outdoor resting malaria mosquitoes. The mosquitoes collected by these methods were used to assess their blood meal origins. The comparison of methods was done only between outdoor clay pot and pit shelters collections to assess the suitability of clay pots to substitute the pit shelters. Hence, the current study aimed to assess the species composition, their biting rhythms and infection rates of malaria mosquitoes.

2. Methods

2.1. Description of study area

This study was conducted in two malaria endemic rural villages namely Chano Mille and Fura in Gamo Zone (Fig. 1) close to Arba Minch town. Arba Minch is the capital of Gamo zone which is located 515 km south of Addis Ababa, the capital city of Ethiopia. Most people live in traditional houses constructed with wood, a mud floor, and mud walls, and a roof of either grass thatched or corrugated iron. This days, corrugated iron roof houses are dominating in the area. Rainfall is bimodal, with the heaviest rain falling from April to May and the shorter rainy season occurring from September to December. The majority of malaria transmission occurs following rainfall and transmission peaks twice in a year (Loha and Lindtjørn, 2012). In both villages, IRS of propoxur was deployed by government in August 2019. The two villages were selected purposively.

Chano Mille is located at about 15 km north of Arba Minch town. The altitude is 1206 m above sea level (masl). There are 1388 households and 8121 total population. The east of the village is bounded by the Lake Abaya. The principal source of irrigation water is the Harrae River. The swampy area around the Lake shore is the main mosquito breeding sites (Massebo et al., 2013a, 2013b). The main source of income of the residents is agriculture primarily cultivation of banana, maize and mango mainly by irrigation from the Harrae River.

Fura is also another malarious village located at about 35 km north of Arba Minch town. The total household is 1240 and the total population is about 6078. The southeast of the village is bounded by the Lake Abaya. The main source of income is agriculture primarily cultivation of tomato, maize and banana by irrigation from the Lake Abaya and Tilmato River. The farmers cultivate tomato twice per year and they apply different types of chemicals such as dimethoate 40%, Hamectin 3.6, Cropzeb 80 and Isacop 50 to control tomato pests and fungal diseases. Such practice is not common in Chano Mille village.

2.2. Study design and mosquito collection

Entomological sampling was employed twice per month both indoors and outdoors for six months from June to November 2019. Ten households were randomly selected in each village for clay pot and pit shelter collections. The outdoor resting collection was done using both clay pots and pit shelters. The indoor clay pot collection was done to see whether mosquitoes rest inside clay pot for indoor rest collection, not for the methodological comparison. As the indoor pit shelter construction is not possible, no indoor pit shelter collection was done. The HLC collection was done both indoor and outdoor in two houses other than those selected for clay pots and pit shelters collection in each village. This information is relevant in monitoring the vector control interventions.

2.3. Mosquito collection using HLC

HLC technique was deployed to assess the indoor and outdoor biting activities of malaria mosquitoes. It provides important information about human exposure rates and the malaria mosquito behaviors, which help design tools based on their behavior. Two houses were selected in each village for HLC. *Anopheles* mosquitoes were captured when they attempting to bite a person sitting on a chair by exposing lower legs. Mosquito collectors were trained to minimize the variation between collectors and avoid mosquito bites. One collector was sitting outside for outdoor collection, while the other person was sitting inside for indoor collection until the midnight. The other two team members replaced them in the mid-night. Every hour, the collectors changed their sitting position to avoid bias due to their attractiveness and skills. Mosquitoes collected in each hour were separately placed in a labeled cup. The HLC collection was done between 18:00 to 6:00.

2.4. Pit shelter mosquito collection

A total of 20 pit shelters (10 in each village) were constructed under shade to collect outdoor resting mosquitoes using an aspirator. One pit shelter was constructed in front of the main door at about 10 m distance from the house selected for sampling.

Pit shelter construction was done by digging 1.5 m length, 1.5 m width, and 1.5 m depth (Silver, 2008). In each of the four sides, at about 45–60 cm from the bottom of the pit, there are 30 cm deep cavities in each side of the four sides. These cavities were constructed to create ideal resting sites. These mosquitoes were collected in the early morning from 6:00–8:00. The mouth of the pit shelter was covered by a white sheet to prevent mosquito from escaping the pit shelter (Fig. 2). Mosquitoes inside the pit shelter were collected by mouth aspirator.

2.5. Clay pot collection

Forty clay pots were deployed for sampling outdoor resting mosquitoes and another forty for indoor resting sampling. Clay pots were placed under the shade of the vegetation. One pot was placed outdoor in front of the main door and the other was placed opposite to the main door at equal distance (Fig. 3). Putting a clay pot in shade may attract mosquitoes to rest and increase the efficiency of clay pot to sample mosquitoes (Debebe et al., 2018). Two clay pots were placed inside each house, one close to the bed and the other near to the window of the house. Clay pots were manufactured locally by local potters which are 20 l capacity, with an opening of 20 cm width, a round bottom, and a maximum width of 45 cm. During manufacturing, a 2 cm diameter hole was created at the center of the base to made the pot not to hold water and limit the likelihood of theft (Odiere et al., 2007). Sampling of mosquitoes from clay pot was conducted in the morning between 6:00 and 8:00. During mosquito collection, a mosquito cage was placed over the opening of the clay pot and gently lifting and shaking of the pot was done. Also, the air was blown through the small opening at the bottom of the pot to encourage the mosquitoes to enter into the cage (Fig. 3). The pots were checked for the remaining mosquitoes and were collected using an aspirator to a well-labeled paper cup.

2.6. Mosquito processing

All the collected mosquitoes were transported to the laboratory of the Arba Minch University and the live mosquitoes were killed by freezing. Female *Anopheles* mosquitoes were identified using a morphological key (Coetzee, 2020) under dissecting microscope. All *Anopheles* mosquitoes were subsequently dried over silica gel granules at room temperature and stored in -20 °C freezers after complete desiccation to prevent decay. The head and thorax were dissected for CSPs test, abdomen for blood meal origin and the wing and legs for molecular species identification.

2.7. ELISA test for CSPs and human and bovine blood meal origins

The head and thorax of all female *Anopheles* mosquitoes were tested for the presence of CSPs by using ELISA technique (Beier et al., 1987). The blood meal digestion stages of female *Anopheles* mosquitoes collected by clay pot and pit shelter were identified under a dissecting microscope and then grouped as unfed, freshly fed, half-gravid, and gravid. Then, blood meal sources of freshly fed *Anopheles* mosquitoes from pit shelter and clay pot were identified using ELISA technique (Beier et al., 1988).

2.8. Species identification using PCR technique

Legs and wings of *An. gambiae* complex group were screened for farther speciation. Molecular species identification for screened members of *An. gambiae* complex was identified by polymerase chain reaction (PCR) technique (Scott et al., 1993).



Fig. 2. Construction of pit shelter for outdoor resting mosquito collection under the shade (left) and collecting mosquitoes by closing the mouth of pit shelters by white sheet (right).



Fig. 3. Outdoor (left) and indoor (middle) and collection of resting mosquitoes (right) using clay pots.

2.9. Data management and analysis

Data were entered into Microsoft excel and transferred to SPSS version 20 for analyses. *An. arabiensis* collected from clay pot and pit shelter were described as the mean number of *An. arabiensis* per collection method. A Generalized Estimating Equations (GEE) with a negative binomial error distribution was used to account for variations in the number of mosquitoes between collection months, and repeated catches made in the same clay pot and pit shelter. The mean number of *An. arabiensis* in clay pots and pit shelters were used for comparison and identify the efficient method for outdoor resting collections. The hourly biting rhythm of *An. arabiensis* was measured using the mean difference of mosquitoes per person/h. Clay pots to pit shelter ratio was 2:1. Chi-square test was also used to compare indoor and outdoor biting rhythm of mosquito from HLC in both villages.

3. Results

3.1. Anopheles mosquito species composition

A total of 904 female *Anopheles* mosquitoes were collected by HLCs, clay pots and pit shelters. Five species, namely *An. arabiensis* (95%; 858), *An. pharoensis* (2.9%; 26), *An. tenebrous* (1.9%; 18), *An. dencalicus* (0.1%; 1) and *An. demelloni* (0.1%; 1) were documented in the two study villages. Majority (577, 64%) of *Anopheles* mosquitoes were collected by HLCs. *Anopheles arabiensis* was the dominant species in all collection methods (Table 1).

Majority (84%) of *Anopheles* mosquitoes were collected from Chano Mille while the rest 16% were from Fura village (Table 2). *Anopheles arabiensis, An. pharoensis* and *An. tenebrosus* were documented in both study villages. *Anopheles arabiensis* was the dominant species in both villages.

3.2. Identification of the sibling species

Of 233 An. gambiae complex screened for molecular confirmation, 224 (96%) were found to be An. arabiensis, and 9 (4%) were not amplified for An. gambiae complex.

3.3. Monthly distribution of Anopheles mosquitoes

The monthly distribution of *An. arabiensis* was indicated in Fig. 4. Majority of the *An. arabiensis* were collected from July to August. There was a decrease in number of *An. arabiensis* in all collection methods after August 2019.

3.4. Clay pot and pit shelter comparison for outdoor resting mosquitoes

The overall mean monthly density of *Anopheles* mosquito for HLC was 6 and it was 5.6 for *An. arabiensis*, 0.25 for *An. pharoensis* and 0.17 for *An. tenebrosus*. The overall mean monthly density of *Anopheles* mosquito from pit shelter was 0.64 and it was 0.72 from clay pot. The density of *An. arabiensis* from outdoor pit shelter was 0.63 (95% CI: 0.39–0.91) and it was 0.71 (95% CI: 0.46–0.99) in clay pot.

Table 1

Species compositions of Anopheles mosquitoes in malaria endemic villages of southern Rift Valley, Ethiopia.

Collection techniques	# Anopheles mosquitoes					
	An. arabiensis	An. pharoensis	An. tenebrosus	An. dencalicus	An. demelloni	Total
Clay pot	172	1	0	0	1	174
Pit shelter	151	1	1	0	0	153
HLC	535	24	17	1	0	577
Total	858	26	18	1	1	904

Table 2

Overall Anopheles mosquito species collected in malaria endemic villages of southern Rift Valley, Ethiopia.

Anopheles species	Study villages and number	Total, n (%)	
	Chano Mille, n (%) Fura,		
An. arabiensis	735 (96.6)	123 (86)	858 (95)
An. pharoensis	16 (2.1)	10 (7)	26 (2.9)
An. tenebrosus	9 (1.2)	9 (6.3)	18 (1.9)
An. dencalicus	1 (0.1)	0	1(0.1)
An. demelloni	0	1(0.7)	1(0.1)
Total	761	143	904



Fig. 4. The monthly distribution of Anopheles arabiensis mosquitoes in malaria endemic villages of southern Rift Valley, Ethiopia.

There was no statistical significant difference between the two outdoor collection methods pit shelter and clay pot (F = 0.15; DF = 1; *P* value = 0.69).

3.5. Human biting patterns of Anopheles arabiensis from HLCs

Anopheles arabiensis in the two study villages were slightly tended to bite human outdoor than indoor (Fig. 5). About 53.6% of *An.* arabiensis were collected outdoors, while 44.4% were collected indoors. The variation between indoor and outdoor number of biting mosquito was statistically significant ($\chi 2 = 5.7$, P = 0.017).

Anopheles arabiensis were active throughout the night. The maximum bite a person received was 6 bites/person/night. They were most active from 7 to 11 pm with peak biting hour of 10–11 pm (Fig. 6). This variation however was not statistically significant (F = 1.67; DF = 11; *P* value = 0.07).

3.6. CSPs test and human and bovine blood meal origins detection

904 Anopheles mosquitoes were tested for CSPs and none of them were found to be positive. Also, a total of 264 fresh blood-fed Anopheles mosquitoes were tested for blood meal origins. The overall human blood meal index (HBI) of An. arabiensis, including mixed blood meals was 0.1, and the bovine blood meal index (BBI), including the mixed blood meal was 0.5. The HBI of An. arabiensis from the outdoor clay pot was 0.04 and it was 0.07 from pit shelter. A substantial number of Anopheles mosquitoes were found to be



Fig. 5. Monthly indoor and outdoor distribution of human biting Anopheles arabiensis in malaria endemic villages of southern Rift Valley, Ethiopia.



Fig. 6. The overall biting rhythm of An. arabiensis in malaria endemic villages of southern Rift Valley, Ethiopia.

negative for human and bovine blood meal origins (Tables 3 and 4).

4. Discussion

Anopheles arabiensis was the common species which predominantly fed on the bovine blood meal origin. The species was tending to bite humans at early hours and outdoors and their peak biting hours was 10-11 pm. Clay pot could be suitable for outdoor resting mosquito collections. Few studies also recommended clay pot for outdoor resting collection (Degefa et al., 2019; Odiere et al., 2007). Debebe et al. (2018) collected *An. gambiae* complex by clay pot in the same region. No mosquitoes were sampled indoor by clay pots, unlike the study reported in Kenya (Bijllaardt et al., 2009). Claiming for the suitability of clay pot for indoor resting collections. Moreover, given the small number of mosquitoes caught in the study sites, it is less likely to conclude that the mosquitoes were not resting indoors in clay pots. Construction of pit shelter is difficult. It is also not durable and hard to collect mosquitoes in the rainy seasons. Unlike pit shelter, clay pot can be used in different places by transporting and collect no water for mosquito breeding in rainy seasons. This implies that clay pot could substitute the laborious pit shelter at least for outdoor resting collection.

Anopheles arabiensis was the only species of *An. gambiae* complex confirmed by molecular technique. Other studies in the region documented similar reports (Massebo et al., 2013a, 2013b; Abrahama et al., 2017; Esayas et al., 2020). Some specimens were PCR negative for *An. gambiae* complex. This could be due to the un-amplification of DNA or morphological misidentification of other *Anopheles* mosquitoes as *An. gambiae* complex. Morphological identification of the species might be more difficult if specimens have lost important external features. Moreover, the skill of the entomology personnel could determine the efficiency of morphological identification of the species. Therefore, it is vital to do molecular assays in conjunction with morphological identification to minimize the problem related with misidentification. The species is the primary malaria vector of malaria in Ethiopia (Abrahama et al., 2017; Esayas et al., 2020); and is responsible for more than 90% of malaria transmission. It continues as the primary vector in Ethiopia though the vector control intervention initiated long years and intensified much more in recent years (Taffese et al., 2018). *An. pharoensis* and *An. tenebrous* were documented in both villages. Abrahama et al. (2017) reported CSP *An. pharoensis* in the southern rift valley.

The density of *Anopheles* mosquito documented in Fura village was lower than Chano Mille village. There are number factors causing this variation in mosquito density. The extensive use of different insecticides, fungicides and pesticides for tomato cultivation might be one reason. The farmers use Dimethoate 40% (organophosphate insecticide), Abamectin belongs to Avermectins and Cropzeb 80 (fungicide) to control tomato pests and diseases. These chemical may drain into water bodies and might kill the aquatic stages of malaria mosquitoes and affect mosquito population (Chouaïbou et al., 2016). Conversely, the application of such insecticides may favor the malaria vectors to develop behavioral and physiological resistance to public health insecticides (Reid and McKenzie, 2016). There was no such an intensive use of chemicals for agricultural pests in Chano Mille village. Insecticide resistance monitoring could be recommended to assess the resistance status of malaria mosquitoes in the two sites.

Blood meal origins were determined for freshly fed *Anopheles* mosquitoes by ELISA. A very low proportion of malaria mosquitoes fed on human blood meal origin. That means they predominantly feed on bovine blood meal source. The overall HBI was much lower than BBI in the study villages. It was consistent in clay pot and pit shelter collections. Studies in the same region also documented similar results (Massebo et al., 2013a, 2013b; Massebo et al., 2015). This feeding behavior could be linked with the way how people kept animals at night and the abundance of the animals. Usually, animals are kept outdoor the entire night within the compound of human dwelling. Blood meal origins of some malaria mosquitoes were not identified. These mosquitoes might feed on other available animals like donkey, sheep and goats. Malaria mosquitoes feed in diverse hosts depending on their availability (Lefèvre et al., 2009).

The HLCs of malaria mosquitoes revealed that the *Anopheles* mosquitoes tend to bite humans outdoors than indoors. *Anopheles* mosquitoes were very active during the early hours of the night both indoor and outdoor. In these hours, people in these villages are also active for outdoor activities like tomato cultivation, and playing in early evening. These activities might expose them to host seeking malaria mosquitoes. A study in Tanzania documented the link between human and vector behaviors (Finda et al., 2019). These mosquitoes couldn't be targeted by the indoor based intervention like IRS and ITNs. Though the excito-repellent effect of insecticide

Table 3

Overall blood meal origin of Anopheles mosquitoes in malaria endemic villages of southern Rift Valley, Ethiopia.

	Blood meal origins					
Anopheles species	No. tested	\neq positive human (HBI)	\neq positive bovine (BBI)	\neq mixed (Human/bovine)	\neq negative	
An. arabiensis	260	12 (0.046)	119 (0.46)	12 (0.046)	117 (0.45)	
An. pharoensis	2	1 (0.5)	(0)	(0)	1	
An. tenebrosus	1	(0)	(0)	(0)	1	
An. demelloni	1	(0)	(0)	(0)	1	
Total	264	13 (0.05)	119 (0.45)	12 (0.045)	120 (0.45)	

Table 4

The blood meal origins *Anopheles* mosquitoes collected by pit shelter and outdoor clay pot in malaria endemic villages of southern Rift Valley, Ethiopia.

Collection methods		Blood meal origins			
	No. tested	\neq positive human (HBI)	\neq positive bovine (BBI)	\neq mixed (Human/bovine)	Negative (%)
Pit shelter	122	8 (0.07)	55 (0.45)	5 (0.02)	54 (44.3)
Clay pot	142	5 (0.04)	64 (0.44)	7 (0.03)	66 (46.5)
Total	264	13 (0.05)	119 (0.45)	12 (0.045)	120 (45.5)

provides some protection for non-users, ITN provides protection mainly for those people inside the nets. The outdoor biting and early hour activity of malaria mosquitoes could contribute for malaria transmission regardless of the coverage and use of the existing vector control tools (Carnevale and Manguin, 2021). This implies that the current vector control intervention tools might not be sufficient to stop malaria transmission without addressing outdoor and early hours biting mosquitoes.

5. Conclusions

Clay pots could be an alternative tool for pit shelter for outdoor resting malaria mosquito collection. According to the current blood meal sources analysis, most *Anopheles* mosquitoes displayed the tendency to feed on bovine blood meal origin. The early hours and outdoors biting tendency of malaria mosquitoes could be a challenge for vector control and contribute for residual malaria transmission. They also prefer bovine blood meal than human regardless of the resting collection techniques. Insecticides susceptibility monitoring could be done in Fura village as there is an intensive use of various chemicals for tomato pest control. Farther studies needed to assess the effectiveness of clay pot in different regions and for indoor sampling.

Consent for publication

Not applicable.

Availability of data and materials

All analyzed data are available here in the body the article.

Funding

The project was funded (ETH-13/0025) by the Norwegian Programme for Capacity Development in Higher Education and Research for Development.

Ethical consideration

This study was reviewed and approved by ethical review board of Arba Minch University (IRB/109/2011). Verbal and written consents were obtained from all collectors. Malaria prophylaxis was given for mosquito collectors. There was a strict supervision to minimize exposure risk. No report of yellow fever, dengue and lymphatic filariasis were documented in the area.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

We are grateful to Mr. Elias Afamo and Tefer Manguda for participating in the field work.

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