

Population dynamics, biting activities and resting behavior of *Anopheles* mosquitoes in Jabi Tehnan district, Northwest Ethiopia: implications for indoor malaria vector control

Alemnesh Hailemariam^{1,2}, Sisay Dugassa³, Eliningaya J. Kweka^{4,5} and Habte Tekie^{2*}

¹Ethiopian Public Health Institute, PO Box 1242, Addis Ababa, Ethiopia

²Department of Zoological Sciences, College of Natural and Computational Sciences, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

³Aklilu Lemma Institute of Pathobiology, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

⁴Department of Medical Parasitology, School of Medicine, Catholic University of Health and Allied Sciences-Bugando, P.O. Box 1464, Mwanza, Tanzania

⁵Division of Livestock and Human Disease Vector Control, Tropical Pesticides Research Institute, P.O.Box 3024, Arusha Tanzania

*Corresponding author: habte_tm@yahoo.com

Abstract

Background: Effective malaria control programs rely on the implementation of evidence-based interventions. Careful assessment of *Anopheles* mosquito behavior like population dynamics, biting and resting behavior of mosquito vectors strengthen the sustainability and efficacy of the deployed vector control tools.

Methods: The study was conducted from September 2016 to August 2017. Adult mosquitoes were collected both indoors and outdoors in selected villages of Jabi Tehnan District, North West of Ethiopia. Adult mosquitoes were collected from indoor and outdoor using human landing catch (HLC), and CDC light traps (CDC-LT);, from indoor using pyrethrum spray catch (PSC) () and outdoor using pitfall shelters (PS).. A total of 35 houses were selected for sampling mosquitoes, 10 houses each for CDC-LT, PSC and PS, and five houses for HLC. Mosquito collection was carried out twice a month for 12 months.

Results: In the 12 months sampling period, 9,258 mosquitoes were collected and morphologically identified to species level using standard keys. Of these, 5,098 (55.1%) were *Anopheles* mosquitoes belonging to five species, and the rest 4,160 (44.9%) were *Culex* spp. The five *Anopheles* species comprised *Anopheles gambiae* s.l. (58.5%, n=2,982), *An. pharoensis* (11.9%, n=605), *An. funestus* (3.4%, n=176), *An. coustani* (15.2%, n=774) and *An. cinereus* (11%, n=561). The peak biting time for *An. gambiae* s.l. was between 22:00 to 00:00 hours. However, the activity decreased sharply after 23:00 - 24:00 hours and stopped completely after 04:00. *Anopheles gambiae* s.l., *An. pharoensis* and *An. coustani* s.l exhibited different peak biting activities throughout the night. *Anopheles gambiae* s.l. was the most abundant malaria vector species in the district. The *Anopheles* mosquito population peaked right after the long rains in October and during the short rainy season in March.

Conclusions: The results indicate that *An. gambiae* s.l. (primarily *An. arabiensis*) is the most abundant malaria vector in Jabi Tehnan District and showed marked high biting activities during peak sleeping hours. Thus, in addition to the indoor-based interventions (LLINs and IRS) in place, additional vector control methods should be designed to address the problem of outdoor biting mosquitoes.

Keywords: Abundance, *Anopheles* spp., human landing catch, Jabi Tehnan district, vector control

Introduction

In Ethiopia, even though malaria incidence continued to decline following the concerted efforts of control put together by the government and partner agencies, it continued to be among major health threats, as 60% of the population remains at risk (FMOH 2011; WHO 2020). The disease incidence rate remains moderately high with 18.6 per 1000 population (FMOH 2014), and it is among the top causes of mortality for under-five children at 55 per 1000 live births (Fikru et al. 2019). There are two peak malaria transmission seasons in Ethiopia, with the first peak occurring between September and December and the second between April and June (Messenger et al. 2017). Both seasons are preceded by rainy months which create a conducive

environment for the proliferation of malaria vectors (EPHI 2015). Unlike most African countries, in Ethiopia, malaria is seasonal and unstable leading to sporadic outbreaks (PMI 2020).

Two *plasmodium* species namely *Plasmodium falciparum* (70%) and *P. vivax* (30%) are the major malaria parasites. *Anopheles arabiensis* is the primary malaria vector in Ethiopia, with *An. funestus*, *An. pharoensis*, and *An. nili* playing minor transmission roles (Henderson et al. 2020). *Anopheles arabiensis*, a member of the *An. gambiae* complex, is the main malaria vector with a wide geographical distribution (Sinka et al. 2010). Other malaria vectors such as *An. funestus*, *An. Pharoensis* and *An. nili* are considered

secondary malaria vectors (Krafsur ES 1978). Indoor residual house spraying (IRS) and Long-lasting insecticidal nets (LLINs) are the key front-line malaria vector interventions against *Anopheles arabiensis*, the primary malaria vector in Ethiopia (FMOH 2011).

Distribution of LLINs, targeted implementation of indoor residual house spraying (IRS), larval source management (LSM), and community-based education of key malaria messages through mass media and health extension workers are key malaria prevention strategies implemented by the National Malaria Control Program of Ethiopia (FMOH 2011). Prompt diagnosis through Rapid Diagnostic Test (RDT) and treatment using Artemisinin Lumefantrine combination therapy (ACT) is being implemented to contain the disease in endemic areas. Despite the National Malaria Control Program (NMCP) set objective to ensure the universal coverage of LLINs (1 ITN for two individuals) and ensuring coverage of IRS for all structures in targeted endemic areas, both LLINs and IRS coverage remain substantially below the intended target (EPHI 2015). According to the Malaria Indicator Survey (MIS) conducted between October and December 2015, 64% of all households owned at least one ITN in malarious areas, and ITN universal coverage stands at 32% of areas (Henderson et al. 2020). An equally worrisome challenge as a low rate of access and utilization to the vector control interventions is, the development and spread of vector insecticide resistance to chemicals used in ITN impregnation and IRS application in Ethiopia (Messenger et al. 2017).

Thus, the National Malaria Control Programme (NMCP) of Ethiopia developed an insecticide resistance management strategy (Messenger et al. 2017) to slow down the development and spread of insecticide resistance and mandated the Ethiopian Public Health Institute (EPHI) to regularly monitor the vector insecticide resistance profile (resistance mapping), insecticide decay rate, vector species composition, vector species dynamics, vector behavioral information, the entomological inoculation rate, and related entomological parameters to make informed decision (Taye et al. 2016; Lelisa et al. 2017). This may lead to changes in the species composition and their susceptibility to insecticides and possibly changes in behavior (Bayoh et al. 2010). Determining the presence of vectors and estimating their population fluctuations are the basis for developing a risk assessment that in turn can be used to predict the transmission of malaria and disease outbreaks (Degefa et al. 2017).

The lack of data on malaria vectors species composition and relative abundance, resting and biting behavior, as

well as insecticide resistance, make the development of best control measures problematic. Hence understanding the local vector host-seeking behavior, its outdoor/ indoor biting preference, and nocturnal biting periods is essential for effectively selecting, applying, and improving vector control tools, such as LLINs and personal protective measures.

Materials and Methods

Study duration and area: The present investigation was conducted for a period of one year from September 2016 to August 2017 in Jabi Tehnan District, West Gojjam Zone, North west Ethiopia. Jabi Tehnan District is malaria-endemic area, which is located about 387 Km from Addis Ababa, and 176 Km Southwest of Bahir Dar City (Getahun 2015). The area lies between 10° 24' 36" to 10° 55' 48"N latitude; and 37° 4' 12" to 37° 30' 36"E longitude. The altitude ranges from 1,345 to 2,572 m.a.s.l. A considerable part of the area lies in ranges closer to 2,000 m.a.s.l where malaria is endemic. The area receives a rainfall of about 1250 mm per annum, and the mean minimum and maximum temperatures are 14°C and 32°C, respectively. The rainfall distribution in the area is unimodal, and the rainy season lasts from June to mid-September (Ayalew 2016). The total population of the district was about 277,590 of which 139,616 were males and 137,974 were females, with an average annual growth rate of 2.8%. Malaria transmission in the area is low, seasonal, and unstable and though the epidemiology has been described, there is relatively little data on the entomological determinants of malaria transmission (Dejenie et al. 2012) (Figure1).

Study design, sampling and sampling collection techniques: A longitudinal study that lasted for one year was deployed to conduct entomological monitoring of *Anopheles* mosquitoes species composition, density, vector population dynamics, behavior, peak biting time and biting places. This district was purposively selected on the reported malaria cases over the years (Jabi Tehnan District Health office, unpublished data), human population densities and implementation of vector control strategies such as LLINs and IRS. Study villages were selected some distances close to mosquito breeding activities were presumed to occur, and the study houses were randomly selected within the villages using a random number generator. The villages were all similar in their attitudes, house types, use of vector control tools and socio-economic status of households, although some villages may have had more houses than others. The global positioning system (GPS) coordinates for house locations were recorded for all surveyed households.

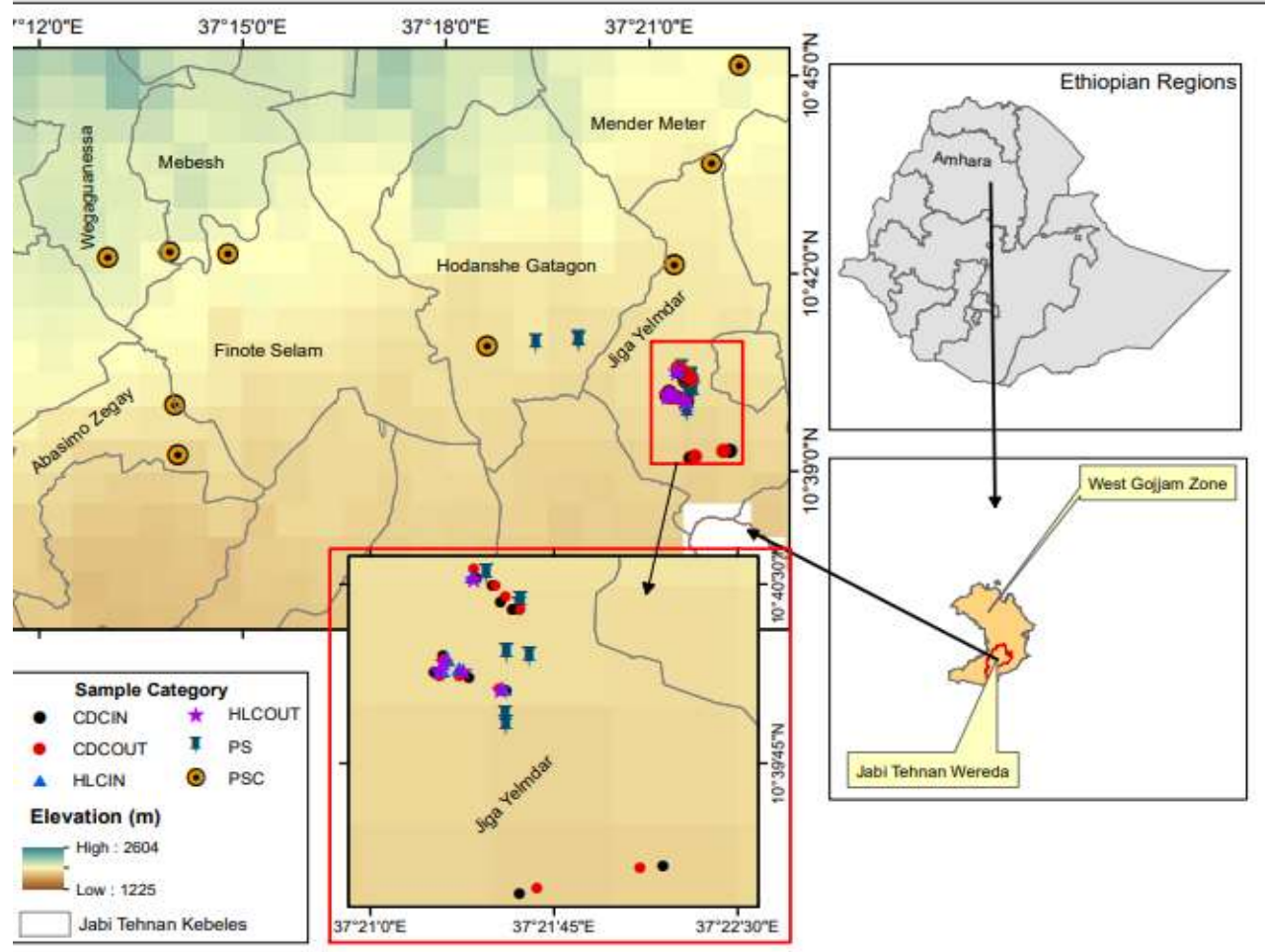


Figure 1: Map of the study sites showing HLC OUT/IN-Human landing catches outside and inside, PSC-Pyrethruspray catches, PS-Pit shelter collections ,CDC Light Traps (CDC-LT IN DOOR/OUTDOOR), 2016-2017

Human landing catches (HLC): In each study site, five houses were randomly selected as sentinel houses for human landing catches. In each of the five houses, indoor and outdoor mosquito collections were carried out from 6:00 pm to 6:00 am for four successive nights. To conduct HLC, each adult male collector exposed his lower limbs and collected the mosquitoes when they landed on his legs with an aspirator (Service MW) (Figure 2a). HLC was conducted by a single catcher at each station for 45 minutes each hour, allowing 15 minutes break for rest. To obtain full hourly biting densities, the catches for each hour were therefore divided by 0.75 (Geissbühler 2007).

In each house, a group of 4 data collection teams was deployed. Once the team members arrive at the collection household, they were divided into two groups. One group occupied an indoor setting and the other group occupied the outdoor setting. Outdoor mosquito collections were carried out about 8-10 meters from each of the three sentinel houses selected for

indoor mosquito collection by HLC. There were two collection shifts: one team of collectors worked from 06:00 pm to 12:00 pm followed by the second team from 01:00 AM to 06:00 AM. Every hour, two volunteers rotated between indoor and outdoor positions and carried out the work to reduce position bias. A full dose of mefloquine was provided as chemoprophylaxis to the volunteers who participated in human landing collections. Daily follow-up was also done on all volunteers for any malaria symptoms and prompt access to treatment with effective anti-malarial drugs if any of the collectors get malaria. Captured mosquitoes were transferred to labelled paper cups and each hourly mosquito collection was recorded separately. Thus, a total of 24 labelled paper cups (12 labelled cups for indoor and 12 labelled cups for outdoor collection) were used for each night collection per house.

At the end of each collection (night), all paper cups with mosquitoes were brought to the field identification center where mosquitoes were identified to species level

using taxonomic keys (Gillies and Coetzee 1987) and then into respective physiological statuses (Unfed, Fed, Half gravid, Gravid).

Pyrethrum spray catches: Ten houses were randomly selected for PSC. Mosquitoes were collected from 6:00 am to 7:00 am early in the morning from each house for two consecutive nights in the 10 selected houses. Before PSC, all the eaves, windows, and other exit points were closed or covered, all animals were removed, all food covered and small furniture removed from the room where the collection took place. White cloth sheets were spread on the floor. A commercially available insecticide spray (Baygon aerosol, SC. Johnson and Son Inc, USA) was sprayed in the entire space of the room (Figure 2b), and the house was then closed for 15 minutes after spraying. Before spraying, the heads of households were informed about the purpose and the time of spray and given clear instructions as to what they had to do before and after their houses are sprayed. After fifteen minutes, all the knocked down mosquitoes on the white sheets were collected carefully with forceps and placed in paper cups. WHO: Manual on practical entomology in Malaria part 2: methods and techniques. Geneva: World Health Organisation; All *Anopheles* mosquitoes collected by spray sheet were identified to species using taxonomic keys and the abdominal stage of each mosquito was also recorded as unfed, freshly fed, half gravid, or gravid (WHO 1975).

Pit shelter collections: Outdoor resting mosquitoes were collected monthly in the mornings (06:00 to 09:00 h.) from ten artificial outdoor pit shelters selected house (Figure 2c). A rectangular pit was dug in the ground (1.5 m in depth, 1.2 m in length, and 1 m in width) within 20 m of each selected house using a mouth-suction aspirator according to the method (Muirhead-Thomson 1958). The collections were repeated using the same pit shelters each month. The captured mosquitoes were killed with chloroform, sorted out, and processed similarly to that of indoor resting collections. The pits were about 0.5m high from the bottom of each pit shelter and 30 cm deep horizontal cavity on all four sides. The mouth of each pit shelter was covered with an untreated net during collection time to prevent mosquitoes from flying out of the pit shelter (WHO 1975).

CDC Light Traps (CDC-LT): CDC miniature light traps (Gladwick St., Rancho Dominguez, C.A., U.S.A., and J.W. Hock Ltd, Gainesville, FL, U.S.A) powered by GS premium high power (6N11-2D; 6v-11Ah/10Hr Japan storage battery CO.LTD Japan), rechargeable battery after every use were used on ten houses which had been selected randomly to give an equal chance of probability as mentioned by (Zaim 1986) (Figure 2d).. This was set

inside selected houses near an occupied bed at a height of 1.5 m from 18:00 to 06:00 h in the night to collect indoor host-seeking mosquitoes. For the outdoor host-seeking mosquito sampling, a CDC light trap was also set in the vicinity (within 2 m) of sentinel houses from 18:00 to 06:00 h. Ten houses were selected for indoor and outdoor light trap catches from the same village based on their relative location from the breeding site in a similar way to houses selected for HLCs. Mosquitoes were collected indoors and outdoors from 6:00 pm to 06:00 am from each selected house using standard battery-operated CDC light traps. Traps were hung from the ceiling or from roof support at the foot end of the bed where people sleep at night and each trap was suspended about 1.5 meters from the floor. Traps were also hung outdoors under the eaves of the house for outdoor mosquito collection. Each trap was set by trained research team members. Collection bags were attached to the traps by stretching the open end of the bag over the bottom rim of the trap and a label marked with the date and the site number was placed inside the collection bag. Collection bags were retrieved from traps in each house in the morning between 06:00 am and 07:00 am. Mosquitoes were identified by morphological characteristics using taxonomic keys (Gillies and Coetzee 1987) and classified into blood-fed, empty (unfed), male/female, gravid, and semi-gravid.

Species identification: Mosquitoes were sorted and identified using morphological features with the aid of an identification manual (Gillies and Coetzee 1987). Female anopheline mosquitoes and those which belong to the *An. gambiae* complex was individually preserved in labelled tubes containing silica gel and stored at room temperature until further processing. The collected mosquitoes were also sorted according to their physiological status as unfed, fresh fed, half-gravid, and gravid following the WHO manual (WHO 2013).

Data analysis: Data were entered in a database created using the 2010 Microsoft Excel and analyzed using IBM® SPSS® Statistics version 26 (Armonk, New York: IBM Corporation) soft wares. Univariate analysis was used to compare the monthly total and the house density of *An. gambiae s.l.* was used to compute mean differences among species and collection methods. In these methods, the main and interaction effects of collection methods and species composition were analyzed by adjusting method and species as fixed effects. The post hoc univariate analysis was done on multi comparison of the observed means and significant levels were separated by HSD-Tukey's test used to distinguish the months and the houses with the maximum mean density of mosquitoes for a mean number of mosquitoes, method of collection, species abundance and months of sampling and paired sample

T-test was used to compute mean differences between indoor and outdoor collections.

Ethics approval and consent to participate: This study was framed within a project known as, “Distribution and Malaria vector behavioural studies in four selected sentinel sites in Ethiopia” (EPHI 6.13/976. Project in agreement between the Ministry of Health and Global Fund) which was approved and supervised by Scientific and Ethical Review Committee (SERC) in Ethiopia Public Health allowing the mosquito collection for

scientific research purposes. The project collected baseline entomological data at all four sentinel sites. Verbal consent was obtained from the heads of households before commencing mosquitoes collection in houses. Free and informed consent was obtained from collectors performing HLC and LT/N. Permission was sought from inhabitants to perform collections in their rooms. The heads of households surveyed were informed that participation in the study was absolutely voluntary and that even after consenting they would be free to withdraw from the study at any time.



Figure 2: Human landing catches (HLC), Pyrethrum spray sheet (PSC) pit shelter (ps), CDC Light Traps (CDC), 2016-2017

Result

Anopheles species composition and abundance:

Overall, 9,258 mosquitoes were collected using all collection methods. The number of *anophelines* and *culicine* mosquitoes were 5,098 (55.1%) and 4,160 (44.9%), respectively. Four trapping methods were compared for monitoring potential vectors whereby 3,467 (37.4%) were collected by CDC light trap, 2,969 (32.1%) by HLC, 2,076 (22.4%) by PSC, and 746 (8.1%) were collected by PS (pit shelters). *Anopheles gambiae* s.l. was the principal malaria vector and most abundant in the study area that make up 2,982 (32.2%) of the total collection (Table 1).

Relative catch of anopheles mosquito per traps:

The mean *Anopheles* mosquito catches per trap night for each species is given in (Table 1). A CDC light trap captured on average 55.0 (95% CI 45.96 – 64.04) mosquitoes per trap per night, The average density of female *Anopheles* collected by HLC was 13.6 (95% CI 4.54 – 22.63) mosquitoes per man per night, PSC was 22.9 (95% CI 13.87 – 31.96), PS was 7.1 (95% CI - 1.95 – 16.13) mosquitoes per trap per night, respectively. Moreover, the p-value indicated that there is a significant difference within a species in each method ($p < 0.001$) (Table 2).

Table 1: Descriptive (Proportions) summary of mosquitoes caught in the surveys using four collection methods from Jabi Tehanan district, 2016-2017

Method	Mosquito Species						Total (%)
	<i>An.coustani</i> no(%)	<i>An. cinereus</i> no(%)	<i>An. Funestus s.l</i> no(%)	<i>An. gambiae</i> s.l no(%)	<i>An. pharoensis</i> no (%)	<i>Culicines</i> no(%)	
CDC	114(3.3)	180(5.2)	48(1.4)	953(27.5)	128(3.7)	2044(58.9)	3467(100)
HLC	338(11.4)	233(7.8)	51(1.7)	852(28.9)	216(7.3)	1279(43.1)	2969(100)
PFS	109(14.6)	73(9.8)	37(4.9)	64(8.9)	67(3.3)	396(53.0)	746(100)
PSC	213(10.3)	75(3.6)	40(1.9)	1113(53.6)	194(9.3)	441(21.2)	2076(100)
Total	774(8.4)	561(6.1)	176(1.9)	2982(32.2)	605(6.5)	4160(44.9)	9258 (100)

Table 2: Numbers of mosquitoes caught using different trapping methods, 2016-2017

Method	Species	Mean	95% Confidence Interval		P-value
			LB	UB	
CDC-LT	<i>Anopheles gambiae</i>	55.0	45.96	64.04	<0.001
	<i>Anopheles pharoensis</i>	6.5	-2.54	15.54	
	<i>Anopheles funestus</i>	7.1	-1.96	16.13	
	<i>Anopheles coustani</i>	3.8	-5.21	12.88	
	<i>Anophelescinereus</i>	5.0	3.75	6.25	
	<i>Culicine spp</i>	216.9	207.88	225.96	
HLC	<i>Anopheles gambiae</i>	13.6	4.542	22.63	<0.001
	<i>Anopheles pharoensis</i>	13.6	4.542	22.63	
	<i>Anopheles funestus</i>	2.0	-7.042	11.04	
	<i>Anopheles coustani</i>	.4	-8.625	9.46	
	<i>Anophelescinereus</i>	9.7	7.77	11.63	
	<i>Culicine spp</i>	256.6	247.542	265.63	
PSC	<i>Anopheles gambiae</i>	22.9	13.875	31.96	<0.001
	<i>Anopheles pharoensis</i>	.2	-8.875	9.21	
	<i>Anopheles funestus</i>	2.2	-6.875	11.21	
	<i>Anopheles coustani</i>	3.1	-5.958	12.13	
	<i>Anophelescinereus</i>	6.3	4.73	7.87	
	<i>Culicine spp</i>	78.4	69.375	87.43	
PFC	<i>Anopheles gambiae</i>	7.1	-1.958	16.13	<0.001
	<i>Anopheles pharoensis</i>	3.2	-5.875	12.21	
	<i>Anopheles funestus</i>	1.3	-7.708	10.38	
	<i>Anopheles coustani</i>	3.0	-6.042	12.04	
	<i>Anophelescinereus</i>	6.1	4.40	7.80	
	<i>Culicine spp</i>	74.7	65.625	83.71	

Outdoor and indoor host-seeking mosquitoes: The relative proportion of *An. gambiae* s.l., *An. funestus*, *An. coustani* s.l., *An.cinereus* and *An. pharoensis* mosquitoes collected indoors and outdoors by CDC and HLC are presented in Table 3. , Density of collections made indoor and outdoor of *An. gambiae* s.l.were 33.3 ± 7.7 and 21.7 ± 5.8 , respectively. The density trend in *An. pharoensis* showed revers with significantly higher catches ($P = 0.002$) documented from an outdoor setting. The outdoor and indoor

collections of *An. pharoensis* were $5.5\% \pm 1.3$ and 0.17 ± 0.1 respectively. HLC collections showed that there was no statistically significant difference ($p=0.208$) between mean indoor and outdoor catches of *An. gambiae* s.l. with a mean density of 33.4 ± 7.7 and 21.7 ± 5.8 per person-hour, respectively. Similarly, there was no significant difference between indoor and outdoor catches in the rest of the three species i.e. *An. funestuss.l.*, *An. coustani* s.l., and *An. Cinereus*.

Monthly mosquito density dynamics: In the year-long sampling period, the highest biting activity was recorded in October and March while the lowest biting activity was recorded between May and July (Figure 3). The variations observed during the study period were found to be statistically significant among the

sampling month (DF=11, F= 2.7, P=0.002). The effect was consistent in every collection month from November to February and April to July, with the greatest differences observed in October and March. Important variation in *Anopheles* density was observed between August to October.

Table 3: Number of host-seeking and biting mosquitoes caught using Centre for Disease Control light traps (CDCLT) and human landing catches (HLC) in the study area, 2016-2017

Method	Species	location	Mean $\pm$ SE	P-Value
CDC-LT	<i>An. gambiae</i> s.l.	Indoor	33.3 $\pm$ 7.7	0.208
		Outdoor	21.7 $\pm$ 5.8	
	<i>An. pharoensis</i>	Indoor	.17 $\pm$ 0.1	0.002
		Outdoor	5.5 $\pm$ 1.3	
	<i>An. funestuss</i> .l.	Indoor	2.4 $\pm$ 1.4	0.252
		Outdoor	4.8 $\pm$ 1.1	
	<i>An. Coustani</i> s.l.	Indoor	1.8 $\pm$ 0.5	0.806
		Outdoor	2.0 $\pm$ 0.5	
	<i>An.cinereus</i>	Indoor	4.42 $\pm$ 0.5	0.120
		Outdoor	6.20 $\pm$ 0.5	
	<i>Culicine</i> spp	Indoor	113.9 $\pm$ 4.7	0.116
		Outdoor	105.3 $\pm$ 4.5	
HLC	<i>An. gambiae</i> s.l.	Indoor	6.6 $\pm$ 1.7	0.601
		Outdoor	7.0 $\pm$ 1.5	
	<i>An. pharoensis</i>	Indoor	6.6 $\pm$ 1.7	0.601
		Outdoor	7.0 $\pm$ 1.5	
	<i>An. funestuss</i> .l.	Indoor	.3 $\pm$ 0.2	0.133
		Outdoor	1.8 $\pm$ 0.9	
	<i>An. coustani</i> s.l.	Indoor	.3 $\pm$ 0.2	0.674
		Outdoor	.2 $\pm$ 0.1	
	<i>An.cinereus</i>	Indoor	7.75 $\pm$ 0.3	0.070
		Outdoor	11.66 $\pm$ 0.7	
	<i>Culicine</i> spp	Indoor	133.1 $\pm$ 3.7	0.026
		Outdoor	123.5 $\pm$ 0.7	

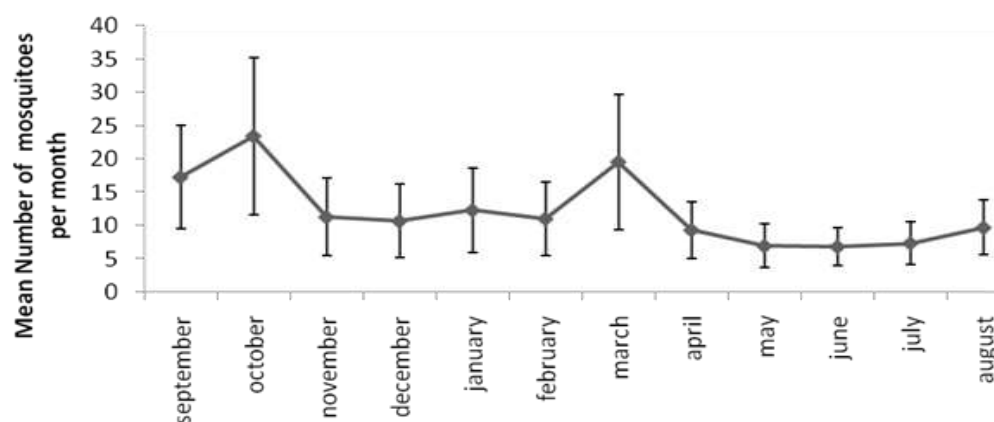


Figure 3: Monthly mean variation of mosquito densities, 2016-2017

Number and proportion of human biting *Anopheles* species: During the study period, a total of 5,098 mosquitoes were collected in all traps of which morphologically five species, *An. gambiae s.l.*, *An. funestus*, *An. pharoensis*, *An. cinereus* and *An. Coustani*, were identified (Figure 4). In terms of

species abundance within the study period, it was found that *An.gambiae s.l* has the highest peak while *An. funestus* had the least. The variation of species abundance was found to have statistical significance throughout the study duration and differences in mean annual catch rate ($P<0.001$) (Figure 4).

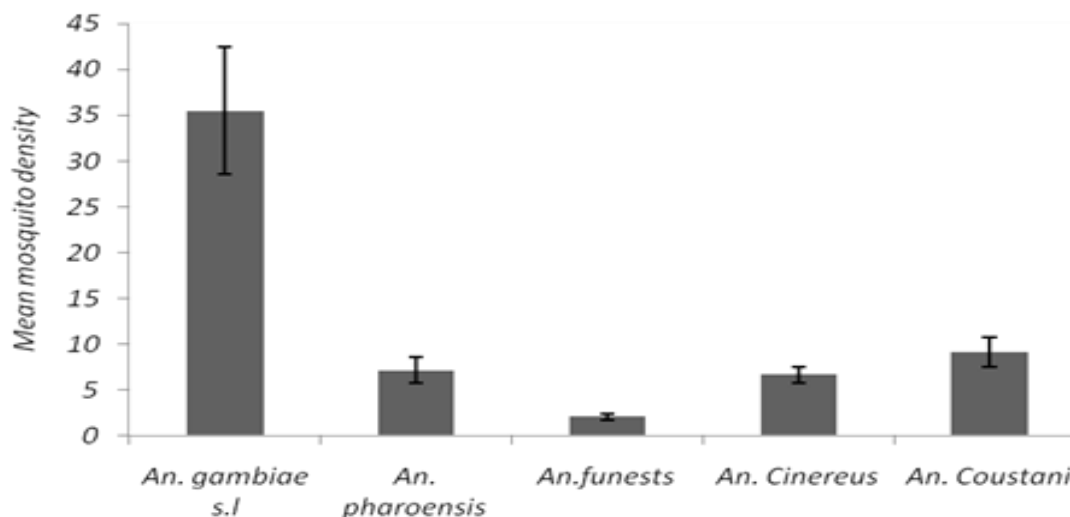


Figure 4: Mean anopheline mosquito density collected indoor and outdoor using CDC, HLC, PSC, and PF methods, Jabi Tehnan District, Northwest Ethiopia. Error bars indicate a 95% confidence interval. *Significant at $P<0.001$, 2016-2017

Human biting rates by *Anopheles* species: A total of 1,705 *Anopheline* and 4,303 *Culicine* mosquitoes were collected using HLC over one year period of the experiment. Out of the 1,705 *Anopheline*, 825 (48.4%) were *An. gambiae s.l.*, 295 (17%) were *An. pahroensis*, 331 (19%) were *An. coustani* and the rest 254 (14.9%) were other *Anopheline*. In this experiment, the peak biting time for major malaria vector *An. gambiae s.l.* was found to be between 22:00 to 00:00 hours with 54.8% of total collections caught in the time interval (Figure 5). The three primary malaria vectors in the area, namely, *An. gambiae s.l.*,

An. pahroensis and *An. coustani* exhibit different biting activities throughout the night. *An. gambiae s.l.* and *An. coustani* most of their peaked activity between both indoor and outdoor (22:00 to 00:00 h) and (23:00 to 00:00) simultaneously. The peak biting activity of *An. pahroensis* found indoor appeared to have started as early as 20:00 to 21:00 and remained constant from 22:00 to 23:00 which was gradually decreased. However, biting peak time of *An. pahroensis* in outdoor started at 20:00 to 21:00, got constant at 22:00 to 00:00 and later gradually decreased.

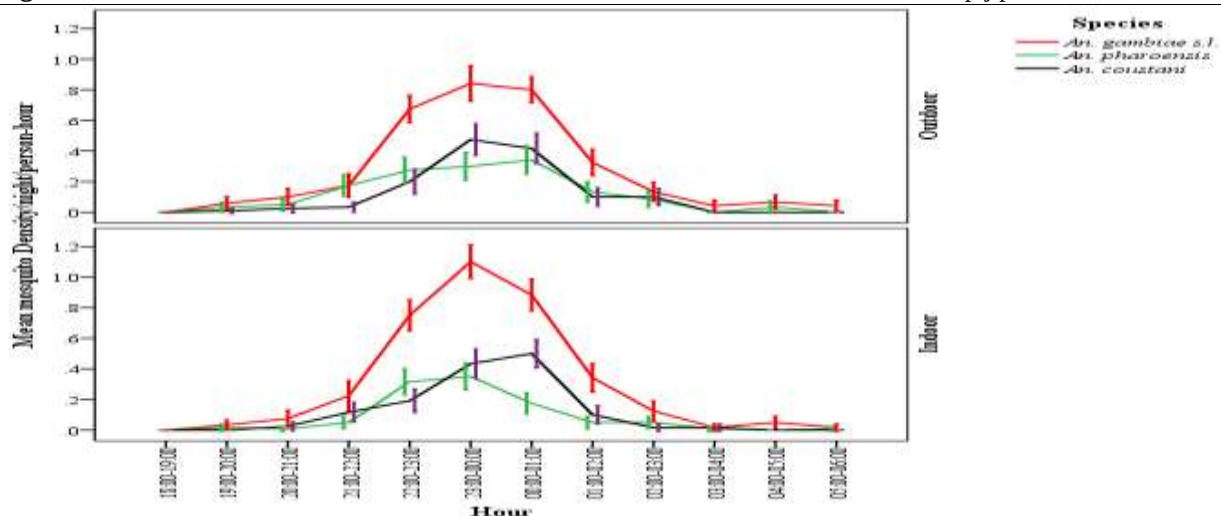


Figure 5: Overall hourly biting activities of the three *Anopheles* species in Jabi Tehnan District, Northwest Ethiopia, 2016-2017

Discussion

Monitoring malaria vectors requires accurate, safe, and reliable collection methods that can be deployed at scale. It is particularly difficult to collect representative samples of field populations and nearly all sampling tools will have some bias (Service 1977). Moreover, Continuous monitoring of malaria vector species behavior and their monthly dynamics helps to ring alarm bells before the disease poses any credible health threat in a certain locality.

This study identified five *Anopheles* species belonging to *An. gambiae* s.l. presumably *An. arabiensis*, *An. funestus*, *An. pharoensis*, *An. coustani*, *An. cinereus* and *Culex* species were recorded in the area with four collection methods. The richness of *Anopheles* species in Jabi Tehnan district may be explained by the existence of different mosquito oviposition sites, which are close to the river and human habitats that may provide more food sources and convenient ecological niches for *Anopheles* species. *Anopheles gambiae* s.l. was the predominant vector species collected using all methods and was documented from different parts of the country including Southern Ethiopia (Massebo et al. 2015), East central Ethiopia (Kibret et al. 2011), South central Ethiopia (Lelisa et al. 2017), Northern Ethiopia (Yohannes and Boelee 2012) and Western Ethiopia (Jaleta et al. 2013).

The suitability of mosquito sampling techniques may vary alongside geographic and ecological differences, necessitating the calibration of sampling tools for use in different settings so we did compare the

performance of different traps to be a function of *Anopheles* mosquito density across all seasons. *Anophelesgambiae* s.l. was found to be the major *anopheline* species demonstrated predominantly in all the four methods and significant differences were apparent in five *Anopheles* species in every sampling method. Similar study findings were reported from other parts of the region, Jimma of Ethiopia (Degefa et al. 2015); Uganda (Kabbale et al. 2013) and Kenya (Kweka et al. 2011).

Many studies have evaluated the performance of the CDC-LT technique relative to the HLC technique. The present study demonstrated that there are no significant differences in host-seeking in both CDC-LT and HLC collections ($p>0.05$) by all *anopheles* mosquitoes except *An. pharoensis* in CDC, which showed higher density at outdoor. Though not supported by this study, increased outdoor feeding and outdoor resting behaviour of vector mosquitoes would be expected since there is growing evidence from across the continent that shows increased exophagy of vector population following widespread use of LLINs and IRS. The excessive use of indoor-based vector interventions is driving vector species toward more flexible feeding and resting behaviors (Govella and Ferguson. 2012). The increased outdoor feeding behavior of *An. pharoensis* documented in this study was also reported by Taye et al. (2017) from Southwestern Ethiopia.

Overall, *Anopheles* mosquito density peaked after the end of the main rainy season in October. Mosquito density also started to increase in June and

reached its peak in October. This finding agrees with Kibret et al. (2017), who found a higher density of *Anopheles* mosquitoes between October and November in Ethiopia. A report by Abrham et al. (2017) showed slightly earlier peak of mosquito density, during the main rainy season of August to September. Higher peak of mosquito density in October could be due to the presence of permanent breeding sites together with elevated temperature. These conditions provide a very conducive breeding ground for *Anopheles* mosquitoes.

In this study, the peak biting time for the major malaria vector, *An. gambiae* s.l., was found to be between 22:00 to 01:00 hours with 54.8% of total collections. The peak biting hour for *An. gambiae* s.l. (*An. arabiensis*) coincided with that of *An. pharoensis* and *An. coustani* i.e increases during midnight. One study from Jimma area showed similar biting pattern and a study conducted in central Ethiopia (Ziway area) showed slightly different behavior with biting activity for *An. pharoensis* progressively decreasing as the night falls with peak biting activity registered between 19:00 to 20:00 hours (Kenea et al. 2016; Degefa et al. 2021). However, studies in Papua New Guinea and Tanzania showed shift in time of biting. when mosquito biting occurred earlier in the evening as hosts had not yet gone to bed and were easily accessible (Kabbale and Akol 2013; Thomsen et al. 2017).

Conclusions

The five *Anopheles* species identified in this study were *An. gambiae* s.l., *An. pharoensis*, *An. funestus*, *An. cinereus* and *An. coustani*. *Anopheles gambiae* s.l. was the most abundant vector species identified in Jabi Tehnan District, Northwestern Ethiopia. The primary malaria vector in Ethiopia, *An. gambiae* s.l., was found to be biting throughout the night with its peak biting hours between 18:00 to 22:00 and 04:00 to 06:00. The national malaria control program should closely follow shifting behavior of vector mosquitoes as the observed outdoor feeding tendency of the vector population could pose challenges to the indoor intervention tools, use of IRS and LLINs.

Competing interest: The authors declare that they have no competing interests.

Acknowledgments

We thank all the community health workers who assisted during entomological surveys and the

communities in all the study villages for their continued support. This work was supported by the Ministry of Health (Global fund). All the financial support for data collection, statistical analysis, and writing up of the manuscript was obtained from the grant of the Federal Ministry of Health (Global Fund) as part of the project approved by the Ethiopian Public Health Institute with reference number EPHI 6.13/976. The funder had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

References

- Abraham M, Massebo M & Lindtjørn B (2017). High entomological inoculation rate of malaria vectors in an area of high coverage of interventions in southwest Ethiopia: implication for residual malaria transmission. *Parasite Epidemiology and Control*, 2(2): 61-69.
<https://doi.org/10.1016/j.parepi.2017.04.003>
- Animut A, Negash Y & Kebede N (2014). Distribution and utilization of vector control strategies in a malarious village of Jabi Tehnan District, northwestern Ethiopia. *Malaria Journal*, 13:356
<http://doi.org/10.1186/1475-2875-13-356>
- Ayalew S, Mamo H, Animut A & Erko B (2016). Assessment of current malaria status in light of the ongoing control interventions, socio-demographic and environmental variables in Jiga Area, Northwest Ethiopia. *PLoS One*, 11(1):11. e0146214. doi:10.1371/journal.pone.0146214
- Bayoh MN, Mathias DK, Odiere MR, Mutuku FM, Kamau L, Gimnig JE, et al. (2010). *Anopheles gambiae*: historical population decline associated with regional distribution of insecticide-treated bed nets in western Nyanza Province, Kenya. *Malaria Journal*, 9:62. Doi: 10:1186/1475-2875-9-62.
- Degefa T, Githeko AK, Lee MC, Yan G & Yewhalaw D (2021). Patterns of human exposure to early evening and outdoor biting mosquitoes and residual malaria transmission in Ethiopia. *Acta Tropica*, 216:105837.
- Degefa T, Yewhalaw D, Zhou G, Lee MC, Atieli H, Githeko AK, et al. (2017). Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. *Malaria Journal*, 16:443. DOI: 10.1186/s12936-017-2098-z
- Degefa T, Zeynudin A, Godesso A, Michael YH, Eba K, Zemene E, et al. (2015). Malaria incidence and assessment of entomological indices among

- resettled communities in Ethiopia: a longitudinal study. *Malaria Journal*, 14:24. DOI: 10.1186/s12936-014-0532-z
Doi:10.1016/j.actatropica.2021.105837
- Dejenie T, Yohannes M & Assmelash T (2012). Adult mosquito populations and their health impact around and far from Dams in the Tigray region, Ethiopia. *Momona Ethiopian Journal of Science*. 4(2): 40-51. DOI:10.4314/mejs.v4i2.80115
- EPHI (2015). Ethiopia National Malaria Indicator Survey 2015. Ethiopian Public Health Institute. Pp 112.
- Fikru C, Masrie C & Shaweno T (2019). Proximate determinants of under-five mortality in Ethiopia: using 2016 nationwide survey data. *Pediatrics Health, Medicine and Therapeutics*, 10:169-176.
- FMOH (2011). Malaria Program Review. The Federal Democratic Republic of Ethiopia. Ministry of Health, Addis Ababa, Ethiopia. Pp 32.
- FMOH (2014). National Malaria Strategic Plan: 2014–2020. Federal Ministry of Health [Online] Addis Ababa. Pp 113. Available from <http://www.moh.gov.et/documents/26765/0/National+Malaria+Program+Plan+2014-2020/>
- Geissbühler Y, Chaki P, Emidi B, Govella NJ, Shirima R, Mayagaya V, et al. (2007). Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania. *Malaria Journal*, 6:126.
Doi: 10.1186/1475-2875-6-126
- Getahun M (2015). Characterisation of agricultural soils in Cascape intervention Woredas of Amhara Region. *BDU-CASCADE working paper*, Pp 90.
- Gillies MT & Coetzee M (1987). A supplement to the Anophelinae of Africa South of the Sahara. *African Institute of Medical Research*, 55:1-143. <https://doi.org/10.1046/j.1365-294X.1997.00177x>
- Govella NJ & Ferguson H (2012). Why the use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination? *Frontiers in Physiology*, 3:199.
<https://doi.org/10.3389/fphys.2012.00199>
- Henderson SJ, Belemvire A, Linn A, Moriarty LF, Brofsky E, Diaw M, et al. (2020). Advancing malaria prevention and control in Africa through the PeaceCorps-President's Malaria Initiative partnership. *Global Advances in Health and Medicine*, 9:1-8.
DOI: 10.1177/2164956120976107
- Jaleta K, Hill SR, Seyoum E, Balkew M, Gebre-resistance monitoring. *Malaria Journal*, 16(1):1-14. 16:469.
- Michael T, Ignell R, et al. (2013). Agro-ecosystems impact malaria prevalence: large-scale irrigation drives vector population in western Ethiopia. *Malaria Journal*, 12(1):1-11.
- Kabbale FG, Akol AM, Kaddu JB. & Onapa AW. (2013). Biting patterns and seasonality of *Anopheles gambiae sensu lato* and *Anopheles funestus* mosquitoes in Kamuli District, Uganda. *Parasites & Vectors*, 6(1):1-9.
- Kenea O, Balkew M, Tekie H, Gebre-Michael T, Deressa W, Eskindir L, et al. (2016). Human-biting activities of *Anopheles* species in south-central Ethiopia. *Parasites & Vectors*, 9:527. DOI: 10.1186/s13071-016-1813-x
- Kibret S, Alemu Y, Boelee E, Tekie H, Alemu D & Petros B (2010). The impact of a small-scale irrigation scheme on malaria transmission in Ziway area, Central Ethiopia. *Tropical Medicine & International Health*, 15(1):41-50.
DOI:10.1111/j1365-3156.2009.02423.x
- Kibret S, Wilson GG, Ryder D, Tekie H & Petros B (2017). Malaria impact of large dams at different eco-epidemiological settings in Ethiopia. *Tropical Medicine & Health*, 45:4. DOI: 10.1186/s41182-017-0044-y
- Krafsur ES & Armstrong JC (1978). An integrated view of entomological and parasitological observations on *falciparum* malaria in Gambela, Western Ethiopian Lowlands. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 72:348–56. Doi: 10.1016/0035-9203(78)90125-6
- Kweka EJ, Zhou G, Lee MC, Gilbreath TM, Mosha F, Munga S, et al. (2011). Evaluation of two methods of estimating larval habitat productivity in western Kenya highlands. *Parasites & Vectors*, 4:110. <https://doi.org/10.1186/1756-3305-4-110>
- Lelisa K, Asale A, Taye B, Emanu D & Yewhalaw D (2017). Anopheline mosquitos' behaviour and entomological monitoring in southwestern Ethiopia. *Journal of Vector Borne Diseases*, 54(3):240.
- Massebo F, Balkew M, Gebre-Michael T & Lindtjørn B (2015). Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia: an opportunity for malaria vector control. *Parasites & Vectors*, 8(1):1-9. <https://doi.org/10.1186/s13071-015-1264-9>.
- Messenger LA, Shililu J, Irish SR, Gedeon YA, Tesfaye AG, Ye-Ebiyo, et al. (2017). Insecticide resistance in *Anopheles arabiensis* from Ethiopia
- Muirhead-Thomson R (1958). A pit shelter for sampling outdoor mosquito populations. *Bulletin*

- of World Health Organization, 1958(19):1116–8. <https://apps.who.int/iris/handle/10665/265386>
- Service MW (1977). A critical review of procedures for sampling populations of adult mosquitoes. *Bulletin of Entomological Research*, 67(3):343-382. Doi: 10.1017/S0007485300011184
- Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe, and the Middle East: occurrence data, distribution maps, and bionomic précis. *Parasites & Vectors*, 3:117. Doi: 10.1186/1756-3305-3-117
- Taye B, Lelisa K, Emana D, Asale A & Yewhalaw D (2016). Seasonal Dynamics, Longevity, and Biting Activity of Anopheline Mosquitoes in Southwestern Ethiopia. *Journal of Insect Science*, 16(1):6.
- Taye B, Mohammed S & Adugna G (2017). Entomological study on species composition, behavior, longevity, and probability of surviving sporogony of *Anopheles* mosquitoes in Lare District, Ethiopia. *Journal of Parasitology and Vector Biology*, 9(9):137-145. <https://doi.org/10.5897/JPVB2017.0295>
- Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa, S, Ura Y, Keven JB, et al. (2017). Mosquito behavior change after distribution of bednets (2012–2016): a nationwide study for insecticide results in decreased protection against malaria exposure. *The Journal of infectious diseases*, 215(5):790-797.
- WHO (1975). Manual on practical entomology in malaria. Part II. Methods and techniques. Manual on Practical Entomology in Malaria. Methods and Techniques. 2(13):186-191.
- WHO (2011). The fourth meeting of the Regional Scientific and Technical Advisory Committee of the WHO / UNEP project. World Health Organization. Pp 31.
- WHO (2020). Health policy and system support to optimize community health worker programmes for HIV, TB, and malaria services: an evidence guide. Pp 24.
- Yohannes M & Boelee E (2012). Early biting rhythm in the afro-tropical vector of malaria, *Anopheles arabiensis*, and challenges for its control in Ethiopia. *Medical and Veterinary Entomology*, 26(1): 103-105. <https://doi.org/10.1111/j.1365-2915.2011.00955.x>
- Zaim M, Ershadi MR, Manouchehri AV & Hamdi MR (1986). The use of CDC light traps and other procedures for sampling malaria vectors in southern Iran. *Journal of the American Mosquito Control Association*, 2(4):511-15.