Anopheles gambiae s.l. exhibits overnight biting activities in Wonji Sugar Estate, Eastern Oromia Ethiopia: a challenge to the current intervention tools

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Abstract

Background

Although the behavioral flexibility of *Anopheles* mosquitoes poses challenge to the indoor-based vector control strategies in Ethiopia, this was not well studied in irrigated areas for sugar cane plantations. Hence, the local *Anopheles* species composition, biting behaviors, feeding site preference, monthly density, and weather variability were evaluated in Wonji Sugar Estate.

Methods

Adult *Anopheles* mosquitoes were sampled using Centers for Disease Control and Prevention light traps (CDC LT), Pyrethrum spray catches (PSC), handheld mouth aspirators and artificial pit shelters. Mosquitoes were identified to species using morphological keys. ANOVA was used to compare mean monthly mosquito densities. Correlation was used to test the relationship between hourly density of *Anopheles* and human activities. Effect of weather variability was tested against *Anopheles* density.

Results

A total of 3,504 *Anopheles* comprising: *Anopheles gambiae s.l., An. pharoensis*, and *An. coustani* complex were collected during the study periods. *Anopheles gambiae s.l.* was the dominant species (75.26%, n = 2,637). Higher number of *Anopheles* mosquitoes were collected using CDC LT (59.80%, n = 2,098) than those collected using PSC, Pit shelter, and Handheld mouth aspirators (mean = 1.83, CI = 1.68–1.97, P = 0.000). *Anopheles gambiae s.l.* exhibits overnight biting pattern with peak biting hours of 7:00 to 10:00 PM (Mean = 0.20 ± 0.02, CI = 0.16–0.24, p = 0.000) and 3:00 to 05:00 AM (Mean = 0.13 ± 0.02, CI = 0.09–0.16, p = 0.000) that has a positive correlation with occupants being on activities (r = 0.135, p = 0.00). The regression analysis reveals an increase in one sleeping householder leads to a lower hourly biting density of *Anopheles* (β -0.037, t= -1.7, p = 0.000). Peak density of *Anopheles* species was noted in July 2019 followed by June 2019. There exists a positive correlation between mean monthly minimum temperature, rainfall and relative humidity and the mean monthly density of *Anopheles* mosquitoes at p-value < 0.05. The overall mean densities of host seeking *Anopheles* mosquitoes indoors (1.97per trap /night) and outdoors (2.58per trap/night) locations (t=-2.113, p = 0.072) were not statistically different. However, greater number of *Anopheles gambiae s.l.* was collected indoors than outdoors (t = 1.565, p = 0.001) and significant numbers of *Anopheles pharoensis* were collected outdoors as compared to indoors (t= -5.962, p = 0.000) which signals the differential host seeking behaviors between the two species.

Conclusion

The peak biting time of *Anopheles gambiae s.l.* coincides with the active working time of the Estate's workers (from 6:00 PM to 6:00 AM) and this ensures the year-round availability of malaria vector that might result in perennial transmissions of malaria in such ecological settings. This calls for interventions on malaria and its vectors across all months of the year. Moreover, attention on outdoor based mosquito control measures as to be sought.

Introduction

Anopheles gambiae s.l., the primary malaria vector in Ethiopia, is also responsible in residual malaria transmission in the country(FMoH 2020). Consequently, malaria remains the biggest public health problems in Ethiopia, notably since COVID-19 pandemic; where increment of malaria cases and deaths observed between 2019 and 2021(FMoH 2020). This could hinder the elimination plan being implemented in the country. Furthermore, the success achieved in the last two decades is threatened by wide spread insecticide resistance development by the vectors (WHO 2022; Ojuka et al. 2015; Riveron et al. 2018; Munywoki et al. 2021; Demissew et al. 2022). Apart from insecticide resistance, vectors' behavioral change has also threatens the indoor based vector control tools (IRS and ITNs) (Kibret and Wilson 2016). Malaria vectors showed a tendency of changing their behavior from indoor feeding and resting to outdoor feeding and/or resting according to studies from Uganda, Kenya, Ethiopia, and Burkina Faso (Ojuka et al. 2015; Kibret and Wilson 2016; Kabbale et al. 2013; Ototo et al. 2015; Lelisa et al. 2017; Bedasso et al. 2022; Perugini et al. 2020; Ondeto et al. 2022).The are also other reports in Africa that indicated the modifications of vectors feeding hours from dusk and dawn (Bedasso et al. 2022; Taye et al. 2016; Demissew et al. 2020). These are times when people actively engage in social affairs indoors and outdoors and not protected by ITNs and IRS (Kabbale et al. 2013).

Several studies showed that the shift in feeding behavior of mosquitoes was pronounced in sugar cane farm areas as the workers spend outdoors most of the night times (Kibret and Wilson 2016; Perugini et al. 2020; Kibret et al. 2014; Yohannes et al. 2005; Yohannes and Boelee 2012; Killeen 2014; Janko et al. 2018). Moreover, studies from different countries in Africa including Ethiopia on *Anopheles* mosquito's bionomics in sugar cane farm indicated that the farm creates new habitats. These favor *Anopheles* mosquito breeding habitats and nutrient availability (Hawaria et al. 2021), increased vector human contact rate (Amaechi et al. 2018), changed *Anopheles* seasonality to year-round presence (Frake et al. 2020), enhance vector longevity (Jaleta et al. 2013), enhance species richness and abundance (Kabbale et al. 2013; Demissew et al. 2020), and increased infective bites (Haileselassie et al. 2021). According to Jaleta *et al.* (Jaleta et al. 2013), for instance, two-fold higher in a human biting rate has been noticed around sugar cane farm as compared to rain-fed based agriculture of non-irrigation communities. The same study reported a four to six-fold increase in the annual inoculation rate of *Anopheles arabiensis* in communities living around sugar cane farming areas as compared in communities living in non-irrigation areas (Jaleta et al. 2013) from Ethiopia. Other studies conducted on Koka reservoir, Gilgel Gibe power generator dams and on micro dams in Tigray reported that the water resources increased the availability of suitable breeding habitats of malaria vectors than the areas located at distant from the dams (Yewhalaw et al. 2009; Ghebreyesus et al. 1999).

Hence, Wonji Sugar Estate is one of the irrigation-based project with suitable climate and altitude for malaria and various studies (Tufa 2021; Lemma 1969; Kloos 1985) reported that malaria is among the leading public health problem. On the other hand, vector control tools like IRS and ITNs have been in use, although malaria cases are not significantly reduced (Lelisa et al. 2023). In Wonji Sugar Estate, the primary malaria vector's behavior and ecology had not been studied to our knowledge. Hence, this study was initiated to determine local *Anopheles* species composition, biting behaviors, monthly density and feeding site preferences.

Methods

This study was carried out at Wonji Sugar Estate, Ethiopia's sugar business, which was established in the 1950s by the collaboration between Dutch private company and the Ethiopian government. The Wonji plain is situated in Eastern Oromia in Ethiopia's central Rift Valley, 110 kilometers southeast of Addis Ababa (Finfinnee). It is situated at 8^{o} 21' to 8^{o} 29'N and 39^{o} 12' to 39^{o} 20'E, at an elevation of 1223–1550 meters above sea level (masl) the study area map described in Lelisa *et al.*, (Lelisa et al. 2023). The average yearly bimodal rainfall pattern in the area is 850 mm per year with mean maximum and minimum temperatures of 27^{0}C and 15^{0}C, respectively (Wonji Sugar Estate meteorological office data). The Estate cultivates sugarcane plantations on 12,800 hectares, with the neighboring community cultivating 7,000 hectares on their own tenure as out growers. Water for irrigation is obtained from the Koka Dam of upper Awash River that discharges after electric generation, and various irrigation utilities(Tufa 2021).

The Wonji sugar estate has 48,000 residents; of this about 38% were office workers. The professional office workers live in facilitated house types made of thin roof and cement walls in two central villages. While the vast majority of the residents were seasonal and casual labor workers living in house structures made of thin roof, woody and mud-plastered walls in 9 villages. There are one primary hospital, one polyclinic, and two post-basic health facilities serving the residents(Lelisa et al. 2023). The employees come from various geographic, educational, and economic backgrounds(Tufa 2021).

Study design and period

A longitudinal entomological monitoring was conducted to investigate the *Anopheles* species composition and behaviors from July 2018 to June 2020.

Meteorological data of the study area

The meteorological station at Wonji Sugar Estate was used to gather metrological data. In addition, the mean monthly maximum and minimum temperature, rainfall, and relative humidity were compared to the mean monthly *Anopheles* density, leading to a correlation analysis.

Anopheles mosquito collection

Adult *Anopheles* mosquitoes were sampled from two randomly selected villages (Wonji Shoa and Bikiltu villages) in Wonji Sugar Estate. Thirty households were chosen randomly and used for entomological sampling based on mosquito flight ranges and proximity to the cane plantation's irrigation system. Adult *Anopheles* mosquitoes were sampled using Centers for Disease Control (CDC) light trap captures (LTC), pyrethrum spray catches (PSC), handheld mouth aspirators tools, and artificially prepared pit shelter (PIT) in accordance with the study's objectives. During the research periods, 10 households were consistently used to sample host-seeking mosquitoes both indoors and outdoors at fortnightly intervals, deploying a total of 40 CDC-LTs each month. Similarly, 20 householders different from CDC-LT surveyed householders were inspected consistently every month; to detect mosquitoes on the gonotrophic cycle. Resting adult sampling was done once per month from indoors and outdoors in the residential houses at potential shady spots and from a prepared pit shelter.

Biting activities of Anopheles mosquitoes

Five households were used for CDC- LT from each village. Accordingly, pair of CDC-LT were set to operate between 18:00 and 06:00 hrs. to sample indoor and outdoor host-seeking *Anopheles* mosquitos. CDC LT was hung at a 1.5m off the floor near the inhabitant's bedroom, who was secured by Long-Lasting Insecticide Impregnated Nets (LLINs)(Silver 2008). The outdoor collection was set in the radius of 15–20m surrounding the house assigned for the indoor collection(Silver 2008). Concurrently, of the ten sampled households, one was selected for overnight hourly biting activity monitoring of mosquitoes in both villages. The hourly biting behaviors of *Anopheles* species were recorded from the 192 CDC-LT set indoors and outdoors throughout 48 sampling nights. The trapped *Anopheles* mosquitoes in each CDC LT were collected every hour and transferred into labeled paper cups with hours, sites, houses and dates of collection.

Assessment of indoor and outdoor resting Anopheles mosquito

Indoor and outdoor resting mosquitoes were inspected once a month from 6:00 AM to 07:30 AM. Pyrethrum spray catch (PSC) was employed to collect indoor resting *Anopheles* mosquitoes. Household food, water, domestic animals, and other items were brought out before the implementation of PSC.

Any openings that could enable mosquitoes to escape were checked and sealed. The floor was completely covered with a white cotton sheet. The house was sprayed with Mobil flit (KillitMT insecticide aerosol) containing pyrethroids by the protected sprayer (person) and then locked for 10 minutes. After ventilating the houses for 10 minutes, the sheet was gently removed from the room and knocked down mosquitoes were collected with forceps (Silver 2008).

Hand-held mouth aspirators were used to collect *Anopheles* species from a naturally available outdoor resting place on the same day and time as indoor resting mosquito collections. Accordingly, any available shady used for resting was assessed within the compound where PSC was conducted. Leftover household materials damped in the compound, tree bark, tree hole, and wall side were inspected for the presence and collection of adult mosquitoes using torch and aspirator.

Artificial pit shelters with depth of 1.5m and opening of 1.2m by1.2m widths were prepared within the compounds where outdoor resting mosquitoes where sampled. Each pit shelter had four digs/pockets at depth of 30cm on each side(WHO 1975; Massebo et al. 2013). The pit was covered with LLINs while mosquito collection to prevent escaping.

Species identification of Anopheles mosquito

Sampled adult *Anopheles* mosquitoes were identified morphologically using standard key(Vernoene 1962) and were labeled and preserved with silica gel in 2.5 ml Eppendorf tube labeled with information including species type, date of collection, place of collection, method of collection and abdominal states. Specimens were stored in freezer at -40˚C at Aklilu Lemma Institute of Patho Biology Insectary laboratory for subsequent activities.

Data analysis

Data were analyzed using SPSS statistical software package version 26 (IBM, Corp. Chicago IL.). Probability (P-value) at < 0.05 was considered statistically significant. Prior to analysis, data cleaning and log(x + 1) transformation were done to normalize the count data distribution. Hourly biting density of *Anopheles* species were correlated with house holders night time activities. Regression analysis was also used to evaluate the effects of weather variability on the mean densities of *Anopheles* species. Multiple, mean comparisons of *Anopheles* species' mean monthly densities were evaluated using ANOVA, and significant means were separated using the Tukey post-hoc test. The student t-test was used to examine differences in the densities of *Anopheles* by place of biting, season, and village. F-test was used to examine variation along *Anopheles* across collection tools and collection months.

Results

Anopheles mosquito species composition and abundance

A total of 3,504 *Anopheles* mosquitoes that belongs to three species were collected: *Anopheles gambiae s.l., Anopheles pharoensis*, and *Anopheles coustani cx.* as morphologically identified. *Anopheles gambiae s.l.* accounted for 75.23% (n = 2,636, CI = 1.255–1.491, P = 0.000) of the total, while the *Anopheles coustani* cx. represented 9.25% (n = 324, CI = 0.322–0.134, P = 0.000). The highest number (59.80%, n = 2,098 CI = 1.682–1.968, P = 0.00) of *Anopheles* mosquitoes was collected using CDC LT which was followed by PSC, Pit shelter, and Handheld mouth Aspirator (N) collection methods. The least number of *Anopheles* mosquitoes was collected by Pit shelter/PIT 340 (n = 9.70%). *Anopheles gambiae s.l.* was the most abundant species trapped using all collection methods while *Anopheles coustani cx.* was the least in all collection tools (Table 1).

Table 1
Anopheles species composition and abundance in Wonji Sugar Estate

	Sampling tools							
Species	CDC LT	PSC	PIT	N	Total	Mean	*CI	P-value
	2,098 (59.87%)	422 (12.04%)	340 (9.70%)	644 (18.38%)	3,504 (100%)	1.83	1.68–1.97	0.000
An. gambiae s.l.	1,543 (44.04%)	387 (11.04%)	241 (6.88%)	465 (13.27%)	2,636 (75.23%)	1.37	1.26–1.49	0.000
An. pharoensis	300 (8.56%)	33 (0.94%)	89 (2.54%)	122 (3.48%)	544 (15.53%)	0.28	0.25–0.32	0.000
An. coustani cx.	255 (7.28%)	2 (0.06%)	10 (0.29%)	57 (1.63%)	324 (9.25%)	0.17	0.13–0.20	0.000

*CI = confidence interval

Hourly biting activities

Anopheles species had their highest biting activity between 6:00 PM and 10:00 PM both indoors and outdoors which was statistically significant compared to midnight and early morning biting hours (Mean = 0.26, CI = 0.21–0.31, p = 0.000). *Anopheles gambiae s.l.* had shown overnight biting pattern, with peak densities from 7:00 PM to 10:00 PM and 3:00 AM to 5:00 AM and the lowest biting activity between 11:00 PM and 2:00 AM, the variation was statistically significant (p- value = 0.000) detail presented in supplementary information (table SI 1). *Anopheles pharoensis* biting activity decreased sharply between 10:00 PM and 4:00 AM both indoor and outdoor (Fig. 1).

Figure 2 shows the night time activities of the householders. Most of them go to sleep at 11:00 PM, but some of them are still awake at 10:00 PM or 12:00 AM. The hourly biting density of *Anopheles* mosquitoes is positively correlated with the number of active householders before and after bedtime (r = 0.135, P = 0.00). On the other hand, the biting density is negatively correlated with the number of sleeping householders (Table 2). The regression analysis also shows that the activities of the householders before and after bedtime explain 2.8% of the variation in the biting density (R^2 = 0.028, F = 16.78, P = 0.000). Moreover, the beta coefficients indicate that an increase in one active householder leads to a higher biting density of *Anopheles* mosquitoes (β = 0.038, t = 1.74, p = 0.08), while an increase in one sleeping householder leads to a lower biting density (β = -0.037, t= -1.7, p = 0.000).

Table 2
Correlation analysis of occupants' activities and *Anopheles* mean density

Number of occupants	**Mean ± SE**	**CI**	***Anopheles***	**CI**	**r**	**P**
Mean density of occupants on activities	1.38 ± 0.05	1.29–1.47	0.06 ± 0.005	0.05–0.07	0.13	0
Mean density of occupants slept	4.78 ± 0.04	4.69–4.86	0.02 ± 0.003	0.02–0.03	-0.07	0.02
Mean density of occupants awake	0.48 ± 0.02	0.44–0.53	0.04 ± 0.005	0.03–0.05	0.05	0.01

Anopheles species spatiotemporal dynamics

As determined by morphological identification, *Anopheles gambiae s.l., Anopheles pharoensis* and *Anopheles coustani cx.* were collected from Wonji Shoa and Bikiltu villages. Although greater number of *Anopheles gambiae s.l.*, was collected from Wonji Shoa village, it was not different from that of Bikiltu villages (t = 0.81; P = 0.42) (Table 3). Similarly, there was no significant mean variation between the two study sites for the *Anopheles pharoensis* and *Anopheles coustani cx.* (t= -1.05; p = 0.08 and t = 1.06; p = 0.29, respectively).

Table 3
Anopheles species distribution in the study villages

Species	**Study village**		**t-test**	**P- value**
	Wonji Shoa	**Bikiltu**		
	Mean ± SE	Mean ± SE		
An. gambiae s.l.	0.23 ± 0.01	0.22 ± 0.01	0.81	0.42
An. pharoensis	0.06 ± 0.01	0.07 ± 0.01	-1.05	0.08
An. coustani cx.	0.04 ± 0.01	0.03 ± 0.00	1.06	0.29

Temporal dynamics of the mean density of *Anopheles* mosquito species in the Wonji Sugar Estate over 24 months of entomological monitoring is presented in Table 5. There were statistically significant monthly mean density differences among *Anopheles gambiae s.l.* (F = 17.71, P = 0.000), *Anopheles pharoensis* (F = 5.53, P = 0.000), and *Anopheles coustani cx.* (F = 10.85, P = 0.000). *Anopheles gambiae s.l.* outcompeted other species in both monthly abundance and availability; the difference was statistically significant at p = 0.000. The highest density of *Anopheles* species was noted in July 2019 followed by June 2019. *Anopheles gambiae s.l.* density was peaked in July 2019 while the least in December 2018. Higher mean densities of *Anopheles pharoensis* were noted in July 2019 and March 2019. The lowest density of was recorded through all the months, whilst non was collected in *Anopheles coustani cx. was* not collected in December 2018 and January 2019 (Table 4).

Table 4

Multiple comparisons of *Anopheles* species mean monthly density

Month & year	***An. gambiae* s.l.**	***An. pharoensis***	***An. Coustani cx.***	**Total**
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Jul-18	0.236 ± 0.042	0.032 ± 0.014	0.008 ± 0.008	0.253 ± 0.043
Aug-18	0.186 ± 0.034	0.086 ± 0.021	0.004 ± 0.004	0.254 ± 0.037
Sep-18	0.302 ± 0.047	0.012 ± 0.028	0.004 ± 0.004	0.377 ± 0.048
Oct-18	0.191 ± 0.036	0.087 ± 0.025	0.015 ± 0.007	0.239 ± 0.041
Nov-18	0.185 ± 0.031	0.088 ± 0.021	0.004 ± 0.004	0.243 ± 0.035
Dec-18	0.047 ± 0.012	0.032 ± 0.011	0.000 ± 0.000	0.074 ± 0.017
Jan-19	0.057 ± 0.015	0.094 ± 0.019	0.000 ± 0.000	0.137 ± 0.024
Feb-19	0.053 ± 0.016	0.019 ± 0.011	0.01 ± 0.007	0.078 ± 0.020
Mar-19	0.112 ± 0.022	0.121 ± 0.025	0.057 ± 0.019	0.250 ± 0.033
Apr-19	0.1454 ± 0.027	0.086 ± 0.021	0.184 ± 0.036	0.350 ± 0.041
May-19	0.267 ± 0.031	0.033 ± 0.012	0.029 ± 0.012	0.308 ± 0.032
Jun-19	0.543 ± 0.043	0.104 ± 0.023	0.035 ± 0.016	0.600 ± 0.041
Jul-19	0.567 ± 0.047	0.168 ± 0.028	0.011 ± 0.006	0.627 ± 0.047
Aug-19	0.416 ± 0.045	0.038 ± 0.011	0.008 ± 0.005	0.432 ± 0.046
Sep-19	0.329 ± 0.040	0.102 ± 0.023	0.149 ± 0.029	0.411 ± 0.049
Oct-19	0.263 ± 0.032	0.104 ± 0.021	0.091 ± 0.024	0.380 ± 0.037
Nov-19	0.161 ± 0.026	0.046 ± 0.014	0.043 ± 0.016	0.226 ± 0.030
Dec-19	0.057 ± 0.015	0.011 ± 0.006	0.029 ± 0.011	0.088 ± 0.019
Jan-20	0.206 ± 0.028	0.044 ± 0.012	0.004 ± 0.004	0.237 ± 0.029
Feb-20	0.129 ± 0.019	0.029 ± 0.011	0.023 ± 0.010	0.175 ± 0.022
Mar-20	0.155 ± 0.026	0.027 ± 0.011	0.031 ± 0.013	0.194 ± 0.029
Apr-20	0.209 ± 0.033	0.023 ± 0.010	0.014 ± 0.008	0.222 ± 0.035
May-20	0.237 ± 0.036	0.021 ± 0.009	0.009 ± 0.007	0.355 ± 0.037
Jun-20	0.301 ± 0.044	0.046 ± 0.016	0.043 ± 0.016	0.346 ± 0.045
Total	0.223 ± 0.007	0.065 ± 0.004	0.034 ± 0.003	0.282 ± 0.008
Mean square	1.53	0.14	0.17	

Month & year	***An. gambiae* s.l.**	***An. pharoensis***	***An. Coustani cx.***	**Total**
F- test	17.71	5.37	10.85	
df	23	23	23	23
P- value	0	0	0	0

The density of *Anopheles* species was significantly higher in the wet (April through September) than in the dry (October through March) seasons (P- value = 0.000). The mean density of *Anopheles gambiae s.l.* was high within the dry seasons (Table 5); however, there was no significant difference in mean density of *Anopheles coustani cx.* across the seasons.

Table 5
Seasonal mean density variation of *Anopheles* species in Wonji sugar estate, July 2018 to June 2020.

***Anopheles* species**	**Wet season**	**Dry season**	**t- test**	**P -value**
	Mean ± SE	**Mean ± SE**		
Anopheles gambiae s.l.	0.31 ± 0.01	0.12 ± 0.01	13.66	0.000
Anopheles pharoensis	0.07 ± 0.01	0.05 ± 0.00	2.57	0.010
Anopheles coustani cx.	0.03 ± 0.01	0.03 ± 0.00	-0.45	0.66
Total	0.36 ± 0.01	0.19 ± 0.01	11.27	0.000

The correlation analysis revealed that *Anopheles* species means monthly density with climate variability has strong positive correlation with monthly; mean minimum temperature and relative humidity (r = 0.24, p = 0.000&r = 0.173, p = 0.000) respectively (Fig. 3). The maximum temperature has shown significant negative correlation (r=-0.050, p = 0.029) with the density of *Anopheles* species. *Anopheles gambiae s. l*'s mean monthly density has demonstrated a strong positive correlation with mean: rainfall, minimum temperature, and relative humidity but a strong negative correlation with maximum temperature (r = 0.57, p = 0.013, r = 0.264, p = 0.000, r = 0.211, p = 0.000, and r = -0.077, p = 0.001, respectively).

The variation in mean monthly *Anopheles pharoensis* density has shown no correlation with: relative humidity, mean maximum and minimum temperatures (r = 0.11, p = 0.623, r = -0.039, p = 0.087, and r = -0.009, p = 0.694, respectively).

The monthly catch density of *Anopheles coustani cx.* has a positive correlation with mean maximum and minimum temperatures (r = 0.055 p = 0.016 & r = 0.102 p = 0.000), but a very weak correlation with average rainfall and relative humidity (r = 0.003 p = 0.909 & r = 0.016 p = 0.491). The regression analysis result justifies the minimum temperature, rainfall and relative humidity predicts the variability in mean density of *Anopheles* species at p-value < 0.05.

Indoor and outdoor host seeking

A total of 2,098 adult *Anopheles* mosquitoes were collected by CDC-LT over 24 months for 48 sampling nights from 960 light trap nights indoors and outdoors (Table 6). The overall mean density of *Anopheles* mosquitoes searching for a host outdoors was 2.58 *Anopheles* per trap/night while indoors was 1.97 *Anopheles* per trap /night. The indoor and outdoor host seeking density did not shown variation by villages (t = -2.113 p = 0.072). The overall outdoor host seeking density was not consistent from month to month over the study periods (SI 2). Higher proportion of *Anopheles gambiae s.l.* was trapped from indoor CDC LT than its counterpart (F = 11.954, P = 0.001), however, the trend was not consistent along all the study months (SI 2). The number of *Anopheles pharoensis* collected outdoors was higher than indoor collection (F = 88.689, P = 0.000).

Table 6
Indoor and outdoor mean density of *Anopheles* species per CDC LT trap/night

	***Anopheles* species**	**n**	**Indoor(M ± SE)**	**Outdoor (M ± SE)**	**t-test**	**P-value**
1	*Anopheles gambiae s.l.*	1,543	0.487 ± 0.023	0.432 ± 0.026	1.565	0.001
2	*Anopheles pharoensis*	300	0.079 ± 0.011	0.199 ± 0.017	-5.962	0.000
3	*Anopheles coustani cx.*	255	0.049 ± 0.009	0.155 ± 0.018	-5.382	0.000
Total		2,098	0.539 ± 0.023	0.612 ± 0.023	-2.113	0.072

Discussion

Anopheles gambiae s.l., Anopheles pharoensis and *Anopheles coustani cx.* were identified in the study area where *Anopheles gambiae s.l*. was the most abundant species. This is consistent with other studies(Lelisa et al. 2017; Demissew et al. 2020; Bedasso et al. 2022; Getachew et al. 2019; Haileselassie et al. 2021; Kindu et al. 2018) which reported that *Anopheles gambiae s.l.*is the most widely distributed species in Ethiopia. Among the contributing factors for the dominance of *Anopheles gambiae s.l.* may related to the availability of open and permanent breeding habitats due to cane farm. Studies conducted in different countries where irrigation-based cane farming practiced concluded that the scenario greatly favors the major malaria vector *Anopheles gambiae s.l.* density (Diakite et al. 2015; Frake et al. 2020; Kibret et al. 2018). However, some studies (Kenea et al. 2016; Degefa et al. 2021) indicated that *Anopheles pharoensis* and *Anopheles ziemanni* were the most abundant species in Bulbul village Kersa district and Edo Kontola small irrigation based mixed faming in Adami Tullu central Ethiopia respectively. Several studies from different countries in Africa also revealed that *Anopheles gambiae s.l.* was dominant in the area of its distribution (Machani et al. 2020; Doumbe-Belisse et al. 2021).

This study indicated that high density of *Anopheles* species was collected by CDC LT compared to PCS, PIT and hand capture; several other studies also agree with this finding that CDC LT was productive tool (Bedasso et al. 2022; Lelisa et al. 2017). The low number of mosquitoes collected in PSC might be an indicative of the decreased density of indoor resting mosquitoes due to indoor based malaria vectors control intervention tools (IRS and ITNs). Moreover, studies conducted in Ethiopia, Kenya and Nigeria before the intervention of indoor based malaria vectors control tools found out high density of indoor resting *Anopheles* species collected by PSC tool(Amaechi et al. 2018). This strengthens the idea that IRS and ITNs intervention affect behavior of *Anopheles* locating their feeding and resting places. Interestingly, in the presence of IRS and ITNs studies conducted in Ethiopia at three altitudinal transect sampled higher density of indoor resting *Anopheles arabiensis* from PSC compared to CDC LT(Animut et al. 2013)due to the miss-uses of ITNs.

Based on the findings of this study, it could be witnessed that night-time work activities were common in the community. This was supported by the Wonji Sugar Estate labor division office, which confirmed that overnight working activities were regular and occur through shifts. This might result in the *Anopheles* overnight biting activities. The overnight biting activities of *Anopheles gambiae* s.l., *Anopheles pharoensis, Anopheles coustani cx.* in irrigation-based cane farms were also reported from Ethiopia (Demissew et al. 2020), Kenya (Ototo et al. 2015), Uganda (Ojuka et al. 2015; Kabbale et al. 2013). This current study also documented two sharply increased peak biting density at early night and early morning this overlap with the human activities before bed and after bed that has high probability of human vector contact. This is consistent with some other studies (Kenea et al. 2016; Degefa et al. 2021)..

Species distribution in both study villages was similar this may be due to; presence of similar breeding habitats and no geographic barriers exist. The co-occurrences of *Anopheles gambiae s.l., Anopheles pharoensis* and *Anopheles coustani cx.* was registered elsewhere in Ethiopia where irrigation based intensive agricultural activities practiced (Haileselassie et al. 2021; Kenea et al. 2016; Getachew et al. 2019). Similar studies in Kenya, Nigeria, reported the co-occurrences of these species in the geography they distributed (Amaechi et al. 2018; Degefa et al. 2017).

The highest mean density of *Anopheles* species (mean = 0.627) was recorded in July 2019 months, the middle of heavy and elongated rainy season. Moreover, the current result indicates increasing in density of *Anopheles* as of onset of long rain fall in June throughout September. This finding is similar with some other reports (Ototo et al. 2015; Amaechi et al. 2018; Tarekegn et al. 2022; Kenea et al. 2016; Ejeta 2017; Shililu et al. 2OO4). In contrast to our findings, there are reports on a peak density in the months of September through November (Kibret et al. 2014; Kibret et al. 2017; Kibret et al. 2012; Massebo et al. 2013). Over the 24 study months of entomological monitoring, *Anopheles gambiae s.l.* and *Anopheles pharoensis* never get zero number their seasonality was changed to perineal. The year-round availability of *Anopheles* in the study community might be contributed due to availability of permanent breeding habitats as indicated by other studies from water resource development for cane farm in different parts of the country(Ejeta 2017; Kibret et al. 2017; Ndiath et al. 2012).

The monthly, availability trends of the major malaria vector, *Anopheles gambiae s.l.* may increase the risk of malaria transmission particularly during the dry season where transmission is not expected under normal condition (Kibret et al. 2017). Even though year-round availability of the *Anopheles gambiae s.l.*

and *Anopheles pharoensis* (the primary and secondary malaria vectors) was noticed, they showed significant seasonal density variation. Similar to this finding, studies from Senegal, Kenya, Nigeria also indicated *Anopheles gambiae s.l.* and *Anopheles pharoensis* density was affected by seasons (Amaechi et al. 2018; Frake et al. 2020; Ndiath et al. 2012).

In this study both the maximum and minimum temperature shown impact on *Anopheles* density variation in which one unit increases of maximum temperature above optimum negatively influenced the number of *Anopheles* species. The observed minimum temperature was in the optimum requirements of *Anopheles* species that may has contributed in the increases in the mean density of *Anopheles* this was supported (Kabbale et al. 2013; Taye et al. 2016; Lunde et al. 2013; Abiodun et al. 2016).

The *Anopheles* mosquito species displayed outdoor host seeking behavior than indoor venues. This may be the result of indoor-based vector control techniques that cause change in host seeking behavior to outdoor venues. According to study by (Kibret and Wilson 2016) which supports our assertion, more unfed host-seeking *Anopheles gambiae s.l.* were found outdoor than indoor venues in a community where ITNs and IRS were in use. Immense studies on *Anopheles gambiae s.l.* in the country strength our finding that shift in feeding venue from indoor to outdoor which could be revealed as insecticide avoidance strategy of the species exhibited (Kibret and Wilson 2016; Lelisa et al. 2017; Kabbale et al. 2013; Taye et al. 2016; Shililu et al. 2OO4). The current study results indicated that *Anopheles pharoensis* and *Anopheles coustani cx.* mainly bite outdoor similar to other previous studies (Taye et al. 2016).

Conclusions

Three *Anopheles* species, *Anopheles gambiae s.l.*, *Anopheles pharoensis* and *Anopheles coustani cx.*, were well established in Wonji Sugar Estate. *Anopheles gambiae s.l.*, the major malaria vector in Ethiopia, was found during all the months of entomological monitoring. Biting occurs throughout the night with two peaks (6:00 PM – 10:00PM and 3:00AM – 6:00AM). Outdoor host seeking behavior of the mosquitoes in similar proportion as indoor might challenge the current indoor based malaria vector control interventions. Moreover, the peak biting behavior during dusk and dawn that overlap with active time of the local people in the sugarcane plantation may sustain high malaria transmission. The availability of *Anopheles gambiae s.l.* during dry season and throughout a year might expose humans to malaria at all seasons. The malaria and malaria vector control tools should be implemented at all seasons of a year. Moreover, a due attention should be given to seek for complementary outdoor mosquito control measures to be implemented in malaria control programs.

Declarations

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Authors' contributions

KL conceived the study design, undertook the field study, performed data analysis, interpreted data and drafted the manuscript. SD involved in the study design, revision of the manuscript, and facilitation of administrative issues. SD, YW and LG take part in critically review of the manuscript for intellectual content. All authors read and approved the final manuscript.

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Availability of data and material

The data supporting the results reported in this article are well addressed within this article and in supplementary information annexed.

Ethical approval and Consent to participate

Ethical clearances were obtained from Addis Ababa University, Aklilu Lemma Institute of

Pathobiology, Institutional Review Board (IRB) (Reference number:

ALIPB/IRB/012/2017/2018**),** and from Oromia Regional Health Bureau Ethical Clearance

Committee (Reference number: BEFO/DBTHL/1-8/422**)**. Letter of permission were also sought from East Shoa zone and Nanawa Adama district's health office for the commencement of the study. Wonji Sugar Estate provides us permission and led us to community level where we sought their consent. Every month of data collection permission was sought from each house owner before the commencement of entomological sampling and during PSC they were consented to stay outside for about 20 minutes post-spray.

Consent for publication

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

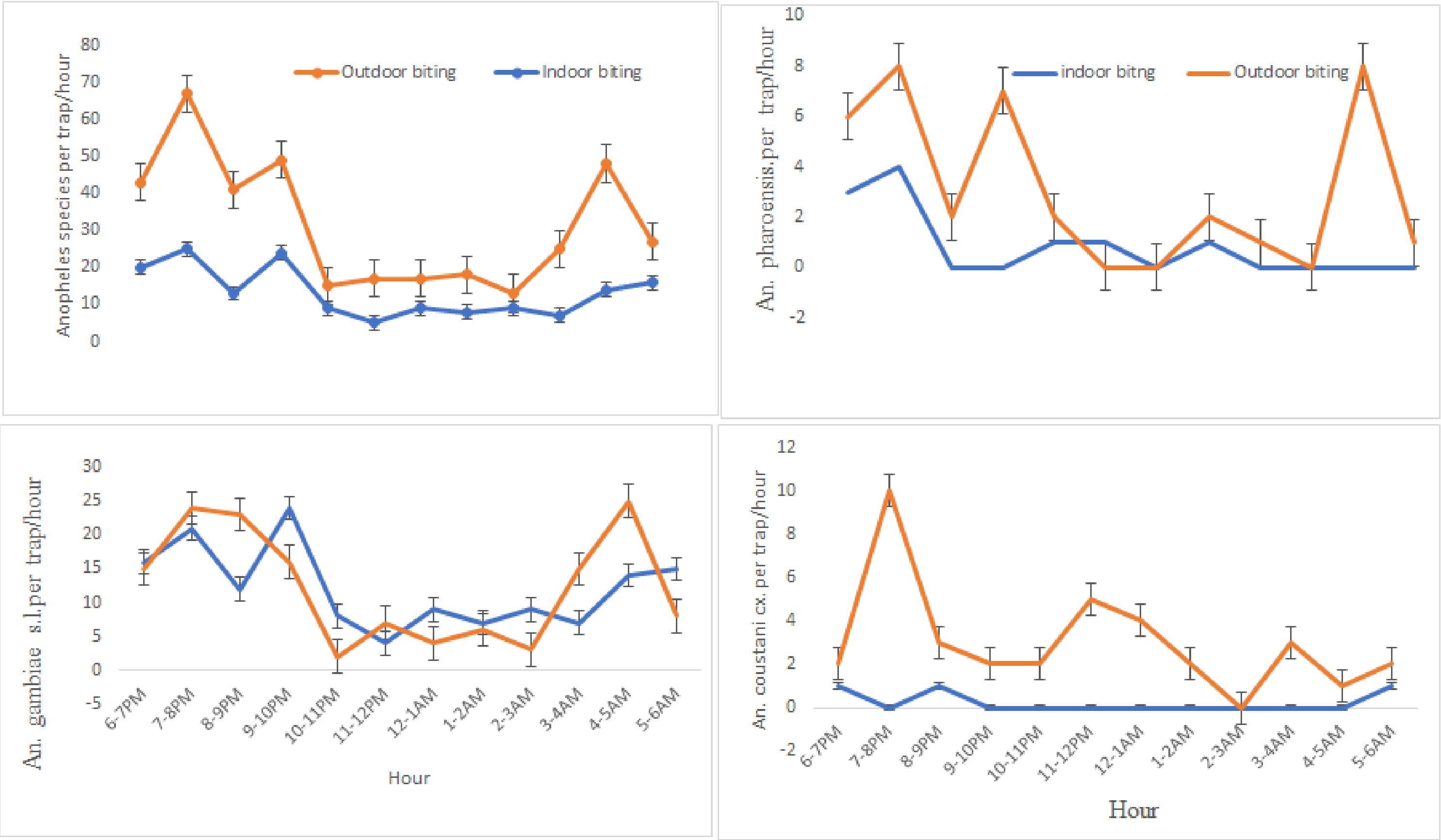

Figure 1

Hourly biting activities of *Anopheles*species in Wonji sugar estate

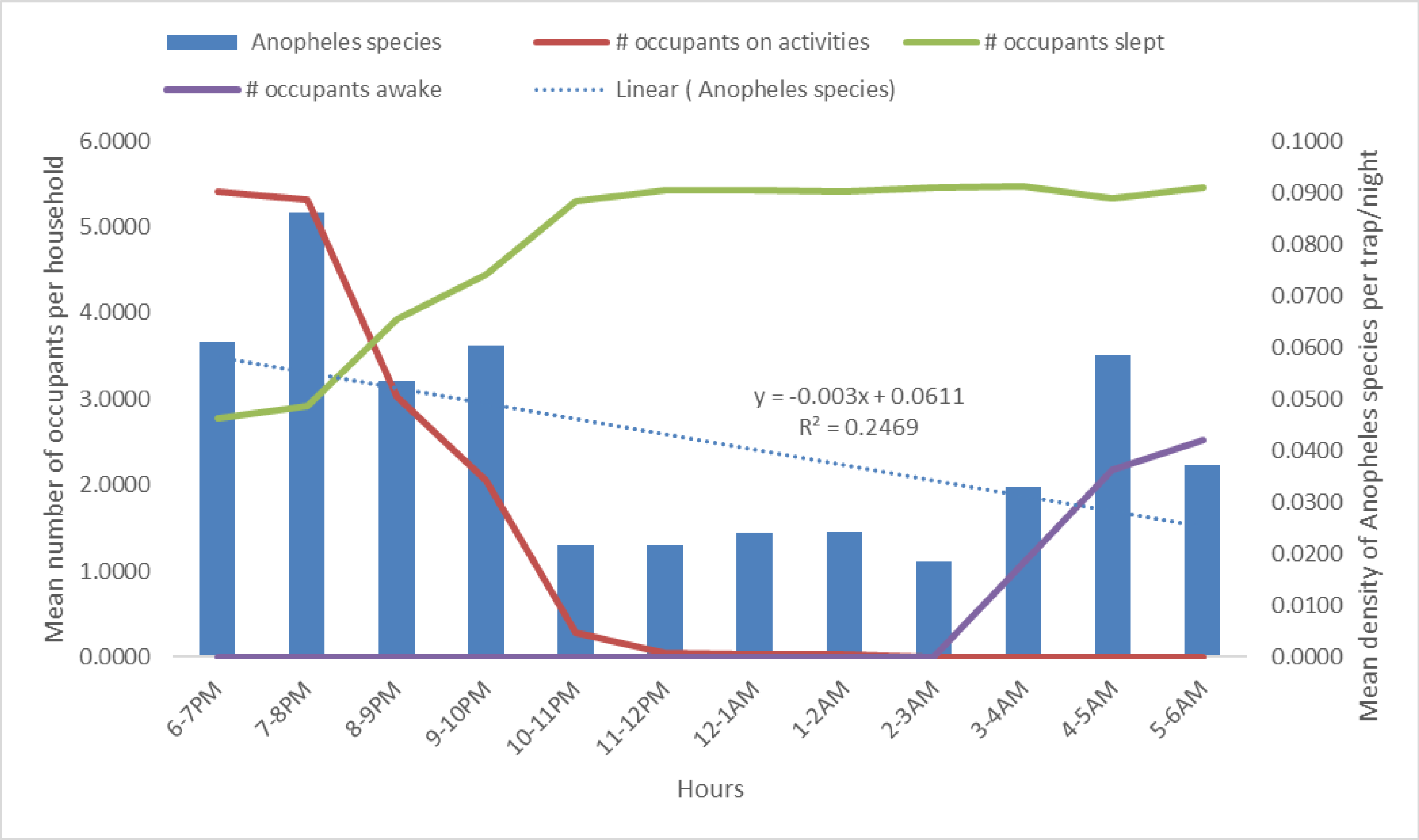

Figure 2

Night time occupants' activities and mean biting density of *Anopheles* species in Wonji Sugar estate

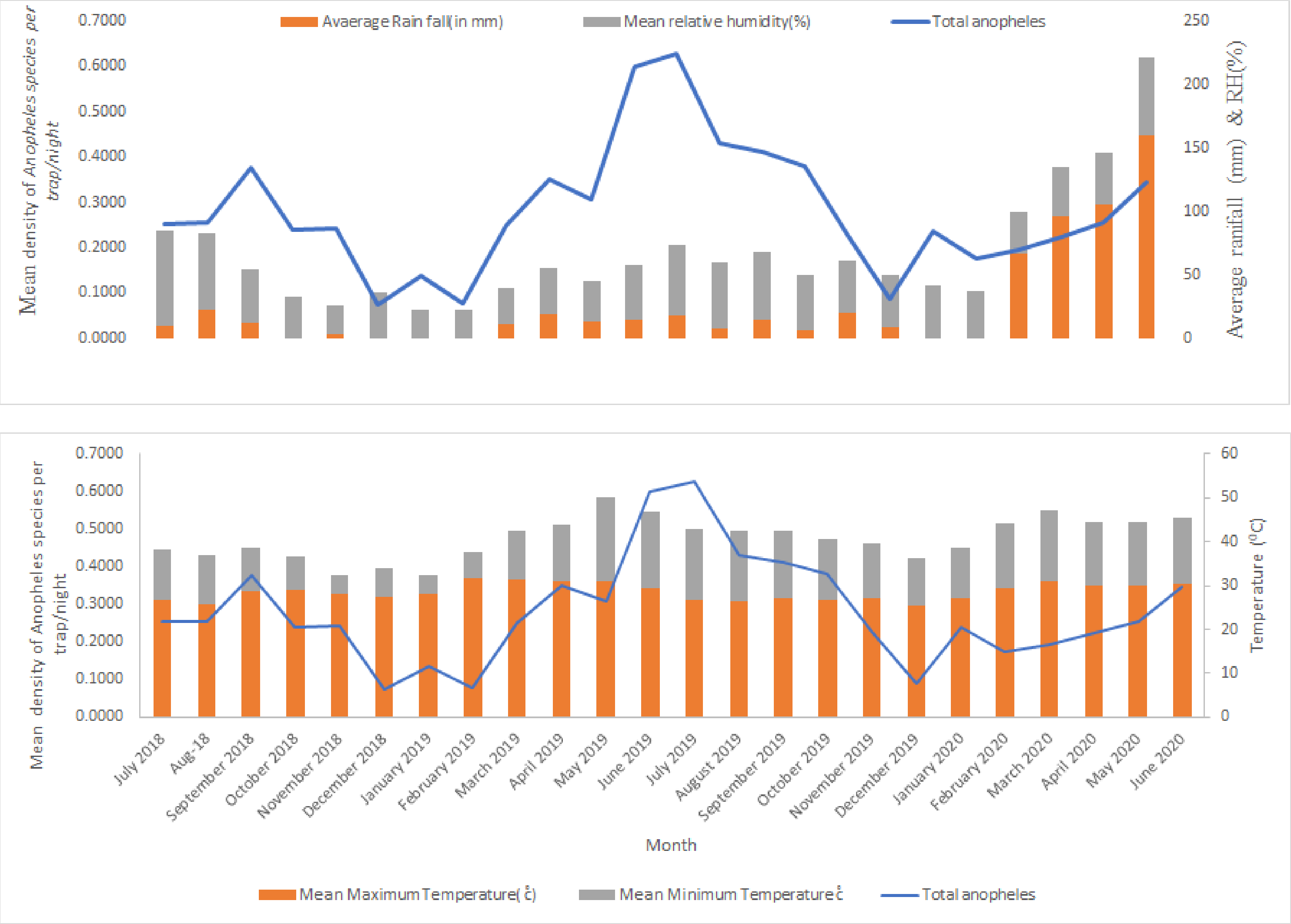

Figure 3

Mean monthly density of *Anopheles* species against mean monthly maximum and minimum temperature, mean Rain Fall (RF in MM) and Relative humidity (RH%)

Supplementary Files

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