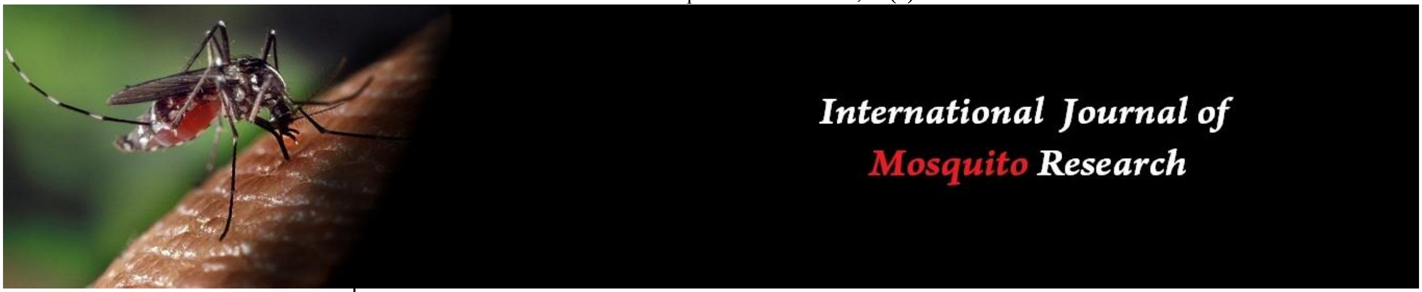

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Insecticide resistant status, larval species composition and habitat characterization of *Anopheles gambiae* (S.L.) in malaria endemic districts of Sidama region, South Ethiopia

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Abstract

Introduction: The prevalence of malaria in Ethiopia has decreased somewhat in recent decades, but it is currently increasing as a result of the introduction of insecticide-resistant *Anopheles* mosquitoes. Therefore, this study was aimed to assess entomological factors related to malarial disease transmission in the study population.

Methods: Socio-demographic characteristics from 48 households were collected by using structured questionnaires. Anopheline immature stages of mosquito were collected by dipping methods and reared to adult in a laboratory to determine the species composition and drug susceptibility test. Using WHO papers and bioassay tubes, 150 adult female *Anopheles* mosquitoes, two to three days old, not fed blood, were exposed for one hour to varying dosages of 0.05% Deltamethrin, 0.45% Alpha-cymethrin, and 0.1% Propoxur. Logistic regression was used to identify local risk factors using SPSS version 25 for further analysis. Statistical significance was declared at p-value<0.05.

Results: The presence of *Anopheles* larvae was confirmed in all water-holding habitats. Insecticide susceptibility test was performed, 100 adult *Anopheles* mosquitoes were exposed to each insecticide as World Health Organization recommended (propoxur 0.1%, deltamethrin 0.05%, and alpha-cypermethrin 0.45%). The knockdown times (KDT), percentage knockdown, and percentage mortalities of the exposed mosquitoes were noted. *Anopheles gambiae* (S.L)., the species of mosquito that was identified, was resistant to propoxur (0.1%) and delthametrin (0.05%), but it was completely susceptible to alpha-cypermethrin (0.45%) across all study sites. *Anopheles gambiae* (S.L) prefer to breed in habitat which exposed to sun light, had stone substrate and had no canopy cover.

Conclusion: There is a need to employed integrated vector management approaches to malaria vector control.

Keywords: Alpha-cypermethrin, *Anopheles gambiae*, Delthametrin, insecticide susceptibility, Propoxur

Introduction

Malaria is the most prevalent serious mosquito-borne disease worldwide [1]. The female *Anopheles* mosquito, which varies depending on the region, is the vector of this infectious disease, which is caused by the parasite Plasmodium and spreads from infected to healthy individuals [2]. The main *Anopheles* species that spread malaria are *An. Gambiae*, *An. stephensi*, *An. dirus*, *An. coluzzii*, *An. albimanus*, *An. funestus*, and *An. Arabiensis* [3].

The WHO's African Region accounts for the majority of the increase in malaria cases, which is expected to reach 247 million worldwide in 2021 from 245 million in 2020 in 84 malaria-endemic countries (including the French Guiana territory) [4]. Over 50% of the global population is susceptible to the illness, which is made worse by failing healthcare systems, rising drug and pesticide resistance, natural disasters, and climate change [5].

These days, the subtropics bear the brunt of the malaria epidemic. *Plasmodium falciparum* is the most dangerous parasite species that infects humans in the African Sahara; it is estimated to cause roughly 250 million cases and approximately 1 million deaths annually [1]. Different *Anopheles* mosquito species carry malaria throughout sub-Saharan Africa, and the risk of infection and illness varies greatly between locations [6, 7].

Malaria has been a persistent public health issue in Ethiopia for a number of years. *Anopheles arabiensis* is the primary vector of malaria, with Plasmodium falciparum and Plasmodium vivax being the two most common parasites [8]. Temperature, precipitation, and altitude all affect the seasonality of transmission [9, 10]. An estimated 68% of the population is at risk of contracting malaria, with over half (60%) of the country's population living in malaria-prone areas [11]. The primary methods for preventing and controlling malaria in Ethiopia are the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [12]. Malaria continues to be the leading cause of morbidity and mortality in Ethiopia despite the achievements [13], and efforts made thus far. According to estimates, the nation would be responsible for 1.7% of cases and 1.5% of deaths from malaria in 2021, making it a major public health concern [14].

Pyrethroids are the primary class of insecticide approved for use in net impregnation, out of five main classes: carbamates, neonicotinoid, organochlorines, organophosphates, and pyrethroids [4]. October 2017 saw the prequalification of the first neonicotinoid product for indoor residual spraying [15]. The majority of pesticides were applied by residual spraying, which was followed by larviciding and space spraying. All of the insecticide classes Organochlorine, Organophosphate, Carbamate, Pyrethroid, and Neonicotinoid are used for residual spraying; Organophosphate, Carbamate, and Pyrethroid are used for space spraying; and Organophosphate is used for larviciding. Pyrethroids are the most effective insecticide for treating nets [15].

Numerous studies have revealed that *Anopheles* species found in various parts of Ethiopia exhibit high levels of resistance to insecticides such as deltamethrin, permethrin, pirimiphos-methyl, bendiocarb, propoxur, DDT, and others [16-20].

All efforts to control vectors rendered useless if insecticide-resistant mosquito species emerge. If an organization fighting malaria sprays insecticides hundreds of times while an insecticide-resistant mosquito variety emerges in a particular area, significant change will not occur. As a result, this study was carried out to assess the presence of insecticide-resistant *Anopheles* mosquitoes and the characteristics of their habitat in the Sidama region's malaria endemic districts, Aleta Chuko, Dale and Loka Abaya which are well known for their malariousity.

Materials and Methods

Description of the study area

The study was carried out in Aleta Chuko, Dale and Loka Abaya districts which are situated in the southwest of the Sidama region. The Districts are well-known for their flat terrain and close proximity to Ethiopia's Abaya Lake. The Districts are situated between 1650 and 2100 meters above sea level. The distance from Aleta chuko, Dale and Loka Abaya to Addis Ababa is 281, 261 and 283 Km respectively. Mean annual rainfall of Aleta chuko, Dale and Loka Abaya is 1961.9, 1264.2 and 999.4 respectively. Mean annual temperature 19.1 ^{0}c, 18.3^{0}c and 20.5 ^{0}c is belong to Aleta chuko, Dale and Loka Abaya respectively. There are 1 primary hospital and 24 health posts at Aleta chuko districts, 10 health centers and 32 health posts at Dale district and 1 primary hospital, 5 health center and 22 health posts at Loka Abaya district.

Study design

Cross-sectional study was employed that includes entomological data collection of the study area.

Sample size determination and sampling methods

For entomological survey 12 different households but similar characteristics (based on usage of malaria control intervention, wealth index constructed by principal component analysis and other factors) was selected in each survey month during the study period in each four study sites in three districts. The survey study was mainly focus on assessment of the available mosquito control practice, assessment of farmers' awareness on vector, socio-economic contribution of malaria control to the households and major constraints on vector control.

Entomological data collection

Immature stages of Anophyles mosquito was made to determine the species composition and drug susceptibility test. Samples were collected from breeding sites. Larval collection was performed from suspected breeding sites using standard dippers (350 ml) monthly during the major malaria transmission season (September- November, 2022), in dry season (January, 2023), minor transmission season (May,2023) and rainy season (July, 2023).

Each temporary and permanent breeding site such as ponds, drainage canal, rain pools, stream bed, water from broken pipes, terracing, water harvesting pools, marshes was searched for the presence of anopheline egg, larvae and pupae. If the breeding habitat contains immature stages (Particularly larvae), sampling was done by dipping method. Larval density in each breeding site was calculated as the number 3rd and 4th instars larvae collected per dip or 10 dips (per 100 dips if the density is to low). The number of dips was dependent upon the size of the habitats. The surface area of each potential mosquito breeding site was estimated in square meter (m^2). One 'sample' which is defined as 30 dips was taken for sites in the range of 5-10 m^2 and two samples was taken from sites in the range of 11-20 m^2. However, 6 dips per m^2 for small sites and a maximum of 6 samples (180 dips) for sites greater than 50m^2 was taken [21]. All immature stages (larvae and pupae) collected during data collection period was transported safely to Addis Ababa University Aklilu Lemma Institute of Patho Biology laboratory and bred in an insectary until adult stage emerges. The adults emerged was used for Species identification and Insecticide susceptibility test using standard keys. The collected specimens were carried to Aklilu Lemma Institute of Patho biology and reared to adult and identified to species level on morphological basis using Gillies and Coetzee [22, 23] for adults and larvae.

Physical characteristics of the larval habitats, including habitat type, habitat stability, water depth, turbidity, presence of floating and/or emergent vegetation and distance to the nearest homestead was recorded. Turbidity of breeding habitat was determined through visual examination of water and categorized into 4 classes namely clear, low, medium and high based on water color on a white background. The proportion of the larval habitat covered by vegetation was estimated visually. Habitat stability was expressed in terms of the length of time the habitat contained water. A habitat was considered temporary if it held water for 2 weeks or less and permanent if it held water for more than 2 weeks. Distance between the breeding habitats and sampled household was obtained by ArcGIS software after recording breeding habitats coordinates and sample households by handheld GPS. Water pH, conductivity, and temperature was measured using hand-held multi parameter device. Water harvesting pools and terracing was observed for the presence of mosquito larvae. Agricultural land covers such as presence of maize fields and other crops and nonagricultural land covers was carefully recorded around mosquito breeding habitats.

Insecticide susceptibility tests

Mosquito larvae and pupae was collected from various breeding sites of the selected localities was reared into adults. Two to three days old, non blood fed adult females morphologically identified as *An. Gambiae* s. l was exposed to discriminating dosages of 4% DDT, 0.05% deltamethrin and 0.75% permethrin using WHO papers and bioassay tubes for 1 hour. Number of mosquitoes knocked down during exposure was recorded at intervals of 5 minutes and the proportions of survivors and dead mosquitoes was recorded 24 hours post exposure. The same number of mosquitoes was exposed to insecticide free papers as controls [25].

Data Analysis

SPSS statistical for Windows (IBM Corp., Armonk, NY), version 25, was used to analyze the Habitat characterization and insecticide susceptibility test data. For every test, values were deemed significantly different if $p<0.05$.

Tables were used to describe the number of immature *Anopheles* mosquitoes sampled and the larval breeding habitats. The relationship between pH, temperature, and water depth and the density of *Anopheles* larvae was examined using correlation analysis. The number of *Anopheles* larvae (early or late) divided by the total number of dips taken from each larval habitat was used to calculate the *Anopheles* larval density. Log transformed log10 (x+1) larval density was used to increase distribution normalcy. The environmental factors linked to the presence of *Anopheles* larvae were found using multiple regression analysis. The presence or absence of algae, the permanence of the habitat (temporary or permanent), the presence or absence of surface debris, the intensity of light (sunlit or shaded), and the movement of the water (still or fowing) were the two variables that were compared between the samples using the Mann-Whitney U test. The water turbidity, water perimeter, distance to the closest house, canopy cover, emergent plant coverage, habitat type, and substrate type were all compared between samples in more than two groups using the Kruskal-Wallis H test. Larval densities from sites with various habitat characteristics were compared using these non-parametric tests. IBM

Insecticide susceptibility test

Insecticide susceptibility data were entered into Microsoft Excel spreadsheets after being recorded on the corresponding laboratory data forms in the insectary and laboratory. These steps included error checks and corrections. For all test runs with matching negative and positive controls, the WHO bioassay knockdown was recorded every 10 minutes for one hour, and the final mortality was recorded at twenty-four hours. When the negative control mortality was between 5% and 20%, experiments with negative control mortality greater than 20% were discarded, and if the control mortality was less than 5%, the formula was ignored. In these situations, Abbot's formula was used to adjust the percentage mortality. 98%–100% mortality in the sample population suggested that the tested insecticide was susceptible to the population. Less than 90% of deaths in the tested species indicated resistance, while deaths between 90 and 98% suggested potential resistance [4]. The Statistical Package for the Social Sciences (SPSS) software (Version 25) was used to perform a probit analysis on the knockdown time 50% (KDT_{50}) of all tested female mosquitoes for each insecticide.

Quality Control

Other predator insects were removed from collected water containing sample mosquito larvae, sufficient air was allowed to enter in to larva containing container during transportation to prevent death of larvae due to shortage of air and temperature & humidity was controlled at insectary room to create suitable environments for larvae taken from field.

Ethical approval

This study was conducted after reviewed and approved by Institutional Ethical Committee Review Board of College of Natural Sciences, through the Department of, Biology, Hawassa University (Ref.no. RERC/12/2023).

Results

Insecticide resistance status of *A. gambiae* in study sites

In this study, 7908 *An. Gambiae* mosquitoes were tested to ascertain their level of insecticide resistance using WHO guidelines. According to [26], mortality falling between 98 and 100% indicates susceptibility, 90 to 97% indicates potential resistance for which more research is necessary, and less than 90% indicates resistance, necessitating the management of resistance against insecticides used to control malaria vectors.

Insecticide resistance was observed in the Dale, Aleta Chuko, and Loka Abaya districts primarily due to deltamethrin and propoxur of the pyrethroid and carbamate insecticide classes, respectively (Table1). Aleta Chuko mosquitoes demonstrated pyrethroid resistance (deltamethrin, 0.05%; carbamate, propoxure, 0.1%) (89% and 18% mortality, respectively), whereas Loka Abaya mosquitoes demonstrated notable carbamate resistance (propoxure, 0.1%) (22% mortality). In addition, Dale showed resistance to carbamate (propoxur 0.1%) and pyrethroid (deltomethrin 0.05%) with mortality rates of 88% and 38%, respectively. In the Loka Abaya district, potential resistance (90–97% mortality and 91% mortality) to deltamethrin 0.05% was confirmed (Table 1). Mosquitoes from every district were found to be susceptible to pyrethroids (alpha-cypermethrin 0.45%) with a mortality rate of approximately 98% (Table 1). The alpha-cypermethrin and pyrethroid susceptibilities of Loka Abaya and Aleta Chuko mosquitoes (0.45% and 98% mortality, respectively) were similar. Mosquitoes in the Dale district were found to be susceptible to pyrethroids, with alpha-cypermethrin having a 0.45% mortality rate (Table 1)

Table 1: A summary of percentage mortality 24 h after a 1-h exposure to different classes of insecticides on field collected F1 progeny of *An. Gambiae* (n=100 per insecticide) from Loka Abaya, Aleta chuko and Dale districts, Sep,2022 to Dec,2023.

Insecticides		Districts and Resistance status		
Class	**Name**	**Loka Abaya**	**Aleta Chuko**	**Dale**
Pyrethroid	Alpha-cypermethrin (0.45%)	98(S)	98(S)	99(S)
	Deltamethrin (0.05%)	91(PR)	89(R)	88(R)
Carbamate	Propoxur (0.1%)	22(R)	18(R)	38(R)

Letters in the parentheses indicate resistance status of tested mosquitoes (S: susceptible, PR: potential resistance and R: resistant)

Knockdown Time KDT_{50}

Propoxur (0.1%) had the highest mosquito KDT_{50} (Fig. 1) among the districts sampled. Aleta Chuko recorded the highest value (56.25 minutes), while Dale recorded the lowest value (51.95 minutes). Compared to other insecticide classes, pyrethroids (deltamethrin, 0.5%; alpha-cypermethrin, 0.45%; Fig. 1) showed the lowest KDT_{50}. Deltamethrin (0.5%) had the highest values (23.57 min, 24.4 min, and 26.23 min) in Loka Abaya, Aleta Chuko, and Dale districts, whereas alpha-cypermethrin (0.45%) had the lowest values (20.82 min, 18.67 min, and 24.23 min, respectively) in Aleta Chuko.

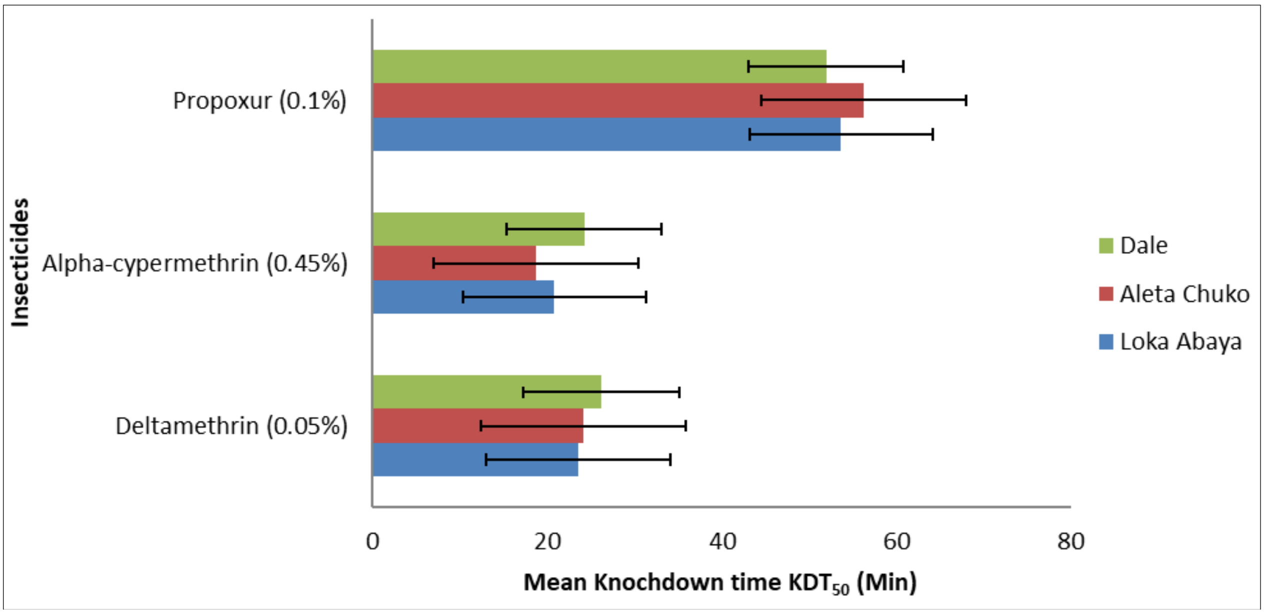

Fig 1: Mean±95% CL summary results of knockdown time (KDT_{50}) (minutes) of field collected *Anopheles gambiae* F1 progeny (n=100 per insecticide) from malaria endemic districts tested against different classes of insecticides

***Anopheles* larvae species composition**

A total of 4489 adult *Anopheles* mosquitoes from single species were identified based on their morphology. *An. Gambiae* sensu lato (s.l.) was identified from Loka Abaya, Aleta Chuko, and Dale study sites out of the entire *Anopheles* species. *Anopheles gambiae* (s.l.) comprised 100% across all study sites.

***Anopheles* larval productivity in different habitat types**

Larval sampling findings and types of productive larval habitat in the study area are presented in Table 2. In Aleta Chuko, seven different types of habitat have been identified, including ditches, swamps, burrow pits, water canals, rain pools, and road puddles. Except for the water canal, all of these types of habitats were also found in Loka Abaya and Dale. Larval habitats were ditches (Loka Abaya, n = 10, Aleta Chuko, n = 17, and Dale, n = 12), road puddles (Loka Abaya, n = 9, Aleta Chuko, n = 13, and Dale, n = 11), and rain pools (Loka Abaya, n = 23, Aleta Chuko, n = 27, and Dale, n = 23). *Anopheles* mosquitoes were used to sample a total huge populations of immature *Anopheles* mosquitoes were gathered from ditches and burrow pits at Loka Abaya. Larvae were abundant in rain pools and ditches at Aleta Chuko, with 11.5 and 10.7 larvae per dip and 0.1 and 0.2 pupae per dip. Large populations of immature *Anopheles* mosquitoes were collected from ditches and burrow pits at Dal.

Association between larval density and habitat variables

According to the multiple regression model, temperature at the time of collection (p = 0.000), water depth (p = 0.000), substrate type (0.001), canopy cover (p = 0.011), and habitat type (p = 0.041) were the best predictors of *Anopheles* larval densities (Table 3). The ANOVA table's F-ratio demonstrated a statistically significant correlation between the larval densities and the physicochemical parameters observed during the larval sampling, with F14, 58 = 46.235, $p<0.0001$, and $R^2 = 0.918$.

Table 2: Density of *Anopheles* larvae in different habitat types in Aleta Chuko, Dale and Loka Abaya districts study sites

Study site	Habitat type (n)	Total no. of dips	Total Larval count	No. of larvae/dip (mean ±se)	Total pupal count	No. of pupae/dip (mean ±se)
Aleta Chuko district	Rain pool (27)	136	1545	11.5±1.2	13	0.1±0.1
	Road puddle (13)	46	374	8.0±1.1	5	0.1±0.2
	Ditches (17)	55	582	10.7±1.3	12	0.2±0.3
	Swamp (4)	20	205	10.3±0.7	2	0.1±0.2
	Burrow pits (6)	61	321	5.3±0.4	20	0.3±0.2
	Marshes (4)	19	159	8.3±0.6	1	0.1±0.1
	Water canal (4)	28	32	1.2±0.3	0	0±0
	Total	365	3218	8.8	53	0.1
Dale district	Rain pool (23)	138	706	5.2±0.4	5	0.04±0.1
	Road puddle (11)	52	360	7.1±1.5	2	0.04±0.1
	Ditches (12)	56	505	9.1±1.1	6	0.1±0.2
	Swamp (3)	27	84	3.1±0.2	0	0±0
	Burrow pits (7)	65	590	9.2±0.8	9	0.1±0.2
	Marshes (3)	15	125	8.3±0.4	0	0±0
	Total	353	2370	6.7	22	0.06
Loka Abaya district	Rain pool (23)	133	685	5.2±0.9	5	0.1±0.2
	Road Puddle (9)	58	365	6.5±1.4	2	0.04±0.1
	Ditches (10)	59	510	8.9±1.5	6	0.1±0.2
	Swamp (3)	29	86	2.9±0.3	0	0±0
	Burrow pits (5)	47	510	11±1	9	0.2±0.2
	Marshes (4)	20	164	8.2±1.1	0	0±0
	Total	346	2320	6.7	22	0.06

Table 3: Multiple regression analysis showing the key predicting factors for *Anopheles* larvae density

Variable	B	95% C.I. for B		t	Sig
		Lower	Upper		
Temperature	0.861	0.749	0.972	15.319	0.000
PH	-2.124	-3.971	-0.277	-2.3	0.025
Turbidity	0.503	-0.239	1.244	1.356	0.180
Intensity of Light	0.76	-1.163	2.683	0.79	0.432
Canopy Cover	1.317	0.307	2.327	2.609	0.011
Surface debris	0.093	-1.381	1.568	0.127	0.9
Algae	0.708	-0.495	1.911	1.177	0.244
Emergent Plants	0.342	-0.334	1.019	1.013	0.315
Water depth	-0.033	-0.045	-0.022	-5.67	0.000
Substrate type	1.345	0.549	2.14	3.381	0.001
Water Perimeter	-0.017	-0.964	0.930	-0.036	0.972
Habitat stability	0.426	-0.637	1.489	0.802	0.426
Distance to nearest house	-0.017	-0.77	0.736	-0.044	0.965
Habitat type	-0.183	-0.358	-0.007	-2.076	0.041
Constant	-8.676	-15.735	-1.616	-2.46	0.017

CI confidence intervals, B the unstandardized coefficient value, Sig significant at $p<0.05$

Larval densities and water temperatures were positively correlated (r = 0.936, p = 0.045) (Table 4). A strong negative correlation was observed between the depth and density of *Anopheles* larvae (r = 0.485, p = 0.000). However, pH and *Anopheles* larval densities were not correlated (r = 0.116, P = 0.323).

Table 4: Correlation of some larval habitat characteristics with the average *Anopheles* larval density sampled

Physicochemical characteristics	Mean±SD	Correlation coefficient (R^2)	p-value
Water Temperature	27.096 ±2.832	0.936**	0.045
Water depth	44.27±42.08	-0.495**	0.000
Water pH	7.173±0.302	-0.116	0.323

SD standard deviation *Correlation is significant at the 0.05 level

For *An. Gambiae* there were sufficient numbers of late instar larvae available for the examination of environmental factors. *An. Gambiae* larval density did not significantly differ between permanent and temporary habitats (U=486.5, p=0.060), habitats with or without algae (U=582, p=0.854), with or without surface debris ($p>0.05$), between habitats of small and large water perimeter ($p>0.05$), between habitats closer and farther from human habitation ($p>0.05$), or between clean, moderately turbid, or turbid water ($p>0.05$), according to the results of the Mann-Whitney U tests and the Kruskal-Wallis H test (Table 5). It was, however, significantly higher in habitats with sun lit (U=136.5, p=0.024), in habitats with stone substrate (($\chi2$=19.534, df=2, p=0.000), and in habitats with no canopy cover close to the larval habitat ($\chi2$=11.216, df=2, p=0.004).

Table 5: Environmental variables and distribution of *Anopheles* larvae at Aleta Chuko district (2024)

Environmental factors	***An. Gambiae* (s. l.)**			
	Mean rank		U	P
Presence of algae				
Present	37.69		582	0.854
Absent	38.7			
Intensity of light				
Sunlit	54.44		136.5	0.024
Shaded	36.04			
Surface debris				
Present	43.03		407.5	0.279
Absent	36.53			
Habitat Stability				
Permanent	34.35		486.5	0.060
Temporary	44.13			
	An.gambiae (s.l.)			
	Mean rank	X^2	df	p
Turbidity				
High	44.46	1.795	2	0.408
Medium	38.46			
Low	34.37			
Canopy cover				
Open	33.29	11.216	2	0.004
Shrub	53.25			
Tree	55.7			
Emergent plant				
Absent	37.56	2.112	3	0.550
Grass	35.03			
Weeds	51.38			
Grass+weeds	44.5			
Substrate type				
Mud	31.6	19.534	2	0.000
Stone	60.39			
Gravel	53.7			
Water perimeter (m)				
<10 m	38.72	1.321	2	0.517
10 to 100 m	35.8			
>100 m	48.63			
Distance to nearest house (m)				
≤100 m	38.57			
101 to 200 m	37.62			
201-300 m	30.25	1.041	2	0.594

Discussion

In this study, the most commonly observed larval habitats were rain pools, ditches, and road puddles. These small water bodies hold water and can serve as larval sites only during the rainy season [27]. A similar cross-sectional study conducted in the same country indicates that rain pools, ditches, and tire tracks/road puddle were the major contributors during the long rainy season [28,29] ditches, burrow pits, and rain pools contribute a large number of *Anopheles* larvae in the study area This is in line with other studies that showed that ditches burrow pits and rain pools supported the greatest number of mosquito larvae [30,31].

In the present study, larval breeding habitats such as road puddles and swamps have lower larval densities compared to other sites. This is in contrast with other findings that show road puddles and swamps had higher larval density [29,32]. This could be due to less number of larvae in the habitat at the time of sampling which could be attributed to habitat disturbance by human and animal activity.

Temperature, pH, water depth, canopy cover, and habitat types were the most important variables for *Anopheles* larval density at the time of sampling. Densities of *An. Gambiae* were found to be significantly associated with sunlit habitats, no canopy cover, and stone substrates. This is in line with studies conducted elsewhere [33,34]. Generally, *Anopheles* larval density was not significantly associated with water pH, water temperature, water turbidity, algal content, and distance to the nearest house. The mean water temperature was 27.1 ± 2.8 which may have at a range of suitable water temperature for the *Anopheles* larvae to survive and develop into an adult as was stated by [35]. The distance to the nearest house was measured only from habitats containing *Anopheles* larvae; therefore, most habitats were close (300 m) to the nearest house, and a statistically significant association was not found between distance to the nearest house and larval density.

The results presented in the present study showed that *An. Gambiae* s.l. from all study districts had a high resistance status for Deltamethrin (0.05%) with a possible resistance rate (91%) in Loka Abaya and the least resistance rate (88%) in Dale. This resistance development for Deltamethrin (0.5%) in Loka Abaya, Aleta Chuko and Dale district as had been showed in Ethiopia [18-20] and Uganda [36]. This resistance of Anopheline mosquitoes to deltamethrin may be due to its impregnation on LLIN. This is in agreement with earlier findings [18,37]. These findings are consistent with those reported by [38] that *An. Gambiae* (*sensu stricto*) is highly resistant to deltamethrin insecticidal agents. The resistance to deltamethrin was observed in *An. Gambiae* populations in five different localities from Lagos, South-Western Nigeria [39].

A similar study done by [40], *An. Gambiae* were resistant to deltamethrin and alpha-cypermethrin insecticides in most of the sites. In contrast, other study reported that approximately 87.2% of *An. Gambiae* in Lake Tana, northwest Ethiopia, died by using deltamethrin insecticide with the lowest percentage of resistance [18]. And also similar study also suggested that no confirmed resistance was recorded when *An. Gambiae* (s.l.) populations tested for deltamethrin but suspected resistance was observed [41]. *An. Gambiae* from Loka Abaya showed possible resistance to Deltamethrin insecticide; this may be the result of climate change, particularly the effects of temperature and the previous use of pyrethroids in this community.

Anopheles gambiae s.l. with Propoxur insecticide had its highest resistance rate (38%) in Dale and its lowest resistance rate (22%) in Loka Abaya. Propoxur insecticide had the highest mortality rate (78%) in Loka Abaya and the lowest (62%) in Dale. This study showed that *Anopheles gambiae* s.l develops resistance to propoxure in all study sites. This is in line with findings in Nigeria [42] and West Africa [43]. In contrast, mosquito population susceptible to propoxur was reported from different parts of Ethiopia from 2012 to 2024 [19,20,44]. This contradictory result may be the first evidence that *An. Gambiae* has resistance to propoxur in Ethiopia. These findings are somewhat agreed with that of [39], who reported that mortality rates between 25-77% from propoxur exposure in some localities.

The lowest mortality rate of *An. Gambiae* was recorded in all three districts (Loka Abaya, Aleta Chuko, and Dale) through Alpa-cypermethrin and Deltamethrin insecticides. Alpha-cypermethrin (0.45%) treated *An. Gambiae* s.l. are susceptible (≥ 98% mortality) in all study sites. Susceptibility to alpha-cypermethrin may be attributed to the fact that alpha-cypermethrin is new for the mosquito population in the study area. In contrast, populations of *An. Gambiae* s.l. proved resistance to apha-cypermethrin at Gambela and Gondar respectively [19, 45]. Similar findings have also been reported from Côte d'Ivoire [46] that *An. Gambiae* is resistant to alpha-cypermethrin.

The existence of resistance to two classes of insecticides in populations of *An. Gambiae* in three districts of the Sidama region is worrisome and an indication of the threatened sustainability of malaria vector control programs utilizing any of these insecticides. The major challenge arising from these results is that the choice of Pyrethroid and Carbamate may not be a suitable alternative in the event of switching to another class of insecticide. According to [47]. *An. Gambiae* (in broad sense) was identified as the identification key. Further molecular investigations were not conducted. This is consistent with other studies which reported that *An. Gambiae* s. l. is the most widely distributed species in Ethiopia.

Assessment and selection of pesticides based on their time of action for vector control is an essential component that has a bearing on the management of insecticide resistance. The KDT_{50} determines the time that enables 50% of the mosquito population to be knocked down by an insecticide. As reflected by the trend in KDT_{50} in the findings, knockdown was more rapid for pyrethroids i.e. deltamethrin and alpha-cypermethrin [48,49], reported similar observations. Propoxur showed a delayed effect of knockdown to 50% of the mosquito population within 1 hour and it is less effective to kill mosquitoes in the study area. In contrast, Propoxur has a knockdown of 100% after 1 h of exposure [19], and is a candidate insecticide for vector control activities in Ethiopia.

Conclusions

Anopheles gambiae s.l is most predominating species found in malarious districts of Sidama region .It is susceptible to Alpha-cypermethrin (0.45%) insecticide and resistant to deltamethrin (0.05%) and propoxure (0.1%). Ditches, burrow pits, and rain pools were habitat types in which *Anopheles* larvae found in large number. Larvae of *Anopheles gambiae s.l* in study area occur in habitat with stone substrate, exposed to sunlight and had no canopy cover. Hence, there is a need to incorporate and use integrated vector management approaches to malaria vector control.

Ethics approval and consent to participate

Approval for this study was done by ethical institutional review board (IRB), College of Natural Sciences, through the Department of, Biology, Hawassa University (Ref.no. RERC/12/2023). The purpose, benefits, confidentiality and the voluntary nature of participation were explained and informed consent was obtained from participants.

Competing of interests

The author participated in the manuscript confirms that I have no competing interests.

Authors' contributions

MB conceived the project idea, designed the study protocol, collected the data, Both MB and MT analyzed the data, interpreted and drafted the manuscript.

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References

1. WHO. World Malaria Report; c2008. WHO Global Malaria Programme [http://www.who.int/malaria/wmr2008/].
2. Pimenta PF, Orfano AS, Bahia AC, Duarte AP, Ríos-Velásquez CM, Melo FF, *et al*. An overview of malaria transmission from the perspective of Amazon *Anopheles* vectors. Memórias do Instituto Oswaldo Cruz. 2015;110(1):23-47.
3. Saraiva RG, Kang S, Simões ML, Angleró-Rodríguez YI, Dimopoulos G. Mosquito gut antiparasitic and antiviral immunity. Developmental and Comparative Immunology. 2016;64:53-64.
4. WHO. World Malaria Report 2022. World Health Organization; c2022.
5. Marten P, Hall L. Malaria on the move: Human population movement and malaria transmission. Emerging Infectious Diseases. 2000;6:28-45.

6. De Silva PM, Marshall JM. Factors contributing to urban malaria transmission in sub-Saharan Africa: A systematic review. Journal of Tropical Medicine; c2012. https://doi.org/10.1155/2012/819563.
7. Doumbe-Belisse P, Kopya E, Ngadjeu CS, Sonhafouo-Chiana N, Talipouo A, Djamouko-Djonkam L, *et al*. Urban malaria in sub-Saharan Africa: dynamic of the vectorial system and the entomological inoculation rate. Malaria Journal. 2021;20:1-18.
8. Ashine T, Eyasu A, Asmamaw Y, Simma E, Zemene E, Epstein A, *et al*. Spatiotemporal distribution of *Anopheles stephensi* in different eco-epidemiological settings in Ethiopia; c2023. PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-3793340/v1].
9. Bødker R, Akida J, Shayo D, Kisinza W, Msangeni HA, Pedersen EM, *et al*. Relationship between altitude and intensity of malaria transmission in the Usambara Mountains, Tanzania. Journal of Medical Entomology. 2003;40(5):706-717.
10. Alemu A, Abebe G, Tsegaye W, Golassa L. Climatic variables and malaria transmission dynamics in Jimma town, South West Ethiopia. Parasites and Vectors. 2011;4:1-11.
11. PMI. President's Malaria Initiative - Ethiopia. Malaria Operation Plan Financial Year 2018. 2019.
12. Bugssa G, Tedla K. Feasibility of malaria elimination in Ethiopia. Ethiopian Journal of Health Sciences. 2020;30(4).
13. WHO. World Malaria Report. Geneva: World Health Organization. 2014.
14. Berhe B, Mardu F, Legese H, Negash H. Seasonal distribution and seven year trend of malaria in North West Tigrai: 2012–2018, Ethiopia. Tropical Diseases, Travel Medicine and Vaccines. 2019;5:1-7.
15. WHO. World Report, World Health Organization (WHO, 2020), Geneva, Switzerland, https://www.who.int/news-room/fact-sheets/detail/malaria. 2021.
16. Yared S, Gebressielasie A, Damodaran L, Bonnell V, Lopez K, Janies D, *et al*. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. Malaria Journal. 2020;19(1):1-7.
17. Kinfe E, Irish S, Hailemariam A, Wuletaw Y, Abate S, Tekie H. Susceptibility of *Anopheles gambiae* s.l. and *Anopheles funestus* s.l. to seven insecticides in southern Ethiopia. Ethiopian Journal of Public Health and Nutrition. 2021;4(2):153-159.
18. Kendie FA, Wale M, Nibret E, Ameha Z. Insecticide susceptibility status of *Anopheles gambiae* s.l. in and surrounding areas of Lake Tana, northwest Ethiopia. Tropical Medicine and Health. 2023;51(1):3.
19. Barasa S, Jejaw A, Aemero M, Woldesenbet D, Abebe W. Assessing insecticide susceptibility status of *Anopheles* mosquitoes in Gondar Zuria District, Northwest Ethiopia. 2023.
20. Woyessa D, Yewhalaw D. Bionomics, seasonal abundance and insecticide susceptibility of *Anopheles* (Diptera: Culicidae) in low and high malaria transmission settings in Ethiopia. 2024. https://doi.org/10.21203/rs.3.rs-3307406/v2.
21. Amenya DA, Naguran R, Lo TC, Ranson H, Spillings BL, Wood OR. Over expression of a cytochrome P450 (CYP6P9) in a major African malaria vector, *Anopheles funestus*, resistant to pyrethroids. Insect Molecular Biology. 2008;17(1):19-25.
22. Gilles MT, Coetzee M. A Supplement to Anophelinae of Africa South of the Sahara. South African Institute for Medical Research, Johannesburg. 1987, 55.
23. Verrone G. Outline for the determination of malaria mosquitoes in Ethiopia. Part I. Adult female *Anopheles* mosquitoes in Ethiopia. Mosquito News. 1962a;22:38-39.
24. Verrone G. Outline for determination of malaria mosquitoes in Ethiopia. Part II. Anopheline larvae. Mosquito News. 1962b;22:394-401.
25. WHO. Training Module on Malaria Control. Malaria Entomology and Vector Control. Guide for Participants. WHO, Geneva, Switzerland; c2012.
26. WHO. Larval source management: A supplementary malaria vector control measure: An operational manual; c2013.
27. Getachew D, Balkew M, Tekie H. *Anopheles* larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin, southwestern Ethiopia. Malaria Journal. 2020;19:1-13.
28. Hawaria D, Demissew A, Kibret S, Lee MC, Yewhalaw D, Yan G. Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site, Southwest Ethiopia. Infectious Diseases of Poverty. 2020;9:1-11.
29. Tsegaye A, Demissew A, Hawaria D, Abossie A, Getachew H, Habtamu K. *Anopheles* larval habitats seasonality and environmental factors affecting larval abundance and distribution in Arjo-Didessa sugar cane plantation, Ethiopia. Malaria Journal. 2023;22(1):350.
30. Nicholas K, Bernard G, Bryson N, Mukabane K, Kilongosi M, Ayuya S, *et al*. Abundance and distribution of Malaria vectors in various aquatic habitats and land use types in Kakamega County, highlands of Western Kenya. Ethiopian Journal of Health Sciences. 2021, 31(2).
31. Tarekegn M, Tekie H, Wolde-Hawariat Y, Dugassa S. Habitat characteristics and spatial distribution of *Anopheles* mosquito larvae in malaria elimination settings in Dembiya District, Northwestern Ethiopia. International Journal of Tropical Insect Science. 2022;42(4):2937-2947.
32. Babale SK, Salim H, Yakudima II, Kabir BM, Mamman R, Chiroma UM, *et al*. Seasonal assessment of pupal habitat productivity of malaria vector: *Anopheles gambiae* s.l. as influenced by physico-chemical conditions at selected breeding habitats in Niger, Nigeria. EUREKA: Life Sciences. 2023;(2):38-55.
33. Gimnig JE, Ombok M, Kamau L, Hawley WA. Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. Journal of Medical Entomology. 2001;38(2):282-288.
34. Kenea O, Balkew M, Gebre-Michael T. Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, central Ethiopia. Journal of Vector Borne Diseases. 2011;48(2):85-92.
35. Bayoh MN, Lindsay SW. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae sensu stricto* (Diptera: Culicidae). Bulletin of

Entomological Research. 2003;93(5):375-381.

36. Oruni A, Lynd A, Njoroge H, Onyige I, van't Hof AE, Matovu E, *et al*. Pyrethroid resistance and gene expression profile of a new resistant *Anopheles gambiae* colony from Uganda reveals multiple resistance mechanisms and overexpression of Glutathione-S-Transferases linked to survival of PBO-pyrethroid combination. Wellcome Open Research. 2024;9:13.
37. Dagne A, Taye B, Yeshanew S. Susceptibility status of malaria vector *Anopheles gambiae* in settlement villages of southwestern Ethiopia. Indian Journal of Entomology. 2020;82(2):209-212.
38. Chukwuekezie O, Nwosu E, Nwangwu U, Dogunro F, Onwude C, Agashi N, *et al*. Resistance status of *Anopheles gambiae* s.l. to four commonly used insecticides for malaria vector control in South-East Nigeria. Parasites and Vectors. 2020;13:1-10.
39. Oduola AO, Idowu ET, Oyebola MK, Adeogun AO, Olojede JB, Otubanjo OA *et al*. Evidence of carbamate resistance in urban populations of *Anopheles gambiae* s.s. mosquitoes resistant to DDT and deltamethrin insecticides in Lagos, South-Western Nigeria. Parasites and Vectors. 2012;5:1-8.
40. Kouassi BL, Edi C, Tia E, Konan LY, Akré MA, Koffi AA, *et al*. Susceptibility of *Anopheles gambiae* from Côte d'Ivoire to insecticides used on insecticide-treated nets: evaluating the additional entomological impact of piperonyl butoxide and chlorfenapyr. Malaria Journal. 2020;19:1-11.
41. Rakotoson JD, Fornadel CM, Belemvire A, Norris LC, George K, Caranci A, *et al*. Insecticide resistance status of three malaria vectors, *Anopheles gambiae* (s.l.), *An. funestus* and *An. mascarensis*, from the south, central and east coasts of Madagascar. Parasites & Vectors. 2017;10:1-17.
42. Shehu IK, Ahmad HB, Olayemi IK, Solomon D, Ahmad AH, Salim H. Insecticide susceptibility status in two medically important mosquito vectors, *Anopheles gambiae*, and *Culex quinquefasciatus* to three insecticides commonly used in Niger State, Nigeria. Saudi Journal of Biological Sciences. 2023;30(2):103524.
43. Minwuyelet A, Yewhalaw D, Sciarretta A, Atenafu G. Susceptibility Status of Principal Malaria Vectors to Insecticides Commonly Used for Malaria Control in Africa: Meta-analysis and Systematic Review; c2023. PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-3020410/v1].
44. Abraham M, Massebo F, Lindtjørn B. High entomological inoculation rate of malaria vectors in areas of high coverage of interventions in southwest Ethiopia: implication for residual malaria transmission. Parasite Epidemiology and Control. 2017;2(2):61-69.
45. Chanyalew T, Natea G, Amenu D, Yewhalaw D, Simma EA. Composition of mosquito fauna and insecticide resistance status of *Anopheles gambiae* sensu lato in Itang special district, Gambella, Southwestern Ethiopia. Malaria Journal. 2022;21(1):125.
46. Ekra AK, Edi CA, Gbalegba GCN, Zahouli JZ, Danho M, Koudou BG. Can neonicotinoid and pyrrole insecticides manage malaria vector resistance in high pyrethroid resistance areas in Côte d'Ivoire?; c2023.
47. Coetzee M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malaria Journal. 2020;19(1):1-20.
48. Okafor MA, Ekpo ND, Opara KN, Udoidung NI, Ataya FS, Yaro CA, *et al*. Pyrethroid insecticides susceptibility profiles and evaluation of L1014F kdr mutant alleles in *Culex quinquefasciatus* from lymphatic filariasis endemic communities. Scientific Reports. 2023;13(1):18716.
49. Muhammet M, Elcin E. Evaluation of insecticide resistance and biochemical mechanisms of *Culex pipens* L. in four localities of east and middle Mediterranean basin in Turkey. International Journal of Mosquito Research. 2015;2(3):39-44.