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RESEARCH

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Composition, habitat preference and seasonal variation of malaria vector larval and pupa stage in Akure North Local Government Area of Ondo State, Nigeria

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Abstract

Background: The study of habitat preference and identification of malaria vector is one of the important steps in malaria control. Knowledge of local *Anopheles* mosquitoes capable of transmitting malaria parasites has contributed largely to the reduction in the menace caused by malaria infection. This present study examined the habitat nature and identified the species of *Anopheles* mosquitoes involved in malaria parasites transmission in the study area. Monthly collection and identification of the fourth larval stage was carried out from October 2018 to September 2019.

Result: The prevalence of *An. gambiae* complex and *An. funestus* larvae was 95.86 and 4.15%, respectively. The highest (14.17%) and the least (4.25%) number of larvae were collected in the month of November and May, respectively. Out of the total number of *Anopheles* mosquito larvae collected during the wet season, 69.77% of the larvae was collected from the clean habitat, while 30.23% was collected from the dirty habitats. During the dry season, the larvae dwell more in dirty aquatic habitat, with 64.74% of the larvae collected from the dirty habitats, while 35.27% was recorded from clean habitats. Statistically, there was no significant difference in the electrical conductivity when comparing both seasons ($P=0.19$; $X^2=53.14$). The average recorded electrical conductivity in dry and wet seasons were 350.76 µS/cm and 178.91 µS/cm, respectively. The pH recorded in dry and wet seasons were 6.78 and 7.04, respectively. There was no significant difference in the pH when both seasons were compared ($P=0.13$; $X^2=54.89$). The total dissolve solid where not significant different ($P=0.58$; $X^2=13.35$) when both seasons were compared. The temperature ($P=0.04$; $X^2=43.54$) and dissolve oxygen ($P=0.00$; $X^2=30.09$) were significantly different comparing dry and wet seasons in all the habitats where the immature stages of *Anopheles* mosquitoes were collected.

Conclusion: The study revealed major vector of malaria parasite in the study location, also the pattern of their breeding during dry and rainy season which is influenced by some selected ecological factors.

Keywords: *Anopheles gambiae*, *Anopheles funestus*, Habitat, Dry and rainy seasons

Background

Anopheles mosquito is the most studied and well-known genus of mosquito, largely because of their great impact on human health. As vector of etiologic agent of malaria and filariasis, *Anopheles* mosquitoes have affected the lives of more humans than any other insect. Human malaria is caused by either one or more of the protozoan parasites: *Plasmodium falciparum*, *P. malariae*, *P. vivax* and *P. ovale* (Antinori et al. 2012). Among these, *P. falciparum* causes the deadliest form of human malaria and the most prevalent in tropical parts of the world. The genus *Anopheles* contain over four hundred recognized species of *Anopheles* and about 50 members of *Anopheles* species involved in transmitting human malaria

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parasites (Tabue et al. 2017). The most important species of malaria vector in Africa, where incident of malaria disease is highest, are *Anopheles gambiae*, *An. funestus*, *An. arabiensis* and *An. colluzzi* (Wiebe et al. 2017).

Proper understanding of malaria vector oviposition behavior and habitat characterization study will help in combating and reducing the burden of malarial disease (Muema et al. 2017). The habitats of *Anopheles* are specifically different compared to other genus of mosquitoes. The immature stages of *Anopheles* mosquito occur in many different types of large and more or less permanent habitats, ranging from freshwater, mangrove swamps, rice fields, edges of streams, drainage pits, rivers and ponds (Walshe et al. 2017). They are also found in small and often temporary breeding places such as puddles, hoof prints, well, discarded tins and sometimes water-storage pots. Each species has different biological behavior, which made them distinct from each other (Nandi et al. 2000). The larvae of *Anopheles* mosquitoes are more abundant in temporary habitat characterized with direct sunlit and low emergent vegetation (Getachew et al. 2020). These habitats may have negative influence in supporting the life of other organisms that may serve as predators or compete with the immature stages of the mosquito (Roux and Robert 2019). The depth knowledge of oviposition sites and distribution pattern of *Anopheles* larvae and pupae will help in managing the vector. Control of the larvae and pupae of malaria vectors can be advantageous because the immature stages are relatively immobile and occupy a specific habitat, compared to the adults that are active and readily mobile. The information on larval habitats, species composition and distribution is among the easiest way in strategic planning of malaria control (Tabbabi et al. 2015). Thus, the implementation of control plan against immature stages (larvae and pupae) of the malaria vector will reduce adult emergence and indirectly suppress the transmission of malaria parasites. Therefore, this study evaluated the composition, habitat preference and seasonal variation in physiochemical factors of *Anopheles* mosquito larvae habitat in Akure North Local Government of Ondo State, Nigeria.

Methods

Study area

Akure North Local Government has an area of 660 km^2 and a population of 131,587 (National Population Commission 2009). The five locations where *Anopheles* mosquito samples were collected are Oba-Ile, Igoba, Isinigbo, Ita-Ogbolu and Iju (Fig. 1). These locations were selected for the study due to the increasing in the population and expansion of the area, which as result involved numerous activities which support the breeding of mosquitoes. The breeding sites were identified by random sampling of stagnant water in drainages, pond, river edges and open soil surfaces. The geographical positioning coordinate of the collection sites was taken using geographical positioning system mobile device.

Collection of *anopheles* mosquito larvae and pupae

Larvae and Pupae were randomly collected between 8 and 11am in the morning from different breeding sites identified in the study area during dry season (October 2018 to February 2019) and wet season (March 2019 to September 2019). The breeding sites were characterized according to their nature (dirty and clean). *Anopheles* larvae were identified by their characteristic horizontal positioning on the surface of the water. The larvae and pupae were carefully collected into plastic containers of 750 ml from each of the identified breeding habitats by scooping gently to avoid injury; this was carried out thrice a month. The same process of collection was considered for both dry and wet seasons to avoid bias. Each sample was labeled indicating date, site and locality of collection. The containers were loosely capped to avoid suffocation and immediately transported to Biology laboratory at Federal University of Technology Akure.

Morphological identification of mosquitoes

The larvae were identified under dissecting microscope using standard morphological characters keys supplied by Das et al. (1990) and Nagpal and Sharma (1995). Species identification was done based on taxonomic keys published by Gillies and Coetzee (1987).

Determination of physicochemical parameters of *Anopheles* mosquitoes immature stage habitats

Physicochemical parameters such as pH, temperature, electrical conductivity, dissolve oxygen, temperature and total dissolve solid were determined *in situ* during sampling three times per month for a period of twelve months. Electrical conductivity (range 0–9999μS/cm) and temperature (range 0–80 ℃) were determined using Aqua-pro water tester (model-AP2). The pH was determined using Aquarium pool IA digital meter (model-PH 009; range 0.0–14.0pH). Amtast dissolved oxygen meter (model-AMT08; range 0.0–19.9 mg/L) was used to determine dissolve oxygen. The total dissolved solids of the habitat were determined using Hanna instrument total dissolved meter (model-DIST 1; range 10–1990 ppm).

Data analysis

The variation in seasonal and habitat nature was statistically analyzed using statistical package for social science version 22 software. Chi-square test was use to determined difference between season variation that exist between the physicochemical parameter both in dry and

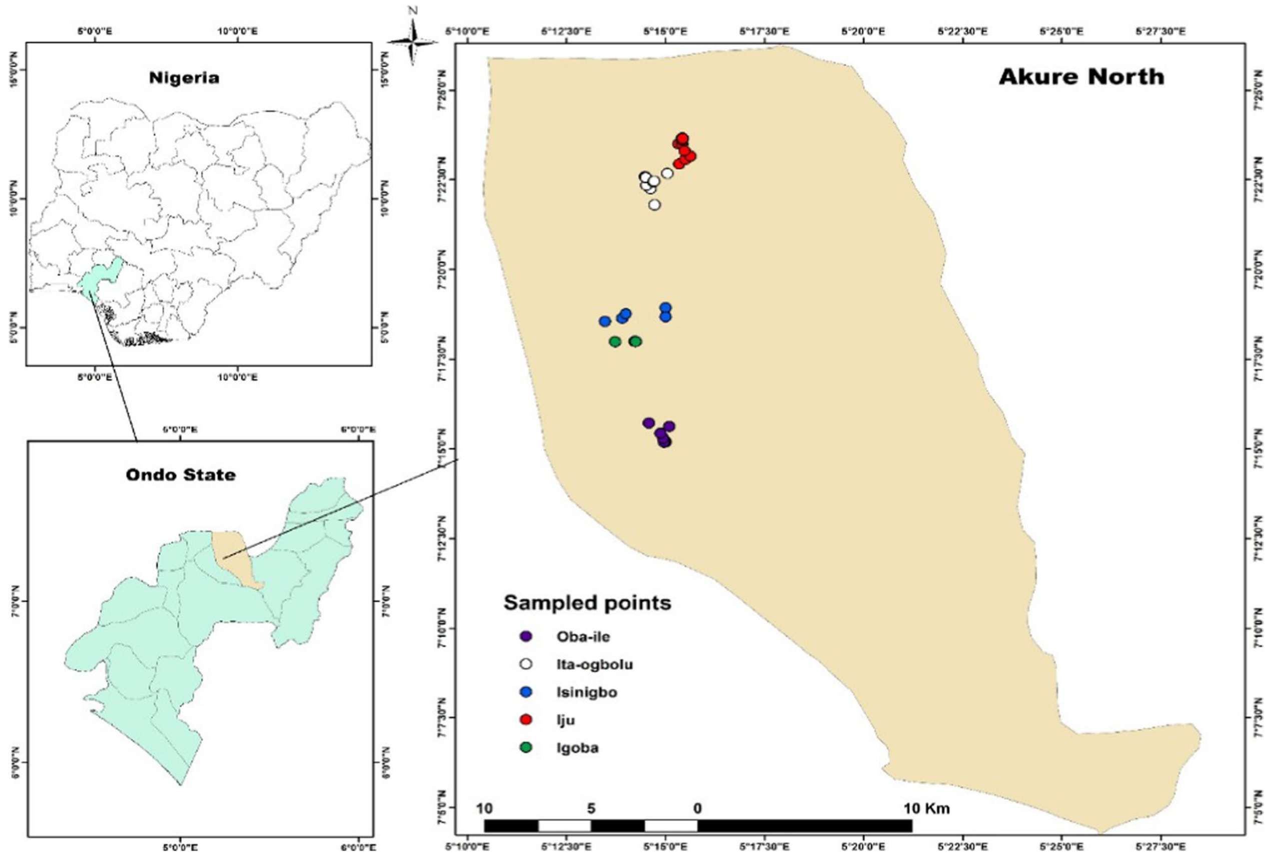

Fig. 1 Map of Akure North Local Government Area showing the sample collection points

wet season. Frequency distribution graph was use to compare the abundant of the vectors in both seasons and nature of the habitats.

Results

Monthly collection of *Anopheles* mosquito larvae in the study areas

The total monthly collection of *Anopheles* mosquito larvae is shown in Table 1. The highest percentage of *Anopheles* larvae was collected in the month of November (14.17%) followed by 13.71% in December, while the least was recorded in the month of May (4.25%). The larvae and pupae of *An. gambiae* complex constituted 95.86% and 95.99% of all the collected samples, while 4.15% and 4.00% were recorded for *An. funestus.* Figure 2 shows the distribution of malaria vector larvae and the nature of the breeding sites. The percentage of *Anopheles* larvae collected from dirty habitats were high in month of November (68.51%) during the dry season. The habitat shifted during the raining season, while the highest percentage of *Anopheles* mosquito larvae was collected from clean habitant in the month of May (87.69%).

The overall seasonal distribution and composition of *Anopheles* larvae are presented in Fig. 3. During the dry season, *Anopheles* mosquito larvae have more preference for dirty habitats than clean habitats. A higher percentage of 64.74% of *Anopheles* mosquito larvae were collected from dirty habitat, while 35.27% were recorded from clean habitat. In rainy season, a higher percentage of *Anopheles* mosquito larvae occupy clean habitat compared to dirty habitat with 69.77% and 30.23% larvae recorded for clean and dry habitats, respectively. Table 2 showed the habitats characterized in the study area. Pond bed, River bed and Rivers edges are potential breeding sites for *Anopheles* mosquito larvae and pupae during dry season while pot-hole, Tyre racks, abandon containers and Rain pool accommodate immature stages of *Anopheles* mosquitoes during the rainy season.

Table 3 shows that the Electrical conductivity of the habitats is higher during the dry season (350.76 ± 60.07) than the rainy season (178.91 ± 48.65) with no significant difference between the two seasons ($P = 0.19$). The pH of the habitats was neutral during the rainy season (7.04 ± 0.33), while that of the dry season was slightly acidic (6.78 ± 0.20). There was a slight change

Table 1 Monthly collection of *Anopheles* mosquito larvae and pupae from habitats in the Study Areas (750 ml of water per collection)

Months	*An. gambiae* complex			*An. funestus* group			Total		
	Larvae (%)	Pupa (%)	Total (%)	Larvae (%)	Pupae (%)	Total (%)	Larvae (%)	Pupa (%)	Total (%)
October	742 (12.19)	81 (6.49)	823 (11.23)	34 (12.93)	12 (23.08)	46 (14.60)	776 (12.23)	93 (7.16)	869 (11.13)
November	858 (14.12)	96 (7.69)	954 (13.02)	41 (15.59)	18 (34.62)	59 (18.73)	899 (14.17)	114 (8.78)	1013(13.25)
December	824 (13.55)	112 (8.98)	936 (12.77)	46 (17.49)	–	46 (14.60)	870 (13.71)	112 (8.62)	982 (12.85)
January	591 (9.72)	124 (9.94)	715 (9.76)	68 (25.89)	05 (9.62)	73 (23.18)	659 (10.39)	129 (9.93)	788 (10.31)
February	433 (7.12)	162 (12.99)	595 (8.12)	54 (20.52)	12 (23.08)	66 (20.95)	487 (7.68)	174 (13.39)	661 (8.65)
March	482 (7.93)	154 (12.35)	636 (8.68)	10 (3.81)	05 (9.62)	15 (4.76)	492 (7.75)	159 (12.24)	651 (8.52)
April	578 (9.50)	116 (9.30)	694 (9.47)	–	–	–	578 (9.11)	116 (8.93)	694 (9.08)
May	249 (4.09)	76 (6.09)	325 (4.43)	–	–	–	249 (3.92)	76 (5.85)	325 (4.25)
June	344 (5.66)	101 (8.09)	445 (6.07)	–	–	–	344 (5.42)	101 (7.78)	445 (5.82)
July	321 (5.28)	28 (2.25)	349 (4.76)	–	–	–	321 (5.06)	28 (2.16)	349 (4.57)
August	297 (4.88)	108 (8.66)	405 (5.53)	–	–	–	297 (4.68)	108 (8.31)	405 (5.29)
September	363 (5.97)	89 (7.14)	452 (6.17)	10 (3.81)	–	10 (3.18)	373 (5.88)	89 (6.85)	462 (6.04)
Total	6082 (95.86)	1247 (95.99)	7329 (95.88)	263 (4.15)	52 (4.00)	315 (4.12)	6345	1299	7644

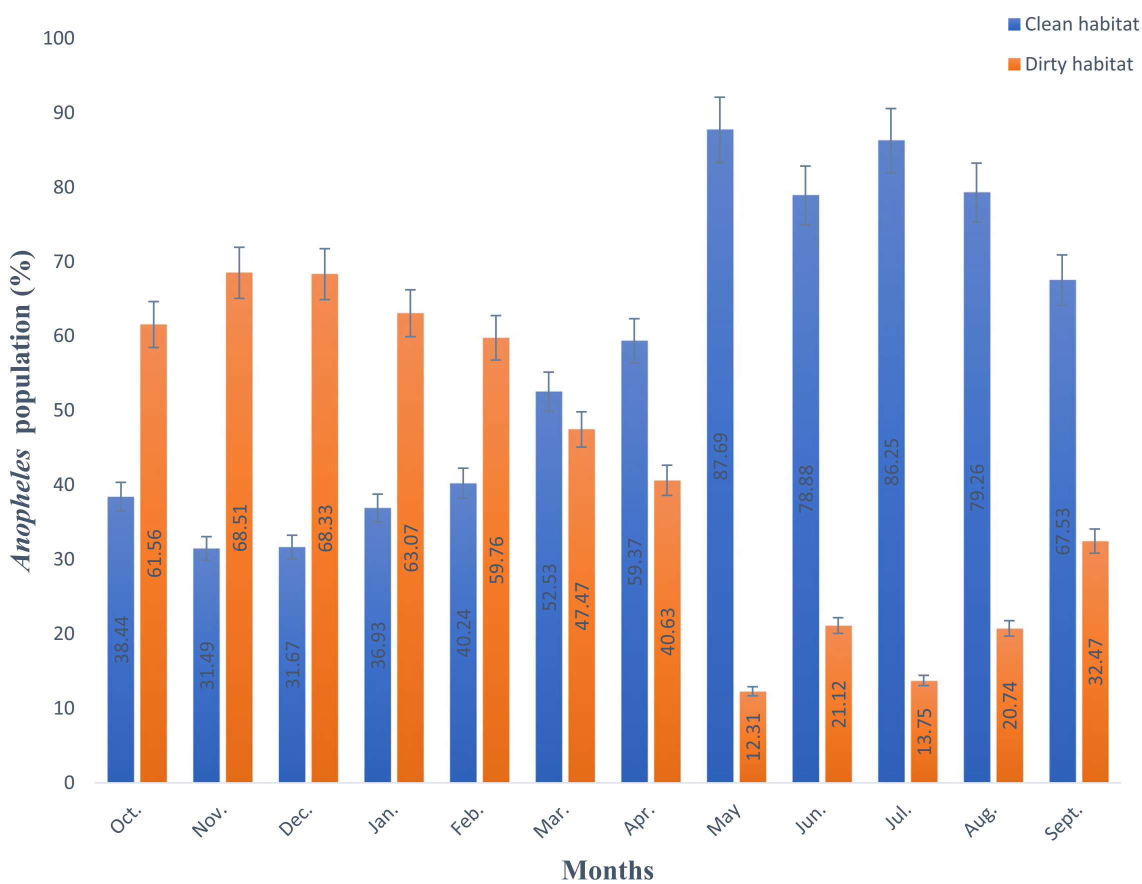

Fig. 2 Monthly distribution of *Anopheles* larvae and pupae in relation to habitat nature preference

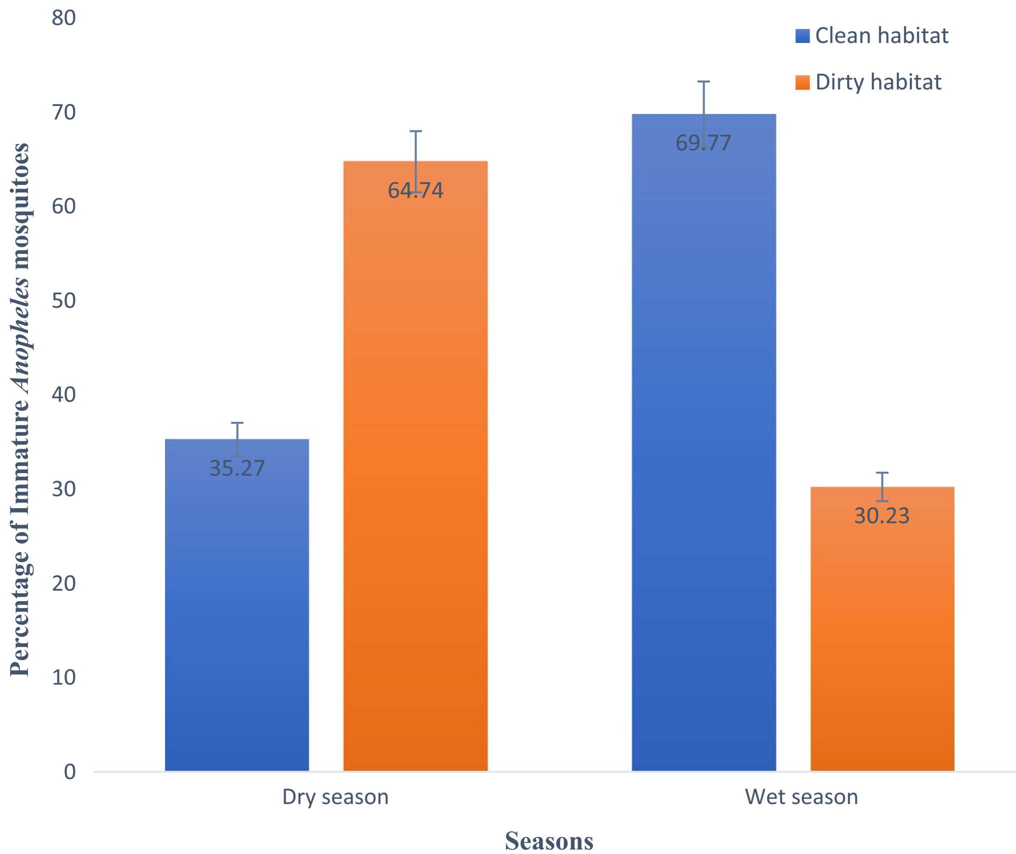

Fig. 3 Seasonal distribution and composition of immature *Anopheles* mosquitoes in the study areas

Table 2 Habitat preference and seasonal variations of *Anopheles* mosquitoes larvae and pupae in the study area (750 ml of water per collection)

Habitat	*Anopheles gambiae* complex		*Anopheles funestus* group	
	Dry	Wet	Dry	Wet
Pond bed	++	−	+	−
River bed	++	−	+	−
Spoor	++	++	+	+
Concrete gutter	+	++	−	−
River edges	++	−	+	+
Canal	++	+	+	−
Abandon tyre	−	+	−	−
Pot – hole	−	++	−	−
Gutter	+	++	+	−
Tyre tracks	−	+	−	−
Abandon containers	−	+	−	−
Rain pool	−	++	−	−

Plus sign (+): present, minus sign (−): absent, double plus sign: multiples of hundreds, single plus sign: multiple of tens

in temperature and dissolved oxygen in the mean value recorded from *Anopheles* mosquito larvae and pupae habitat in both seasons. The temperature recorded for dry and rainy seasons was 27.13℃ and 25.25℃, respectively. There was significant different in temperature comparing the seasons ($P = 0.04$). The dissolved oxygen from both seasons was significantly different ($P = 0.00$).

Discussion

The immature stages of *An. gambiae* complex and *An. funestus* group encountered in this study showed high preference for clean habitats during the rainy season and dirty habitats during the dry season. There was a shift in habitat preference during the dry season where most of the *Anopheles* larvae collected were found in dirty or muddy habitats, the highest number of *Anopheles* immature stages were collected in month of November in dry season. This may be due to disappearance of suitable habitats for oviposition during this period. Charlwood et al. (2000) and Tantely et al. (2016) reported that *Anopheles* mosquito larvae breed in different habitat during the dry season compare to the rainy season, most especially

Table 3 Seasonal variation of the physicochemical parameters (mean ± S.D) of *Anopheles* larvae habitats

Parameter	Season		Total mean ± S.D	X^2	P-value
	Dry	Wet			
Electrical conductivity	350.76 ± 60.07	178.91 ± 48.65	250.52 ± 104.19	53.14	0.19
pH	6.78 ± 0.20	7.04 ± 0.33	6.93 ± 0.30	54.89	0.13
Total dissolved solid	17.48 ± 4.23	15.97 ± 4.05	16.60 ± 4.01	13.25	0.58
Temperature	27.13 ± 0.77	25.25 ± 0.81	26.03 ± 1.22	43.54	0.04
Dissolved oxygen	7.91 ± 0.11	8.21 ± 0.12	8.09 ± 0.19	30.09	0.00

vector of malaria parasites (*An. gambiae* s. s., *An. arabiensis* and *An. funestus*) in Afro-tropical region. Mattah et al. (2017) compared the seasonal variation of the distribution of *Anopheles* mosquito larvae; it was reported that rainy season has a significant high proportion of the *Anopheles* mosquito immature stages compared to dry season. The present study showed that the immature stages of *Anopheles* mosquitoes increased during dry season, though there was no significantly different when comparing the proportion of the immature stages of *Anopheles* mosquitoes in both seasons. Minakawa et al. (2001) and Magombedze et al. (2018) reported that *An. gambiae* Giles and *An. arabiensis* survive in large number during the dry season in a way that remain unknown.

The appearance of *An. funestus* during dry season shows that the vector contributed to the malaria infection rate during this season. The appearance could be as a result of reduction in potential breeding sites for oviposition since most of the habitats are dried up. Charlwood et al. (2000) and Getachew et al. (2020) suggested that since the habitat suitable for *Anopheles* species breeding dried up during the dry season, the malaria vector could migrate several kilometers in searching for suitable breeding sites, it was also suggested that the vector that transmits malaria during this season is immigrants from several kilometers away during this period. The outcome of this study was supported the report of Ondiba et al. (2019) where it was reported that the suitable period for most mosquito species to thrived was during the dry season, though it was reported that there was no significant different in survival rate of mosquito larvae between the two seasons (rainy and dry season). The result of this research also supported the findings of Zogo et al. (2019), who reported that the percentage of *Anopheles* mosquito larvae was higher during the dry season compare wet season. However, it is contrast to the report of Mattah et al. (2017), who reported high proportion of Anopheline mosquito larvae during the raining season compared to the dry season.

The positive habitats for *Anopheles* mosquitoes in the study area include Pond bed, River bed, Animal spoors, Concrete gutter, River edges, Canal, abandon tires, Pot-holes, Gutter, Tyre tracks, abandon containers and Rain pool, and this was correlated with what was reported by Elmalih et al. (2018) in Sudan. Other mosquitoes larval and organisms found in the habitat are *Aedes* larval, *Culex* larval, *Culiseta* larval, Tadpoles and Dragon fly larval, while some habitats were characterized with vegetation. Among the habitats, pond bed, river bed, spoors/foot tracks, gutter and canal are positive for *Anopheles* mosquitoes during dry season and all these habitats are within human dwelling, Dida et al. (2018) and Getachew et al. (2020) reported some of these habitats for *Anopheles* mosquitoes larvae. During rainy season, rain pool, abandon containers, spoors, gutter and abandon tyres are home of the immature stages of this vector in the study area.

The electrical conductivity of the *Anopheles* immature stages habitat is high in dry season than in rainy season. Ajani et al. (2018) reported that water bodies containing the immature stages of *Anopheles* mosquitoes are higher in electrical conductivity during dry season than in rainy season. *Anopheles* mosquitoes larval has been reported to thrived more in habitat with high electrical conductivity (Othaman et al. 2020). The total dissolved solid recorded from the habitats in dry season was a little bit high more than what was recorded in rainy season. The total dissolved solid recorded in this study was extremely low compared to what was reported from other findings (Emidi et al. 2017). In rainy season, the pH of the habitats was a bit higher than neutral compare to the pH in dry season which is slightly lower and below neutral. The pH recorded in all the habitats where immature *Anopheles* mosquitoes were collected are in conformity with the report of Tirado et al. (2017). There was a significant different in temperature of the *Anopheles* mosquito (larvae and pupae) habitats in both seasons, the temperature of the habitat is slightly higher in dry season than in rainy season. The temperature recorded in both seasons supported the development of immature stages of *Anopheles* mosquitoes, and this was in agreement with the report of Christiansen-Jucht et al. (2014) and Ajani et al. (2018).

Conclusion

This study has exposed the breeding pattern of malaria parasites vectors in the study location. All *Anopheles* mosquitoes identified in the study area are epidemiologically important for malaria transmission. The immature stages of *Anopheles* mosquito mostly prefer clean aquatic environment during the raining seasons, though, immature stages have high population during the dry season and positively associated with dirty habitats.

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Authors' contributions
OTA, SIA and AAV contributed to the research design and involved in field and laboratory work. AAV carried out statistical analysis and interpret the result of the study. AAV write the first draft of the manuscript. OTA and SIA review the manuscript. All author read and approved the final manuscript.

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Available of data and materials
All analyzed data involved in this study are included in this manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing for interests
The authors declare that they have no competing interests.

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