FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 2, April, 2024, pp 331 - 337
DOI: https://doi.org/10.33003/fjs-2024-0802-2255

LARVAL ABUNDANCE, IDENTIFICATION AND PHYSICOCHEMICAL PROPERTIES OF THREE BREEDING HABITATS OF *Aedes aegypti* IN KANO METROPOLIS, KANO STATE

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ABSTRACT

Aedes aegypti is the primary vector for some arboviral diseases such as Yellow fever, dengue, zika and chikungunya. This specie of invasive mosquito has gained attention globally because of their fast-spreading trend and successful adaptation. This study was designed to determine the larval abundance and physic-chemical parameters of *Aedes aegypti* from three breeding sites within Kano metropolis. Determination of larval abundance was performed using ovitraps according to the protocol described by Wan-Norafikah *et al.*, 2012. Twenty ovitraps were placed BUK old site, BUK New site and SOT from April – September, 2023. Physico-chemical parameters such as pH, Temperature, Electrical conductivity, Dissolved Oxygen, Total Dissolved Solids, Turbidity and Total Suspended Solids were measured for all breeding habitats. A total of 2959 *Aedes aegypti* larvae were collected in the study areas within Kano metropolis which comprised of BUK old site 31.80%, BUK New Site 32.75% and School of Technology (Kano Poly) 35.45%. The study revealed that temperature ranges from 29.98ºC to 31.05ºC, pH range of 6.37 – 6.74, TDS level of 109.80 – 305.09 mg/L and Electrical Conductivity ranges from 270 – 390 µS/cm. It can be concluded from this study that *Aedes aegypti* are abundant in the study area and also physic-chemical parameters of the breeding habitats showed significant variations among the three breeding habitats. This data may help in management of *Aedes aegypti* especially in eliminating stagnant water which serves as the breeding habitat.

Keywords: Aedes, Physicochemical, Temperature, Vector

INTRODUCTION

Mosquitoes are tiny arthropods that are known to be vectors of many diseases like malaria, yellow fever, dengue fever and filaria (Wilkerson *et al.*, 2021). Mosquito vectors transmit more than 17% of mosquito-borne diseases globally and kill more than 700,000 people annually (WHO, 2020).

Aedes aegypti is the primary vector for some arboviral diseases such as Yellow fever, dengue, zika and chikungunya. This specie of invasive mosquito have gained attention globally because of their fast-spreading trend and successful adaptation from feeding on animals in the natural forest ecosystems to preferentially feeding on humans in anthropogenic modified habitats (Akhir *et al.*, 2022). This urban mosquito transmits various types of arboviral diseases, impacting over 120 countries, including many low-income and middle-income nations. These diseases have a devastating impact on human health and economic development (Wilder-Smith *et al.*, 2020). Aedes species transmit various clinically important mosquito-borne flaviviruses that are significant human pathogens. These include Dengue virus (DENV), Japanese encephalitis virus (JEV), West Nile virus (WNV), yellow fever virus (YFV), and Zika virus (ZIKV). Among these, dengue has been ranked as the most prominent mosquito-borne viral disease globally by the World Health Organization (WHO, 2020).

In Nigeria, six viruses that cause haemorrhagic fever have been isolated. These include the dengue virus, yellow fever virus, and Rift valley fever virus. In the 1980s, Adekolu-John and Fagbami discovered the presence of the dengue virus in the Kainji Lake area of Nigeria (Adekolu and Fagbami, 1983). Since then, many researchers have confirmed the existence of the dengue virus in different regions of the country, including northern Nigeria. Additionally, in the Guinea Savannah region of Nigeria, dengue virus antibodies (anti-DENV IgG) have been found with a prevalence rate of 20.5%. Similarly, in Kano, located in the north-western part of Nigeria, a high prevalence of dengue IgM has been observed in patients with febrile fever (Muhammad and Sulaiman, 2022). In November, 2023, the Nigeria Center for Disease Control and Prevention (NCDC) reported 71 suspected cases, 13 confirmed cases and zero deaths in Sokoto state. The virus has been reported in three (3) local government areas (LGAs): Sokoto South (60 cases), Wamako (3 cases) and Dange Shuni (1 case) (NCDC, 2023).

The global prevalence of yellow fever virus is estimated to be around 200,000 cases per year, resulting in approximately 30,000 deaths of which 90% of these cases occur in Africa. For more than six decades, Nigeria has been experiencing outbreaks of Yellow Fever, particularly in the southern region where the initial cases were identified. Recently, these outbreaks have been observed in over half of the local government areas across the entire nation, with infections in the states of Bauchi, Benue, Delta, Ebonyi, and Enugu. In the first eight months of 2021 alone, Nigeria recorded a staggering total of 1,312 confirmed cases of Yellow Fever Virus (WHO, 2020).

A crucial control strategy to combat major mosquito-borne diseases involves specifically targeting mosquito vectors in order to effectively disrupt disease transmission. (Asma'u *et al.*, 2021). Indiscriminate and prolonged use of insecticides in agricultural practices alters the natural environment of mosquitoes, leading to the development of resistance (Alhassan *et al.*, 2015). Understanding the ecology and spatial distribution of mosquito larvae is crucial for ensuring the effectiveness of vector control measures (Mereta *et al.*, 2013). Breeding habitats can be classified as either artificial or natural, and further categorized based on their stability as temporary, permanent, or semi-permanent in nature (Adel *et al.*, 2024). Places with stagnant water are ideal for mosquito reproduction and the growth of their larvae. Additionally, breeding sites typically consist of various types of vegetation, whether natural or cultivated, dense or sparse, and submerged

or emerged. Research has shown that mosquitoes tend to prefer habitats with shorter vegetation rather than those with taller vegetation (Sowilem *et al.*, 2017).

The physicochemical parameters greatly affect the species, density, and development of mosquito larvae, and these parameters can vary across breeding sites. These parameters include temperature, pH, dissolved organic and inorganic materials, salinity, turbidity, and electrical conductivity (Gopalakrishnan *et al.*, 2013). Changes in these parameters of the breeding sites can have both positive and negative effects on the vectors. For instance, when the temperature drops below 14°C or rises above 30°C, the larval growth rate in most vector species decreases. Additionally, mosquito larvae are typically found in environments with pH levels ranging from 3.3 to 10.5 (Liu *et al.*, 2021). Monitoring and knowledge of environmental factors like physicochemical parameters are important in tackling insecticide resistance challenges (Ononamadu *et al.*, 2020). Understanding the environmental characteristics of mosquito breeding sites as well as their prevalence has been demonstrated to be a successful strategy in reducing mosquito-borne diseases. Hence this study was therefore designed to determine the larval abundance and physic-chemical parameters of *Aedes aegypti* breeding sites in Kano metropolis.

MATERIALS AND METHODS

Study Area

The study was conducted in three breeding habitats within Kano metropolis. These include Bayero University Kano old site (11º58'46"N, 8º28'42"E), Bayero University, Kano New Site (11º58'15"N, 8º26'5"E), and School of Technology Kano State Polytechnic (11º59'29" N, 8º32'26"E) (Figure 1).

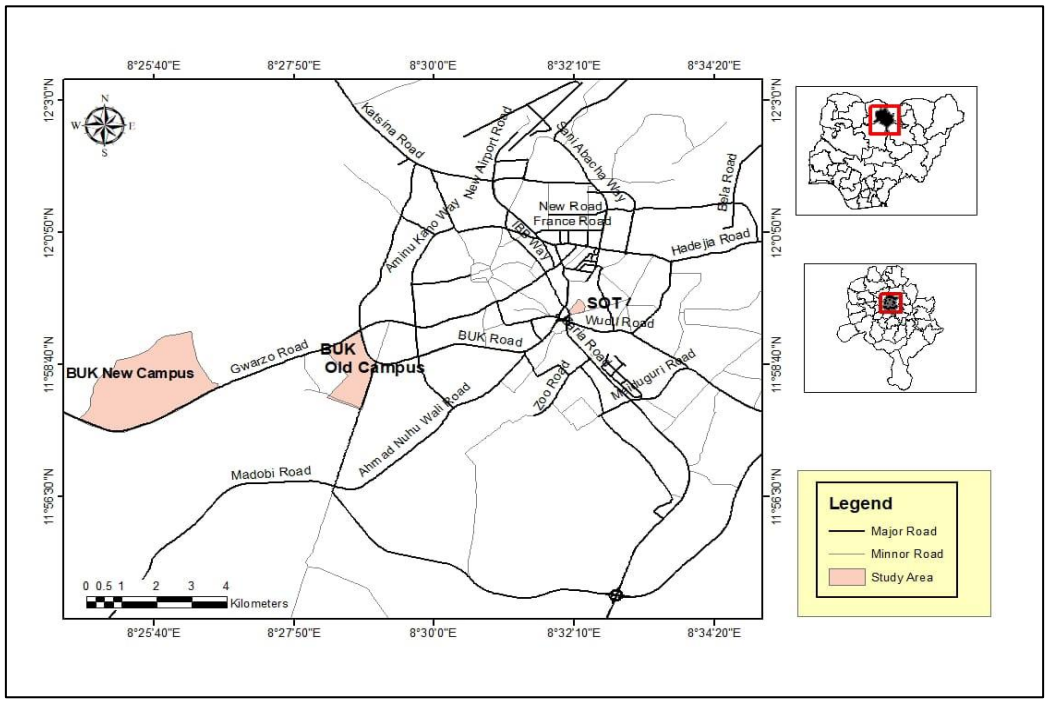

Figure 1: Map of Kano Metropolis showing study areas

Determination of *Aedes aegypti* Larval Abundance

The distribution of Aedes mosquito larvae were performed using ovitraps according to the protocol described by Wan-Norafikah *et al.*, 2012. Twenty ovitraps which were obtained from Vector Control Unit (VCRU), Universiti Sains Malaysia were placed in each collection site. Each ovitrap was filled with water in each collection site which was either partially or totally shaded to avoid from direct sunlight and heavy rain that may cause water spillage. The ovitrap surveillance was conducted weekly from April – September, 2023.

The larvae and eggs collected were transported to the insectary at the Department of Biochemistry, Bayero University, Kano and maintained on chinchilla pellets, supplemented with brewer's yeast tablet. All larvae were identified as Aedes using the present or absent of siphon and counted fourth instar larvae using a microscope. The larval numbers were recorded individually for each collection site. The larvae were fed with grounded biscuits and yeast in mosquito plastic cage where they were reared to adults for morphological and molecular identification. The adult emergence numbers were recorded individually for each collection site.

Identification of Mosquitoes to Species Level

Morphological Identification

A total of 210 (50 from BUK old site, 72 from BUK New Site and 88 from SOT) adults *Aedes aegypti* were identified using the morphological and pictorial keys (Rueda, 2004). They were identified using a 7X–45X, Trinocular XTL Stereo Zoom microscope.

Molecular Identification

Genomic DNA Extraction

DNA was extricated according to the protocol described by Livak, 1984. Livak grind buffer was first prepared using 1.6 ml 5mM NaCl, 5.48g sucrose, 1.57 g Tris, 10.16 ml 0.5M EDTA, 2.5 ml 20% SDS and sterile water to make the final volume to 100 ml. The solution was then sterilized, filtered and stored at -20ºC until used.

Genomic DNA was extracted from 30 mosquitoes (10 mosquitoes from each location). The livak grind buffer was first pre heated in a water bath at 65ºC until clear. A single adult mosquito was put in a 1.5ml effendorf tube and homogenized in 100 ul of pre-heated livak grind buffer with plastic pestle. The samples were incubated for 30 minutes at 65ºC and microfuge briefly to collect condensation. Then 14

ul of 8M potassium acetate was added into each tube and mixed before incubation on ice for 30 minutes. The samples were then centrifuge at 13,000 rpm at 4ºC for 20 minutes. The supernatant was transferred to a new effendorf tube and 200 ul of 100% ethanol was added. The samples were centrifuged again at 13,000 rpm for 15 minutes at 4ºC. The supernatant was then discarded and the DNA pellet was rinsed with 100 ul of 70% ethanol. The pellet was left on the bench to dry overnight and then re-suspended with 30 ul of nuclease free water and incubated at 65ºC for 10 minutes. DNA concentration was measured using Nanodrop and stored at –20ºC until use.

Molecular Identification

Molecular identification of *Aedes aegypti* to species level was done using polymerase chain reaction based on the method described by Beebe *et al.,* (2007). The primers used are: forward primer ITS1A, 5-CCTTTGTACACACCGCCCGTCG, and reverse primer ITS1B, 5- ATGTGTCCT GCAGTTCACA. The final 12.5 ul of PCR mixture consist of 2 ul of genomic DNA, 6.25 master mix, 1 ul of each forward and reverse primer and 2.25 ul of nuclease free water. The PCR cycling conditions were 95ºC 5 minutes and 35 cycles of 94ºC for 30 seconds, 51ºC for 40 seconds and 72ºC for 45 seconds, followed by the final extension step of 72ºC for 10 minutes. PCR products were size separated on a 1.5% agarose gel to confirm product size. Restriction analysis was carried out in a 0.5 ml microfuge tube containing 6.25 ul of PCR product and 0.5 ul of RsaI buffer. The mixture was incubated at 37ºC for 2 h and then size separated on a 1.5% agarose gel stained with ethidium bromide at 100 V for 55 min. The gel was visualized on gel imaging system.

Determination of Physico -chemical Parameters

Water samples from each collection site were collected and analyzed using water testing kit (model no. ISO9001, 1575-02-1504) for the following physicochemical parameters: pH, Temperature, Electrical conductivity, Dissolved oxygen, Total Dissolved Solid, Turbidity and Total Suspended Solid.

Data Analysis

Data analysis was done using R Software version 4.3.1. Physico-chemical parameters were analyzed using one way ANOVA. Larval abundance was calculated as percentage using this formula as described by Olajide et al., 2019.

$$\text{Larval abundance} = \frac{\text{Number of larvae per site}}{\text{Total number of larvae collected}} \text{ x } 100$$

RESULTS AND DISCUSSION

Larval Abundance

A total of 2965 *Aedes aegypti* larvae were collected in the study areas within Kano State which comprised of BUK old site 31.80%, BUK New Site 32.75% and School of Technology (Kano Poly) 35.45% .

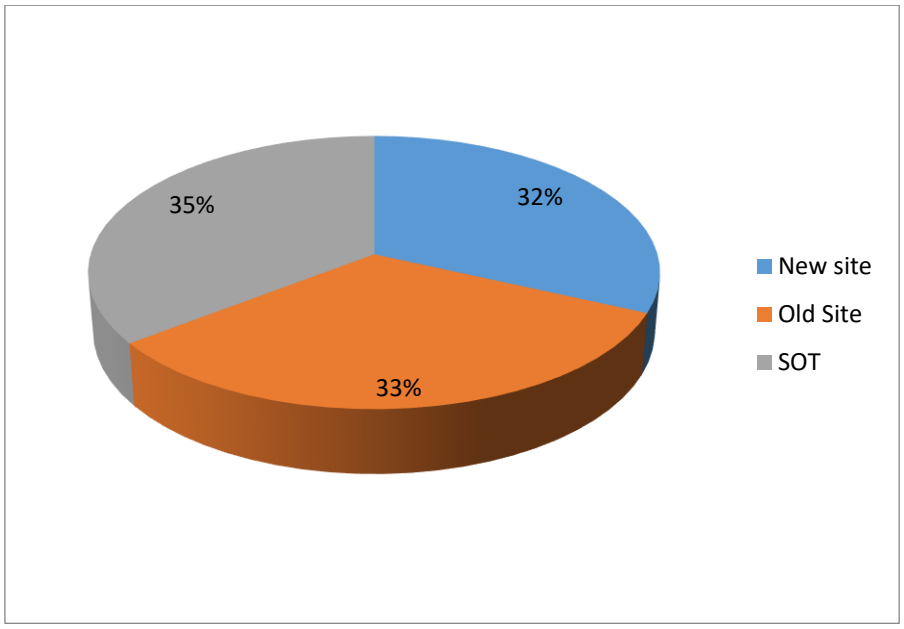

Figure 2: *Aedes aegypti* larvae Collected from Kano state

Table 1 presents the monthly distribution of *Aedes aegypti* larvae collected in Kano State for the months of July, August, and September. In July, the collected data indicates that there were 1144 Aedes mosquito larvae, the number of mosquito larvae decrease to 570 in August. In September, the data indicates an increase in the number of mosquito larvae compared to August. There were 1251 Aedes mosquito larvae. There were total of 2965 *Aedes aegypti* larvae collected across the three location in Kano State (Table 1).

Table 1: Monthly Distribution of Mosquito larvae collected in Kano State

Month	Aedes
April	0
May	0
June	0
July	1144
August	570
September	1251
Total	**2965**

Morphological identification of *Aedes aegypti*

Morphological identification confirmed that all collected Aedes mosquitoes belonged to the Aegypti species. The head region displays white flat-scale patches on the clypeus and vertex, with only a few erect forked scales present on the occiput. The proboscis was entirely dark without any white band. The legs had a white stripe on the femoral knee-spot and the front part of the mid-femur, as well as on the thorax. The scutum was black with two white stripes running along the center.

Molecular identification of *Aedes aegypti*

After Polymerase Chain reaction for specifies identification, gel electrophoresis was carried out to view the results. A band of 750 bp PCR product was observed on 1.5% agarsoe gel confirming that these mosquitoes belong to genus Aedes (Fig. 2). The digestion of the PCR product using RsaI determined the species of the sample. The gel images obtained after incubation with the RsaI enzyme show clear bands indicating the correct base pair sizes for *Aedes aegypti* (bands at 120, 180 and 400 bp) (Figure 3).

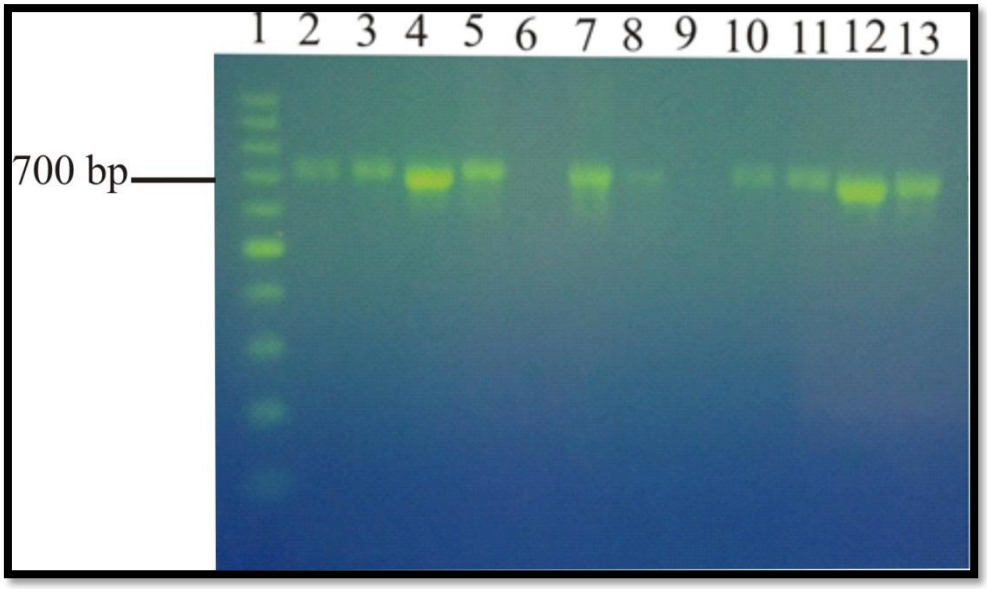

Figure 2: Gel electrophoresis picture showing bands observed after conducting PCR using internal prescribed spacer region 1 primer (ITS1) (Step one of a two-step PCR protocol) for species identification.

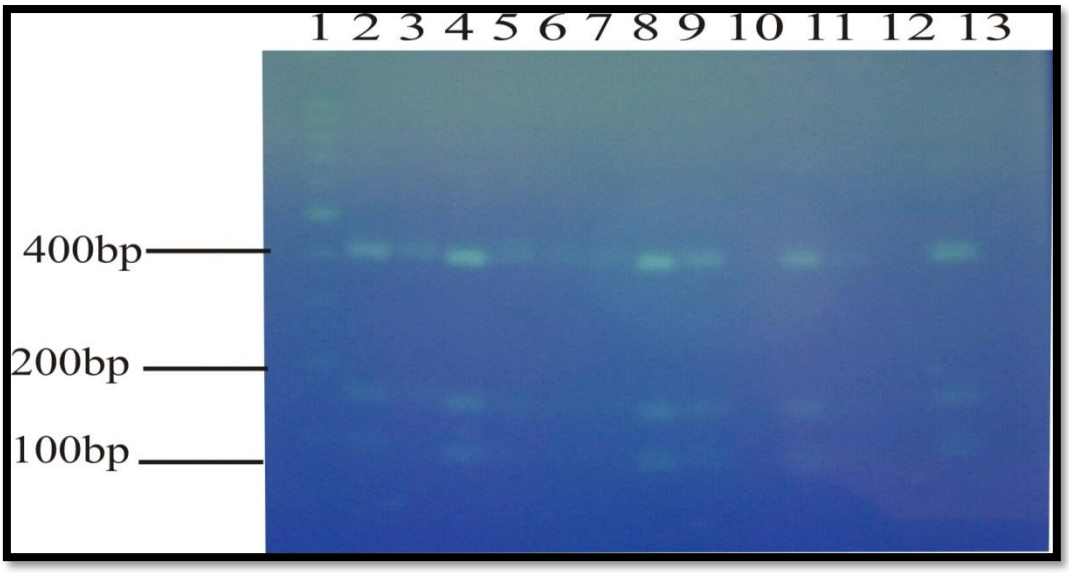

Figure 3: Gel electrophoresis picture showing the bands after digestion the PCR product using RsaI enzyme. (Step two of a two-step PCR protocol) for species identification.

Physico-chemical Parameters

The result for the physicochemical parameters of *Aedes aegypti* breeding sites from Kano State is presented in Table 2. For pH water sample from SOT (6.41 ± 0.39) has the highest pH level while water sample from BUK old (6.74 ± 0.4) site has the lowest pH level (Table 3). For Temperature, water sample from BUK New Site has the highest temperature (33.18 ± 1.64) and water sample from SOT recorded the lowest temperature (29.98 ± 1.58). For Electrical Conductivity and Dissolved Oxygen, water samples from BUK Old Site showed the highest levels than the water water samples with 390.0 ± 0.0 and 109.08 ± 11.0 respectively (Table 2). For Turbidity and Total, water samples from BUK New Site showed higher values than BUK Old site and SOT.

Table 2: Physicochemical Parameters of *Aedes aegypti* Breeding Sites from Kano State

Parameter	BUK old site	BUK New Site	SOT
pH	6.74 ± 0.4^{a}	6.37 ± 0.4^{a}	6.41 ± 0.39^{a}
Temperature (°C)	31.05 ± 1.65^{a}	33.18 ± 1.64^{b}	29.98 ± 1.58^{a}
EC (µS/cm)	390.0 ± 0.04^{a}	270.0 ± 0.05^{a}	320.0 ± 0.04^{b}
DO (mg/L)	5.63 ± 0.63^{a}	4.65 ± 0.61^{b}	4.96 ± 0.63^{b}
TDS (mg/L)	109.08 ± 11.0^{a}	179.98 ± 11.27^{a}	305.09 ± 10.92^{b}
Turbidity (NTU)	77.54 ± 11.98^{a}	226.16 ± 11.99^{b}	97.56 ± 11.86^{a}
TSS (mg/L)	301.68 ± 11.77^{a}	337.2 ± 12.14^{b}	351.54 ± 11.94^{b}

Values are expressed as Mean ± SEM of Three Replicates. Values with the same Alphabet within the same row are not significantly different. BUK = Bayero University Kano, SOT = School of Technology (Kano Poly)

Discussions

This study was designed to determine the larval abundance, identification and physico-chemical parameters of *Aedes aegypti* breeding habitats in Kano metropolis, Kano State Nigeria. Morphological and molecular identification of the *Aedes* mosquitoes in this study confirms the presence of *Aedes aegypti*. This study confirms that ovitraps are suitable for the oviposition preferences of female *Aedes aegypti* mosquitoes, as they tend to lay their eggs in black containers like cans and discarded tires. This is in agreement with the findings of Rydzanic *et al.,* (2003). This study reveals that larval abundance was found during rainy season (July – September) and no any larvae was found during the months of April – May (Table 1). This sheds light on the absence of *Aedes aegypti* during dry seasons which could be because there was no enough water to favor unlimited suitable habitats that leads to massive *Aedes aegypti* production, abundance and distribution. This is in conformity with the results of many reports which indicated that *Aedes aegypti* population density is strongly influenced by rainfall and temperature (Lourenço-de-Oliveira *et al.,* 2004; Brown *et al.,* 2014).

Table 1 showed the monthly larval abundance of *Aedes aegypti* in the study area. The month of September recorded the highest larval abundance followed by July. However, the lowest larval abundance was recorded during the month of August. This could be attributed to the increase in the frequencies of rain falls in August that might facilitate the washing away of the developing larvae from the ovitraps thereby leading to the decline in the population of mosquitoes. This is in agreement with the report of Barde *et al.,* (2019).

The physicochemical properties showed significant variation amongst the three breeding sites of *Aedes aegypti* (Table 2). The results indicated variation in temperature from 29.98ºC to 31.05ºC, and there were no much variation in pH values ranging from 6.37 – 6.74. This is In agreement with other studies which report that a wide range of pH levels from 6.5 to 8.0 is suitable for *Aedes* mosquito survival and breeding (Waewwab *et al.,* 2019). According to Umar and Donpedro (2008), the pH of the breeding habitats of mosquitoes interferes with osmoregulation and oxygen transport mechanisms. An increased in alkalinity, salinity and pH may lead to quicker mosquito growth larvae up to 9.0 pH. After pH reaches the substantial alkalinity range, pH inhibits the further

growth of *Aedes* larvae at the field level. So, at the household level, basic actions like common salt may be used to reduce *Aedes* breeding (Rasika *et al.*, 2022).

Total dissolved solids (TDS) is a measure of the combined dissolved content of all inorganic and organic substances present in water - molecular, ionized, or micro-granular suspended form. Natural sources, wastewater, municipal and agriculture run-off and industrial effluents are all sources of TDS in bodies of water (Bernard and Ayeni, 2012). Overgaard *et al.* (2017) reports that immature *Aedes aegypti* infestation was positively associated with total dissolved solids but negatively associated with dissolved oxygen. This is in conformity with the current research as can be seen in Table 2. The highest TDS level was observed in SOT and the same site has the highest percentage of *Aedes aegypti* larvae (Figure 1).

CONCLUSION

The findings from this study revealed the presence of *Aedes aegypti* in the studied area. It can also be concluded that physicochemical parameters of Aedes mosquito breeding habitats showed significant variations among the three breeding sites. Controlling Aedes mosquitoes in artificial breeding habitats such as cans and discarded tyres are critical for controlling diseases associated with *Aedes aegypti*. This study highlighted the importance of vector control interventions especially by eliminating stagnant water which serves as *Ae. Aegypti* breeding sites.

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