Biological and Environmental Sciences Journal for the Tropics
Volume 21, Number 2, August 2024. Pp 130 - 138
https://dx.doi.org/10.4314/bestj.v21i2.14

Research article

Effect of Salinity on the Development and Emergence *of Anopheles* Larvae

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Submission: 18/03/2024
Accepted: 09/08/2024

Abstract

Mosquitoes are insects in the order Diptera with the two main subfamilies *Anophelinae* and *Culicinae*. Mosquitoes exploit all kinds of lentic habitats for breeding: natural breeding habitats (e.g. ponds, swamps, streams etc.) or artificial breeding habitats (e.g. ditches, pits, construction sites, gutters, tyre tracks, cans etc). Prevailing physicochemical variables in these breeding habitats are essential factors for the survival, development and adult emergence of mosquitoes. This study aimed to investigate the impact of salinity on the emergence and development of *Anopheles* larvae. *Anopheles* larvae were collected at Lamingo, in Jos-North Local Government Area (LGA) and physicochemical parameters of the habitats were measured to establish a range for salinity treatments. Larvae were exposed to four salt concentrations (0.125g/ml, 0.25g/ml, 0.375g/ml and 0.5g/ml) to assess the survival, mortality, development of the larvae and emergence of the adult mosquitoes in every of the treatment solutions. A generalized linear mixed model (GLMM) with poison link distribution was used to test the impact of pH, temperature and salinity on the survival, development and emergence of adult mosquitoes. There was no significant influence of salinity on the survival of *Anopheles* larvae ($p>0.05$). However, as salinity increases, survival of *Anopheles* larvae decreases. However, there was a positive significant influence ($p<0.05$) of salinity on the mortality of *Anopheles* larvae, showing an increased mortality with increasing salinity levels. Furthermore, it was observed at a salinity concentration of 0.375g/ml, that the rate of larval development and adult emergence was significantly high ($p<0.05$) compared to the other concentrations. The results of this study suggest further research in this area to harness the possibility of using salinity levels as a mosquito control tool.

Keywords: *Anopheles* larvae, Salinity, Mortality, Survival, Development, Emergence

Introduction

Globally, malaria incidence continues to claim millions of human lives aside from its attendant cost on human efforts and productivity (World Health Organization, 2023). Nigeria accounts for the highest percentage of the global malaria burden compared to any other country, with 27% of the global estimated malaria cases and 31% of the estimated deaths due to malaria (WHO, 2023). The disease is spread by female *Anopheles* mosquitoes which have a cosmopolitan distribution and breed in freshwater habitats. These species have been reported in various microhabitats and the concern to invade different human communities in Nigeria (Goselle et al, 2017 & Lapang *et al,* 2019). However, the dominant malaria vector species *Anopheles gambiae* consists of several sibling species that can breed in diverse habitats with varying salinity concentrations (Gillies &De Meillon, 1968; Coluzzi & Sabatini, 1969 & Mouchet et al., 2004). Salinity tolerance refers to the physiological and molecular mechanism by which an organism can balance off the ionic components of salinity pressure (Munns & Tester, 2008). Mosquitoes genetically have a level of saltwater tolerance which has epidemiological and ecological outcomes and plays a vital role in establishing the use of habitat as well as ecological distribution; hence contribute to mosquitoes' spread of disease (White et al., 2013). Mosquito larvae can be found in numerous types of water bodies, such as brackish, saline and fresh waters (Clark et al., 2004) and classified by their tolerance to salt concentrations as < 0.5 parts per thousand [ppt], 0.5–30 ppt, and > 30 ppt and respectively (Beadle, 1939).

Mosquito's survival to adulthood has apparent fitness implications, the influence of environmental factors on the more subtle component of growth as well as the development of a mosquito are also essential in figuring out the fitness of the mosquitoes (Clark & Renold, 2004). The larvae of mosquitoes are faced with a great number of threats, including predation and desiccation of breeding habitats (Obi et al, 2019). Nonetheless, they are usually known to rapidly complete their life cycle. Rapid development accords multivoltine insects, like mosquitoes, to complete more generations during the breeding season and gain more explosive growth in population during suitable seasons (Clark et al., 2004). Studies on the breeding potentials of mosquitoes in salty water have been conducted (Garrett & Bradley, 1984; Blanco-Sierra et al., 2024). These studies revealed that species such as *Ae. albopictus*, traditionally thought to be limited to freshwater environments, are capable of thriving in brackish waters, contrary to previous assumptions. Notably, this adaptation occurs even in the absence of specialized features typically associated with euryhaline species, which are known for their ability to tolerate a wide range of salinity levels. These adaptations potentially empower such species to pioneer new breeding grounds and expand their reach into previously unfavourable territories, facilitating the colonization of new environments. In the genus *Anopheles*, species such as *Anopheles stephensi* are known to have the capacity to breed in brackish water (Jude et al., 2022), while others such as *An. gambiae* (*s.s), An. arabiensis*, *An. Fontenillei* and *An. coluzzii*, are obligate freshwater species.

As mosquito vectors continue to expand their reach into new habitats, it is essential to investigate the effect of salinity on the emergence and development of *Anopheles* larvae. By doing so, we can identify key factors that either promote or limit population growth, and develop targeted strategies to manipulate these factors, ultimately leveraging habitat-based mosquito control methods to reduce the burden of mosquito-borne diseases.

MATERIAL AND METHODS

Study Design

A laboratory-based study was conducted. Larvae of Mosquito were collected from various mosquito breeding habitats which include ditches, pools, and rice fields in Lamingo, Jos-North Local Government Area of Plateau State, Nigeria having an elevation of 1187.06m with geographical coordinates of latitude 09.88334ºN and longitude 008.92294ºE. The site is large enough to provide the required sample size for the study owing to the different breeding habitats of mosquitoes encountered. The physicochemical parameters (pH, salinity and temperature) of the bodies of water were taken using a digital multi-parameter (PCS Testr 35, Eutech/Oakton Instrument, USA) tester. These physicochemical parameters have been reported to have significant influence on the occurrence and larval abundance among mosquito species (Emidi *et al*., 2017).

Mosquito Larval Collection

Water bodies three (3) each of ponds, ditches, pools, and rice fields were randomly selected and surveyed and subsequently sampled twice in July and August, 2022. The third instar (L3) immature mosquito larvae were collected daily from the numerous breeding sites until the required number of larvae was gained by standard dipping technique. The collection tool (dipper) was gently lowered at an angle of 45º (degrees) to avoid disturbance of the water bodies. However, this resulted in the larvae flowing into the dipper and to avoid water spillage the dipper was subsequently raised slowly and mosquito larvae were assessed per dip and transferred to a small plastic bucket (Williams & Pinto, 2012; Lapang *et al*., 2019). Afterwards, the pH, temperature of the water and Salinity were established at the sites of the larval collection with the help of a Multimeter and coordinates of the site of the collection were also taken. Larvae collected were identified as anopheline then conveyed immediately to the disease surveillance laboratory, University of Jos for further studies.

Experimental Media

A range finding test (RFT) was conducted where five (5) definitive concentrations of 0.125g, 0.25g, 0.375g, and 0.5g were obtained according to the method described by Organization for Economic Cooperation and Development, (OECD, 1992).These were weighed using a digital weighing balance, and dissolved separately in 250 ml of distilled water resulting in solutions with respective concentrations equivalent to 0.5 g/L, 1.0 g/L, 1.5 g/L and 2.0 g/L respectively. These solutions were kept in a 280 ml plastic container. Using a glass measuring cylinder 50ml of each of the preparation was drawn and placed into a 250 ml plastic container with each treatment having four replicates and a control for each treatment containing 50ml of distilled water only. These preparations were left for a few minutes before introducing the mosquito larvae into them.

A total of 10 *Anopheles* third instar (L3) larvae were placed in each of the plastic containers containing salt solutions (treatments) and control treatments, larvae were exposed to the media for 6 days to observe for mortality and developmental changes. Larvae were fed using yeast solution while solute concentration was monitored and recorded daily as described by WHO, (2005).

Statistical Analysis

The response variable survival, development and emergence were all subjected to the test of normality using histogram and Shapiro-Wilks test using R statistical software version 3.6.3. Assumptions of normal distribution even after logarithm transformations were not met. Thus, non-parametric tests were conducted. Also, generalized linear mixed models with Poisson link distribution were used to test the impact of salinity, pH and temperature on the survival, emergence and development of *Anopheles* larvae using the function glmer () from the "lme4" package in R. The variable days were set as a random factor to control for temporal auto-correlation. A chi-square test was conducted to test the differences in the mortality of *Anopheles* larvae across the various concentrations. Also, a generalized linear mixed model was used to investigate the influence of salinity on the mortality of *Anopheles* larvae where P values were seen as significant at $p<0.05$.

RESULTS

Effect of salinity on survival of Anopheles larvae

Figure 1 shows the influence of salinity on the survival of *Anopheles* larvae. Salinity had a negative influence on the survival of *Anopheles* larvae such that for every 1 unit increase in the concentration of salinity there was a corresponding decrease in survival by 0.05 ± 0.04 (Figure 1). However, the effect observed was not significant ($z = -1.48$, $p = 0.14$).

Effect of salinity on mortality rate of Anopheles larvae

Although generalized linear mixed model analysis showed that salinity did not have any significant influence on larval survival ($z = 0.08$; $p = 0.94$), it however had a positive effect on the mortality of *Anopheles* larvae such that for every 1 unit increase in salinity, mortality increased by 0.004 ± 0.05 (Figure 2). but, when mortality was compared across different concentrations, there was no significant effect observed ($\chi^2_{(20)} = 14.11$; $p = 0.83$). (Figure 3).

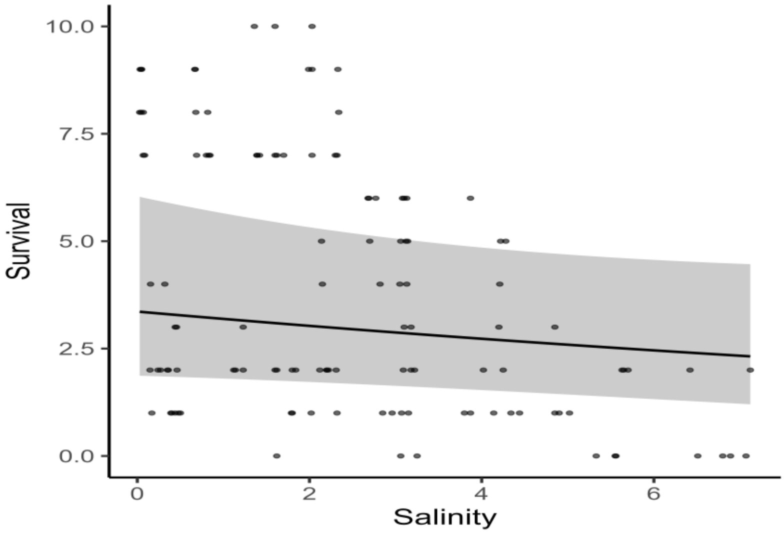

Figure. 1: Effect of salinity on the survival of Anopheles larvae

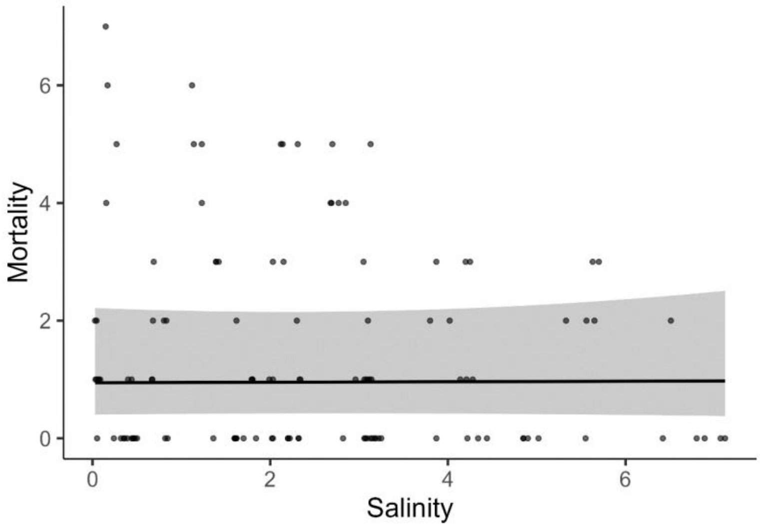

Figure 2: Effect of salinity on mortality rate of *Anopheles* larvae

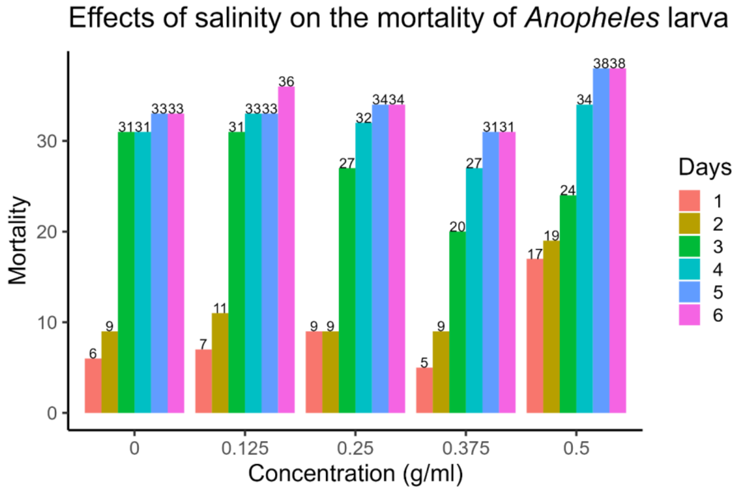

Figure 3: Effect of Salinity on Mortality Rate of *Anopheles* Larvae under Different Salinity Concentrations

Effect of physicochemical parameters on the development of Anopheles larvae

The physicochemical parameters (pH, salinity and temperature) of experimental waters under laboratory conditions were also evaluated to assess their correlation with the development of *Anopheles* larvae. However, pH did not significantly change thereby maintaining the same range as recorded from the natural breeding site. As expected, there was an increase in salinity from 0.569ppt – 7.12ppt. The result showed that salinity significantly influenced the development ($z = -2.27$; $p = 0.02$) of *Anopheles* larvae negatively. Such that for every 1 unit increase in salinity, development decreased by 0.27 ± 0.12 (Figure 4). Also, temperature had a significant influence on the development ($z = -2.45$; $p < 0.01$) of *Anopheles* larvae such that for every unit increase in temperature, development decreased by 0.53 ± 0.22. (Figure 5).

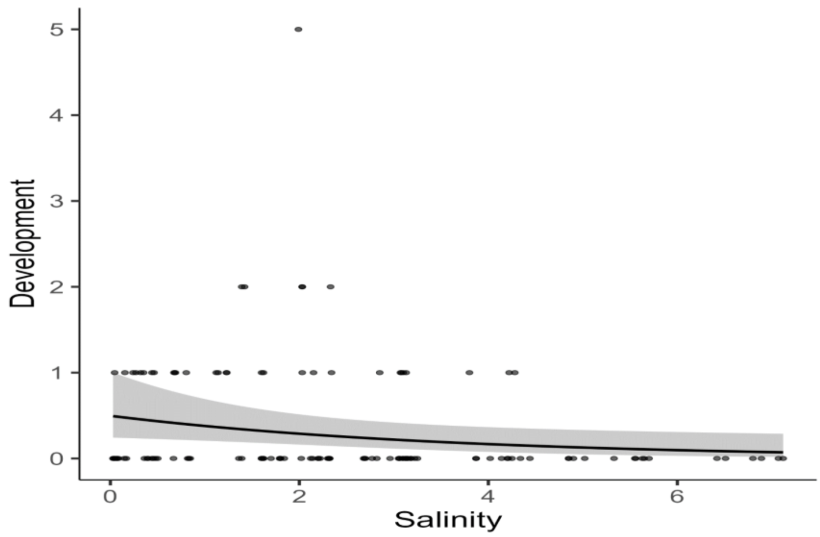

Figure 4: Effect of Salinity (ppt) on the Development of *Anopheles* Larvae under Laboratory Conditions

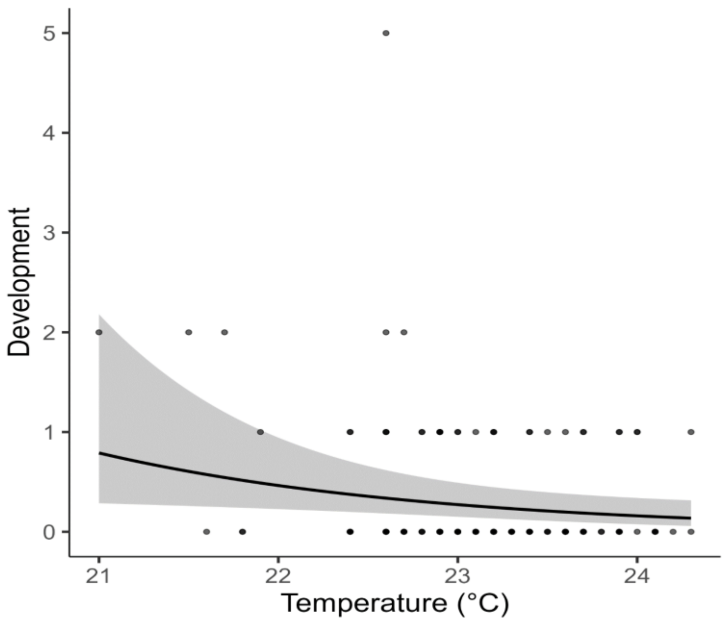

Figure 5: Effect of temperature (°C) on the development of *Anopheles* larvae under laboratory conditions

Effect of salinity on the development and emergence of Anopheles larvae

The impact of salinity on the development and emergence of *Anopheles* larvae was presented in a histogram (Figures 6 &7). The result showed that at 0.375g/ml (1.99 - 4.02ppt) there was a high rate of development recorded, signifying a favourable salinity concentration for development. Also, there was a lower rate of development at 0.5g/ml, which is indicative of an unfavourable condition for *Anopheles* larvae development (Figure 6).

The result for emergence is also similar to that of development which revealed that at 0.375g/ml, there was a high record of emergence of *Anopheles* larvae which seems to prove a more favourable condition for emergence of *Anopheles* larvae. However, there was also a lower record of emergence at a salinity concentration of 0.5g/ml which seems to be an unfavourable condition for both development and emergence. (Figure 7).

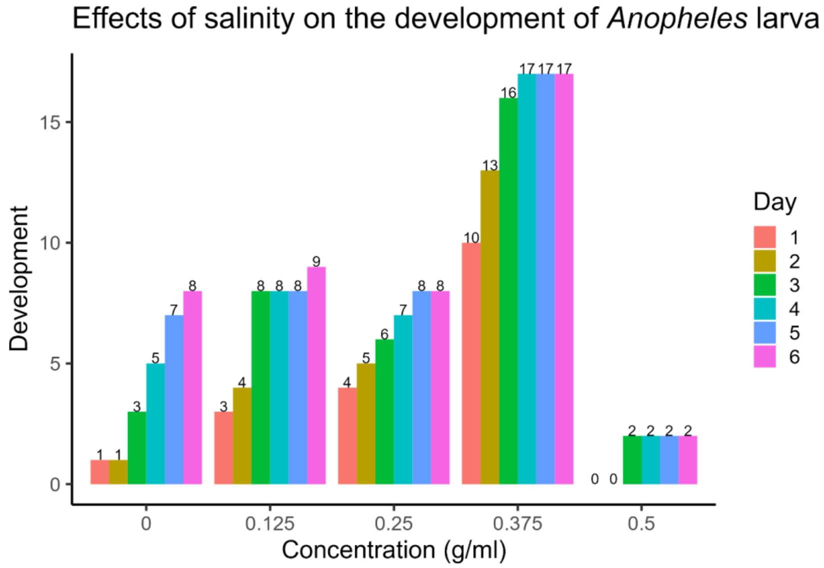

Figure 6: Histogram Representation showing the Effect of Salinity at Different Concentration Levels on Development of *Anopheles* Larvae

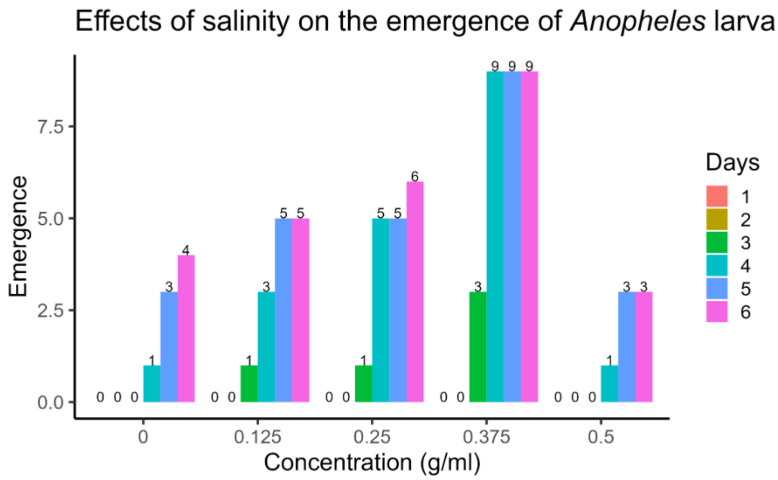

Figure 7: Histogram Representation of the Effect of Salinity at Different Concentration Levels on the Emergence of *Anopheles* Larvae

DISCUSSION

This study was carried out to establish the impact of salinity on the survival, mortality, development and emergence of *Anopheles* larvae. The results presented showed that with an increase in salinity, there was a decrease in the survival of *Anopheles* larvae, which is in contrast with the findings of Emidi *et al*. (2017) who recounted that upper percentile (high concentrations) of salinity significantly related with the increased in density of *Anopheles* larvae. However, in a study by Nwaefuna *et al*. (2019) it was observed that with increased saline concentrations, there is less likelihood of females of *Anopheles coluzzii* laying eggs in water. Thus, the oviposition decreases in relation to increasing salinity gives reasons why this particular species evades unsuitable oviposition sites. However, several studies have reported the survival of *Anopheles* larvae in higher levels of salinity compared to what has been used in this study. For instance, Ould *et al*. (2020) reported

75% – 86.5% survival of other sibling species of *Anopheles* at a salinity level of 17.5 g/l. However, the findings of Ekechukwu & Ekeh (2011) showed that *Aedes aegypti* survived at lower salinity concentrations and up to 100% mortality was recorded at higher concentrations. This was so stated because larvae were hypertonic in low salinity to the medium but hypotonic in higher concentration (Nielsen, 1995).

Pierre *et al*. (2019) reported increased mortality with increasing levels of salinity in their study. Lukwa *et al*., (2017) recounted an increase in mean per cent larval mortality of *Anopheles gambiae* with increasing salinity level. Almost equal level of mortality was recorded in the control of this study, although this disputes the findings of Lukwa et al., (2017) who recorded no mortality in the control, reporting that a lesser concentration of salinity did not kill *Anopheles* larvae. This could be attributed to differences in environmental and climatic factors which could have impacted the survival of the mosquito larvae.

Data obtained from this study showed that salinity and temperature had a measurable effect on the development of *Anopheles* larvae. The results showed that an increase in salinity and temperature levels decreased the development of *Anopheles* larvae. Slower development was observed at high levels of salinity and temperature, this indicates that based on the quantity of ions in the water bodies, the growth of larvae as well as metabolism may be affected especially during moulting, therefore the extent and speed of their development (Mamai *et al*., 2021) might be altered. Imam & Deeni (2014) and Garba & Olayemi (2015) who in their separate works observed that the growth and development of *Anopheles* larvae increases with increasing temperature.

It was observed in this study that a salinity concentration of 0.375g/ml within the range of 1.99ppt – 4.02ppt encouraged the development of *Anopheles* larvae and 3.05ppt – 4.02ppt supported the emergence of adult *Anopheles* mosquitoes. Although the development and adult emergence of *Anopheles* mosquitoes were observed at other salinity levels, the treatment concentration of 0.375g/ml appeared to be more favourable for the development of larvae and the emergence of adult *Anopheles* mosquitoes. In another study, it was gathered that *Anopheles* larvae completed their development in saline solutions of concentrations ranging from 0-100% with optimum development occurring at saline concentrations of 0%, 40%, and 60% (Coetzee *et al*., 1988; White et al., 2013). Bell *et al*., (1999) also found out that salinities of up to 2.2% - 2.5% were suitable for survival and adult emergence, from the fourth instars. This also suggested that higher salinity levels may slow down larval development.

Conclusion

This result has shown that as salinity levels increase, the survival of *Anopheles* larvae decreases, thereby, resulting in a corresponding increase in the mortality of *Anopheles* larvae. It was also observed that increasing levels of salinity led to a decreased/slower rate of *Anopheles* larvae development as well as adult emergence. However, the optimum level of salinity that was favourable to the developmental rate and emergence of *Anopheles* mosquito larvae in this study was 0.375g/ml, ranging from 1.99ppt-4.02ppt and 3.05ppt - 4.02ppt respectively.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

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