



# Evaluation of the influence of homogenate filtrates of some larval habitat-inhabiting Flora and Fauna species on immature development and adult body size of *Culex quinquefasciatus* mosquitoes (Diptera: Culicidae)

Abubakar Nafisat Eleajo, Olayemi Israel Kayode, Ukubuiwe\* Azubuike Christian

Applied Entomology and Parasitology Research Unit, Department of Animal Biology,  
Federal University of Technology, Minna, Nigeria

\*For correspondence E-mail: a.ukubuiwe@futminna.edu.ng

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## ABSTRACT

This study was carried out to determine the influence of breeding sites flora and fauna on mosquito developmental success (survival rate and duration of development) and adult fitness attributes. Homogenate filtrate of four organisms: tadpole (*Rana temporaria*), Nile Tilapia (*Oreochromis niloticus niloticus*), water lily (*Nymphaea lotus*), and a Fresh water algae (*Spirogyra porticalis*), were prepared and tested against freshly hatched-out mosquito larvae. Analyses of results revealed high (>90.00 %) immature survival rate, with no significant ( $p < 0.05$ ) difference among the filtrates. Larval survival rates ranged from  $96.73 \pm 7.82$  (Tilapia) to  $99.99 \pm 0.83$  (water lily), pupal survivorship,  $87.57 \pm 10.48$  (water lily) to  $98.96 \pm 2.09$  (tadpole), and Average Immature survivorship,  $96.71 \pm 6.47$  (water lily) to  $99.09 \pm 3.22\%$  (tadpole). Developmental periods of immature populations of *Cx. quinquefasciatus* mosquitoes significantly ( $p < 0.05$ ) varied among the homogenate filtrates, being as short as  $8.02 \pm 0.76$  days (tadpole) and as long as  $9.73 \pm 1.29$  days (*Spirogyra* filtrate). There was, however, no significant difference ( $p > 0.05$ ) in post-emergence longevity (range =  $3.27 \pm 0.38$  to  $3.60 \pm 0.43$  days) and Fluctuating Asymmetry ( $0.01$  mm). Wing length measurement and volumes of adult mosquitoes in *Spirogyra* and *Tilapia* filtrates were significantly ( $p < 0.05$ ) lower than individuals from other filtrates: water lily filtrate producing mosquitoes with the longest wings and volume ( $3.73 \pm 0.45$  mm and  $51.90 \pm 0.09$  mm<sup>3</sup>). The results of this study provide a better understanding of the breeding ecology of mosquitoes, as a prelude for effective biological control of mosquito larvae.

**Keywords:** Biological fitness, Tilapia, Tadpole, Spirogyra

## 1. INTRODUCTION

Most of the world's life-threatening and debilitating parasites and viral diseases are transmitted by mosquitoes which includes malaria (*Anopheles*), filariasis (*Culex*), and Dengue fever and Zika virus (*Aedes aegypti*) [1, 2]. More than 700 million people around the world are infected with these mosquito borne diseases annually [3]. Among the three genera of vector mosquitoes, *Culex* mosquitoes are perhaps the least study, however, they continue to constitute serious public health challenge to man. Their catholic behaviour [4, 5], ubiquitous [6] and ability to oviposit in any available water-retaining receptacle [7, 8] have been reported to enhance their vectorial tendencies.

*Culex* mosquitoes are responsible for transmission of important diseases like Japanese encephalitis and lymphatic filariasis. More than 120 million people are infected currently with lymphatic filariasis with about 40 million of them disfigured and incapacitated by the disease [9]. The success of these species of mosquito is hinged on their dynamic ovipositional strategies [6, 10] and immature developmental successes [11].

The oviposition preferences of adult female mosquitoes are affected by biotic and abiotic factors within a water body [7]. These significantly influence mosquito density and distribution [12]. Biotic factors such as presence of organisms like algae and bacteria in larval habitat significantly influence oviposition attraction and success of gravid mosquitoes [10, 13]. Gravid mosquitoes can discriminate between different biotic and abiotic factors using visual, semiochemical and physico-chemical cues [14].

Physical objects like vegetation can influence oviposition soil or water substrates [15]. Bond *et al.* [13] investigated that filamentous algae serve as food source and provide refuge from predators for mosquito larvae and form floating mat in rivers side pools, which is ideal for the development of *Anopheles* mosquitoes. Therefore, ovipositing females select habitat based on the presence of this algae. Tadpoles co-occur in a range of habitats with mosquito larvae [16, 17] and consume mosquito larvae as source of food [19, 20], while frogs can reduce mosquito population by preying on adult mosquitoes [18].

Understanding how these breeding site inhabiting flora and fauna enhances or reduce developmental activities of mosquitoes will help develop appropriate larval control strategies. Hence, this study was designed to determine how the presence of certain Flora and Fauna species (as homogenate filtrate)

influence oviposition site selection and subsequent development success of mosquito using *Culex quinquefasciatus* as model.

## 2. MATERIALS AND METHODS

### 2.1 Collection and processing of mosquito – breeding habitat Flora and Fauna

This involved the collection of tadpoles of *Rana temporaria*, *Tilapia* Sp (*Oreochromis niloticus niloticus*), an alga (*Spirogyra porticalis*) and Water lily (*Nymphaea lotus*) from conventional mosquito breeding sites in Minna (longitude 6° 33' E and latitude 9° 27' N), North central Nigeria.

All flora and fauna were obtained in fresh state during the morning hours and immediately transported to the Laboratory of Animal Biology, Federal University of Technology, Minna. They were crushed separately in a mortar and filtered in distilled water (at the ratio of 50 g to 500 ml) using No 1 Whatman filter paper. The filtrate was preserved at 4 °C until needed.

### 2.2 Bio-assay of Homogenate Filtrates against Mosquito Immature Stages

Approximately, day old egg rafts of *Culex quinquefasciatus* were obtained from the same breeding sites where larval habitat flora and fauna species were collected, by setting ovitraps. The egg rafts were placed, individually, in 150 ml of distilled water for hatching. Upon hatching, the identity of cohorts of larvae from each egg raft was authenticated as *Cx. quinquefasciatus* using taxonomic keys of Hopkins [21].

Upon identification, the larvae were sorted into earthen pots (6.5 Liters) containing 2 Liters of borehole water at the rate of 25 healthy larvae per pot. The earthen pots were arranged in four treatments constituted by addition of 2 ml homogenate filtrates of tadpole, *Tilapia*, *Spirogyra* and water lily, respectively. A control experiment, similar to the treatment but devoid of homogenate filtrate was also set up. Each treatment and control experiment had four replicates. The water and homogenate filtrate content of the earthen pots were changed every 48-hours, to ensure freshness of the larval culture media and consistency of effects on the development of the immature life stages.

The larvae were fed and maintained following standard techniques of Olayemi *et al.* [22], under laboratory condition of 28°C and 12: 12 hours (light: dark) regimen. The experiment was monitored daily

at 0700 and 1900 hrs for larval mortality, metamorphosis (duration of development) pupation and adult eclosion. The whole experiment was repeated within 2 weeks of the termination of the first for enhanced replication.

### 2.3 Determination of Survivorship of Immature Life Stages

Survival rates during the life stages were determined as the proportion of individuals at the beginning of a life stage that successfully transformed to the next stage according to the formula of Olayemi and Ande [23]:

$$S_i = \frac{n_i}{(X_{ni} - 1)} \times 100$$

Where:

$S_i$  - Survival rate of life stage  
 $n_i$  - No of individuals entering a life stage  
 $X_{ni} - 1$  - No of individuals that entered the preceding life stage.

### 2.4 Determination of Duration of Immature Life Stages

The duration of immature life stages was also determined using the formula of Olayemi and Ande, [23] which estimates mean larval instar and pupae stage duration in days.

$$D_i = T_i - (t_i - 1)$$

Where,

$D_i$  - Duration of a life stage.  
 $T_i$  - Present mean age  
 $t_i - 1$  - Previous mean age at ecdysis

### 2.5 Determination of Adult Body Size (Wing Length), Volume and Fluctuating Asymmetry

At 24-hours post-eclosion, the wings were carefully detached from the adult mosquitoes and those of each sides (i.e., right and left) were preserved separately in envelopes. The length of each wing was measured from the apical margin to the alular notch, under a dissecting microscope fitted with an ocular micrometer gauge as described by Ye-Ebiyo *et al.* [24] and Ukubuiwe *et al.* [25]. Volume of adult was expressed as cube values of wing lengths, while fluctuating asymmetry was difference between right and left wings.

### 2.6 Data Analysis

Data obtained were analyzed using one-way Analysis of Variance (ANOVA) to determine the level of significance of difference in means among the treatments; Post hoc tests were then carried out, using Duncan's Multiple Range Test (DMRT), to separate the means where necessary. All data were analyzed at 0.05% level of significance, using Statistical Package for Social Sciences (SPSS) software version 20.

## 3. RESULTS

### 3.1 Effects of Homogenate filtrates on Immature Survivorship of *Culex quinquefasciatus*

The effects of homogenate filtrates of the biota species on survivorship of the immature *Cx. quinquefasciatus* mosquitoes are highlighted in Table 1. Average Immature Survival rate (survivorship from L1 to L4) was generally very high i.e. greater than 95.00% and did not vary significantly ( $P > 0.05$ ) among the four filtrate culture media (Range =  $96.71 \pm 6.47$  in water lily medium to  $99.09 \pm 3.22$  % those of tadpole). Similar trends of effect of filtrates culture media were also obtained for the larval instars (i.e. L1-L4) and Average Larval Survivorship. However, the effects of the filtrate culture media on the pupal stage of the mosquito species were significantly different ( $p < 0.05$ ), notably reduced mortality from  $98.96 \pm 2.09\%$  among larvae reared in tadpole filtrate media to  $87.57 \pm 10.48\%$  in those cultured water lily-infused media (Table 1).

### 3.2 Effects of Homogenate filtrates on Duration of Development and post emergence longevity of *Culex quinquefasciatus*

The influence of homogenate filtrate of the selected flora and fauna on duration of immature life stages of the mosquitoes is show in Table 2. Unlike the TIS, the filtrate of the species tested had significant effects on Total Immature Duration (TID) but insignificant effects ( $p > 0.05$ ) on the duration of the pupal stage. TID significantly reduced from  $9.73 \pm 1.29$  days among mosquitoes exposed to *Spirogyra* filtrate infused-medium, to  $8.02 \pm 0.76$  days in the tadpole medium. In addition, duration of each larval instar (i.e., L1-L4) as well as, Total Larval Duration (TLD) was significantly ( $p < 0.05$ ) affected by variation in species filtrate-infused culture media. Post-emergence longevity of the unfed adult mosquitoes were however, not affected significantly ( $p > 0.05$ ) by flora/fauna species filtrate-infused culture media of the immature stage, ranging from  $3.52 \pm 0.06$  days in *Tilapia* medium to  $3.60 \pm 0.43$  days among those reared in *Spirogyra* filtrate medium (Table 2).

**Table 1.** Survival rates (%) of Immature Life Stages of *Culex quinquefasciatus* mosquitoes reared in culture media infused with homogenate filtrates of selected homogenate filtrates of selected breeding site-inhabiting flora and fauna

Homogenate filtrate	Larval Stages				Average Larval Survivorship	Pupal Survivorship	Average Immature Survivorship
	L1	L2	L3	L4			
<b>Control</b> (Distilled Water)	100.00±0.00 <sup>a*</sup>	100.00±0.00 <sup>a</sup>	98.00±2.31 <sup>a</sup>	96.96±2.03 <sup>a</sup>	98.74±0.52 <sup>a</sup>	95.79±0.09 <sup>ab</sup>	98.15±2.10 <sup>a</sup>
<b>Tadpole</b> ( <i>Rana temporaria</i> )	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	96.50±7.00 <sup>a</sup>	99.13±1.75 <sup>a</sup>	98.96±2.09 <sup>b</sup>	99.09±3.22 <sup>a</sup>
<b>Tilapia</b> ( <i>Oreochromis niloticus niloticus</i> )	99.00±2.00 <sup>a</sup>	98.00±4.00 <sup>a</sup>	92.39±15.22 <sup>a</sup>	100.00±0.00 <sup>a</sup>	96.73±7.82 <sup>a</sup>	94.27±8.90 <sup>ab</sup>	96.73±7.82 <sup>a</sup>
<b>Algae</b> ( <i>Spirogyra porticalis</i> )	99.00±2.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	97.00±3.83 <sup>a</sup>	99.00±0.82 <sup>a</sup>	97.00±6.00 <sup>ab</sup>	98.60±3.25 <sup>a</sup>
<b>Water lily</b> ( <i>Nymphaea lotus</i> )	100.00±0.00 <sup>a</sup>	99.00±2.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	96.96±2.03 <sup>a</sup>	99.99±0.83 <sup>a</sup>	87.57±10.48 <sup>a</sup>	96.71±6.47 <sup>a</sup>

\*Values followed by same superscript alphabets, in a column, are not significantly different at p<0.05; L= Larval instars

**Table 2.** Developmental Duration (Days) and Post emergence Adult Longevity (Days) of *Culex quinquefasciatus* Mosquitoes exposed to Homogenate Filtrate of Selected Breeding-Site Flora and Fauna species

Homogenate filtrate	Larval Stages				Total Larval Duration	Pupal Stage Duration	Total Immature Duration	Post Emergence Longevity**
	L1	L2	L3	L4				
<b>Control</b> (Distilled Water)	1.59±0.13 <sup>c*</sup>	1.23±0.13 <sup>ab</sup>	1.50±0.20 <sup>b</sup>	2.79±0.15 <sup>ab</sup>	7.11±0.61 <sup>b</sup>	2.35±0.51 <sup>a</sup>	9.47±1.12 <sup>b</sup>	3.27±0.38 <sup>a</sup>
<b>Tadpole</b> ( <i>Rana temporaria</i> )	1.06±0.06 <sup>a</sup>	1.07±0.44 <sup>a</sup>	1.13±0.05 <sup>a</sup>	2.51±0.09 <sup>a</sup>	5.77±0.67 <sup>a</sup>	2.25±0.09 <sup>a</sup>	8.02±0.76 <sup>a</sup>	3.60±0.43 <sup>a</sup>
<b>Tilapia</b> ( <i>Oreochromis niloticus niloticus</i> )	1.39±0.32 <sup>bc</sup>	1.56±0.57 <sup>b</sup>	1.46±0.12 <sup>b</sup>	3.10±0.14 <sup>b</sup>	7.51±1.15 <sup>b</sup>	2.17±0.93 <sup>a</sup>	9.68±2.08 <sup>b</sup>	3.52±0.06 <sup>a</sup>
<b>Algae</b> ( <i>Spirogyra porticalis</i> )	1.53±0.06 <sup>c</sup>	1.50±0.08 <sup>ab</sup>	1.83±0.07 <sup>c</sup>	2.46±0.33 <sup>a</sup>	7.32±1.03 <sup>b</sup>	2.41±0.26 <sup>a</sup>	9.73±1.29 <sup>b</sup>	3.36±0.40 <sup>a</sup>
<b>Water lily</b> ( <i>Nymphaea lotus</i> )	1.18±0.09 <sup>ab</sup>	1.24±0.13 <sup>ab</sup>	1.45±0.19 <sup>b</sup>	2.96±0.36 <sup>b</sup>	6.83±0.77 <sup>b</sup>	2.25±0.98 <sup>a</sup>	9.08±1.75 <sup>b</sup>	3.58±0.34 <sup>a</sup>

\*Values followed by same superscript alphabets, in a column, are not significantly different at p<0.05; L= Larval instar; \*\* Without Feeding

**Table 3:** Wing Length (mm) and Fluctuating asymmetry (mm) of *Culex quinquefasciatus* mosquitoes reared in media infused with homogenate filtrates of selected flora and fauna species

Homogenate Filtrate	Wing Length (mm)		Mean Wing Length (mm)	Volume of Adult (mm <sup>3</sup> )	Fluctuating Asymmetry (mm)
	Right	Left			
Control (Distilled Water)	3.54±0.13 <sup>ab*</sup>	3.54±0.12 <sup>ab</sup>	3.54±0.13 <sup>ab</sup>	44.36±0.00 <sup>a</sup>	0.00±0.01 <sup>a</sup>
Tadpole ( <i>Rana temporaria</i> )	3.66±0.66 <sup>ab</sup>	3.65±0.65 <sup>ab</sup>	3.66±0.66 <sup>ab</sup>	49.03±0.29 <sup>b</sup>	0.00±0.01 <sup>a</sup>
Tilapia ( <i>Oreochromis niloticus niloticus</i> )	3.49±0.10 <sup>a</sup>	3.48±0.12 <sup>a</sup>	3.49±0.11 <sup>a</sup>	42.51±0.00 <sup>a</sup>	0.00±0.01 <sup>a</sup>
Algae ( <i>Spirogyra porticalis</i> )	3.48±0.16 <sup>a</sup>	3.48±0.14 <sup>a</sup>	3.48±0.15 <sup>a</sup>	42.14±0.00 <sup>a</sup>	0.00±0.01 <sup>a</sup>
Water lily ( <i>Nymphaea lotus</i> )	3.73±0.08 <sup>b</sup>	3.72±0.08 <sup>b</sup>	3.73±0.08 <sup>b</sup>	51.90±0.09 <sup>b</sup>	0.00±0.00 <sup>a</sup>

\*Values followed by same superscript alphabets, in a column, are not significantly different at p=0.05

### 3.3 Effects of Homogenate filtrates on Immature Survivorship of *Culex quinquefasciatus*

Table 3 presents the wing length (proxy for adult body size), body volumes and fluctuating asymmetry (vectorial fitness) of the *Culex quinquefasciatus* mosquitoes as influenced by homogenate filtrates of some selected breeding-site flora and fauna species. Variations in homogenate filtrate-infused culture media significantly ( $p < 0.05$ ) influenced wing length in the mosquitoes. Mean wing length ranged from  $3.48 \pm 0.51$  mm (right wing,  $3.49 \pm 0.10$  mm; left wing,  $3.48 \pm 0.12$  mm) in mosquitoes cultured in *Tilapia* filtrate-infused media to  $3.73 \pm 0.08$  mm (right wing,  $3.73 \pm 0.08$ ; left wing,  $3.72 \pm 0.08$  mm) in those reared in water lily media. However, significantly bigger adult mosquitoes were produced from culture media infused with water lily filtrate, with mean wing length of  $3.73 \pm 0.45$  mm. Volumes of adult mosquito ranged from  $42.14 \pm 0.00$  to  $51.90 \pm 0.09$  mm<sup>3</sup>. There was, also, no significant ( $p < 0.05$ ) in the fitness of all the mosquitoes in all filtrate infusions, as a reflected by the low fluctuating asymmetry ( $0.00 \pm 0.00$  to  $0.00 \pm 0.01$  mm).

## 4. DISCUSSION

Generally, the homogenate filtrates of the biota species tested had no significant effect on survival rates of larval stage of the mosquito species; however, the reverse was the case with the pupal stage. Further, these filtrates serving as proxies for the presence of the live organisms played little or no role in survivorship of the species. Although, field studies have suggested that these organisms significantly affect dynamics of larval densities [16] due to predation by tadpole and fishes including *Tilapia* [26] and increased dissolved oxygen as a result of the release of oxygen during photosynthesis by *Spirogyra* and water lily [27].

However, the significant reduction in the survivorship of the pupal stage by the water lily filtrate may be due to growth and /or developmental disruptive activities of inherent phytochemicals of this plant species on the mosquitoes [28]. According to Imam and Tajudeen [29], water lily contains a toxin, which is toxic against aquatic invertebrates. On the other hand, while the biota filtrates significantly affected total duration of immature and larval development, the pupae were not significantly affected. Total Immature Duration (TID) was significantly shortest among the mosquitoes raised in culture media infused with tadpole filtrate. These findings, probably, indicate that these biota filtrates may contain growth-enhancing substances that

interfere, positively, with cell division, growth, feeding tendencies [30] and, perhaps, teneral reserve accumulation [31]: physiological processes that accelerate growth and development characteristic of this life stages.

Duration of the pupal stage of the mosquitoes was not significantly affected by the biota filtrates, perhaps, due to the relatively short non-feeding interval and non-ingestion of bioactive metabolites inherent in the filtrates [32, 33]. More so, according to David *et al.* [34], in mosquito larvae, exposure to xenobiotics from toxic leaf litters in breeding habitats elicit enzymatic responses (e.g. by cytochrome P450 monooxygenases, P450s), which is stimulates higher tolerance [33]. Thus, metabolic enzymes in the pupae could have neutralized the metabolites ingested during the larval life stages.

The biota filtrates had no significant effect on post-emergence longevity of the adult mosquitoes. However, these periods were relatively short in the mosquito cohorts utilized in the present study. Interestingly, significantly bigger adult mosquitoes (as indicated by wing length) were produced from culture medium infused with water lily filtrate. Generally, the biota species filtrate reduced fluctuating asymmetry (FA) between the right and left wings of the mosquitoes considerably, thus, enhancing the fitness of the mosquitoes as vectors. In mosquitoes, FA results from endo or exogenous stresses during immature development [35], and is reliably used as bio-indicator of suitability of breeding-sites for larval development [36] and fitness of adult mosquitoes for disease transmission [37]. Thus, it seems that the active substances in the filtrates of the larval habitat biota species tested served as anti-stress agents in mosquito larval habitats. These may encourage oviposition [38, 39] and help extend the range of highly productive habitats to include polluted sites, ordinarily avoided by ovipositing mosquitoes, because of inherent mitigating factors associated with the live organisms in such habitats.

### Conflicts of Interest

There are no conflicts of interest.

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