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VECTOR MOSQUITO DIVERSITY AND HABITAT VARIATION IN A SEMI URBANIZED AREA OF KELANIYA IN SRI LANKA

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A B S T R A C T

Mosquito larval sampling was performed at 30 locations, maintaining a distance of at least 200 m radius between two locations in a semi-urban area of the city of Kelaniya in Sri Lanka. The analysis of similarity test identified the abandoned rice field mud flats as the habitat type revealing the highest diversity of *Culex* mosquito species, showing the presence *C. quinquefasciatus*, *C. bitaeniorhynchus*, *C. gelidus* and *C. whitmorei* and one *Armigeres* species. *Culex gelidus* and *C. whitmorei* were restricted to such habitats occupied with live vegetation with high water conductivity and turbidity. Blocked drains were associated with a significantly higher occurrence of *C. quinquefasciatus* when compared with *C. bitaeniorhynchus* or *Armigeres* spp. This study revealed that water quality requirements of the habitat of the culicine mosquito larvae varied among the species. The larval habitats of *C. quinquefasciatus* were characterized by low dissolved oxygen and high biological oxygen demand compared with those of the other culicine mosquitoes. Such habitats were associated with tubificid worms (Annelida) and chironomid larvae (Diptera). *C. quinquefasciatus* larval survival rapidly declined at pH level of 9.4.

Keywords: *Culex*; container breeders; marshy lands; rice field habitats; turbidity.

INTRODUCTION

The type of water required for mosquito oviposition is more or less specific to each species. Mosquito larvae are found in habitats possessing a wide range of physicochemical factors including salinity, dissolved organic and inorganic matter, degree of eutrophication, turbidity, air and water temperature, pH and sunlight (Muturi *et al*., 2008; Hamid *et al*., 2009; Yadav, 2009). Abundance and species distribution are also determined by the geographic location, water body dimensions, climate, predatory abundance, anthropogenic activities and human and animal population distributions (Okogun *et al*., 2003; Yadav, 2009). The presence of vegetation and floating plants provide optimal breeding conditions by acting as food sources as well as shelter from predators, for some mosquito species. Vegetation also creates stagnant conditions by decreasing the water flow (Walton, 1990; Yadav, 2009).

Mosquito borne diseases are one of major health problems in almost all tropical and subtropical countries. Apart from being vectors their bites become the serious nuisance to people living around. Lack of proper maintenance in urban areas has created polluted and abandoned water puddles that are ideal for culicine mosquito breeding in many areas in Sri Lanka. The objective of the present study therefore was to examine the mosquito vector diversity in varying breeding habitat types and effect of water quality parameters on their relative abundance in a semi urbanized area in Sri Lanka.

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MATERIALS AND METHODS

Site selection and sampling: The present study was conducted in Wanawasala, a semi-urban area of the city of Kelaniya (6°58'0"N and 79°53'30"E) in the Western province of Sri Lanka, covering an estimated area of 4.1 km^2 with a human population of 6935 (http://www.ds.gov.lk). Climate wise, this area is warm and humid for the major part of the year having a relative humidity of 80%, an average annual air temperature of 27 °C and an average annual rainfall of 2390 mm. In all, thirty mosquito breeding sites were chosen, maintaining a distance of at least 200 m radius between two sites. Each site was geo-referenced (GARMIN-etrex SUMMIT). The following locations were explored while capturing the larvae: blocked drains carrying domestic wastes; open drains carrying polluted water contaminated with sewage; large polluted water bodies directly receiving sewage discharge; abandoned rice fields with irrigation canals, abandoned rice fields with mud flats covered with *Salvinia,* water hyacinth and weed grasses; polluted water bodies into which poultry effluents and domestic wastes directly get discharged, marshy areas covered with live vegetation; stagnant water bodies; flowing drains; clean water bodies, irrigation channels; discarded coconut shells, discarded pots and plastic cups accumulated with rain water. Sampling for mosquito larvae was conducted using the dipping method employing a 250 ml scoop. Water in small containers was poured into rearing containers and the volume was adjusted. The contents of each sample were carefully examined and larval counts were recorded. These larval samples were brought into the laboratory and reared in containers covered by a small sized mesh of nylon net until the emergence of adults. Species identification was done using the morphological keys of adult mosquitoes (Chelliah, 1984; Reuben et al., 1994). Sampling was performed on a monthly interval between February and July 2011. Sampling in March was not done as most of the habitats were dry due to the hot weather.

Physicochemical and biotic factors: The pH level (model: PH 315i/SET), dissolved oxygen content (DO) (using standard equipment and Winkler's method), total dissolved solid content (TDS) and the water conductivity (model: Cond 340i/SET) of the mosquito breeding sites were measured at the time of sampling, at each sampling site. Water samples were collected into 250 ml dark stoppered bottles (n=3) to determine a five-day biological oxygen demand (BOD_{5}). These water samples were brought into the laboratory and stored in the dark for five days and the final oxygen concentration of the dark bottles was measured as the method described for DO.

Effect of the different pH levels on the survival of *Culex quinquefasciatus* larvae: A stock of water was collected from a blocked drain where *Culex quinquefasciatus* was highly abundant (pH = 7). A series of sub-samples possessing varying pH levels from 7 to 11 were made by adding soap solution as required. Then 200 ml of pH-adjusted drain water was poured into a transparent plastic container, 12 cm diameter x 8 cm height (n=3). Five individuals of the fourth instar larvae of *C. quinquefasciatus* collected from the same habitat were directly introduced into each container and kept covered using a small-meshed mosquito net. Containers were maintained at 28 °C $\pm$ 2 room temperature. Larval survival was recorded and the containers were maintained until the emergence of the adult or death of the insects, whichever occurred first.

Data analysis: The statistical data analysis was done using MINITAB 14 version. The relative abundance of the mosquito larvae was expressed as the number of mosquito larvae and pupae per dip (one scoop). The data between species abundance and the physicochemical parameters were analyzed using the one-way ANOVA. Tukey's pair wise test was performed to determine whether any significant difference in the abundance of the different vector mosquito species was present. The diversity of the mosquito species in the different breeding habitats was analyzed using the Shannon Weiner diversity index. These species were grouped by means of dis-similarity using cluster analysis (average linkage clustering). The groups thus formed were depicted in a dendrogram. Multidimensional scaling (MDS ordination) was performed to show the separate clusters between the container breeding habitats and others. The input data used in the analysis were seven species/taxa of mosquitoes and 11 habitat types. The analysis of similarity (ANOSIM) test was used to determine the variation between the different habitat types.

RESULTS AND DISCUSSION

The total collection yielded seven mosquito species that included four *Culex* species, *C. bitaeniorhynchus* (5.4%), *C. gelidus* (13.7%), *C. quinquefasciatus* (55.2%), and *C. whitmorei* (9%); two *Aedes* species, *A. aegypti* (3.1%) and *A. albopictus* (2.1%); and one *Armigeres* spp. (11.5%). The one-way ANOVA revealed that the monthly mean relative abundance of *C. quinquefasciatus* was significantly higher than that of the other mosquito species found in this area (F= 17.49, df = 6, P=0.000) throughout the study (Table 1). Further, these results demonstrated that both *C. gelidus* and *C. whitmorei* were prevalent in the same aquatic habitats such as rice field mud flats and marshy lands. Rice field mud flats were found to be the highest diverse habitat type resulting in five species of mosquitoes that included four *Culex* species and an *Armigeres* species (Shannon wiener diversity index / H' = 0.6648). Rice field irrigation canals were the next most diverse breeding habitat supporting *C. bitaeniorhynchus, C. gelidus, C. quinquefasciatus* and *C. whitmorei* (Shannon wiener diversity index / H' = 0.4798), while container breeding habitats were identified as the lowest diverse breeding habitats, which revealed the presence of *Aedes aegypti* and *A. albopictus*. Polluted water habitats encouraged the breeding of *C. quinquefasciatus* and *Armigeres* mosquitoes.

Table 1. The relative abundance (Number of larvae / dip) of mosquito larvae recorded in Wanawasala area of Kelaniya.

Species	February	April	May	June	July	Mean ±SE
Cu. Quinquefasciatus	235	354	255	268	432	308.80 ± 36.89^{a}
Cu. Whitmorei	67	61	65	50	31	55.80 ± 5.76^{b}
Cu. Gelidus	2	11	36	93	160	61.60 ± 29.04^{b}
Cu. Bitaeniorhynchus	16	12	35	66	9	27.60 ± 10.61^{b}
Armigeres	25	78	91	116	104	82.80 ± 15.78^{b}
Ae. Aegypti	15	27	28	0	47	23.40 ± 7.78^{b}
Ae. Albopictus	0	0	0	0	187	37.40 ± 37.40^{b}

(Same superscript in the table are not significantly different P>0.05)

Cluster analysis performed on mosquito larval abundance with respect to breeding habitats, revealed six easily distinguishable clusters in a 30% similarity level (Fig. 1). Blocked drains that carried domestic waste water (W1), large polluted water bodies (W7) and stagnant water bodies (W8) were clustered together at a 35% similarity level, whereas marshy lands (W3), rice field mud flats (W4) and rice fields with irrigation canals (W5) were represented by a separate cluster at a 40% similarity level. According to the cluster analysis, the container habitats such as discarded water filled pots (W9) and plastic cups (W10) showed a mixed 82.5% similarity level. Coconut shells (W11), clean large water bodies (W6) and flowing drains (W2) were clustered separately as unique clusters, at almost 10% similarity level. The two dimensional view of the MDS ordination of breeding habitats based on the relative abundance of mosquito larvae shows that the container breeding habitats (W_9, W_{10}, and W_{11}) were clustered separately from the rest of the other habitats (Fig. 2**)**. According to ANOSIM these two clusters are significantly different from each other (P= 0.006 < 0.05).

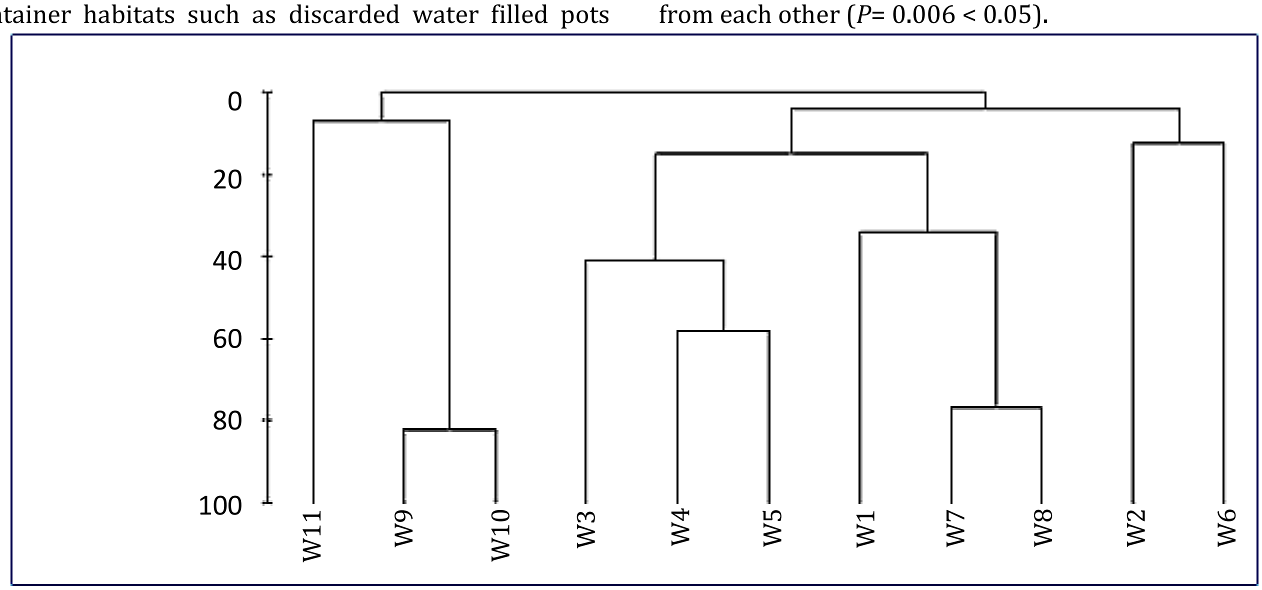

Figure 1. Dendrogram showing the clustering of breeding habitats respect to relative larval abundance (Larval count / dip) of different mosquito species in Wanawasala area. (W_1 Blocked drains, W_2 Flowing drains, W_3 Marshy lands, W_4 Rice field mud flats, W_5Rice field with irrigated canals, W_6 Clean large water bodies, W_7 Large polluted water bodies, W_8 Stagnant water bodies, W_9 Pots, W_{10} Plastic cups, W_{11} Coconut shells).

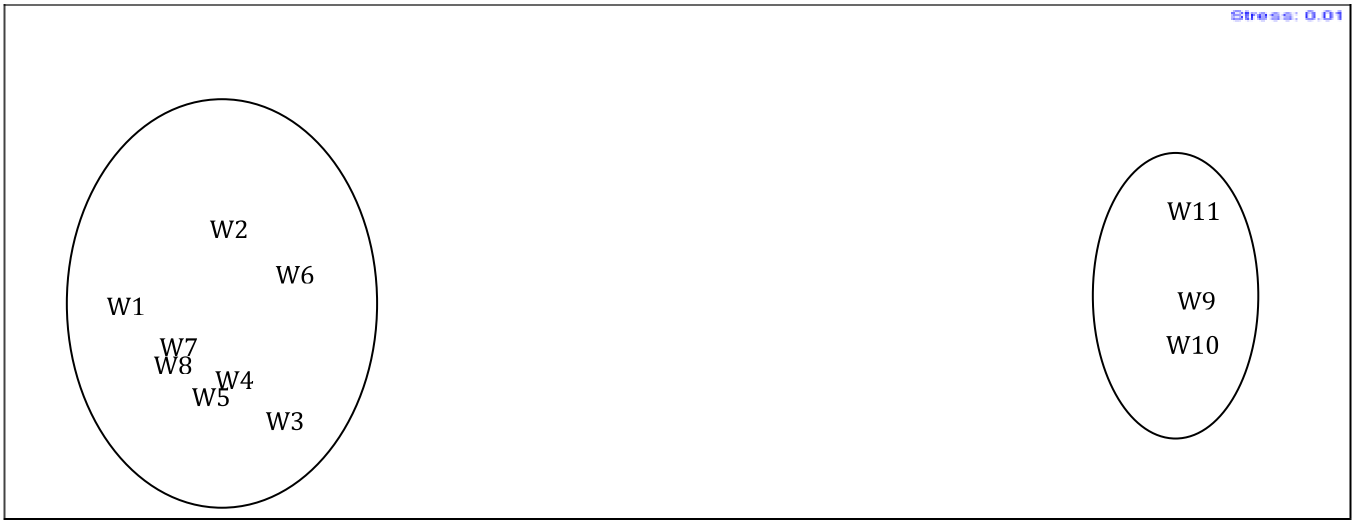

Figure 2. Two dimensional MDS plot showing the clustering of breeding habitats based on the mosquito larval abundance in Wanawasala area.

About 50% of the larval habitats recorded in this area had alkaline water, with low dissolved oxygen content, high conductivity and high dissolved solid content. About 70% of the larvae were recorded in breeding water with a higher biological oxygen demand. The *Aedes* species occurred within a very narrow pH range from 6-8 where 44% of *A. aegypti* and 10% of *A. albopictus* were found in a pH range of 6-7, while 56% of *A. aegypti* and 90% of *A. albopictus* were found in the 7-8 pH range (Table 2). *Culex gelidus* and *C. bitaeniorhynchus* existed in breeding water of 6-9 pH range while *Armigeres* and *C. whitmorei* occurred in the broader pH range of 6-10. The only mosquito species that was recorded within a very broad pH range from 5 to 11, including slightly acidic and extremely alkaline water was *C. quinquefasciatus*. However, the mean pH tolerance value for each individual species did not vary significantly at P>0.05 according to the one-way ANOVA. The average pH of the breeding water for almost all the mosquito species fell in the range between 6 and 8 and this value concurred with those of the earlier studies (Yasuoka, 2007).

Table 2. The relative abundance (as a %) of mosquito species in habitats with different pH levels. Last column indicates mean tolerant pH value.

Mosquito species	5-6pH	6-7pH	7-8pH	8-9 pH	9-10pH	10-11pH	Mean pH ± SE
Cu. quinquefasciatus (%)	0.2	42.9	53.6	2.2	0.9	0.2	7.18±0.13[a]
Cu. whitmorei (%)	0.0	29.4	45.9	21.2	3.5	0.0	7.27±0.19[a]
Cu. gelidus (%)	0.0	71.6	18.0	10.5	0.0	0.0	6.80±0.24[a]
Cu.bitaenyorhynchus (%)	0.0	54.0	40.4	5.60	0.0	0.0	7.08±0.24[a]
Armigeres (%)	0.0	65.8	26.3	4.1	3.8	0.0	7.32±0.15[a]
Ae. aegypti (%)	0.0	44.4	55.6	0.0	0.0	0.0	7.10±0.18[a]
Ae. albopictus (%)	0.0	9.1	90.9	0.0	0.0	0.0	7.46±0.64[a]

(Same superscript in the table are not significantly different P>0.05).

The *Aedes* species was identified in breeding water with low dissolved oxygen levels; 55% of *A. aegypti* occurred in 0-4 mg/l and 45% of the *A. aegypti* in 4-8 mg/l of water, whereas 91% of *A. albopictus* was recorded in 0-4 mg/l and 9% of *A. albopictus* in 4-8 mg/l (Table 3). C*ulex quinquefasciatus* and *C. whitmorei* occurred within a very broad range of dissolved oxygen from 0 to 16 mg/l. The maximum occurrence was noted at 0-8 mg/l DO level, while their prevalence was drastically reduced in habitats with a high DO level. *Culex bitaeniorhynchus*, *Culex gelidus* and *Armigeres* were not reported when DO levels were above 12 mg/l. However, a mean dissolved oxygen tolerance level, between 4.94 ± 0.59 and 5.73±1.24, was noted for seven mosquito species. It did not significantly vary at *P*>0.05 according to the one-way ANOVA. The present study also revealed that about 60% of the mosquito species was distributed within a range between 0-8 mg/l of dissolved oxygen. Of them, the majority of *C. quinquefasciatus* were found in a DO range of 0-4 mg/l. Therefore, a low level of dissolved oxygen may be an indicator of the quantity of decaying organic matter that influences oviposition and/or survival of their larvae.

Table 3. The relative abundance (as a %) of mosquito species in habitats with different DO levels (mg/l). Last column indicates mean tolerant DO value.

Mosquito species	0-4 mg/l	4-8 mg/l	8-12 mg/l	12-16 mg/l	Mean DO ±SE (mg/l)
Cu. quinquefasciatus (%)	60.8	30.4	6.1	2.7	5.20±0.61 [a]
Cu. whitmorei (%)	38.0	37.5	14.7	9.8	4.94±0.59 [a]
Cu. gelidus (%)	3.7	56.3	40.0	0.0	5.73±1.17 [a]
Cu. bitaenyorhynchus (%)	60.5	24.4	15.1	0.0	5.73±1.24 [a]
Armigeres (%)	24.0	52.5	23.5	0.0	5.04±0.70 [a]
Ae. aegypti (%)	55.6	44.6	0.0	0.0	5.11±1.32 [a]
Ae. albopictus (%)	90.9	9.1	0.0	0.0	5.25±1.42 [a]

(Same superscript in the table are not significantly different P>0.05)

The only mosquito larvae that survived in >10.00 mg/l biological oxygen demand *was C. quinquefasciatus* (Table 4). Other mosquito larvae were recorded in a lower BOD range between 0.00-10.00 mg/l. A significant difference was noted between the BOD of the natural breeding water of seven mosquito species (F = 7.94, df = 6, P = 0.000). However, the BOD of the breeding water of *C. quinquefasciatus* was significantly higher (57%) than that of the other mosquito larvae, because high BOD levels are indicative of organically polluted water (Muturi, 2007).

Table 4. The relative abundance (as a %) of mosquito species in habitats with different BOD levels (mg/l).Last column indicates mean tolerant BOD value.

Mosquito species	≤1 mg/l	2-3 mg/l	3-5 mg/l	5-10mg/l	≥10 mg/l	Mean BOD ±SE (mg/l)
Cu. quinquefasciatus (%)	0.0	0.3	2.2	40.0	57.5	9. 33±0.25 [a]
Cu. whitmorei (%)	0.0	9.6	15.4	75.0	0.0	4.93±0.34 [b]
Cu. gelidus (%)	0.0	8.7	91.3	0.0	0.0	4.30±0.83 [b]
Cu. bitaenyorhynchus (%)	7.7	23.3	40.9	28.1	0.0	3.11±0.84 [b]
Armigeres (%)	3.1	18.1	11.5	67.3	0.0	5.31±0.71 [b]
Ae. aegypti (%)	13.2	24.5	62.3	0.0	0.0	3.19±1.35 [b]
Ae. albopictus (%)	0.0	90.0	9.1	0.0	0.0	3.10±*[b]

(Same superscript in the table are not significantly different P>0.05, * unavailability of data)

More than 90% of the *Culex* larvae were recorded in natural breeding water with greater than 5.00 μs/cm conductivity levels. The mean conductivity values of the breeding water of *Culex* and *Armigeres* mosquitoes were always found to remain greater than 10.00μs/cm, although that of the *Armigeres* species was the highest (13.0± 0.51). Water conductivity tolerated by the *Aedes* mosquitoes ranged from <5–10 μs/cm and *A. albopictus* were identified only between 5.00 and 10.00 μs/cm. The percentage of the relative abundance of the *Culex* species increased with the increased water conductivity, although that of *Armigeres* and two *Aedes* species did not (Table 5). The mean dissolved solid (TDS) content of the water in which each mosquito species was bred showed no significant difference (*F*= 1.84, *df*=6, *P*= 0.135) (Table 6). Also, there was no significant difference in the conductivity level of the breeding water for each mosquito species. Over 70% of *C. gelidus,* and *C. whitmorei* were recorded in water with TDS levels greater than 10.00 mg/l and their percentage relative abundance increased with the increase of TDS. Two species *C. quinquefasciatus* and *Armigeres* were recorded within a very broad TDS range from less than 5 to more than 10 mg/l. *Culex bitaeniorhynchus* was recorded in breeding water with low TDS values and the percentage relative abundance greatly declined at the higher TDS levels. Over 90% of *Aedes* mosquitoes were distributed in the more or less clear breeding water. The *C. quinquefasciatus* mosquito larvae were mostly found in habitats having comparatively high TDS levels (> 5.00 mg/l). Muturi *et al*., (2008), recorded that *C. quinquefasciatus* larvae were positively associated with

TDS and that their abundance was higher when TDS levels were high.

Table 5. The relative abundance (as a %) of different mosquito species in habitats with different conductivity levels (µs/cm). Last column indicates mean tolerant conductivity.

Mosquito species	≤5.00 µs/cm	5-10 µs/cm	≥10 µs/cm	Mean Conductivity±SE (µs/ cm)
x *Cu.quinquefasciatus*(%)	7.7	28.1	64.2	10.69±0.31 [a]
Cu. whitmorei(%)	0.0	29.6	70.4	11.01±1.00 [a]
Cu. gelidus(%)	11.4	35.7	52.9	10.11±0.60 [a]
Cu. bitaenyorhynchus(%)	10.1	44.3	45.6	10.43±0.60 [a]
Armigeres(%)	2.2	57.5	40.3	13.00±0.51 [a]
Aedes aegypti(%)	40.8	59.8	0.0	8.73±1.16 [a]
Ae. albopictus(%)	0.0	100.0	0.0	9.10±1.35 [a]

Same superscript in the table are not significantly different P>0.05)

Table 6. The relative abundance (as a %) of mosquito species in habitats with different TDS levels (mg/l).Last column indicates mean tolerant TDS value.

Mosquito species	≤5.00 mg/l	5-10 mg/l	≥10 mg/l	TDS±SE (mg/l)
Culex quinquefasciatus(%)	24.9	37.4	37.7	11.33±0.61 [a]
Culex whitmorei(%)	7.4	22.0	70.6	11.36±0.99 [a]
Culex gelidus(%)	0.0	25.2	74.8	11.81±1.15 [a]
Culex bitaenyorhynchus(%)	4.0	73.3	22.7	10.44±0.57 [a]
Armigeres(%)	16.8	35.9	47.3	13.07±0.48 [a]
Aedes aegypti(%)	0.0	95.7	4.3	10.18±1.30 [a]
Aedes albopictus(%)	0.0	100.0	0.0	9.85±1.80 [a]

(Same superscript in the table are not significantly different P>0.05)

The survival of *Culex quinquefasciatus* larvae in pH adjusted water decreased as the pH increased from 7.3 to 11.00 (F= 37.72, df= 8, P < 0.05). Fig. 3 indicates that the survival and adult emergence of *C. quinquefasciatus* mosquitoes rapidly declined when the pH increased above 9.4 and the mosquito larvae could not survive when the breeding water reached a level of more than pH 11.00. The laboratory investigation revealed that the larval survival and adult emergence of *C. quinquefasciatus* rapidly declined when the pH level rose above 9.4; nor could they survive at a pH level of 11.0. A similar study was done by Clark *et al.*, (2004), for fresh water and euryhaline water, where mosquito larvae of almost all the species were reduced in number under extreme acidic and alkaline conditions.

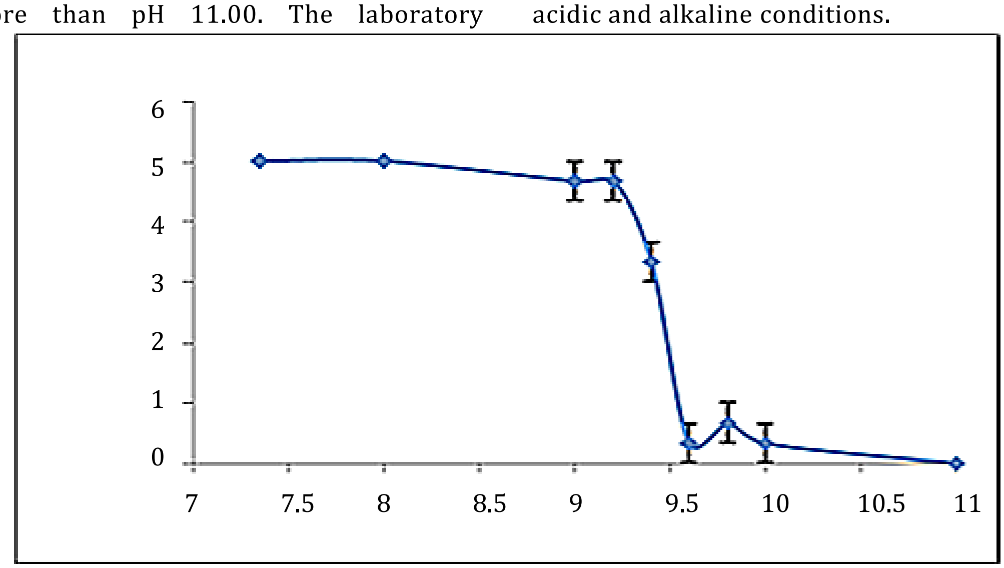

Figure 3. The survival of *Culex quinquefasciatus* mosquito larvae at different level of pH under laboratory condition.

Chironomid larvae (Diptera) and tubificid worms (Annelida) were indicative to *Culex quinquefasciatus* mosquito larval habitats of stagnant drains and polluted water bodies. These proved to be an indicator to distinguish them from other mosquito breeding habitats. These habitats were lacking in vegetation cover and other macro fauna. *Culex whitmorei* and *Culex gelidus* were recorded only from habitats having a thick cover of aquatic vegetation such as rice fields, and wetland marshy plants covered with *Salvinia* and water hyacinth, *Eichhornia* spp. Aquatic insects were the only associated fauna in the *Aedes* mosquito breeding habitats.

The analyses done in this study demonstrated the complexity of the mosquito ecology and highlighted the necessity of mosquito control strategies that should be integrated with the ecological characteristics of the local mosquito vectors and their interactions with environmental and biological factors. On ecological analyses of mosquito breeding it was found that stagnant polluted drains and rice fields were the two major breeding sites that needed to be focused upon for the control of the *Culex* species, whereas container breeding habitats needed to be targeted to control the *Aedes* mosquitoes in a sustainable manner.

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