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LARVAL BREEDING HABITATS AND ECOLOGICAL FACTORS INFLUENCE THE SPECIES COMPOSITION OF MOSQUITO (DIPTERA : CULICIDAE) IN THE PARKS OF DHAKA CITY, BANGLADESH

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Abstract: Mosquito larval ecology is prerequisite for determining the larval abundance and species assemblage in mosquito control program. The study explored the association of five mosquito species with their breeding habitat diversity and species distribution in three selected parks from May to October, 2015. A total of 3217 mosquito larvae were reported from six breeding habitats, namely tree hole, leaf axils, water bottle, tire, drain and coconut shell. The frequency of the three species (*Ae. aegypti*, *Ae. albopictus* and *Ar. subalbatus*) coexistence was higher in tree holes (82.4%) than that of the other coexisting species. Pearson Chi-square result revealed that the association of species was significantly dependent on the breeding habitats. ANOVA further suggested that mosquito density varied across habitats where among the highest density of *Cx. quinquefasciatus* (3.87 ± 0.22) found in drain, followed by both *Ae. albopictus* (2.02 ± 0.17) and *Ar. subalbatus* (0.50 ± 0.09) in tree holes and *Ae. aegypti* (1.25 ± 0.23) in coconut shell. *Cx. tritaeniorhynchus* occurred in drain with the least observed density (0.03 ± 0.01). CCA results suggested that *Aede*s species were likely preferred to oviposit in shaded habitats where pH was associated with *Ae. albopictus* and dissolved oxygen was with *Ae. aegypti* and *Ar. subalbatus*. *Culex* species were positively associated with the habitats characterizing muddy bottom and emerging vegetation but not with any of the physico-chemical parameters. These findings concluded that ecological factors influence mosquito species to favor their breeding habitats can be helpful in controlling targeted vector species as well as the mosquito borne diseases.

Key words: Mosquito, coexistence, breeding habitats, physico-chemical parameters

INTRODUCTION

Mosquito-borne transmittable diseases have been perilous to human health throughout the world including Bangladesh. The world has been observed the outbreak of dengue, chikungunya, Japanese encephalitis and Zika viruses during the past two decades (Mackenzie *et al*. 2004, Rezza 2014, Hennessey *et al*. 2016). Recently, Bangladesh has been witnessing an outbreak of mosquito-borne diseases, including chikungunya and dengue incurring huge losses to human health. In the wake of the rampant occurrence of such diseases, much more attention is being paid to control mosquito-borne diseases in broader scale

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in Bangladesh. To do so, it is certainly inevitable to gather information about several driving forces such as climatic, biological factors and physico-chemical properties of breeding habitats, survival and the spatial and temporal distribution of disease vector (Amerasinghe *et al*. 1995, Gimnig *et al*. 2001).

Mosquitoes utilize almost all types of aquatic habitats for oviposition, larval development, emergence, resting, swarming and mating (Overgaard *et al*. 2002). The coexistence of different mosquito species along with other biotic organisms forms a community in the share habitat requirements (Devi and Jahuri 2007). The *Aedes* vectors exploit natural breeding sites such as tree holes, leaf axils (Lee *et al*. 1987) along with artificial containers for larval growth (Gubler 1998). *Armigeres subalbatus* is a vector for bancroftian filariasis closely linked with artificial habitats and also breeds in tree holes (Carpenter *et al*. 1982).*Culex quinquefasciatus* is the most adaptable species choose to breed in both polluted and clear waters (Forattini 2002), while *Cx. tritaeniorhynchus* prefer water storage containers to breed (Kanojia *et al*. 2010).

In Dhaka city, recreational parks are used for different types of human activities resulted in the deposition of poly bags, coconut barks and discarded bottles etc. which provide favorable environments for mosquito breeding (Dom *et al*. 2013). Early and intermittent rains in 2017, lead to water logging in both natural and artificial habitats which favored increase of *Aedes* population, thereby exacerbating the recent emergence of dengue and chikungunya in Dhaka city. Habitat ecology of mosquito plays an important role in determining larval densities, proliferation and species assemblage and mosquito control programs (Simsek 2004). It is important to have sound knowledge on mosquito breeding site preferences require for formulating an effective mosquito control strategy (Ijumba and Lindsay 2001). However, information on mosquito larval ecology is inadequate and the awareness is lacking in Bangladesh. Despite a few studies on mosquito larvae abundance in urban areas in Dhaka city made by Khan (1980) and Ahmed *et al*. (1990). Bashar *et al.* (2014) and Sultana *et al*. (2016), explicit data about how different types of breeding habitats and their properties influence the occurrence of mosquito species in this area are scare till date.

To do so, we analyzed the abundance of different mosquito species along with the degree of association in both natural and artificial habitats. We also determined the physical and physico-chemical properties that influence on the distribution of mosquito in the parks of Dhaka city.

MATERIAL AND METHODS

Sampling sites: The study was conducted in the three parks of Dhaka city, namely Osmani Uddyan (23⁰43″33.34′ N to 90⁰24″27.17′ E), Suhrawardi Uddyan (23⁰43″57.80′N to 90⁰23″59.01′E), and Baldha Garden (23⁰43″00.21′N to 90⁰25″09.24′E) (Fig. 1). A total of six breeding habitats such as tree hole, leaf axils, drain, coconut shell, discarded tire and discarded water bottle were selected from these parks.

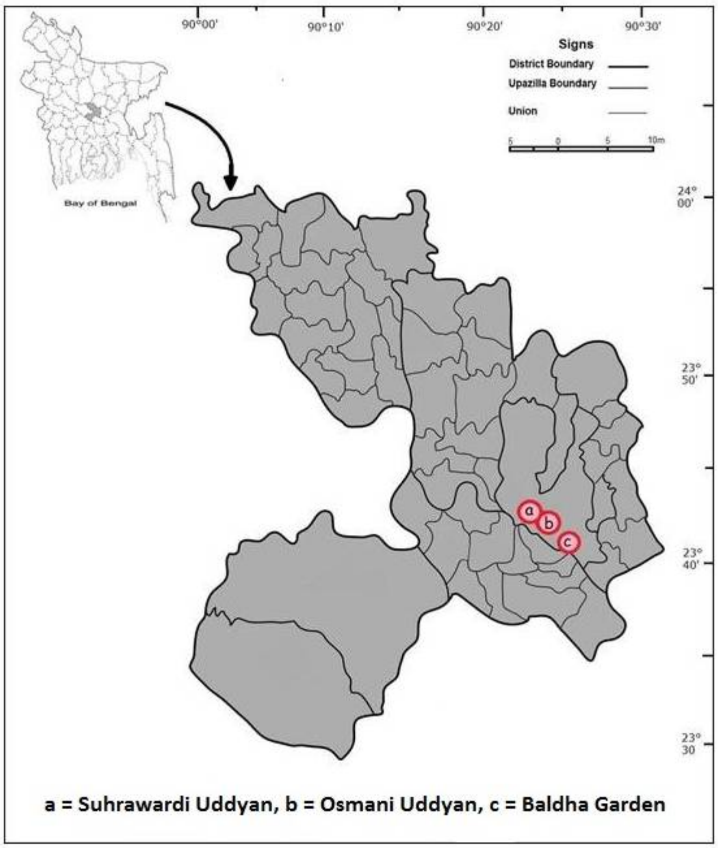

Fig. 1. Map of old Dhaka city in Bangladesh indicating three parks.

Mosquito larval sampling and identification: A total of 182 breeding sites belonging to the following six breeding habitats were randomly sampled: Tree hole (n = 38), leaf axils (n = 30), water bottle (n = 42), tire (n = 23), drain (n = 29), and coconut shell (n = 20). Tree hole and leaf axils were mostly found in the following tree species: *Artocarpus heterophyllus*, *Anthocephalus chinensis*, *Delonix regia*, *Mangifera indica*, *Musa sapientum*, *Litchi chinenses*, *Mimusops elengi*, *Ployalthia longifolia*, *Artabotryss uncinatus*, *Hevea brasiliensis*, *Syzygium* *jambos*, *Syzygium cumini* and *Accacia moniformis*. Larvae were collected from different breeding sites by using dipper (Service 1993) and different size pipettes once in a month during the period of May to October in 2015. The collected larvae were kept in small plastic jars with water in the cages for adult emergence. A few of them were taken into 70% alcohol in vials for further identification. Adults were identified morphologically by using taxonomic methods (Rueda 2004) with the help of a stereoscopic microscope.

Characterization of larval habitats: In order to analyze the physical properties of each breeding habitat, several parameters such as depth, width, vegetation cover, habitat bottom and light condition were recorded. Habitat depth and width were measured using a metal ruler in sampling points. While the presence or absence of vegetation, bottom status and light were visually codified as present = 1 and absent = 0. The nature of habitat bottom was classified into muddy and transparent while light condition was classified into shaded and semi-shaded.

Water chemistry: Water pH and electrical conductivity of mosquito breeding habitats were calculated by pH meter and electrical conductivity meter (Sense Ion, 156; HACH USA), respectively. Water temperature was recorded with a digital thermometer (Fisher, USA). Other parameters including total dissolved solids (2100Q; HACH, USA) and dissolved oxygen (HQ 30 D; HACH, USA) were measured during larval sampling on site following the standard method by APHA (2005).

Data analysis: The percentages of positive habitats were calculated as the number of habitats in which species were counted and divided by the total number of habitats. Levene test of equality was used to evaluate the homogeneity of variance of the mosquito species across all breeding sites. The relationship between species and physical characteristics of mosquito breeding habitats, including sunlight, vegetation and the nature of the bottom determine by a stepwise multiple regression test. One-way ANOVA was performed with density and physico-chemical parameters to determine the differences between the larval density and physico-chemical parameters in all the breeding habitats. When the significant effects were observed, the mean values of larval density and physico-chemical parameters were distinguished by Tukey (HSD) test. All statistical analyses were executed with STASTICA 8.0 (Statsoft2007). To determine whether a linear or unimodal type of relationship was present along environmental gradients, the data set was first analyzed using a detrended correspondence analysis (DCA) in CANOCO 4.5 (TerBraak and Smilauer1998). While, the length gradient of DCA was > 3.0, canonical correspondence analysis (CCA) was preferred to investigate the association between species abundance and physico-chemical factors. In addition, Monte-Carlo permutations were used to identify the statistical importance of Eigen values and species-environment correlations generated by the canonical correspondence analysis (CCA).

RESULTS AND DISCUSSION

Concurrence and distribution of mosquito larvae in breeding habitats: A total of 3217 mosquito larvae belonging to five species under three genera were encountered from six breeding habitat types, namely *Aedes albopictus*, *Aedes aegypti*, *Armigeres subalbatus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Table 1). Of them, *Ae.albopictus*, *Ar. subalbatus* and *Ae. aegypti* were found in both natural and artificial habitats. Coexisting nature of *Ae. aegypti* and *Ae. albopictus* was found in different habitats providing insinuation to the shared habitat (Hawley 1988). The highest frequency of coexistence among three mosquito species (*Ae. albopictus*, *Ae. aegypti* and *Ar. subalbatus*) were observed in tree holes, accounting for 82.4%, followed by 63.2% in coconut shell (*Ae. aegypti*, *Ar. subalbatus* and *Cx. quinquesfasciatus*) and 35.2% in water bottle (*Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus*).

Table 1. Concurrence of mosquito larvae occupied by *Ae. albopictus* (ALB), *Ae. aegypti* (AEG), *Ar. subalbatus* (AS), *Cx. quinquefasciatus* (CQ) and *Cx. tritaeniorhynchus* (CT) in the study sites.

Sites	Species	Positive habitats (%)	Frequencies of occurrence (%)	No. of Larvae
Tree hole (n = 38)	*Ae. albopictus*	3 (7.9)	5.89	63
	Ar. subalbatus	1 (2.6)	2.14	23
	ALB + AS	3 (7.9)	9.52	102(63 + 39)
	ALB + AEG + AS	30 (78.9)	82.4	883(645 + 110 + 128)
Leaf axils (n = 30)	*Ae. albopictus*	28 (93.3)	100	198
Water bottle (n = 42)	*Ae. aegypti*	4 (9.5)	7.23	16
	Cx.quinquefasciatus	5 (11.9)	18.0	40
	AEG + CQ	7 (16.7)	39.3	87(35 + 52)
	ALB + AEG + CQ	17 (40.4)	35.2	78(25 + 28 + 25)
Tire (n = 23)	*Cx.quinquefasciatus*	5 (21.7)	52.0	127
	AEG + CQ	1 (4.3)	4.5	11(6 + 5)
	ALB + AEG + CQ	15 (65.2)	43.4	106(29 + 28 + 49)
Drain (n = 29)	*Cx. quinquefasciatus*	21 (72.4)	67.1	761
	CQ + CT	8 (27.6)	32.9	373 (362 + 11)
Coconut shell (n = 20)	*Ae. aegypti*	3 (15)	10.7	37
	Cx. quinquefasciatus	1(5)	0.2	1
	AEG + CQ	2 (10)	13.1	45 (35 + 10)
	AEG + AS	2 (10)	12.5	43(34 + 9)
	AEG + AS + CQ	12(60)	63.2	217 (145 + 38 + 34)

Ae. albopictus, notably, occurred alone in leaf axils with the highest frequencies of 100% as opposed to the findings by Sultana *et al.* (2012). They found the highest density of *Ae. albopictus* in tree hole (2.02 ± 0.17). In the present study, the highest density of *Ae. aegypti* and *Ar. subalbatus* was observed in coconut shell (1.25 ± 0.23) and tree hole (0.50 ± 0.09) (Fig. 2). In the rainy season, coconut shells remain full of water with the dirty humus, which is preferable for the breeding of *Ae. Aegypti* (Khan *et al*. 2014). Basher *et al.* (2006) found *Ar. subalbatus* in coconut shells. *Cx. quinquefasciatus* occurred with the highest density (3.87 ± 0.22) in drain; whereas, *Cx. tritaeniorhynchus* had the least density (0.03 ± 0.01). *Cx. quinquefasciatus* occurs in a wide variety of aquatic habitats abundantly in which stagnant drains were the most suitable

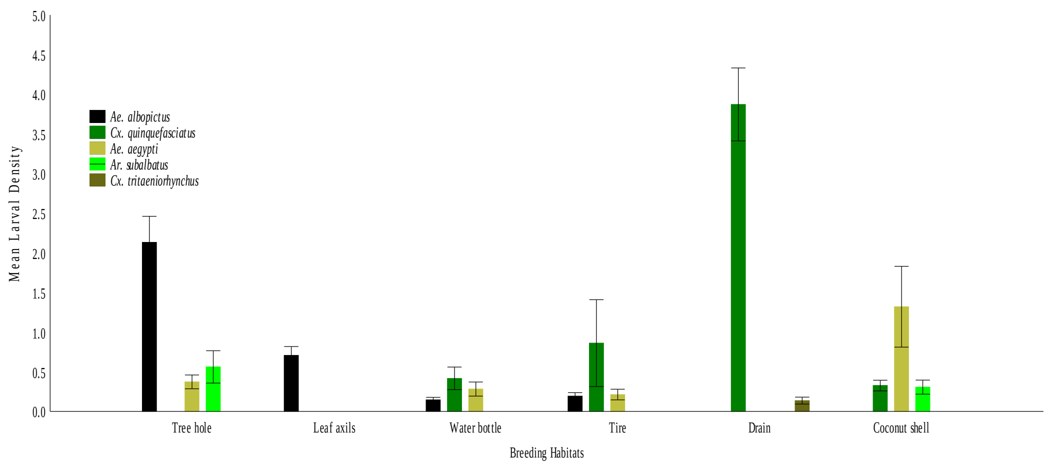

Fig. 2. Larval density of five mosquito species in their breeding habitats.

for its regeneration (Ali *et al*. 1999, Khan *et al*. 2014). *Cx. tritaeniorhynchus* was found in drain, which showed the conformity with the findings of (Bashar *et al*. 2014, Bashar *et al*. 2016, Farjana *et al*. 2015). *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* were in the same habitat might be due to the presence of suitable breeding places with drain water. *Cx. quinquefasciatus* cohabits with the *Aedes* species in tire, water bottle and coconut shell reported by (Juliano *et al.* 2004). ANOVA results showed that density of all species varied significantly across all the habitats (F = 61.70, p = 0.00). Tukey (HSD) test further exhibited that tree hole and drain habitats were the most significant habitats for *Ae. albopictus* and *Cx. quinquefasciatus,* respectively. The densities of these two species were significantly differed within and between species across all the habitats. The Pearson Chi square test suggested that the association of species was significantly dependent on the habitat types (x^2 = 570.09, p = 0.00), inferring that mosquito preferred to occur in a habitat where they would positively cohabit with other species.

Physical characteristics of larval habitats: The results of multiple regression analysis showed that the density of all mosquito species varied significantly across all the breeding habitats for its physical characteristics (Table 2). Among

Table 2. Stepwise multiple regression analysis for physical variables of breeding habitats in relation to mosquito density.

Species	Physical variables	R^2	F	Beta	SE	p
Ae. albopictus	Shaded	0.091	11.419	0.244	0.0723	0.0009
Ae. Aegypti	Semi-shaded	0.076	14.768	-0.275	0.0717	0.0002
Ar. subalbatus	Shaded	0.156	33.326	0.395	0.0685	0.0000
Cx. quiquefasciatus	Vegetation	0.785	657.035	0.751	0.0455	0.0000
	Muddy bottom	0.805	18.61	0.196	0.0458	0.0000
Cx. tritaeniorhynchus	Vegetation	0.215	49.219	0.463	0.066	0.0000

all the physical variables, the degree of light availability appeared to be a predictor factor for the occurrence of *Ae. aegypti*, *Ae. albopictus* and *Ar. subalbatus*. Both *Ae. albopictus* (R^2 = 0.091, p <0.001) and *Ar. subalbatus* (R^2 = 0.156; p < 0.001) showed a significant relationship with shaded environment while *Ae. aegypti* preferred semi-shaded habitats (R^2 = 0.076, p < 0.001). *Aedes* sp. prefers to occur in shaded areas (Bashar *et al*. 2016).Vegetation showed to have a significant relationship with both *Cx. quinquefasciatus* (R^2 = 0.785, p < 0.001) and *Cx. tritaeniorhynchus* (R^2 = 0.215, p < 0.001). *Cx. quiquefasciatus* also showed significant association with the muddy substrates (R^2 = 0.805; p < 0.001). This finding was inconsistent with the results obtained by (Basher *et al*. 2016) stating that *Cx. quiquefasciatus* and *Cx. tritaeniorhynchus* were negatively associated with emergent vegetation. In both natural and artificial habitats, *Cx. quiquefasciatus* was associated with the presence of vegetation (Pires and Gleiser 2010). Likewise, vegetation and urbanized areas are suggested to associate with the occurrences of *Cx. tritaeniorhynchus* larvae (Sallam *et al*. 2013).

Physico-chemical characteristics of mosquito breeding habitats: The highest temperature was recorded in tire water (30.3°C) and the lowest in leaf axils (22.2°C) (Table 3). The pH ranged in between 6.6 (tire and drain) and 7.1 (tree hole and leaf axils) was observed. The highest DO (5.2 mg/l) was observed in leaf axils and the lowest DO (3.6 mg/l) possessed in the drain. Mean conductivity ranging from (256.0 ± 66.8) µS/cm to (929.9 ± 145.9) µS/cm varied greatly in the habitats. Tire water possessed the highest dissolved solids (643.2 ± 42.9

mg/l) and the lowest in leaf axils (88.40 ± 10.1 mg/l). ANOVA results showed that all physico-chemical parameters varied significantly in all the breeding habitats (F = 64.05, df = 25, p = 0.000). Further Tukey HSD results identified that all parameters in leaf axils and tree hole were varied significantly among all habitats.

Table 3. Physico-chemical parameters of the breeding habitats water (Mean +SE).

Habitat types	Temperature (⁰C)	pH	Dissolved oxygen (mg/l)	Conductivity (µs/cm)	TDS (mg/l)
Tree hole	25.2±3.28	7.1±0.16	4.5±0.99	267.6±101.2	89.68±11.5
Leaf axils	22.2±2.80	7.1±0.17	5.2±0.98	256.0±66.8	88.40±10.1
Water bottle	28.1±3.87	6.8±0.19	4.9±1.25	403.3±142.7	239.4±43.0
Tire water	30.3±3.01	6.6±0.23	4.1±1.12	929.9±145.9	643.2±42.9
Drain	28.0±2.44	6.6±0.33	3.6±0.60	577.8±157.2	306.5±10.3
Coconut shell	28.4±1.74	6.8±0.12	5.1±1.27	808.8±65.3	499.1±21.7
All habitats	26.8±3.96	6.9±0.27	4.6±1.18	498.6±269.3	273.5±192.2

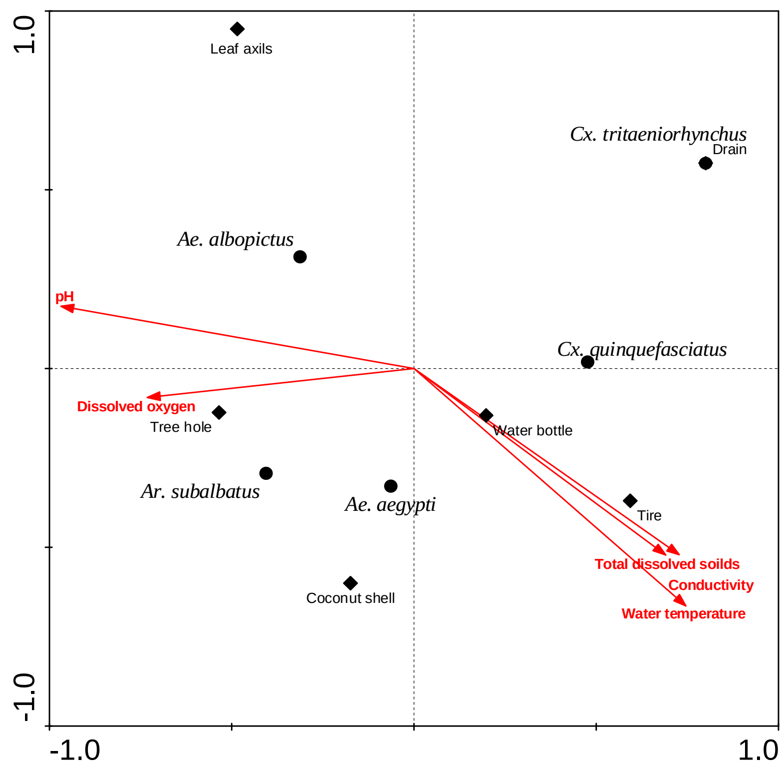

Fig. 3. Ordination plot of mosquito species and physico-chemical variables in habitats on the canonical correspondence analysis (CCA).

Influential role of physico-chemical parameters on larval distributions: From the CCA, the Eigen values of the first (51.9%) and second axes (28.5%) together explained the maximum variation of mosquito species along the physico-chemical parameter gradient (Fig. 3). Species alone showed 51.4% of variation; however, this variation rose up to 60.0% in the presence of physico-chemical factors indicating to having a significant association between species density and physico-chemical parameters. Among five explanatory variables, water temperature, total dissolved solids and conductivity were not linked to the abundance of any mosquito species. Bashar *et al.* (2016) showed that both *Culex* and *Aedes* were not associated with water temperature. However, TDS was found to have a positive relationship with *Cx. quinqueafasciatus* that was also observed by Muturi *et al*. (2008).The density of *Ar. subalbatus* and *Ae. aegypti* were positively related with dissolved oxygen, while higher pH was associated with *Ae. albopictus*. The positive influence of pH and DO on the distribution of *Aedes* mosquito species in the breeding habitats, giving an insight of the suitability of breeding habitats for larval growth and survival. Earlier studies reported that *Aedes* species had an inclination for narrow pH range (6 - 8), while *Culex* and *Armigeres* occurred in the broader range of pH 6-10 (Amarasinghe and Dalapadado 2014). In our previous study, *Ae. aegypti* also showed a significant correlation with DO (Sultana *et al*. 2016). The presence of *Aedes* and *Culex* species in tree hole and drain are influenced by different variables may be useful to identify their actual breeding sites in different parks. Measurements should be implemented to ameliorate the waste management and drainage system in the area. Ecological studies are better to describe the spatial and temporal distribution of vector habitats might help to combat with the recent outbreaks of vector-borne diseases in Dhaka city.

CONCLUSION

Present findings suggested that mosquito larval abundance might be influenced by both physical and physico-chemical variables where each variable has an effect on mosquito breeding habitats preference. The coexistence nature of different mosquito species identified that bolstered an idea about their choice of shared habitats for oviposition. *Ae. albopictus* showed an affinity to shaded habitats with water pH. *Ae. aegypti* and *Ar. subalbatus* preferred shaded and semi-shaded habitats in the presence of the higher dissolved oxygen, respectively. *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus* occurred in the breeding habitats (drain) having vegetation. A strong positive association between mosquito abundance and the physical and physico-chemical characteristics of breeding habitats inferred that these habitats were well suitable for larval oviposition, growth and development. Therefore, present findings might help to further research on mosquito ecology focusing on breeding water chemistry and influencing environmental variables that might be implemented to identify the actual breeding sites for vector control; thus, enhancing the effectiveness to design breeding habitat control strategies.

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