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How dengue vector *Aedes albopictus* (Diptera: Culicidae) survive during the dry season in Dhaka City, Bangladesh?

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ABSTRACT

Background & objectives: In 2000, a dengue outbreak occurred in Bangladesh that included Dhaka City. Both dengue vectors, *Aedes aegypti* and *Ae. albopictus* are present in Bangladesh. *Aedes aegypti* mosquitoes mainly breed in and around houses and *Ae. albopictus* is an outside breeder. There are many old trees throughout Dhaka City in different parks, streets and the university campus which may have holes that can contribute as potential breeding habitat for the dengue vector. Therefore, a survey was conducted to investigate the presence of eggs of the dengue vector mosquitoes in treeholes during the dry season in February 2001 to know their contribution on dengue outbreaks.

Methods: All treeholes in 10 different localities (parks, streets and university campus) of Dhaka City were surveyed. All trees were examined for treeholes up to the height of approximately 3 m and sampled. Debris were collected and packed in poly bags and brought to the laboratory for detailed studies. These were then soaked with tap water to observe egg hatching. The soaked materials were kept up to 20 days covered by a fine mosquito net. After 2–3 days, the eggs started hatching and larvae were separated from the sample for rearing up to IV instar.

Results: A total of 245 treeholes were surveyed in 49 identified tree species and 18 unidentified trees. Altogether, 1365 *Aedes* larvae were found, of which 1096 were *Aedes albopictus* and 269 were other *Aedes* species. The largest number of larvae was observed in *Delonix regia* of Leguminosae family. The number of *Aedes albopictus* found in the treeholes have perfect positive correlation with the number of other *Aedes* species. Not a single egg of *Aedes aegypti* was found in this survey.

Interpretation & conclusion: This information will inform public health workers as well as the national control programme to help to solve mosquito borne diseases specially that of dengue. This is critical in planning for vector control operations due to the diversity of dengue outbreak in the nature.

Key words *Aedes albopictus*; debris; dengue; dry season; treeholes

INTRODUCTION

Mosquitoes are the most prominent of the numerous species of blood sucking insects that annoy human and other warm-blooded animals. Blood sucking mosquitoes play an important role in medical entomology[1]. Mosquitoes are the sole vectors of the pathogens causing malaria, yellow fever, dengue fever/dengue hemorrhagic fever (DF/DHF), Japanese encephalitis and they are of prime importance in spreading filariasis. Apart from yellow fever, all other four above-mentioned mosquito borne diseases prevail in Bangladesh[2–6].

Dengue fever (DF) and especially the more severe manifestation dengue hemorrhagic fever (DHF), rank highly among newly emerging infectious diseases in public health significance and is considered to be one of the most important arthropod-borne viral diseases[7]. There were 22,42,022 dengue cases and 14,031 deaths reported from WHO South-East Asia Region for the period of 2003 to 2012[8]. Dengue viruses are transmitted to humans through the bite of infected female *Aedes* mosquitoes principally *Aedes aegypti* and *Ae. albopictus* (Asian tiger mosquito). It was identified that *Ae. albopictus* was responsible for dengue transmission in Dhaka City, Bangladesh during the outbreak in 2000[3]. In 2001, dengue fever occurred among employees of a recreation club in Dhaka where both vectors *Ae. aegypti* and *Ae. albopictus* were found[4].

In 2000, an outbreak of DF and DHF occurred in Bangladesh with 5555 cases and 93 deaths[9–10]. The outbreak was mainly in Dhaka City[11] where more wealthy populations resided. Since then, every year cases are being reported by the Directorate General of Health Services (DGHS). During the last 10 yr (2003 to 2012)

12,229 cases and 46 deaths were reported from Bangladesh[8].

Treeholes are among the most widely spread class of natural container habitats for mosquitoes; species of several genera of mosquitoes breed in them[12–13]. It is well known that *Aedes* can be collected from dry debris in treeholes[14–18] observed that females of *Ae. albopictus* select treeholes of a wide range of properties for breeding. Dengue antigen positive *Ae. albopictus* were identified from treeholes[19] which represent one of the important natural habitat for dengue vectors in an urban environment. Though, treeholes have been identified as a potential breeding habitat for *Aedes* mosquitoes in other places, we have very little information from Bangladesh. It was expected that the current study would enrich our knowledge on dengue vectors that use treeholes as their breeding habitat. Thereafter, based on the evidence from this study, the national progamme can take action to help control the dengue vector to reduce transmission in high endemic areas. From a national point of view, the present study is highly important as dengue is one of the most common vector borne diseases in the country that has a high impact on public health.

In Bangladesh, the dry season starts in mid-November and continues to early-March. During the dry season, most areas become dry as there is little rain. Therefore, the present study was aimed to investigate how dengue vectors survive during the long dry spell (winter season) in the capital city of Bangladesh in a common natural habitat, the "treehole".

MATERIAL & METHODS

Study area

For the collection of mosquito eggs in the debris of treeholes, altogether 245 treeholes were sampled at 10 different localities in Dhaka City in February 2001. Sampled trees were identified using a botany book[20]. The sampling areas in Dhaka City were Sohrawardhi Uddayan, Ramna Park, Osmani Uddayan, Baldha Garden, Dhaka University Campus, Gulshan Park, Shahid Capt. Mansur Ali Sarani (Mogbazar, Kakrail, Minto Road, Baily Road and High Court Area), Sher-*e*-Bangla Nagar, Chandrima Uddayan and the ICDDR, B premises (Fig. 1).

Collection and processing of the sample

In each area, a survey team was assigned to conduct the survey. A survey team consisted of three research assistants (one female and two males). The members of survey team were given intensive training about the treeholes survey procedure. For sample collection, a procedure was maintained in which a central point was marked in each locality and from this point, the locality was divided approximately into four parts. The collection was started from one part and then the other parts were completed in a cyclic manner. In each part, all trees present were examined and treeholes up to the height of approximately 3 m were sampled. In case of roads, treeholes sample collection was continued from one end to other. Collections were made from treeholes by removing dry debris following procedures as previously described[14–17]. Eggs were collected by scraping the walls of treeholes following the procedure described earlier[21]. In addition, for collecting loose material from the bottom of treeholes, the inside walls were carefully scraped with a metal spoon to recover the maximum number of eggs as previously described as well[15]. From the treeholes, eggs were sometime collected by filling the dry habitats with water and then siphoning or pumping out the contents[22]. Standing water or moist organic matter was also removed from treeholes by siphoning. Collections were packed in plastic bags and were brought to the laboratory for detailed studies.

Fig. 1: Study locations in Dhaka City.

Rearing and identification of the sample

The samples were soaked with tap water to observe egg hatching. The soaked materials were kept up to 20 days covered by a fine mesh mosquito net. After 2–3 days, the eggs started hatching and larvae were separated from the sample for rearing up to IV instar. Egg hatching was observed for 20 days but no hatching was found after six days. Then the larvae were counted and identified with the help of a phase contrast stereo binocular microscope. For identification, the larvae were temporarily mounted by glycerine on the slides. Identifications were made as previously described[23–24]. Except *Ae. albopictus*, other *Aedes* species were identified up to genus level.

Statistical analysis

The data were prepared and organized in MS Excel spread sheet and statistical analysis was carried out using STATA/SE 12.0 for windows (2011) package. The linear correlation between variables were calculated using Pearson's correlation coefficient (r) and significance of estimated correlation coefficients were tested using t statistic with appropriate degrees of freedom. The multivariate regression modeling procedure was used to predict a set of dependent variables that have multivariate normal distribution with appropriate mean vector and variance-covariance matrix using the same independent variable and significance of the models was tested using F-statistic and significance of the individual slope coefficients were tested using t-statistic. Whether the frequencies of a categorical variable were uniformly distributed among the categories were tested using non-parametric Chi-square statistic. For each of the hypothesis test, probability value (p) was calculated and compared to either 1% or 5% level of significance (α). If the p-value was less than the α value, than the test was considered as significant.

RESULTS

The numbers of *Ae. albopictus* and other *Aedes* species present in treeholes and types of tree species are shown in Table 1. A total of 245 treeholes were found in 49 identified tree species and 18 unidentified trees. A total of 1365 *Aedes* larvae were observed in the laboratory. Out of the 1365 larvae, 1096 were *Ae. albopictus* and the remaining 269 larvae belonged to other species of the genus *Aedes* (Table 1). Of 245 treeholes surveyed, 30 (12.2%) were *Aedes* positive. Among these positive trees, 10 were from the family of Leguminosae. Under this family, it was observed that the largest number (larvae) was observed for *Delonix regia*. Of these identified larvae, 572 were *Ae. albopictus* and 112 were other *Aedes* species (Table 1). Under the Ebenaceae family, a single tree *Diospyros peregrina* was surveyed and it was *Aedes* positive which contained 196 *Ae. albopictus* and 49 other *Aedes* species (Table 1). In *Azardirachta indica* under the family Meliaceae, four treeholes debris were examined, of which two were *Aedes* positive. The larvae developed from this sample were abundant with 100 *Ae. albopictus* and 40 from other *Aedes* species. In this study, 26 *Mangifera indica* treeholes were surveyed, of which three treeholes were *Aedes* positive. From the total 55 *Aedes* larvae categorised, 42 were *Ae. albopictus* and 13 other *Aedes* species (Table 1). In this survey, two positive treeholes were found from the tree species of *Albizia procera*, *Azardirachta indica, Tectona grandis*, *Lagerstroemia parviflora* with 4, 140, 9 and 9 larvae respectively (Table 1). Only one positive treehole was found in the tree species of *Averrhoa carambola*, *Cyclostamon assamicus*, *Acacia moniliformis*, *Samanea saman*, *Ficus benghalensis*, *Ochna squarrosa*, *Roystonea regia, Litchi chinensis*, *Syzygium cumini,* and *Syzygium samaranese.* Relatively large numbers of larvae were found from trees of *Syzygium samaranese, Litchi chinensis* and *Averrhoa carambola.* No positive treehole was found in the tree species belonging to the families of *Annonaceae, Apocynaceae, Combretaceae, Liliaceae, Magnoliaceae, Oleaceae, Rubiaceae, Rutaceae, Rhamnaceae* and *Sapotaceae* (Table 1).

Out of 50 tree species surveyed, only 18 tree species were found *Aedes* positive (*Ae. albopictus* and other *Aedes* spp). The number of *Ae. albopictus* found in the treeholes have nearly perfect positive correlation with the number of other *Aedes* species [r (48) = 0.98, p <0.01]. The estimated multivariate regression model results that can predict the number of *Aedes* positive treeholes, *Ae. albopictus* and other *Aedes* species from the number of treeholes surveyed are: Number of *Aedes* positive treeholes = 0.0188+ 0.1186 Number of treeholes surveyed (R^2 = 0.59, $F_{1, 48}$ = 69.36, p <0.01); Number of *Ae. albopictus* = –10.8840 + 6.6947 Number of treeholes surveyed (R^2 = 0.30, $F_{1, 48}$ = 20.85, p <0.01); and Number of other *Aedes* species = –1.0854 + 1.3195 Number of treeholes surveyed (R^2 = 0.27, $F_{1, 48}$ = 17.32, p <0.01) (Table 2).

The percentage of positive treeholes for *Aedes* larvae in different spots of Dhaka City is shown in Table 3 and which are not equally distributed [$\chi^2(8) = 159.58, p<0.01$]. The highest number of treeholes was surveyed in Ramna Park, the largest park in Dhaka City. The percentage of positive treeholes and the number of larvae are also high in this locality. On the basis of the larval positivity in treeholes, Sohrawardhi Uddayan ranked in second posi-

Table 1. Number of *Aedes* species found in treeholes by families and their species in Dhaka City in 2001

Family name of surveyed trees	Scientific name of tree species	No. of tree-holes surveyed	No. of *Aedes* positive treeholes	No. of *Aedes* mosquitoes		Total
				Ae. albopictus	Other *Aedes* spp	
Anacardiaceae	*Mangifera indica*	26	3	42	13	55
	Lannea coromandelica	1	0	0	0	0
Annonaceae	*Artabotrys uncinatus*	4	0	0	0	0
Apocynaceae	*Allamanda cathartica*	2	0	0	0	0
	Nerium indicum	2	0	0	0	0
	Plumeria acutifolia	1	0	0	0	0
Averrhoaceae	*Averrhoa carambola*	5	1	24	3	27
Combretaceae	*Terminalia arjuna*	1	0	0	0	0
	Terminalia catappa	1	0	0	0	0
Ebenaceae	*Diospyros peregrina*	1	1	196	49	245
Euphorbiaceae	*Baccaurea ramiflora*	1	0	0	0	0
	Cyclostemon assamicus	5	1	1	0	1
Leguminosae	*Accacia catechu*	1	0	0	0	0
	Accacia moniliformis	9	1	5	0	5
	Acacia nilotica	2	0	0	0	0
	Albizia procera	20	2	4	0	4
	Butea superba	2	0	0	0	0
	Cassia fistula	1	0	0	0	0
	Delonix regia	33	6	572	112	684
	Samanea saman	2	1	1	0	1
	Saraca indica	3	0	0	0	0
	Tamarindus indicus	2	0	0	0	0
Liliaceae	*Dracaena* spp	1	0	0	0	0
Lythraceae	*Lagerstroemia parviflora*	4	2	7	2	9
Magnoliaceae	*Magnolia grandiflora*	2	0	0	0	0
Meliaceae	*Azardirachta indica*	4	2	100	40	140
Moraceae	*Ficus benghalensis*	15	1	4	0	4
	Ficus hispida	1	0	0	0	0
	Artocarpus heterophyllus	20	2	6	1	7
Myrtaceae	*Syzygium cumini*	3	1	1	3	4
	Syzygium jambos	1	0	0	0	0
	Syzygium samaranese	3	1	65	18	83
	Psidium guajava	4	0	0	0	0
Ochnaceae	*Ochna squarrosa*	1	1	2	0	2
Oleaceae	*Nyctanthes arbortristis*	1	0	0	0	0
Palmae	*Areca catechu*	1	0	0	0	0
	Cocos nucifera	3	0	0	0	0
	Roystonea regia	1	1	14	7	21
Rubiaceae	*Gardenia jasminoides*	2	0	0	0	0
	Anthocephalus chinensis	1	0	0	0	0
Rutaceae	*Murraya paniculata*	1	0	0	0	0
	Citrus grandis	1	0	0	0	0
Rhamnaceae	*Alege marmelos*	2	0	0	0	0
	Zizyphus mauritiana	3	0	0	0	0
Sapindaceae	*Litchi chinensis*	9	1	46	18	64
Sapotaceae	*Manilkara achras*	1	0	0	0	0
Verbenaceae	*Tectona grandis*	5	2	6	3	9
Unknown	*Bassia latifolia*	1	0	0	0	0
	Dalbergia sissoo	11	0	0	0	0
	Unknown	18	0	0	0	0
Total		245	30	1096	269	1365

Table 2. Multivariate regression estimates for number of *Aedes* positive treeholes, *Ae. albopictus* and other *Aedes* species using number of treeholes surveyed

Independent variable	Coefficient	Standard error	t	P>|t|	95% Confidence interval
Dependent = Number of positive treeholes					
Constant	0.0188	0.1215	0.15	0.878	(– 0.2254, 0.2630)
Number of treeholes surveyed	0.1186	0.0142	8.33	0.0	(0.0900, 0.1472)
Dependent = Number of *Aedes albopictus*					
Constant	–10.8840	12.5054	– 0.87	0.388	(– 36.0277, 14.2598)
Number of treeholes surveyed	6.6947	1.4663	4.57	0.0	(3.7466, 9.6428)
Dependent = Number of other *Aedes* species					
Constant	–1.0854	2.7038	– 0.40	0.690	(– 6.5218, 4.3509)
Number of treeholes surveyed	1.3195	0.3170	4.16	0.0	(0.6821, 1.9569)

Table 3. Percentage of positive treeholes for *Aedes* larvae in 10 different localities of Dhaka City

Locality	Visited tree-holes	*Aedes* positive treeholes	% of positive treeholes	*Aedes* species		
				No. of *Ae. albopictus*	No. of *Aedes* spp	Total
Ramna Park	36	16	44.4	766	167	933
Sohrawardhi Uddayan	16	5	31.3	145	45	190
Baldha Garden	26	3	11.5	81	25	106
Chandrima Uddayan	21	2	9.5	22	7	29
Dhaka University Campus	33	2	6.1	69	21	90
Shahid Capt. Mansur Ali Sarani	24	1	4.2	2	1	3
Osmani Uddayan	34	1	2.9	11	3	14
Gulshan Park	20	0	0	0	0	0
Sher-*e*-Bangla Nagar	20	0	0	0	0	0
ICDDR, B Premises	15	0	0	0	0	0
Total	245	30		1096	269	1365

tion (Table 3). The number of larvae positive treeholes are comparatively low in the Baldha Garden, Chandrima Uddayan, Dhaka University Campus, Shahid Capt. Mansur Ali Sarani and Osmani Uddayan. Fifty-five treeholes were visited in Gulshan Park, Shere-*e*-Bangla Nagar and ICDDR, B premises, but none had found larvae.

Different sizes of treeholes with larvae by the tree types are shown in Table 4. The highest (36%), second highest/moderate (25%) and lowest (8%) number of *Aedes* larvae positive treehole sizes were of diameter 50.1–62.5, 62.6–75 and 1–12.5 cm, respectively. The percentages of *Aedes* larvae positive treeholes were significantly different in different treehole sizes [χ^2 (6) = 39.33, p <0.01].

Types of debris collected from different treeholes and the number of larvae present is shown in Table 5. *Aedes* positive treeholes found with debris of soil; sand with rotten wood and leaves; and granular type are 13, 8 and 15%, respectively. Treeholes with liquid debris, none of them found larvae positive. The percentages of *Aedes* positive treeholes found in different debris types were significantly different [χ^2 (3) = 15.53, p <0.01] but excluding liquid debris from the analysis, the percentages were nonsignificantly different [χ^2 (2) = 2.65, p = 0.26 >0.05].

DISCUSSION

DF and DHF are major public health concerns in Dhaka City since 2000, and are now gradually spreading to major cities throughout the country. Due to lack of regular vector surveillance on dengue, it is hard to know how the vectors survive during the dry season? In this study, we found vector eggs in the treeholes during dry season. In this form, eggs could survive over several months during the dry season or over winter which is known as diapause condition. In Bangladesh, the dry season includes winter which lasts from November to mid-March[25]. The dry season is characterized by clear skies,

Table 4. *Aedes* mosquito positive treeholes number by tree species and the size of the holes in Dhaka City

Tree name	1–12.5 cm	12.6 –25 cm	25.1–37.5 cm	37.6 –50 cm	50.1–62.5 cm	62.6 –75 cm	> 75 cm
Acacia nilotica	2	0	0	0	0	0	0
Acacia catechu	1	0	0	0	0	0	0
Acacia moniliformis	5	0	2	1 (1)	1	0	0
Aegle marmelos	0	1	0	1	0	0	0
Albizia procera	9 (1)	5	4 (1)	0	0	0	2
Allamanda cathartica	2	0	0	0	0	0	0
Anthocephalus chinensis	1	0	0	0	0	0	0
Areca catechu	1	0	0	0	0	0	0
Artocarpus heterophyllus	4	8 (1)	3	2	0	2	1 (1)
Artabotrys uncinatus	2	0	0	0	0	0	2
Averrhoa carambola	2	1	1	0	1 (1)	0	0
Azadirachta indica	2 (1)	1	0	0	1 (1)	0	0
Bassia latifolia	0	1	0	0	0	0	0
Baccaurea ramiflora	0	0	0	1	0	0	0
Butea superba	0	0	1	1	0	0	0
Cassia fistula	0	0	0	0	0	0	1
Citrus grandis	1	0	0	0	0	0	0
Cocos nucifera	2	0	1	0	0	0	0
Cyclostemon assamicus	3 (1)	2	0	0	0	0	0
Delonix regia	5 (3)	4	5 (1)	5 (1)	2	2 (1)	10
Diospyros peregrina	0	0	0	0	0	0	1 (1)
Dracaena spp	1	0	0	0	0	0	0
Dalbergia sissoo	10	1	0	0	0	0	0
Ficus benghalensis	6	1	1	0	0	2 (1)	5
Ficus hispida	1	0	0	0	0	0	0
Gardenia jasminoides	1	1	0	0	0	0	0
Lagerstroemia parviflora	2 (1)	0	0	1	0	0	1 (1)
Lannea coromandelica	0	0	1	0	0	0	0
Litchi chinensis	0	4 (1)	2	1	2	0	0
Mangifera indica	6	4 (1)	3	3	0	0	10 (2)
Magnolia grandiflora	2	0	0	0	0	0	0
Manilkara achars	1	0	0	0	0	0	0
Murraya paniculata	1	0	0	0	0	0	0
Nerium indicum	2	0	0	0	0	0	0
Nyctanthes arbortristis	0	0	0	0	0	1	0
Ochna squarrosa	1 (1)	0	0	0	0	0	0
Plumeria acutifolia	0	0	0	0	0	1	0
Psidium guajava	2	0	0	2	0	0	0
Roystonea regia	0	0	0	0	0	0	1 (1)
Samanea saman	0	0	1 (1)	1	0	0	0
Saraca indica	3	0	0	0	0	0	0
Syzygium cumini	0	1	0	1	1 (1)	0	0
Syzygium samaranese	0	1	0	1	1 (1)	0	0
Syzygium jambos	0	1	0	0	0	0	0
Tamarindus indicus	0	0	0	1	1	0	0
Tectona grandis	1	1 (1)	0	0	0	0	3 (1)
Terminalia arjuna	1	0	0	0	0	0	0
Terminalia catappa	1	0	0	0	0	0	0
Zizyphus mauritiana	2	1	0	0	0	0	0
Unknown	15	1	0	1	1	0	0
Total	101 (8)	40 (4)	25 (3)	23 (2)	11 (4)	8 (2)	37 (7)

Size = Depth × Diameter of the holes; Figures in parentheses indicate number of larvae positive holes.

Table 5. Number of larvae in treeholes by types of debris and localities in Dhaka City

Locality	Soil*	Sand*	Granular	Liquid*	Total treeholes
Sohrawardhi Uddayan	10 (3)	3 (1)	1 (1)	2	16 (5)
Ramna Park	25 (11)	7 (3)	4 (2)	0	36 (16)
Osmani Uddayan	14	2	18 (1)	0	34 (1)
Baldha Garden	20 (1)	1	5 (2)	0	26 (3)
Dhaka University Campus	17	5 (1)	10 (1)	1	33 (2)
Gulshan Park	9	16	5	0	20
Shahid Capt. Mansur Ali Sarani	15 (1)	4	5	0	24 (1)
Sher-*e*-Bangla Nagar	12	8	0	0	20
Chandrima Uddayan	8 (1)	4	8 (1)	1	21 (2)
ICDDR, B Premises	9	3	3	0	15
Total	139 (17)	43 (5)	59 (8)	4	245 (30)

*With rotten leaves and woods; Figures in parentheses indicate number of larvae positive holes.

almost no rain and minimum daily temperature of <5–10°C.

Among the surveyed areas, Ramna Park appeared to have a greater number (36) of treeholes. A large number (933) of *Aedes* species larvae were found from 16 treeholes in this park. From these identified larvae, *Ae. albopictus* (766) were the most common from the total of 1096 and other species of *Aedes* (167) out of the remaining 269. Most of the treeholes debris was soil with rotten leaves and woods. The Ramna Park area has had plantation for a long time and have various types of trees, i.e. fruit, timber, and flower. Most of the positive treeholes were of *Delonix regia* and *Mangifera indica.* The older trees may have some advantages for mosquito's oviposition. This park is the largest in the city located in the central area, thus, is the major recreational site for city dwellers. It was observed that *Ae. albopictus* attacked humans predominately in open areas near densely populated regions[26].

Sohrawardhi Uddayan was second in larvae positivity and had about half as many larvae positive holes as Ramna Park. Baldha is an oldest (about 200 yr) garden in Dhaka City and most of the trees are rare and exotic varieties. It therefore could be inferred that the lowest positive treeholes were found for the more exotic tree species. Chandrima Uddayan and Osmani Uddayan have also same type of secondary plantations for about 25 yr. For this reason, no ideal breeding habitat was found in these places. A large number of trees were examined in the Dhaka University Campus but the actual reason of the lowest *Aedes* positive condition is not clear. Shahid Capt. Mansur Ali Sarani is one of the busy roads of Dhaka City, which is also a residential area of elite people. Therefore, the frequently use of insecticides and insecticide fogging may disturb the mosquito habitat. No *Aedes* positive treeholes were found in Gulshan Park, Sher-*e*-Bangla Nagar or the ICDDR,B premises. Gulshan Park was established according to the city development plan about 20 yr ago. In Gulshan Park area, most of the city dwellers are aristocrats and they regularly use insecticide like fogging and spraying. So, it could be suggested that the treeholes of this area were not suitable for mosquito breeding. Sher-*e*-Bangla Nagar is the residential area of government high officials. The regular application of insecticide and formal cleaning activities in this area is not suitable for *Aedes* breeding. *Aedes* negative treeholes were also found in ICDDR,B premises. In spite of available *Aedes* breeding habitat the cause of negative condition was not clear.

In this study, 245 treeholes were surveyed with different sizes and these were arranged in seven categories including; 1–12.5, 12.6–25, 25.1–37.5, 37.6–50, 50.1–62.5, 62.6–75 and >75.1 cm. *Aedes* positive treeholes number were eight, five, three, two, three, two and seven, respectively for these sizes. These findings did not suggest any implication regarding *Aedes* breeding habit and treeholes sizes, which could be explained as the trees were different.

Different kinds of treeholes debris were collected which were differentiated into four groups, namely sandy, soil with rotten leaves and wood, liquid and granular. Most of the *Aedes* positive treehole debris were of granular and soil with rotten leaves and wood type. Humus is present in soil and this environment is suitable for the growing of microorganisms. Therefore, the mother mosquitoes deposit their eggs in this type of debris for the survival of their future generation. But the cause of comparatively high amount of larvae in granular debris was not understood. The number of positive treeholes with sand debris was five. In sand, the low amount of humus was present and the result was fewer deposited eggs. Treeholes with

liquid debris in four holes were surveyed but none was *Aedes* positive. The collected liquid debris contained a large amount of decomposing materials which appeared not to be suitable for *Aedes* larvae. Of 245 visited treeholes, there were two dead trees without *Aedes* larvae. The surveyed number of dead trees was too few to predict the reason for the absence of *Aedes* .

The major vector (*Ae. aegypti*) responsible for dengue transmission was not found in this survey. This indicates that the mosquito, *Ae. aegypti* does not prefer treehole habitats for egg laying. This was also supported by the author of Fauna of British India: Ceylon and Burma[23] but the presence of *Ae. aegypti* was found in a study in southern India[27]. From the present finding, it may be assumed that *Ae. albopictus* prefers the treehole habitats for egg lying more than other *Aedes* species.

The results of this study have thrown light on the possible places with diapaused eggs during the dry season. In 1996, similar type of findings has in Chittagong City (Personal communication with Professor Dr Qamar Banu). In this study, it was also observed that there was no other genus of mosquito which deposited their eggs in diapause condition.

The study indicates that treeholes are a significant breeding habitat for dengue vectors other than *Ae. aegypti*. If there is an *Ae. albopictus* in a treehole, it was most likely that other *Aedes* species were present in the same treehole. The number of *Aedes* positive treeholes can be significantly predicted from the number of treeholes. Also, the number of *Ae. albopictus* and other *Aedes* species can be predicted from the number of positive treeholes. If we could survey 100 more treeholes, on an average there would be 12 *Aedes* positive treeholes that can be found. If we could find one more *Aedes* positive treehole, on an average there would be about 60 *Ae. albopictus* and about 13 other *Aedes* species.

Taken together, this information will help public health workers to address the problem of mosquito borne diseases, specifically that of dengue. This is critical in planning for vector control operations due to the diversity of dengue outbreak in nature.

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