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Rabeya Khatun
Entomology Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh

Kazi Shakhawath Hossain
Plant Pathology Laboratory, Department of Botany, Jagannath University, Dhaka, Bangladesh

Sharmin Akter
Entomology Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh

Roland Nathan Mandal
(1). Center for Environmental and Geographic Services (CEGIS), A Public Trust under the Ministry of Water Resources, Dhaka, Bangladesh
(2). Key Laboratory of Aquatic Genetic Resources and Utilization, College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China

Biplab Kumar Mandal
(1). Entomology Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh
(2). Key Laboratory of Aquatic Genetic Resources and Utilization, College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China

Corresponding Author:
Biplab Kumar Mandal
(1). Entomology Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh
(2). Key Laboratory of Aquatic Genetic Resources and Utilization, College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China

Entomopathogenic fungus spores in the larval habitat water of *Culex quinquefasciatus* mosquito in Dhaka city, Bangladesh

Rabeya Khatun, Kazi Shakhawath Hossain, Sharmin Akter, Roland Nathan Mandal and Biplab Kumar Mandal

Abstract
Density of entomopathogenic fungi spores in the larval habitat water of *Culex quinquefasciatus* was conducted at the Entomology laboratory and Plant Pathology laboratory, Jagannath University, Dhaka, Bangladesh. The larvae and pupae of different mosquito species were counted; water was preserved for fungal culture. Potato Dextrose Agar (PDA) medium was used for culture and different levels of dilution were performed for culture of fungus spores. These stagnant dirty drain water samples have been containing a few (10) Aedes larvae; coexisting with a huge *Culex* at the same breeding ground. A total of 4138 mosquito larvae and pupa were recorded in the collected habitat water where 3928 larvae and 200 pupa of *Culex* were observed with different larval densities among collection points. Out of eighteen (18) fungal isolates under 10 genera (*Absidia* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Nigrospora* sp., *Penicillium* sp., *Rhizopus* sp. and *Sclerotium* sp.) were identified, seven (7) of them have been reported as entomopathogenic by scientists; to date. Eight (8) isolates were belonging to the genus Aspergillus. Relationship of larval density of Culex were very weak to total spores of all fungi (r = -0.154) as well as with the number of individual isolates of fungus spores. Culture of entomopathogenic fungi in laboratory condition, and use of them as a biological control agent for the mosquitoes could not be recommended.

Keywords: Mosquito, entomopathogenic fungus (EPF), isolates, habitat, management

Introduction
Mosquitoes (Order: Diptera and Family: Culicidae), one of the cosmopolitan [1] organism, is a familiar parasitic vectors of a number of transmissible and life menacing diseases such as malaria, filariasis, dengue fever, yellow fever and most of the arthropod borne viral types of encephalitis [2, 3]. They are generally adapted to stagnant water [4, 5] for breeding; some are more tolerant of cold [6, 7]; and a very harmful insect for both human and animals. So, it is very important to know the habitat, breeding place, status and prevalence of mosquito fauna to control mosquito and mosquito borne diseases [8]. Out of a world total of more than 3000 species only about 113 species are recorded in Bangladesh [9]. *Culex* mosquitoes lay around 100 eggsin oval rafts and the rafts are loosely cemented together; the eggs normally hatch within 24-30 hours [10]. Adult females need a warm blood meal to lay eggs [11]; a female mosquito can lay up to five rafts of eggs in a lifetime [12]. They need a steady temperature with a non-agitated water habitat to hatch and develop; otherwise they might die [13]. *Culex quinquefasciatus* usually likes to breed in the water surface mostly rich in compounds-either in water tanks or stagnant shallow waterbodies [14]. It has been noticed laying eggs in shallow ponds within streams phytotelmata[15], and some artificial habitats such as drains sumps, wells, oxidation ponds at sewage treatment plants [16], stock drinking troughs, septic tanks, rainwater containers, tires and various other small containers [17, 18]. They have been reported to share the same habitat with other mosquito and arthropod species [18]. The hatched larvae are able to overwinter in the cooler months [14, 18]. Adult mosquitoes like to breed and move around the warm blooded animal blood food sources, and normally cannot fly more than 1km for foraging [19].
High diversity of freshwater fungal spores are evident now-a-days, but huge studies are required to know the biodiversity of freshwater fungi; it is just estimated that there are approximately 1.5 million fungal species on earth [20]. Among them, around 3000 species are known as aquatic and only 465 species have been reported to occur in marine saline waters [21].

Aquatic environment is considered highly potential for survival of many organisms and thus, extensive investigations regarding fungi biodiversity in water is demanded. Aquatic fungi are anamorphic; usually microscopic organisms, never produce visible fruiting bodies and grow asexually. Mosquito-killing fungi studies have been evolving in the recent years. It is thought that the Entomopathogenic fungi (EPF) may contribute in a significant and sustainable manner to the control of mosquito-borne diseases. Anamorphic fungi that have been found on mosquitoes include some species of the genera *Aspergillus, Fusarium, Paecilomyces, Penicilium,* and *Verticillium* [22-27].

Present research investigates the fungal flora present in the mosquito larvae habitat water in Dhaka city, Bangladesh and figure out the entomopathogenic fungus (EPF) reported by other scientists previously; which can help to know the relationship of those with mosquito larva densities; and also can comment on the potentials of using such EPF for biological mosquito management in nature, especially hot and humid habitats in Dhaka city, Bangladesh.

Materials and Methods

Dhaka city (23°42′N latitude 90°22′E longitudes), on the eastern banks of the Buriganga River. The city lies on the lower reaches of the Ganges Delta and covers a total area of 300 square kilometers (120 sq mi). Dhaka city is a hot, wet, humid and has a distinct monsoon season, with an annual average temperature of 26 °C and monthly means varying between 19 °C in January and 29 °C in the month of May. Approximately 87% of the annual average rainfall of 2,123 millimeters (83.6 inches) occurs between May and October. The present study was conducted from November, 2014 to February, 2016. During study period, 49 places from 10 different parts of Dhaka city, Bangladesh were selected for the watwr sampling. The selected locations were Sadarghat, Bangshal, Wari, Jatrabari, Tejgaon, Shahbagh, Badda, Khilgaon, Rampura and Mailbagh of Dhaka City corporation area (Fig. 1).

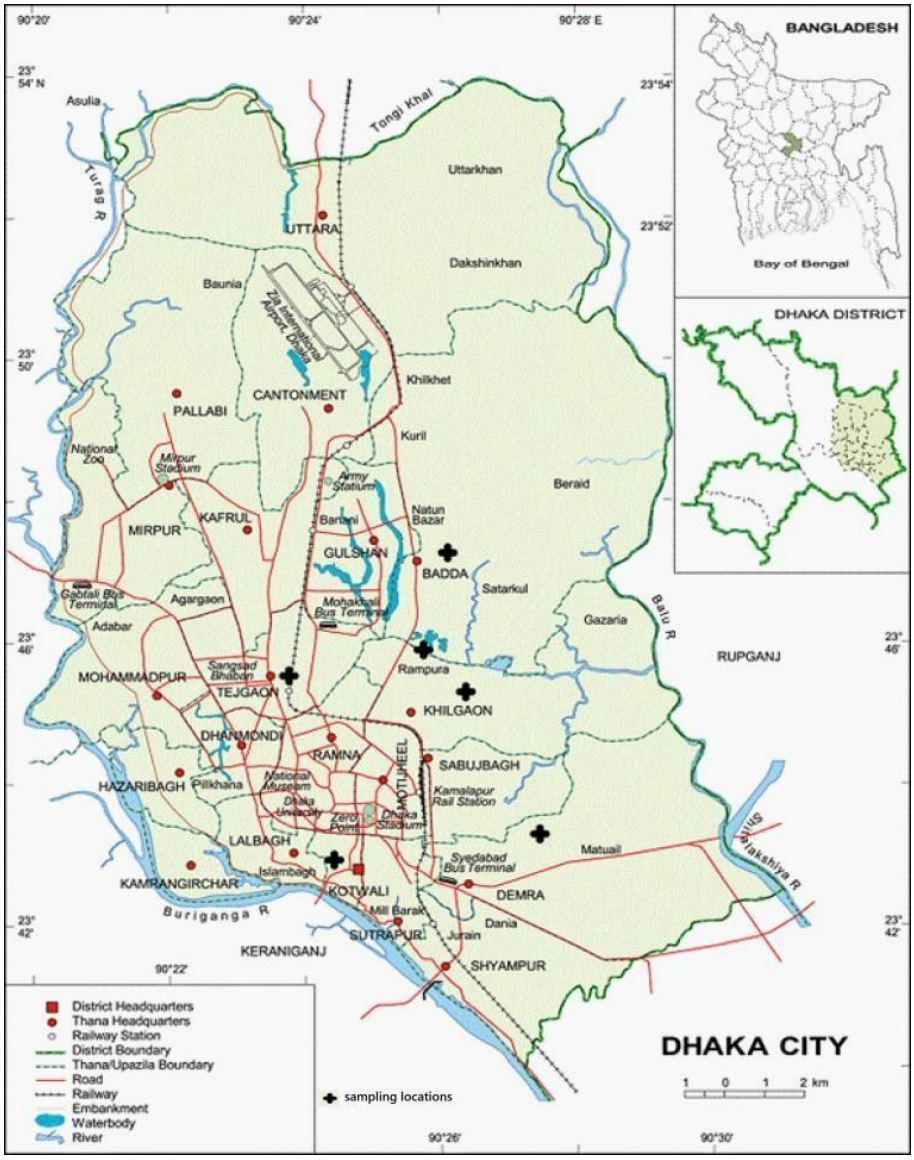

Fig 1: The map of Dhaka city showing different sampling locations [28]

The samples were collected within July, 2015 from different location in the Dhaka city. Further experiments i.e., identification, counting and preserving the mosquitoes, culturing and isolation of preserved water sample was carried in the Entomology laboratory, Department of Zoology and fungal flora has been identified in the Plant Pathology laboratory, Department of Botany, Jagannath University, Dhaka, Bangladesh from August, 2015 to February, 2016.

Sample collection
Water samples were collected from stagnant drains of Dhaka. Water samples were collected by using dipper and transparent plastic jars of specific volume (150 ml). For further analysis, the sampling jars were carried to the Entomology laboratory.

Identifying larvae and pupa
Firstly, 100 ml volume of larvae-containing water was measured by measuring cylinder (100 ml). Then the water was placed on the Petri dish and the numbers of larvae and pupae of mosquitoes were counted carefully. Larvae and pupae of *Aedes* and *Culex* were identified according to the method of the fauna of British-India, including Ceylon and Burma [29, 30].

Isolation of fungi from water sample
After counting larva and pupa of mosquito, water samples were preserved in refrigerator at 4˚C for further analysis. Isolation of required fungi from water samples were done following Serial Dilution Technique. At first, 1 ml of water sample was added into the test tube containing 9 ml of sterile distilled water and shaken well. This suspension was marked as stock suspension. Then 1 ml of stock suspension were transferred by sterile pipette into another test tube containing 9 ml of sterile distilled water for tenfold (1:10) dilution and then further diluted up to 10^5 and 10^6 dilutions and plating in triplicate plates were made from 10^{-5} and 10^{-6} diluted samples. Potato Dextose Agar (PDA) medium fortified with Streptomycin Sulphate (0.1 mg/ml) was poured into petri plates, each containing 15 ml of PDA medium. Then, 1 ml of each of the diluted water samples were transferred into a sterilized PDA petri plate by using sterilized pipettes, and were incubated at room temperature for 3 days. At the end of the incubation period, the developing fungi colonies were counted, identified and calculated for each sample.

Identification of fungi
The fungal colonies appearing on petri plates were sub-cultured into separate petri plates containing PDA medium for identification. Macroscopic characteristics such as size, color and nature of colonies, margin of colonies, presence and absence of concentric ring, sclerotia, colony diameter, exudates, pigmentation on front and back of the culture etc. were observed. Microscopic characteristics including structure and color of mycelia, shape, color and size of spores or conidia and conidiophores, vesicle, metulae, phialides, etc. were studied by mounting small portion of culture on a glass slide with lactophenol-cotton blue and lactophenol separately. The adhesive side of the tape was touched onto the surface of the colony at point intermediate between the center and periphery. Then adhesive side of the tape was adhered over an area on a glass slide with a drop of lactophenol or lactophenol-cotton blue separately. The slides were then examined microscopically for size and shape of vehicle, arrangement of sterigmata and spores, color of spores etc. under 10X, 40X and 100X objective lenses of compound microscope (Novel XSZ-107T). A small portion of the colony was taken into lactophenol-cotton blue solution on a glass slide and spread firmly with the help of sterilized needles. It was covered with a cover glass then examined microscopically. Photograph of the fungal colonies were taken with a digital camera (Nikon COOLPIX-S3500 7X wide) and photomicrographs of the microscopic structures of fungi were taken with the aid of Euromex CMEX-10 digital USB camera, Holland. Measurements of the microscopic character were recorded with the help of Image Focus 4 (version 2.4) software. For proper recording of collected samples, the raw data was recorded in a table which was categorized according to mosquito (larvae and pupae) and fungal flora. The identified fungal isolates were also recorded in a spreadsheet using Microsoft Office Excel.

Results
Mosquito density in sample water
The developing stages of 2 mosquitoes (*Aedes* and *Culex*) were found in the 49 water samples collected from 10 different areas. A total 4138 larvae and pupa of *Aedes* and *Culex* were counted. Among them, 3928 *Culex* larvae and 200 *Culex* pupae were noted. Larvae and pupae of *Aedes* were 10 in total, which might be an exceptional case. Average mosquito density per 100ml water was 56.29 (max 301, min 5).

Fungal isolates and their characteristics
In total, 18 fungal isolates were found within 10 genera of fungal flora from the examined water samples. They are *Absidia* sp., *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Nigrospora* sp., *Penicillium* sp., *Rhizopus* sp. and *Sclerotium* sp. Among them, *Aspergillus* had eight (08) and *Cladosporium* had two (02) different isolates. Theie diagnostic features are given in the Table 1; and are displayed in Fig. 4.

Table 1: The fungus spores of different genera found in mosquito habitat water, and their identifying morphological characteristics

Sl	Fungi	Diagnostic characteristics
1	*Absidia* sp.	Colonies mature rapidly and resemble coarse, gray wool or cotton candy. The reverse is white or light gray. *Absidia* species are similar in microscopic appearance to *Rhizopus*, but rhizoids are internodal. Sporangia are slightly elongated spheres ranging from 20-120µ in diameter. Sporangiospores are round to oval and measure 3-4.5µ.
2	*Aspergillus* sp.	Conidiophores upright, simple, terminating in a globose or clavate swelling, bearing phialides at the apex or radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. Conidia dry, no slime present. Apex of conidiophore enlarged, covered with flask -shaped phialides, conidia in dry chain.
	Aspergillus isolates	a. *Aspergillus* sp. (ash white): Vesicle V-shaped globose. Colony color ash white.
		b. *Aspergillus* sp. (black): Conidiophore wall thick, conidia round, vesicle-sub globose and uniceriad. Colony colour black.
		c. *Aspergillus* sp. (brown): Spore long, spore wall-smooth. Colony color brown.
		d. *Aspergillus* sp. (deep green): Spore wall rough, 2-cili, spore round body. Colony color deep green.
		e. *Aspergillus* sp. (greenish white): Columnar head, single celled. Colony color greenish white.
		f. *Aspergillus* sp. (light yellow): Conidiophore wall thick, conidia round, vesicle-sub globose and uniceriad. Colony colour light yellow.
		g. *Aspergillus* sp. (white): Columnar conidiophore, uniceriad, 1-layer phalids. Colony color white.

		h. *Aspergillus* sp. (yellowish green): Conidiophore wall thick, conidia round, vesicle-sub globose and uniceriad. Colony colour yellowish green.
3	*Cladosporium* sp.	Conidiophores tall, dark, upright, branched variously near the apex, clustered or single; conidia (blastophores) dark, 1 - or 2- celled, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon-shaped; often in simple or branched acropetalous chains; parasitic on higher plants or saprophytic.
4	*Curvularia* sp.	Conidiophores brown, mostly simple, bearing conidia apically or on new sympodial growing points; conidia (porospores) dark, end cells lighter, 3- to 5-celled, more or less fusiform, typically bent, with one of the central cells enlarged; parasitic or Saprophytic. Conidia typically bent by enlargement of one median cell.
5	*Fusarium* sp.	Mycelium extensive and cotton-like in culture, often with some tinge of pink, purple,or yellow in the mycelium on medium; conidiophores variable, slender, and simple, or stout, short, branched irregularly or bearing a whorl of phialides, single or grouped into sporodochia; conidia (phialospores) hyaline, variable, principally of two kinds, often held in small moist heads; macroconidia several-celled, slightly curved or bent at the pointed ends,typically canoe-shaped;microconidiaI-celled,ovoid or oblong, borne singly or in chains; some conidia intermediate,2-or3-celled,oblong or slightly curved; parasitic on higher plants or saprophytic on decaying plant material. A large and variable genus, sometimes placed in the *Tubercularia ceac* because some species produce sporodochia. Thick-walled chlamydospores are common in some species. Hyphae with simple conidiophores, variable Conidiophores, a loose sporodochium formed by branched conidiophores.
	Fusarium isolates	a. *Fusarium* sp.iso.(Crescent like spore):Colony colour orange, spore crescent like. b. *Fusarium* sp.iso.(Coiled forming):Colony color white, mycelium coiled forming.
5	*Geotrichum* sp.	Mycelium is white, septate; conidiophores absent; conidia (arthrospores) hyaline, 1 -celled, short cylindrical with truncate ends, formed by segmentation of hyphae; mostly saprophytic; common in soil. Some basidiomycetes form conidia in this manner.
7	*Nigrospora* sp.	Conidiophores short, mostly simple; conidia (aleuriospores) shiny black, 1-celled, globose, situated on a flattened, hyaline vesicle (cell) at the end of the conidiophore; parasitic on plants or saprophytic. Hyaline vesicle present in tip of conidiophore. Conidia borne on special sporogenous cell; conidia without light germ slit.
8	*Penicillium* sp.	Conidiophores arising from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides; conidia (phialospores) hyaline or brightly colored in mass, 1 -celled, mostly globose or ovoid, in dry basipetal chains; phialides upright, brushlike.
9	*Rhizopus* sp.	Filamentous, branching hyphae that generally lack cross-walls (i.e., they arecoenocytic). In asexual reproduction, sporangiospores are produced inside a spherical structure, the sporangium. Sporangia are supported by a large apophysate columella, the sporangiophore. Sporangiophores arise among distinctive, root -like rhizoids. In sexual reproduction, a dark zygospore is produced at the point where two compatible mycelia fuse.
10	*Sclerotium* sp.	Asexual fruit bodies and conidia lacking; sclerotia brown to black, globose or irregular, compact; mycelium usually light; parasitic, principally on underground parts of plants.

Fungal spore density

The maximum fungal isolates (9) were found in a single sample collected from Wari area. Minimum number of isolates (2) were found in 4 samples collected from Tejgaon and Rampura areas. In total, 635.33×10^5 spores were estimated from total water samples. The 8 *Aspergillus* sp. isolate density has been given in the Table 3. Five fungal genera were found in 11 samples collected from Jatrabari, Shahbagh, Wari, Tejgaon, Badda, Khilgaon and Rampura location. Six fungal genera were found in 11 samples collected from Sadarghat, Jatrabari, Tejgaon, Rampura, Khilgaon and Malibagh areas. *Penicilium* sp. was absent in 10 samples collected from Jatrabari, Bangshal, Tejgaon, Rampura and Malibagh areas. In one sample, *Aspergillus* was absent but *Absidia* sp., *Cladosporium* sp. and *Penicillium* sp. were present. *Geotrichum* sp. was found in only one sample collected from Khilgaon area. *Curvularia* sp. was noted in only one sample from Malibagh area. The detailed results are given in the Table 2.

Correlation of larval density of *Culex* with total fungal spore count, and with EPF count, was both negative and very weak (r= -0.145 and r= -0.197, respectively). Correlations are displayed in Fig. 2 and Fig. 3. Calculations were carried out on the basis of densities of both fungal spores and mosquito larvae density in every 100ml of water samples. Out of 18 fungal isolates, seven (07) were described as entomopathogenic by researchers (Table 4). Densities of the previously described entomopathogenic fungal spores found in the collected samples are given in the Table 3.

Table 2: Number of spores of different fungal isolates in different water samples

Sl.	Sample	Number of Fungal spores of different isolates ($\times10^5$)											Total ($\times10^5$)	FI	NML
		Absd	*Asp* isolates	*Clad.*	*Curv.*	*Fusrm.*	*Fusrm1.*	*Geotrchm.*	*Nigrspr.*	*Pnc.*	*Rhzps.*	*Sclr.*			
1	S-3	--	3.333	0.333	--	--	--	--	--	10	--	--	13.667	3	162
2	S-6	--	1.667	--	--	--	--	--	--	6.667	--	--	8.333	3	18
3	S-7	--	2	--	--	--	--	--	--	0.667	3.333	--	6	4	17
4	S-12	--	4	0.333	--	--	--	--	--	3.333	--	0.333	8	6	106
5	S-13	--	9	--	--	3.333	--	--	--	1	--	--	13.333	5	5
6	S-16	--	3.667	--	--	--	--	--	--	--	3.333	--	7	3	134
7	S-19	--	0.667	1.667	--	3.333	--	--	--	3.333	0.333	--	9.333	5	23
8	S-20	--	8.333	0.333	--	--	0.667	--	--	0.333	--	--	9.667	8	51
9	S-21	--	14	--	--	--	--	--	--	--	--	--	14	2	82
10	S-23	--	7.667	1.667	--	--	3.333	--	--	--	--	--	12.667	4	60
11	S-24	--	10.333	16.667	--	--	0.333	--	--	--	3.333	--	30.667	6	27
12	S-25	--	3	3.333	--	--	1.333	--	--	2	3.333	--	13	6	21

13	S-26	0.333	--	0.333	--	--	--	--	--	0.333	--	--	1	3	19
14	S-29	--	4.333	1	--	--	--	--	--	0.333	--	0.333	6	5	28
15	S-50	--	8	0.333	--	--	0.333	--	--	0.667	--	--	9.333	7	25
16	S-52	--	1	--	--	3.333	--	--	--	--	0.333	3.333	8	6	54
17	S-53	--	9	6.667	--	0.333	--	--	3.333	3.333	--	--	22.667	7	62
18	S-55	--	4	3.333	--	--	0.333	--	3.333	10	0.333	0.333	21.667	8	61
19	S-56	--	1.667	0.333	--	--	--	--	--	1	--	--	3	4	83
20	S-57	--	6.667	--	--	--	--	--	--	--	--	--	6.667	2	33
21	S-59	--	3.333	--	--	--	3.333	--	--	0.333	--	--	7	3	100
22	S-63	--	0.667	--	--	--	3.333	--	--	--	--	--	4	2	186
23	S-64	--	3.667	--	--	--	3.333	--	--	3.333	--	--	10.333	4	267
24	S-68	--	1.667	--	--	--	--	--	3.333	0.333	--	3.333	8.667	6	149
25	S-70	--	1.667	0.667	--	--	--	--	--	4.667	--	0.333	7.333	6	192
26	S-71	--	14.667	0.333	3.333	0.333	--	--	0.667	--	--	--	19.333	7	63
27	S-74	--	8.667	0.667	--	--	0.333	--	0.667	0.333	--	--	10.667	7	37
28	S-78	--	7.333	1.333	--	--	--	--	--	0.667	--	--	9.333	5	90
29	S-79	0.333	5.333	3.333	--	3.333	--	--	0.333	3.333	--	--	16	9	228
30	S-82	--	3.333	0.333	--	--	3.333	--	--	--	--	--	7	3	315
31	S-84	--	2	--	--	--	--	--	--	0.333	--	--	2.333	3	196
32	S-85	--	1	--	--	--	--	--	--	3.333	--	--	4.333	3	149
33	S-90	--	6.667	1.333	--	--	--	--	--	3.333	--	6.667	18	5	122
34	S-92	--	3.667	3.333	--	--	--	--	6.667	3.333	--	3.333	20.333	6	79
35	S-95	--	11.333	0.667	--	--	--	--	--	0.333	--	--	12.333	7	57
36	S-97	--	7.667	--	--	--	6.667	--	3.333	3.333	--	--	21	5	87
37	S-110	--	10.667	--	--	--	--	0.333	--	2	--	--	13	6	17
38	S-111	--	10	--	--	1.667	--	--	--	5.333	--	--	17	4	23
39	S-113	--	3	--	--	--	--	--	--	0.667	0.333	--	4	5	14
40	S-114	--	0.667	--	--	--	--	--	--	48.333	--	--	49	2	13
41	S-116	--	2.333	--	--	--	--	--	--	10	3.333	--	15.667	5	24
42	S-119	--	0.333	0.333	--	--	0.333	--	--	0.333	1	3.333	5.667	6	97
43	S-120	--	4	3.333	--	--	3.333	--	--	--	--	--	10.667	4	57
44	S-122	--	0.333	--	--	--	--	--	3.333	1.333	3.333	--	8.333	4	49
45	S-124	--	5	--	--	--	13.333	--	--	2.667	--	--	21	5	35
46	S-125	--	40	0.333	--	--	--	--	--	0.667	--	0.333	41.333	5	178
47	S-128	--	7.333	1.667	--	--	--	--	--	1	--	--	10	5	58
48	S-129	--	5.667	--	--	--	--	--	--	1.667	--	--	7.333	4	138
49	S-130	--	2.667	0.333	--	--	0.333	--	--	20	0.333	6.667	30.333	6	37
Total		0.667	277	54.333	3.333	15.667	44	0.333	25	164	22.667	28.333	635.333		3928

**Absd= Absidia*sp. isolate, *Asp* isolates = *Aspergillus* sp. isolate, *Clad. = Cladosporium* sp. isolate,
Curv. = Curvularia sp. isolate, *Fusrm. = Fusarium* sp. isolate, *Fusrm1. = Fusarium* sp. isolate,
Geotrchm. = Geotrichum sp. isolate, *Nigrspr. = Nigrospora* sp. isolate, *Pnc.=Penicillium* sp. isolate,
Rhzps. = Rhizopus sp. isolate, *Sclr. = Sclerotium* sp. isolate, FI= Fungal Isolates, NML=Number of mosquito larvae

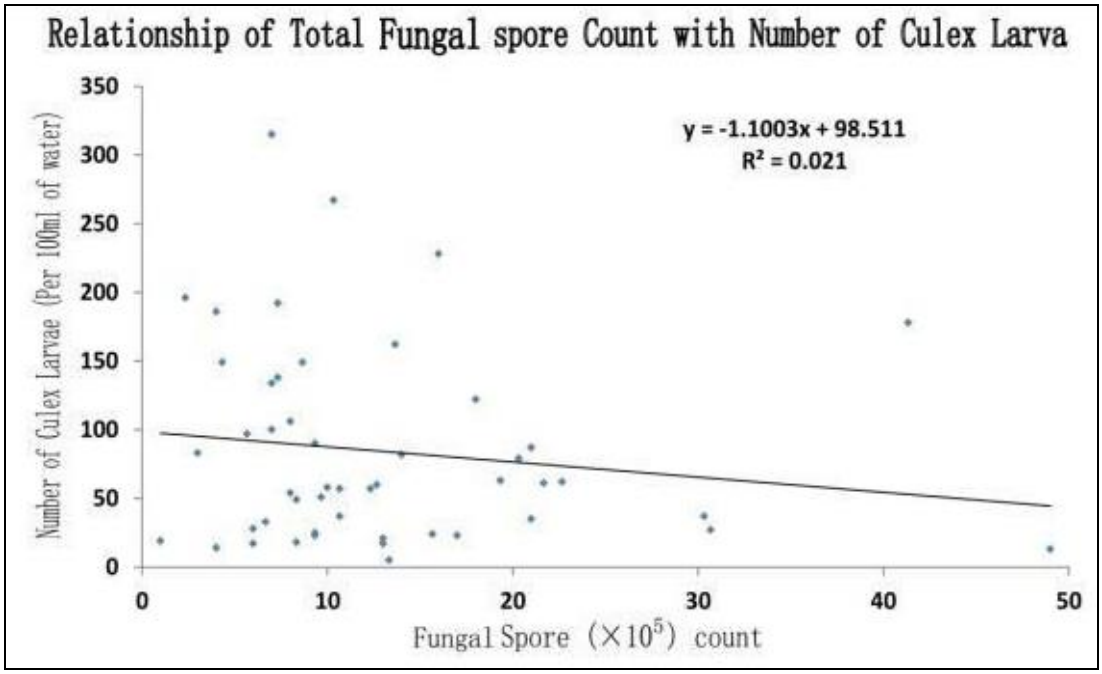

Fig 2: Relationship of Fungal spore density and mosquito larvae density

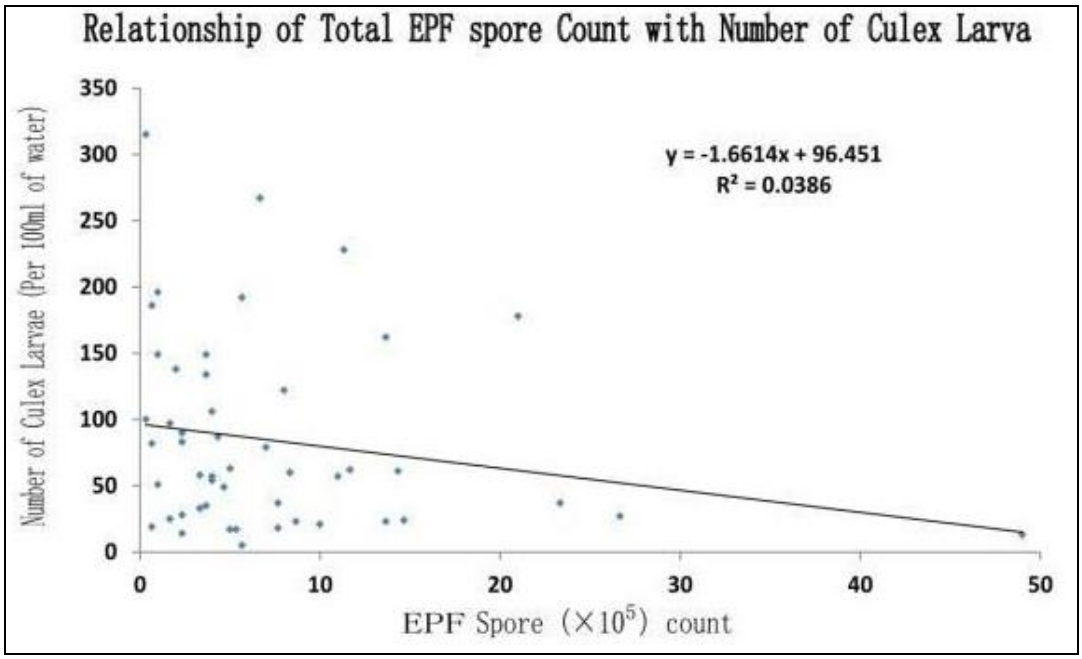

Fig 3: Relationship of Entomopathogenic fungi (EPF) spore density and mosquito larvae density

Table 3: Number of spores of different *Aspergillus* isolates in different water samples

Sl no.	Sample ID	Number of spores found for Different *Aspergillus* sp (×10 ^{5})								Total	*Aspergillus* isolates
		Asp.aw	*Asp*.bl	*Asp*.br	*Asp*.dgr	*Asp*.gw	*Asp*.ly	*Asp*.w	Asp.ygr		
1	S-3	--	3.333	--	--	--	--	--	--	3.333	1
2	S-6	--	1.000	--	0.667	--	--	--	--	1.667	2
3	S-7	--	1.000	--	1.000	--	--	--	--	2.000	2
4	S-12	0.333	0.333	--	3.333	--	--	--	--	4.000	3
5	S-13	--	1.333	--	4.333	--	3.333	--	--	9.000	3
6	S-16	--	0.333	--	--	--	--	3.333	--	3.667	2
7	S-19	--	--	--	0.667	--	--	--	--	0.667	1
8	S-20	3.333	0.333	0.667	--	--	--	3.333	0.667	8.333	5
9	S-21	--	0.667	--	13.333	--	--	--	--	14.000	2
10	S-23	--	6.667	--	1.000	--	--	--	--	7.667	2
11	S-24	--	6.667	0.333	3.333	--	--	--	--	10.333	3
12	S-25	--	1.333	--	1.667	--	--	--	--	3.000	2
13	S-26	--	--	--	--	--	--	--	--	--	0
14	S-29	--	1.000	--	3.333	--	--	--	--	4.333	2
15	S-50	0.333	0.667	--	6.667	--	--	0.333	--	8.000	4
16	S-52	--	0.333	--	0.333	--	--	0.333	--	1.000	3
17	S-53	--	1.333	--	7.333	--	0.333	--	--	9.000	3
18	S-55	--	0.667	--	3.333	--	--	--	--	4.000	2
19	S-56	--	1.000	--	0.667	--	--	--	--	1.667	2
20	S-57	--	3.333	--	--	--	--	--	3.333	6.667	2
21	S-59	--	--	--	--	--	--	--	3.333	3.333	1
22	S-63	--	0.667	--	--	--	--	--	--	0.667	1
23	S-64	--	3.333	--	0.333	--	--	--	--	3.667	2
24	S-68	--	0.667	--	0.333	--	--	--	0.667	1.667	3
25	S-70	--	0.333	0.333	1.000	--	--	--	--	1.667	3
26	S-71	0.333	1.000	--	13.333	--	--	--	--	14.667	3
27	S-74	--	6.667	0.333	1.667	--	--	--	--	8.667	3
28	S-78	--	0.333	6.667	0.333	--	--	--	--	7.333	3
29	S-79	--	1.000	--	0.667	0.333	--	3.333	--	5.333	4
30	S-82	--	--	--	3.333	--	--	--	--	3.333	1
31	S-84	--	0.667	--	1.333	--	--	--	--	2.000	2
32	S-85	--	0.333	--	0.667	--	--	--	--	1.000	2
33	S-90	--	3.333	--	3.333	--	--	--	--	6.667	2
34	S-92	--	0.333	--	--	--	--	3.333	--	3.667	2
35	S-95	0.667	6.667	--	0.333	3.333	--	0.333	--	11.333	5
36	S-97	--	1.000	--	6.667	--	--	--	--	7.667	2
37	S-110	--	3.333	0.667	3.333	--	3.333	--	--	10.667	4
38	S-111	--	6.667	--	3.333	--	--	--	--	10.000	2
39	S-113	--	1.333	--	1.000	--	0.667	--	--	3.000	3

40	S-114	--	0.667	--	--	--	--	--	--	0.667	1
41	S-116	--	1.333	--	0.333	--	--	0.667	--	2.333	3
42	S-119	--	--	--	0.333	--	--	--	--	0.333	1
43	S-120	--	0.667	--	3.333	--	--	--	--	4.000	2
44	S-122	--	--	--	0.333	--	--	--	--	0.333	1
45	S-124	--	1.000	--	0.667	--	--	--	3.333	5.000	3
46	S-125	--	20.000	--	20.000	--	--	--	--	40.000	2
47	S-128	--	0.667	--	3.333	--	--	--	3.333	7.333	3
48	S-129	--	0.333	--	2.000	--	--	--	3.333	5.667	3
49	S-130	--	2.667	--	--	--	--	--	--	2.667	1
Total		5.000	96.333	9.000	122.333	3.667	7.667	15.000	18.000	277.000	

**Asp*.aw = *Aspergillus* isolate (ash white), *Asp*.bl= *Aspergillus* isolate (black), *Asp*.br = *Aspergillus* isolate (brown), *Asp*.dgr= *Aspergillus* isolate (deep green), *Asp*.gw =*Aspergillus* isolate (greenish white), *Asp*.ly = *Aspergillus* isolate (light yellow), *Asp*.w= *Aspergillus* isolate (white), Asp.ygr= *Aspergillus* isolate (yellowish green)

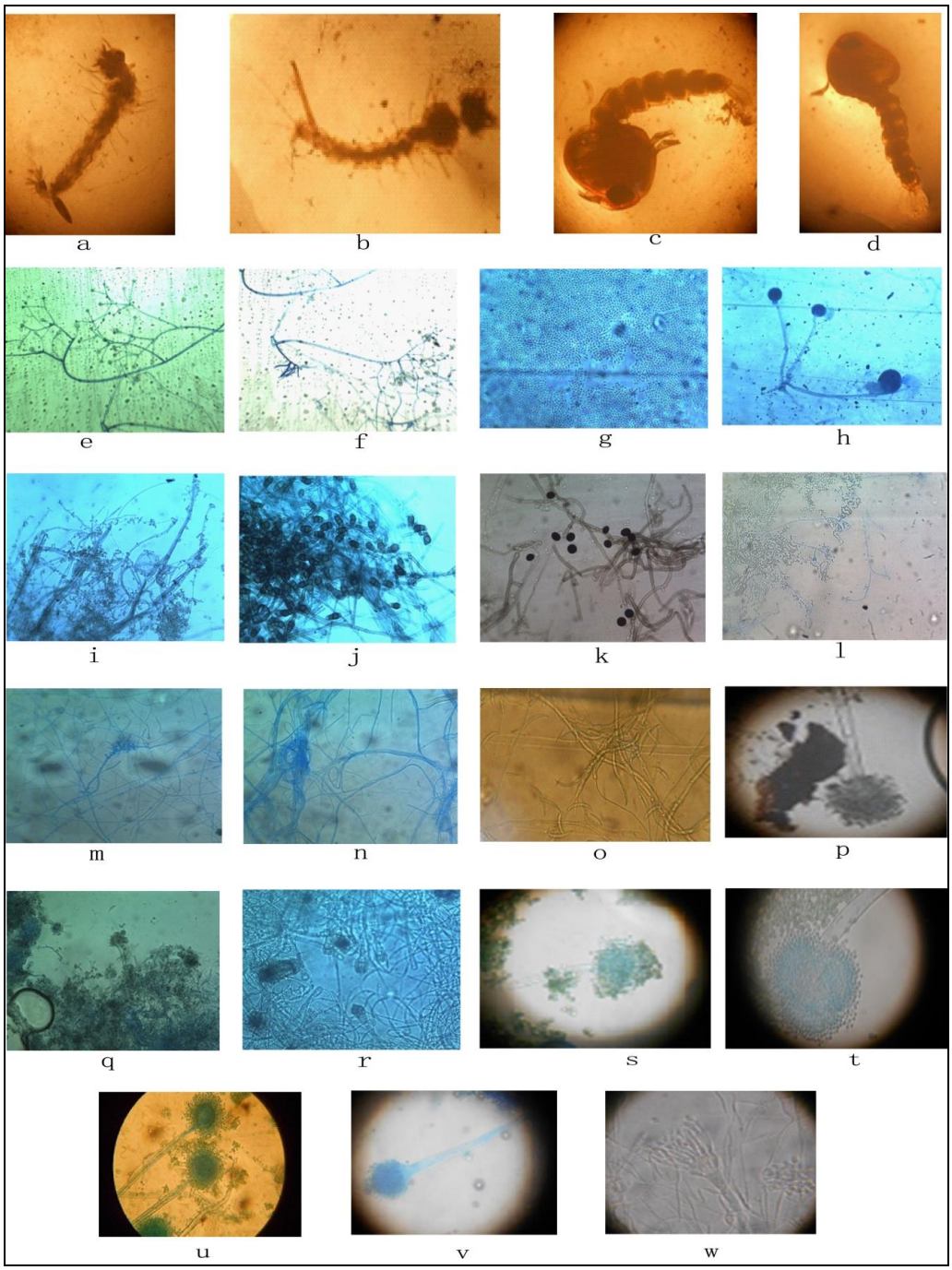

Fig 4: Photomicrographs of the found mosquitoes and fungi spores found in their habitat water samples-(a) *Aedes* sp. Larva; (b) *Cx. quinquefasciatus* larva; (c) *Aedes* sp. Pupa; (d) *Cx. quinquefasciatus* pupa; (e) and (f) *Absidia* sp.; (g) *Geotrichum* sp.; (h) *Rhizopus* sp.; (i) *Cladosporium* sp.; (j) *Curvularia* sp.; (k) *Nigrospora* sp.; (l) *Penicilium* sp.; (m) *Sclerotium* sp.; (n) *Fusarium* sp. isolates; (o) *Fusarium* sp. isolates; (p) *Aspergillus* sp. isolates (black); (q) *Aspergillus* sp. isolates (ash white); (r) *Aspergillus* sp. isolates (greenish white); (s) *Aspergillus* sp. isolates (brown); (t) *Aspergillus* sp. isolates (deep green); (u) *Aspergillus* sp. Isolates (light yellow); (v) *Aspergillus* sp. isolates (yellowish green); (w) *Aspergillus* sp. Isolates (white)

Discussion

Mosquito species found in drain water

Mosquitoes were reported to breed both in the temporary and permanent, from highly polluted to clean, large or very tiny waterbodies. Water-filled buckets, flower vases, tires, hoof prints and leaf axes are reported as potential sources for their breeding [31]. Dhaka is a highly populated city with not very planned drainage system where careless human activities are regularly performed such as deposition of waste into the stagnant water that made the habitats very suitable for mosquito regeneration, especially *Culex*. Some studies [32-34] commented that breeding habitats such as drains and coconut barks were the richest habitats for the mosquitoes in the study areas. In the present study, *Culex* larva and pupa were found in mosquito larvae habitats which were mostly with draining stagnant water. It was observed that *Aedes albopictus* bred most of the recorded breeding habitats except drain, lake, pond, and mud pool [35]. A few studies [36] reported that larvae of Genus *Aedes* were found abundantly in car tires only. In the present study, *Aedes* species was found in same water habitat (only one sample) of *Culex* larvae that means *Culex* species share with same habitat of *Aedes* species while breeding; or, for *Culex*, it is possible to breed in such a clean waterbody where *Aedes* could breed.

Density of larvae and pupa in habitat water

Two genera were identified in the mosquito larval habitats [37]. From present study, same genera were identified in the mosquito larval habitats; the stagnant drains of Dhaka City. It was also reported that the average number of *Cx. quinquefasciatus* larvae obtained per dip varied from 0.04-263.6. In the present study, the average number of *Culex* larvae obtained 80.16 per 100 ml water. Significantly higher larval density was recorded [38] in sewage water (n= 5534; 46.08%) as compared with released water (n = 2903; 24.17%) and drainage water (n= 3573; 29.75%).

According to a few investigations [39-41], the average density of the larvae are higher during the winter months (November to March). A highest of 69 and the lowest 31 larvae (per 100 ml of water) had been found at the densely occurring sites of larvae. This was, in fact, higher than the findings of the previous studies [42]. The highest count of 11283 larvae of *Culex* mosquitoes were found [43] per square meters of watered area. In present study, the highest of 301 and the lowest 5 *Culex* larvae were found per 100 ml of water. Totally, 3928 larvae of *Culex* and a total of 200 pupae of *Culex* were noted in total sampling drain water. The highest of 39 *Culex* pupae and lowest of 1 *Culex* pupa was found by present investigation.

Table 4: Entomopathogenic status of fungal isolates described by different researchers [44-49]

Sl	Fungal isolates	Target host(s)	References
1.	*Aspergillus* sp. Black Prdicted*: A. niger*	*Dolycoris baccarum, Eurygaster integriceps Acrotylus insubricus, Apodiphus* sp. *Coccinella novemnotata*	Assaf *et al.* 2011 [45]
2.	*Aspergillus* sp. Yellowish green Prdicted*: A. flavus*	*Dolycoris baccarum, Aelia acuminate, Apodiphus sp., Coccinella novemnotata, Anopheles* larva.	Assaf *et al.* 2011 [45] Bhan *et al.* 2013 [46]
3.	*Cladosporium* sp. Grey	Silver leaf white fly Castor oil whitefly Aphids	Nagdy *et al.* 2000 [48]
4.	*Curvularia* sp. Greenish	*Dolycoris baccarum*	Assaf *et al.* 2011 [45]
5.	*Fusarium* sp. Orange (crescent like spore)	*Dolycoris baccarum, Eurygaster integriceps, Nazara viridula, Toxoptera aurientii, Heiroglypus banian*	Assaf *et al.* 2011 [45] Dutta *et al.* 2013 [47]
6.	*Penicillium* sp. Olive green	Crustaceans *Dolycoris baccarum, Eurygaster integriceps* Weaver spider	Agus *et al.* 2015 [44] Assaf *et al.* 2011 [45] Yoder *et al.* 2009 [49]
7.	*Rhizopus* sp. White	*Dolycoris baccarum, Eurygaster integriceps, Anomala* sp., *Apodiphus* sp. *Coccinella novemnotata*	Assaf *et al.*, 2011 [45]

Density and roles of entomopathogenic and other fungi

With other fungi, Entomopathogenic fungi were also present in habitat water-reported by this present research and a number of current and previous researchers (Table 4). Fungi live in association with a host and benefit at the host's expenses [50]. Entomopathogenic fungi cause lethal infections and regulate insect and mite population in nature by epizootics [51-53]. Whatever, naturally occurring fungi spores in drain water of Dhaka city is numerous, but of no use in natural control of mosquitoes. It is pretty sure that in natural conditions where no parameter can be controlled, the EPF along with the other fungi spores might kill some mosquito larvae; but that is not enough to manage mosquito menace.

The present research tried to find out the entomopathogenic fungus genera found in *Culex* breeding habitat. Among the fungi spores we found, a number of them are reported as EPF, reported worldwide to be used as a biological control agent against many insects (Table 4). Fungal pathogens are considered as a natural biological enemy of many insects and other arthropods [51-53]. This phenomenon was inaugurated by Chinese [54] due to fungus-induced mortality of the arthropods, including mosqitoes [53, 55]. Approximately, 750 species of entomopathogenic fungi are known from 85 genera were reported [53-54, 56]. These fungi usually cause mycoses in many Arthropods and in most of the insects [56-57]. Occuring in aquatic, terrestrial, and subterranean habitats; they infect all life stage of insects [58]. Fungal pathogens are unique in many ways [58], especially for their mode of action on a living insect [54].

The present research supports the phenomenon of entomopathogenic fungus and suggests some fungi found in the water habitats of *Cx quinquefasciatus* to use against different mosquito species. They are *Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Penicillium* sp. *and Rhizopus* sp. Not necessarily all of them can act against *Culex*, and even the application may face a number of hazards; but, it can be started to practice for natural control of mosquitoes in Dhaka city, if and only if, the human health hazards can be carefully eliminated. But, we do not think it could be possible under natural condition because no parameter could be controlled here and thus, human health risk might not be minimized.

In fact, studies of entomopathogenic fungi started more than 60 years ago [59]. A number of researchers dealt with different

aspects of their virulence, mode of action and killing capacities to a wide range of insects including mosquitoes [51, 60-72] but the matter of regret is that, no one could formulate a safe and diagnostic way to use of such pathogens against mosquitoes for a better management. EPF might cause huge harm to the human and other animals [73-74] and without proper research, no fungus could be prescribed to do so. The present findings show that the presence of a huge EPF could kill the mosquito larvae, but the relationship is too weak to prescribe. Again, in an ecologically balanced open environment, where many control measures are not achievable, use of EPF against mosquito control could be a vague suggestion, rather, entomologists should find some other sustainable management techniques in a populated place as Dhaka City.

Conclusion

The *Culex* mosquito breeding habitats (stagnant water) in Dhaka City are full of fungi spores and many of them are described previously as entomopathogenic. Yet, the intensity of household mosquito bite is quite higher and people need preventive measures; revealing the naturally occurring EPF cannot play significant roles in mosquito management. Again, due to the risks of human health issues, EPF are not suggested in a densely populated city like Dhaka. So, the local authority must think about some alternatives for a sustainable mosquito management.

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