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EFFECT OF VICHETE 5G AND RIFIT 500 EC ON THE OCCURRENCE AND PHYTOSOCIOLOGICAL ATTRIBUTES OF WEEDS IN RICE FIELD

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Abstract

Two herbicides namely Vichete 5G (12.5, 25.0, 50.0 kg/ha) and Rifit 500 EC (0.5, 1.0 L/ ha) were applied in BRRI Dhan 28 in the field to follow their effects on the occurrence and phytosociological attributes of weeds. A total of 29 weed species belonging to 22 genera covering 10 families was found to occur. 28 weed species under 21 genera and 9 families, 16 weed species under 14 genera and 6 families and 26 weed species under 21 genera and 10 families were assessed during 1st, 2nd and 3rd weedings respectively. 12 Species (*Eragrostis unioloides, Marsilea quadrifolia, Cyperus tenuispica, Murdania nudiflora, Fimbristylis miliacea, Rotala densiflora, Cyperus iria, Setaria pumilla, Fimbristylis diphylla, Leptochloe chinensis, Monochoria hastata* and *Oryza rufipogon*) were recorded in first weeding but not found in second weeding. *Cyperus rotundus, Panicum paludosum, Cyperus difformis, Marsilea quadrifolia* and *Echinocholoa crussgalli* were found to be present at control only. They were controlled by the use of both herbicides. However, the number of weed species was found to reduce in the 2nd weeding in comparison to 1st one in all the treatments. Between the two weedicides, 50.0 kg/ha of Vichete 5G was relatively better than other doses of Vichete 5G and Rifit 500 EC and more effective to control weed species in 1st and 2nd weedings. In first weeding phytosociological attributes were highest in *Schoenoplectus erectus* and lowest in *Rotala densiflora* . In second weeding phytosociological attributes were highest in *Eleocharis congesta* and lowest in *Panicum paludosum* and *Lindernia hyssopiodes* while phytosociological attributes were highest in *Fimbristylis miliacea* and lowest in *Marsilea quadrifolia* of third weeding.

Key words: Vichete 5G, Rifit 500 EC, Weeds, BRRI Dhan 28, Phytosociological attributes

Introduction

Weeds cause problems in rice growing areas in the world, weed grasses and broad leaf weed reduces yield and quality of rice (Smith 1970). Losses due to weeds in Aus rice, range from 58% to complete failure of the crops (Mian and Ahasan 1969 and BRRI 1981). The total loss due to weed competition in Aus rice of Bangladesh is 1716 thousands ton of rough rice per year. Crop loss due to weed competition was most severe from 10 to 20 days after emergence. The main problems confronting farmer is that seeds

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of these weeds are set before harvesting of the crop from the field. Amarjit *et al.* (1994) observed that poor weed control is one of the major factors for yield reduction of rice depending on the type of weed flora and their intensity.

The traditional methods of weed control in rice field in Bangladesh are land tillage and hand weeding which are time consuming and expensive as well (Chowdhury *et al*. 1995). In the paddy field due to narrow spacing and hooding, hand weeding and mechanical hoeing is difficult and herbicides offer more practical, effective and economic means of controlling weeds (Nadeem 1998). Therefore, there is a great urge for the use of herbicides to control weeds in paddy field. Information regarding Vichete 5G and Rifit 500 EC is scanty in Bangladesh. So, a field experiment was designed to assess their efficiency on the occurrence and phytosociological attributes of weeds in BRRI Dhan 28 rice field.

Materials and Methods

A field experiment was done following a Complete Randomized Design using six treatments each with three replications (Block). 40 days old seedlings of BRRI Dhan 28 paddy were transplanted from seedbed to the prepared field.Two healthy seedlings were transplanted per hill. Row to row and hill to hill distance were 20 cm × 20 cm. Plot size was 4m × 4m and in each plot there were 400 hills. The recommended basal dose of fertilizers (Urea-160 kg/ha, T.S.P.-110 kg/ ha, M.P.-110 kg/ha and Zinc Sulphate-30 kg/ ha) used by BRRI (2008) was applied to the experimental fields. Herbicides namely Vichete 5G and Rifit 500 EC were applied after 3 days of transplantation as per treatment at the rate of T_o-control, T_1-12.5 kg/ha, T_2-25.0 kg/ha and T_3-50.0 kg/ha of Vichete 5G; and T_4-0.5 L/ ha and T_5-1.0 L/ha of Rifit 500 EC. Irrigation, weeding and other cultural practices were practiced when required.

Weedings were done after 30, 60 and 105 days after transplantation. The weeds were collected and the number of each weed species was recorded. The phytosociological attributes namely Density, Relative Density, Frequency, Relative Frequency, Abundance, Relative Abundance and Importance Value percent (IV %) were assessed from weeds of samples and computed according to Krebs (1978).

Results and Discussion

The occurrence and quantitative estimation of weeds of different treatments at first (W_1), second (W_2) and third (W_3) weedings have been assessed (Tables 1- 6). In first weeding

a total of 28 weed species under 21 genera and 9 families was collected from six different treatments in BRRI Dhan 28 field of the present investigation (Table 1).

Occurrence of weed species: It is revealed from Table 1 that total number of weed species were 614, 456, 345, 188, 163 and 160 in T_1, T_0, T_2, T_5, T_4 and T_3 respectively under Cyperaceae. The highest number of weed species of Cyperaceae was found in T_1. In Poaceae, the highest number of weed species was collected in T_1 (348) followed by T_4 (229),T_3 (160), T_5 (159), T_0 (135) and T_2 (76). In Pontederiaceae, the highest number of weed species was found in T_2 (34) followed by T_1 (33), T_5 (17), T_4 (16), T_3 (14) and T_0 (12). The highest number of weed species was observed in T_1 (09) followed by T_5 (04) under Scrophulariaceae . In T_0 and T_2 the number of weed species was same (03). In Marsileaceae, the maximum number of weed species was found in T_4 (05) followed by T_3 (04) , T_2 (03) and T_5 (01). The highest number of weed species was observed in T_0 (150) followed by T_1 (31) and T_2 (03) under Rubiaceae, where the number of weed species was equal (01) in T_3 and T_5. In Onagraceae, the maximum number of weed species was obtained in T_1 (18). In T_2 and T_5 the number of weed species was similar (14) but in control the number of weed species was 09. The highest number of weed species was observed in T_1 (12) under Commelinaceae. In T_2 , T_4 and T_5 the number of weed species was equal (03) and was almost equal to control (02). In Lythraceae, the number of weed species was observed only in T_1 (01) but not found in any other treatment. However spp. of Scrophulariaceae and Onagraceae in T_3 and T_4, Marsileaceae in T_0 and T_1, Rubiaceae in T_4 and Commelinaceae in T_5 were not found.

Quantitative estimation of weed species showed that application of Vichete 5G and Rifit 500 EC caused a drastic reduction in their abundance. The reduction decreased from 767 to 487 and 339; and 767 to 416 and 387 due to 25.0 and 50.0 kg/ha of Vichete 5G and 0.5 and 1.0 L/ha of Rifit 500 EC respectively (Table 1). However, application of 12.5 kg/ha of Vichete 5G stimulated their growth from 767 to 1060. Similarly the lowest dose of Rifit 500 EC (0.5 L/ha) also promoted the growth of weeds in comparison to higher dose (1.0 L/ha) markedly from 387 to 416 (Table 1). It is also apparent from Table 1 that *Cyperus rotundus, Fimbristylis diphylla, Cyperus difformis, Fimbristylis dichotoma, Paspalum scrobiculatum, Setaria pumilla, Leptochloe chinensis, Oryza rufipogon, Lindernia hyssopiodes, Lindernia antipoda, Marsilea quadrifolia* and *Rotala densiflora* were nil or almost nil or trace due to treatment of Vichete 5G and Rifit 500 EC or due to dormancy or unfavorable conditions of seed germination.

Table 1. Effect of Vichete 5G and Rifit 500 EC on occurrence of weeds in BRRI Dhan 28 grown in Boro season during first weeding.

Species Serial Number	Name of species	Name of family	Number of weed species					
			Treatments					
				Vichete 5G (kg/ha)			Rifit 500 EC (L/ha)	
			T_0 (0.0)	T_1 (12.5)	T_2 (25.0)	T_3 (50.0)	T_4 (0.5)	T_5 (1.0)
01	*Schoenoplectus erectus* Roxb.	Cyperaceae	243	546	330	148	145	177
02	*Cyperus tenuispica* Steudel.		03	00	03	03	04	01
03	*Eleocharis congesta* D. Don.		187	61	00	03	00	00
04	*Fimbristylis miliacea* L. Vahl.		00	01	03	00	04	02
05	*Kyllinga memuralis* J. R.and G. Farster.		18	06	00	00	00	00
06	*Cyperus iria* L.		03	00	04	04	08	06
07	*Cyperus rotundus* L.		02	00	00	00	00	00
08	*Fimbristylis diphylla* Vahl.		00	00	00	00	02	00
09	*Cyperus difformis* L.		00	00	04	00	00	01
10	*Fimbristylis dichotoma* (L.) Vahl.		00	00	01	02	00	01
	Sub-total		**456**	**614**	**345**	**160**	**163**	**188**
11	*Ischaemum rugosum* Salisb.	Poaceae	102	320	66	122	182	133
12	*Eragrostis unioloides* (Retz.) Nees.		08	03	03	17	12	09
13	*Echinocholoa crussgalli* (L.) Beauv.		09	00	00	12	02	09
14	*Leersia hexandra* Swartz.		00	06	04	07	08	06
15	*Panicum paludosum* Roxb.		00	15	08	00	24	01
16	*Paspalum scrobiculatum* Lamk.		15	01	00	00	00	00
17	*Setaria pumilla* (Poir) Roem and Schult.		01	00	00	02	00	00
18	*Leptochloe chinensis* L. Nees.		00	01	01	00	01	00
19	*Oryza rufipogon* Griff.		00	02	00	00	00	01
	Sub-total		**135**	**348**	**82**	**160**	**229**	**159**
20	*Monochoria vaginalis* (Burnt. f.) kirk.	Pontederiacae	12	33	33	14	12	17
21	*Monochoria hastata* Solms.		00	00	01	00	04	00
	Sub-total		**12**	**33**	**34**	**14**	**16**	**17**
22	*Lindernia hyssopiodes* L. Hains.	Scrophulariceae	00	00	00	00	00	04

Contd.

Species Serial Number	Name of species	Name of family	Number of weed species					
			Treatments					
				Vichete 5G (kg/ha)			Rifit 500 EC (L/ha)	
			T_0 (0.0)	T_1 (12.5)	T_2 (25.0)	T_3 (50.0)	T_4 (0.5)	T_5 (1.0)
23	*Lindernia antipoda* (L.) Alston.		03	09	03	00	00	00
	Sub-total		**03**	**09**	**03**	**00**	**00**	**04**
24	*Marsilea quadrifolia* L.	Marsileaceae	00	00	03	04	05	01
25	*Hedyotis corymbosa* (L.) Link.	Rubiaceae	150	31	03	01	00	01
26	*Ludwigia adscendens* L. Hara.	Onagraceae	09	18	14	00	00	14
27	*Murdania nudiflora* (L.) Brenan.	Commelinaceae	02	12	03	00	03	03
28	*Rotala densiflora* (Roth) koehne.	Lythraceae	00	01	00	00	00	00
	Total		**767**	**1066**	**487**	**339**	**416**	**387**

Phytosociological attributes of weed species

Total number of individual species : The highest total number (1589) of individual species was found in *Schoenoplectus erectus*. The total number (05) of individual species was similar in *Monochoria hastata* and *Cyperus difformis*. The lowest total number (01) of individual species was found in *Rotala densiflora* (Table 2).

Density: The density of species ranged from 264.83 (*Schoenoplectus erectus*) to 0.17 (*Rotala densiflora*). The density of other species was in intermediate range in the first weeding (Table 2).

Frequency of occurrence (F%) : The frequency of occurrence was observed 100% in 4 species; 83.33% in 5 species; 66.67% in 5 species; 50% in 4 species; and 33.33% in 6 species. The lowest frequency of occurrence (16.67%) was in case of 4 species. (Table 2).

Abundance: The highest abundance (264.83) was found in all the treatments and it was *Schoenoplectus erectus* . In *Fimbristylis miliacea , Monochoria hastata* and *Cyperus difformis* the abundance (2.5) was found similar. The lowest abundance (1.00) was obtained in *Rotala densiflora* and *Leptochloe chinensis* (Table 2).

Relative Density (RD %): In *Schoenoplectus erectus* the relative density was found to be the highest (45.89%) in all the treatments. It appeared that the lowest RD (0.03 %) was in *Rotala densiflora* and all other species fall into intermediate range (Table 2).

Relative Frequency (RF %) : The highest relative frequency (0.06 %) was obtained in 4 species. The RF was found to be 0.05% in 5 species; 0.04% in 5 species; 0.03% in 3 species and 0.02% in 6 species . The lowest RF (0.01%) was found in 4 species (Table 2).

Relative Abundance (RA %) : In *Schoenoplectus erectus* relative abundance was found highest (39.46%) and in *Fimbristylis miliacea* , *Monochoria hastata* and *Cyperus difformis* the RA (0.37 %) was same. The lowest RA (0.15%) was found in *Rotala densiflora* and *Leptochloe chinensis* (Table 2).

Importance Value (IV%) : It is apparent from Table 2 that among the 28 weed species the maximum IV (28.47%) was observed in *Schoenoplectus erectus* and the lowest IV (0.06%) was found in *Rotala densiflora*.

Table 2. Effect of Vichete 5G and Rifit 500 EC on phytosociological attributes of weeds in first weeding.

Species Serial No.	Total no. of individual species	No. of treatments in which species occurred	Density	Frequency of occurrence (F%)	Abundance	Relative Density (RD%)	Relative Frequency (RF%)	Relative Abundance (RA%)	Importance Value (IV%)
01	1589	06	264.83	100	264.83	45.89	0.06	39.46	28.47
02	14	05	2.33	83.33	2.80	0.40	0.05	0.42	0.29
03	251	03	41.83	50.00	83.67	7.25	0.03	12.47	6.57
04	10	04	1.67	66.67	2.50	0.29	0.04	0.37	0.23
05	24	02	4.00	33.33	12.00	0.69	0.02	1.79	0.83
06	25	05	4.17	83.33	5.00	0.72	0.05	0.75	0.51
07	02	01	0.33	16.67	2.00	0.06	0.01	0.30	0.12
08	02	01	0.33	16.67	2.00	0.06	0.01	0.30	0.12
09	05	02	0.83	33.33	2.50	0.14	0.02	0.37	0.18
10	04	03	0.67	50.00	1.33	0.12	0.03	0.20	0.12
11	925	06	154.17	100	154.17	26.71	0.06	22.97	16.58
12	52	06	8.67	100	8.67	1.50	0.06	1.29	0.92
13	32	04	5.33	66.67	8.00	0.92	0.04	1.19	0.51
14	31	05	5.17	83.33	6.20	0.90	0.05	0.92	0.62
15	48	04	8.0	66.67	12.00	1.39	0.04	1.79	1.07
16	16	02	2.67	33.33	8.00	0.46	0.02	1.19	0.56
17	03	02	0.50	33.33	1.50	0.09	0.02	0.22	0.11
18	03	03	0.50	50.00	1.00	0.09	0.03	0.15	0.09
19	03	02	0.50	33.33	1.50	0.09	0.02	0.22	0.11
20	121	06	20.17	100	20.17	3.49	0.06	3.00	2.18
21	05	02	0.83	33.33	2.50	0.14	0.02	0.37	0.18
22	04	01	0.67	16.67	4.00	0.12	0.01	0.60	0.24
23	15	03	2.50	50.00	5.00	0.43	0.03	0.75	0.40
24	13	04	2.17	66.67	3.25	0.38	0.04	0.48	0.30
25	186	05	31.0	83.33	37.20	5.37	0.05	5.54	3.65
26	55	04	9.17	66.67	13.75	1.59	0.04	2.05	1.23
27	23	05	3.83	83.33	4.60	0.66	0.05	0.69	1.40
28	01	01	0.17	16.67	1.00	0.03	0.01	0.15	0.06
Total	3463								

Note: Species serial number of Table 1 and Table 2 indicate same name of species.

A total of 16 weed species under 14 genera and 6 families was collected in second weeding (W_2) from all the treatments (Table 3).

Occurrence of weed species: It is apparent from Table 3 that total number of weed species were 4736, 323, 154, 117, 93 and 45 in T_0, T_1, T_4 ,T_5 T_2 and T_3 respectively under Cyperaceae. The highest number of weed species of Cyperaceae was found in T_0. In Poaceae, the highest number of weed species was obtained in control (376) and the lowest value (21) was recorded in 50.0 kg/ha of Vichete 5G. The other treatments were in intermediate range. In Pontederiaceae, the highest number of weed species was found in control (264) and the lowest number was in 50.0 kg/ha of Vichete 5G. The maximum

Table 3. Effect of Vichete 5G and Rifit 500 EC on occurrence of weeds in BRRI Dhan 28 grown in Boro season during second weeding.

			Number of weed species					
			Treatments					
Species Serial Number	Name of species	Name of family		Vichete 5G (kg/ ha)			Rifit 500 EC (L/ ha)	
			T_0 (0.0)	T_1 (12.5)	T_2 (25.0)	T_3 (50.0)	T_4 (0.5)	T_5 (1.0)
01	*Schoenoplectus erectus* Roxb.	Cyperaceae	260	129	48	28	66	61
03	*Eleocharis congesta* D. Don.		2975	136	01	04	63	31
05	*Kyllinga memuralis* J. R.and G. Farster.		01	00	01	00	01	01
07	*Cyperus rotundus* L.		00	00	00	00	08	00
09	*Cyperus difformis* L.		1407	00	00	00	00	00
10	*Fimbristylis dichotoma* (L.) Vahl.		93	58	43	13	16	24
	Sub-total		**4736**	**323**	**93**	**45**	**154**	**117**
11	*Ischaemum rugosum* Salisb.	Poaceae	276	70	00	21	58	06
13	*Echinocholoa crussgalli* (L.) Beauv.		69	01	34	00	18	27
14	*Leersia hexandra* Swartz.		21	00	16	00	00	00
15	*Panicum paludosum* Roxb.		01	00	00	00	00	00
16	*Paspalum scrobiculatum* Lamk		09	10	00	00	00	00
	Sub-total.		**376**	**81**	**50**	**21**	**76**	**33**
20	*Monochoria vaginalis* (Burnt.f.)kirk.	Pontederiacae	264	44	15	12	125	85
22	*Lindernia hyssopiodes* L. Hains.	Scrophulariceae	00	00	01	00	00	00
23	*Lindernia antipoda* (L.) Alston.			32	06	00	69	18
	Sub-total			**32**	**07**	**00**	**69**	**18**
25	*Hedyotis corymbosa* (L.) Link.	Rubiaceae	00	00	00	17	00	00
26	*Ludwigia adscendens* L. Hara.	Onagraceae	54	12	00	03	25	10
	Total		**5280**	**448**	**150**	**86**	**324**	**178**

number of weed species was observed in T_0 (114) followed by T_4 (69), T_1 (32), T_5 (18) and T_2 (07) under Scrophulariaceae. In T_3, no weed species was found. In Rubiaceae, the number of weed species was observed only in T_3 (17) but not found in any other treatments. In Onagraceae, the maximum number of weed species was obtained in control (54) and that of the lowest (03) in treatment receiving 50.0 kg/ha of Vichete 5G and treatment of 25.0 kg/ ha of Vichete 5G showed no weed species at all.

Quantitative estimation of weed species showed that application of Vichete 5G and Rifit 500 EC caused a drastic reduction in their abundance. The reduction decreased from 5280 to 150 and 86; and 5280 to 324 and 178 due to use of 25.0 and 50.0 kg/ha of Vichete 5G ; and 0.5 and 1.0 L/ha of Rifit 500 EC respectively (Table 3). However application of lowest dose of Vichete 5G (12.5 kg/ha) and Rifit 500 EC (0.5 L/ha) promoted the growth of weeds better than other higher doses of Vichete 5G and Rifit 500 EC.

Data presented in Table 3 indicates that the number of weeds decreased markedly with the increase of rate of Vichete 5G and Rifit 500 EC during second weeding. The trend of weeds generation showed little difference when compared with that of first weeding. The effect of treatments is very similar to all the species of four families counted. It is interesting to note that *Kyllinga memuralis, Cyperus rotundus, Cyperus difformis, Leersia hexandra, Panicum paludosum, Paspalum scrobiculatum, Lindernia hyssopiodes* and *Hedyotis corymbosa* were either nil or very few due to treatments of Vichete 5G and Rifit 500 EC.

Phytosociological attributes of weed species

Total number of individual species: The highest number (3210) of individual species was observed in *Eleocharis congesta.* The lowest total number (01) individual species was in *Lindernia hyssopiodes* and *Panicum paludosum* (Table 4).

Density: In *Eleocharis congesta* the density was found highest (535) and the lowest density (0.17) was found in 2 species namely *Lindernia hyssopiodes* and *Panicum paludosum.* The rest of the species was in intermediate range (Table 4).

Frequency of occurrence (F %) : The frequency of occurrence was observed to be 100% in 6 species. The frequency of occurrence was 83.33% in 2 species, 66.67% in 1 species and 33.33% in 3 species. The lowest frequency of occurrence (16.67%) was found in 4 species. (Table 4).

Abundance: The highest abundance (703.50) was found in *Cyperus difformis* only and the lowest abundance (1.0) was found in 3 species. (Table 4).

Relative Density (RD%): The relative density was found to be highest (45.58%) in *Eleocharis congesta* and the lowest relative density (0.01%) was recorded in 2 species. (Table 4).

Relative Frequency (RF%): The relative frequency was highest (0.10%) in 6 species. The relative frequency was 0.08% in 2 species, 0.07% in 1 species and 0.03% in 3 species. The lowest relative frequency (0.02%) was recorded in 4 species. (Table 4).

Relative Abundance (RA%): In *Cyperus difformis*, the relative abundance was found to be maximum (41.62%) and that of the lowest (0.06%) was found in *Lindernia hyssopiodes*, *Panicum paludosum* and *Kyllinga memuralis* (Table 4).

Importance Value (IV%): From Table 4 it is apparent that among the 16 weed species the maximum IV (25.78%) was in *Eleocharis congesta*. The lowest IV (0.03%) was found in *Panicum paludosum* and *Lindernia hyssopiodes.*

Table 4. Effect of Vichete 5G and Rifit 500 EC on phytosociological attributes of weeds in second weeding.

Species Serial Number	Total no. of individual species	No. of treatments in which species occurred	Density	Frequency of occurrence (F%)	Abundance	Relative Density (RD%)	Relative Frequency (RF%)	Relative Abundance (RA%)	Importance Value (IV%)
01	592	06	98.67	100	98.67	8.41	0.10	5.84	4.78
03	3210	06	535.00	100	535.00	45.58	0.10	31.65	25.78
05	04	04	0.67	66.67	1.00	0.06	0.07	0.06	0.06
07	08	01	1.33	16.67	8.00	0.11	0.02	0.47	0.20
09	1407	02	234.50	33.33	703.5	19.98	0.03	41.62	20.54
10	247	06	41.17	100	41.17	3.51	0.10	2.44	2.02
11	431	06	71.83	100	71.83	6.12	0.10	4.25	3.49
13	149	06	24.80	100	24.83	2.12	0.10	1.47	0.90
14	37	02	6.17	33.33	18.50	0.53	0.03	1.09	0.55
15	01	01	0.17	16.67	1.00	0.01	0.02	0.06	0.03
16	19	02	3.17	33.33	9.50	0.27	0.03	0.56	0.29
20	545	06	90.83	100	90.83	7.74	0.10	5.37	4.40
22	01	01	0.17	16.67	1.00	0.01	0.02	0.06	0.03
23	239	05	39.83	83.33	47.80	3.39	0.08	2.83	2.10
25	17	01	2.83	16.67	17.00	0.24	0.02	1.00	0.42
26	104	05	17.33	83.33	20.80	1.48	0.08	1.23	0.93
Total	**7011**								

Note: Species serial number of Tables 3 and 4 indicate same name of species.

A total of 26 weed species under 21 genera and 10 families was collected in third weeding from all the treatments. (Table 5).

Occurrence of weed species: From Table 5 it is evident that total number of weed species was 1670, 791, 697, 600, 337 and 181 in T_4, T_0, T_5, T_1, T_3 and T_2 respectively under Cyperaceae . Moreover, the maximum number of weed species of Cyperaceae was found in treatment receiving 0.5 L/ha of Rifit 500 EC. In Poaceae, the highest number of

Table 5. Effect of Vichete 5G and Rifit 500 EC on occurrence of weeds in BRRI Dhan 28 grown in Boro season during third weeding.

Species Serial Number	Name of species	Name of family	Number of weed species Treatments					
				Vichete 5G (kg/ ha)			Rifit 500 EC(L/ha)	
			T_0 (0.0)	T_1 (12.5)	T_2 (25.0)	T_3 (50.0)	T_4 (0.5)	T_5 (1.0)
01	*Schoenoplectus*	Cyperaceae	217	465	82	44	57	88
02	*erectus* Roxb. *Cyperus tenuispica* Steudel.		03	00	11	03	11	00
03	*Eleocharis congesta* D. Don.		09	33	04	20	31	02
04	*Fimbristylis miliacea* L. Vahl.		519	88	81	239	960	451
05	*Kyllinga memuralis* J. R.and G. Farster.		00	00	00	00	19	25
06	*Cyperus iria* L.		21	11	02	24	553	118
07	*Cyperus rotundus* L.		00	00	00	00	05	01
08	*Fimbristylis diphylla* Vahl.		12	00	00	00	05	09
09	*Cyperus difformis* L.		10	03	01	07	29	03
	Sub-total		**791**	**600**	**181**	**337**	**1670**	**697**
11	*Ischaemum rugosum* Salisb.	Poaceae	294	68	45	48	88	461
12	*Eragrostis unioloides* (Retz.) Nees.		52	07	05	44	43	88
13	*Echinocholoa crussgalli* (L.) Beauv.		03	00	00	00	00	00
14	*Leersia hexandra* Swartz.		21	00	00	00	30	30
15	*Panicum paludosum* Roxb.		85	114	114	154	750	68
16	*Paspalum scrobiculatum* Lamk.		03	00	00	00	03	00

Contd.

Species Serial Number	Name of species	Name of family	Number of weed species Treatments					
				Vichete 5G (kg/ ha)			Rifit 500 EC(L/ha)	
			T_0 (0.0)	T_1 (12.5)	T_2 (25.0)	T_3 (50.0)	T_4 (0.5)	T_5 (1.0)
17	*Setaria pumilla* (Poir) Roem and Schult.		00	00	00	02	00	00
18	*Leptochloe chinensis* L. Nees.		01	00	00	01	14	01
	Sub-total		**459**	**189**	**164**	**249**	**928**	**648**
20	*Monochoria vaginalis* (Burnt. f.) kirk.	Pontederiacae	04	07	03	04	04	05
22	*Lindernia hyssopiodes* L. Hains.	Scrophulariceae	00	00	02	00	00	00
23	*Lindernia antipoda* (L.) Alston.		01	00	00	00	03	00
	Sub-total		**01**	**00**	**02**	**00**	**03**	**00**
24	*Marsilea quadrifolia* L.	Marsileaceae	01	00	00	00	00	00
25	*Hedyotis corymbosa* (L.) Link.	Rubiaceae	00	00	02	00	00	00
26	*Ludwigia adscendens* L. Hara.	Onagraceae	03	00	02	05	25	03
27	*Murdania nudiflora* (L.) Brenan.	Commelinaceae	14	00	38	33	198	158
28	*Rotala densiflora* (Roth) koehne.	Lythraceae	331	11	299	209	780	420
29	*Eriocaulon compressum*	Eriocaulaceae	00	00	00	00	00	02
	Total		**1600**	**914**	**688**	**833**	**3604**	**1928**

weed species was estimated in treatment receiving 0.5 L/ha of Rifit 500 EC and other treatments were in intermediate range. In Pontederiaceae the highest number of weed species was observed in T_1 (07) followed by T_5 (05). In T_0, T_3 and T_4 the number of weed species was same (04) but in T_2 the number of weed species was found to be 03. In Scrophulariaceae, the highest number of weed species was recorded in T_4 (03) followed by T_2 (02) and T_0 (01). No weed species was recorded in T_1, T_3 and T_5. In Marsileaceae, the number of weed species was observed only in T_0 (01) but not found in any other treatment. In Rubiaceae, the number of weed species was observed only in T_2 (02) but not

found in any other treatment. In Onagraceae, the highest number of weed species was observed in T_4 (25) followed by T_3 (05). In T_0 and T_5 the number of weed species was same (03) but in T_2 the number of weed species was recorded to be 02. In T_1, no weed species was occurred. In Commelinaceae, the highest number of weed species was obtained in T_4 (198) followed by T_5 (158), T2 (38), T_3 (33) and T_0 (14). In T_1, no weed species was occurred. In Lythraceae, the highest number of weed species was obtained in T_4 (780) followed by T_5 (420), T_0 (331), T_2(299), T_3(209) and T_1(11). In Eriocaulaceae, *Eriocaulon compressum* was observed only in T_5 (02) but not found in any other treatment. *Eriocaulon compressum* was absent in 1st and 2nd weedings but appeared in 3rd weeding .This might be due to dormancy or unfavorable conditions of seed germination.

Quantitative estimation of weed species showed that application of Vichete 5G caused a reduction in their abundance. The reduction was from 1600 to 688 and 833 but application of Rifit 500 EC caused promotion in weed abundance. It might be concluded that Rifit 500 EC was not effective till third weeding. It is apparent from Table 5 that *Kyllinga memuralis, Cyperus rotundus, Fimbristylis diphylla, Echinocholoa crussgalli, Paspalum scrobiculatum, Setaria pumilla, Lindernia hyssopiodes, Lindernia antipoda, Marsilea quadrifolia, Hedyotis corymbosa* and *Eriocaulon compressum* belonging to 6 families were either nil or few due to treatment of Vichete 5G and Rifit 500 EC.

Phytosociological attributes of weed species

Total number of individual species: The highest number (2338) of individual species was observed in *Fimbristylis miliacea*. The total number (02) of individual species obtained was same in 4 species. The lowest number (01) of individual species was found in *Marsilea quadrifolia* (Table 6).

Density: In *Fimbristylis miliacea*, the density was found to be highest (389.67). In 4 species, the density (0.33) was same. The lowest density (0.17) was found in *Marsilea quadrifolia* (Table 6).

Frequency of occurrence (F%) : The frequency of occurrence was observed 100% in 12 species; 83.33% was in 1 species; 66.67% was in 2 species and 50% was in 2 species. The lowest frequency of occurrence (16.67%) was observed in rest of the 6 species (Table 6).

Abundance: The highest abundance (389.67) was found in *Fimbristylis miliacea* . In case of 5 species, the abundance (02) was same. The lowest abundance (01) was found in *Marsilea quadrifolia* (Table 6).

Relative Density (RD%): In *Fimbristylis miliacea*, the highest relative density (24.71%) was recorded. In 4 species, RD (0.02%) was found to be same. The lowest RD (0.01%) was observed in *Marsilea quadrifolia* (Table 6).

Relative Frequency (RF%): The relative frequency was found to be highest (0.06%) in 12 species; 0.05% was in 1 species; 0.04% was in 2 species; 0.03% was in 2 species and 0.02% was in 2 species. The lowest RF (0.01%) was recorded in 6 species (Table 6).

Relative Abundance (RA %): In *Fimbristylis miliacea* the relative abundance was found to be highest (23.87%). In 5 species, the RF (0.12%) was observed same. The lowest RF (0.06%) was in *Marsilea quadrifolia* (Table 6).

Importance Value (IV%): Among the 26 weed species the maximum IV (16.21%) was observed in *Fimbristylis miliacea* . In 4 species, IV (0.05%) was similar. The lowest IV (0.03%) was found in *Marsilea quadrifolia* (Table 6).

Table 6. Effect of Vichete 5G and Rifit 500 EC on phytosociological attributes of weeds in third weeding.

Species Serial Number	Total no. of individual species.	No. of treatments in which species occurred	Density	Frequency of occurrence (F%)	Abundance	Relative Density (RD%)	Relative Frequency (RF%)	Relative Abundance (RA%)	Importance Value (IV%)
01	953	06	158.83	100	158.83	10.07	0.06	9.73	6.62
02	28	04	4.67	66.67	7.00	0.30	0.04	0.43	0.26
03	99	06	16.50	100	16.50	1.05	0.06	1.01	0.71
04	2338	06	389.67	100	389.67	24.71	0.06	23.87	16.21
05	44	02	7.33	33.33	22.00	0.47	0.02	1.35	0.60
06	729	06	121.50	100	121.50	7.71	0.06	7.44	5.07
07	06	02	1.00	33.33	3.00	0.06	0.02	0.18	0.09
08	26	03	4.33	50.00	8.67	0.27	0.03	0.53	0.28
09	53	06	8.83	100	8.83	0.56	0.06	0.54	0.39
11	1004	06	167.33	100	167.33	10.61	0.06	10.25	6.97
12	239	06	39.83	100	39.83	2.53	0.06	2.44	1.68
13	03	01	0.50	16.67	3.00	0.03	0.01	0.18	0.07
14	81	03	13.50	50.00	27.00	0.86	0.03	1.65	0.85
15	1285	06	24.17	100	214.17	13.58	0.06	13.12	8.92
16	06	02	1.00	100	3.00	0.06	0.06	0.18	0.10
17	02	01	0.33	16.67	2.00	0.02	0.01	0.12	0.05
18	17	04	2.83	66.67	4.25	0.18	0.04	0.26	0.16
20	24	06	4.00	100	4.00	0.25	0.06	0.25	0.19
22	02	01	0.33	16.67	2.00	0.02	0.01	0.12	0.05
23	04	02	0.67	33.33	2.00	0.04	0.02	0.12	0.06
24	01	01	0.17	16.67	1.00	0.01	0.01	0.06	0.03
25	02	01	0.33	16.67	2.00	0.02	0.01	0.12	0.05
26	38	05	6.33	83.33	7.50	0.40	0.05	0.46	0.30
27	441	06	73.50	100	73.50	4.66	0.06	4.50	3.07
28	2050	06	341.67	100	341.67	21.67	0.06	20.43	14.22
29	02	01	0.33	16.67	2.00	0.02	0.01	0.12	0.05
Total	**9461**								

Note: Species serial number of Table 5 and Table 6 indicate same name of species.

Among 28 weed species of first weeding, 16 species were recorded at T_0 and T_4, 17 species were at T_1, 18 species were at T_2 and T_5 and 13 species were at T_3. The lowest number of weed species (13) was found at T_3 where 50.0 kg/ha of Vichete 5G was applied. In quantitative analysis of weeds species of first weeding, *Schoenoplectus erectus* was found to occur in all the treatments where the number of individual weed species was highest (1589) and density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were also found to be highest. The number of individual weed species was found to be lowest (01) in *Rotala densiflora* where the density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were lowest.

Among 16 weed species of second weeding, 13 species were recorded at T_0, 10 species were at T_1 and T_4, 9 species were at T_2 and T_5, and 7 species were at T_3. The highest number of weed species (13) was found at T_0 where no herbicide was used. The lowest number of weed species (07) was found at T_3 where 50.0 kg/ha of Vichete 5G was applied. In quantitative analysis of weed species of second weeding, *Eleocharis congesta* was found to occur in all the treatments where the number of individual weed species was highest (3210) and density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were also found to be highest. The number of individual weed species was lowest (01) in *Panicum paludosum* and *Lindernia hyssopiodes* where density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were also lowest.

Among 26 species of third weeding, 20 species were recorded at T_0 and T_4, 10 species were at T_1, 15 species were at T_2, 16 species were at T_3 and 18 species were at T_5. The maximum number of weed species (20) was found at T_0 and T_4 where no herbicide was used and 0.5 L/ha of Rifit 500 EC was applied respectively. The lowest number of weed species (10) was found at T_1 where 12.5 kg/ha of Vichete 5G was applied. In quantitative analysis of weeds species of third weeding, *Fimbristylis miliacea* was found to occur in all the treatments where the number of individual weed species was highest (2338) and density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were found highest. The number of individual weed species was found lowest (01) in *Marsilea quadrifolia* where density, frequency of occurrence, abundance, relative density, relative frequency, relative abundance and importance value were also lowest.

The results of the present investigation agreed favorably well with the works of other workers. Mamun *et al*. (1986) observed that the principal weed in the direct seeded Aus rice field was *Cyperus rotandus* which constituted 68.22% of the weed vegetation but in the present investigation *Cyperus rotandus* was found rare in wet land of paddy in all the treatments of different weedings. The presence species of *Lindernia, Cyperus, Fimbristylis, Ludwigia and Rotala* in the different weedings of this investigation

corroborates with the findings of Rahman *et al.* (1996) who also observed the presence of these weed species in Aman, Boro and Aus paddy. The density of *Echinochloa crusgalli* was found low. In contrast, Radanachaless and Mercado (1980) observed considerable density of *Echinochloa crusgalli* in rice field in multiple cropping pattern. Ahmed and Moody (1982) also reported that *Leptochloa chinensis*, *Monochoria vaginalis* and *Echinochloa* spp. were dominant weed in the upland rice but these species were not found dominant in the present investigation.

Overbeck (1962 and 1964) who noted that all the principle of herbicides now in use appears to act primarily on some phase or another of the growth process. Crafts (1961) also noted that successful herbicidal chemicals are stable or their toxic reaction products in the plant are stable. He also observed that herbicide deranges the physiology of a plant over a period long enough to kill it.

From the above discussion, it may be concluded that the mode of action of two herbicides in controlling weeds in BRRI Dhan 28 field are different. The effects differ with concentrations and nature of herbicides. Between two herbicides, 50.0 kg/ha of Vichete 5G was relatively better and effective to control weeds species in BRRI Dhan 28 rice field.

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MYCOFLORA ASSOCIATED WITH MOMORDICA CHARANTIA L. AND THEIR PATHOGENIC POTENTIALITY

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Abstract

Momordica charantia L. (Bitter gourd) is one of the popular vegetable in Bangladesh as well as in the world. After harvesting the vegetables are contaminated with fungi within 3-4 days of short storage. An investigation was carried out to find out the fungi associated with fruits of two varieties of *Momordica charantia* during the tenure of May 2015 to June 2016. Nine species of fungi namely, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tiegh, *Curvularia brchyspora* Boedijn, *Fusarium* Link, *Mucor* Fresen, *Penicillium* Link, *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill and *Trichoderma viride* Pers. were found to be associated with the selected vegetable. .*Aspergillus niger* was predominating fungus associated with both the varieties of bitter gourd. Among the isolated 9 fungi *A. niger, C. brchyspora, Fusarium* sp., *R. stolonifer* and *T. viride* were found to be pathogenic to both the varieties of *M. charantia.*

Key words: Mycoflora, *Momordica charantia,* Pathogenic potentiality

Introduction

Momordica charantia, known as bitter melon, bitter gourd, bitter squash or balsam-pear is a tropical and subtropical vine of the family Cucurbitaceae. Bitter gourd are available in a variety of shapes and sizes. The cultivar common to China is 20–30 cm long, oblong with bluntly tapering ends and pale green in color, with a gently undulating, warty surface. The bitter melon more typical of India has a narrower shape with pointed ends, and a surface covered with jagged, triangular "teeth" and ridges. It is green to white in color. Between these two extremes there are some intermediate forms. Some bear miniature fruit of only 6–10 cm in length, which may be served individually as stuffed vegetables. These miniature fruit are popular in Bangladesh, India (common name 'Korolla'), Pakistan, Nepal and other countries in South Asia.

Bitter gourd can be eaten as cooked and play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals and fiber. Vegetables are rich and comparatively cheaper source of vitamins and minerals. Its consumption in sufficient quantities provides taste, palatability and increase appetite. Vegetables are currently reckoned as important adjunct for maintenance of food health and beneficial in protecting against some degenerative diseases. They also play a key role in neutralizing the acids that are formed during digestion (Ananya and Sarmistha 2010). Many people in

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urban and rural areas fully depend on vegetable cultivation and selling in the markets. But it is the matter of deep concern that a remarkable portion of harvested vegetables are being lost due to mismanagement of the vegetables during transit and storage, as a result fungal infection occurs and consequently vegetables are spoiled. The major diseases of *M. charantia* are Alternaria rot (*Alternaria alternata*), Belly rot (*Rhizoctonia solani*), Cottony leak (*Pythium* sp.), Rhizopus soft rot (*Rhizopus stolonifer*), Botryodiplodia rot (*Botryodiplodia theobromae*) (Wikipedia 1016) etc. Haque and Shamsi (2011) reported fungal association of five vegetables in Bangladesh. So far there is no report regarding the association of fungi with fresh fruit of *M. charantia* in storage. Present investigation was undertaken to find out the association of fungi with M. *charantia* and their pathogenic potentiality.

Materials and Methods

Fruits of two varieties of *Momordica charantia* L. (Korolla) were used in the present investigation. One is local variety (small in size, bitter in taste) and another is a hybrid variety (larger in size, less bitter in taste). The samples were collected from five different markets of Dhaka city namely, Ananda bazar, Hatirpul bazar, Kawran bazar, Palashi bazar and Siddique bazar. Five markets were visited three times to collect the samples. From each market sufficient amount of fresh vegetables were collected randomly. Samples were collected during May to December 2015. The fungi were isolated from samples following the 'Tissue Planting Method' on PDA medium following Islam and Shamsi (2016). Identification of the isolates were determined following the standard literature (Barnett and Hunter 1972, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997, Thom and Rapper 1945 and Rapper *et al*. 1949).

Per cent frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Welty 1972):

$$\% \text{ frequency} = \frac{\text{Total numbr of inocula from which a fungal isolate was observed}}{\text{Total number of inocula}} \times 100$$

All the fungi isolated from fruits of *M. charantia* were tested for their pathogenic potentiality following detached fruit assay (Shamsi *et al.* 2016).

Pure isolates from *M. charantia* were individually inoculated into healthy fruits of *M. charantia* at the same stage of maturity. The fungi that were isolated from *M. charantia* were grown separately on PDA medium at pH 6.0 and temperature 25 to 28^{o}C for five to seven days. The healthy vegetables were surface sterilized with 10% Chlorox and washed thrice in changes of sterilized distilled water. Then the inoculated vegetable were kept for one hour for drying.

When the vegetables were fully air dried, a cork borer (5 mm diameter) was driven to a depth of 4 mm into the vegetables to make a groove where the isolated organism could be put. The cultured fungal isolates were also cut into round block with the help of

another sterile cork borer (4 mm diameter) from pure PDA culture medium. Some fresh PDA blocks were also cut for the control set. Two sets of fruits of *M. charantia* were prepared where one set is control i,e, fruits inoculated with PDA blocks and another set is treated i,e, fruits inoculated with particular test pathogens. Two round grooves in local variety and three grooves in hybrid variety of *M. charantia* in suitable distance were made. Fresh PDA blocks were placed aseptically into the groove of the control set and the mycelial blocks were placed into the grooves of the treatment set. Then both the control set and the treatment set of vegetables were incubated into separate sterilized plastic boxes of suitable size. Inoculated fruits were incubated for 5 to 7 days at 25 ± 2˚C. Regular observations were made and re-isolation of any pathogenic test fungi were carried out for comparison with the original fungi.

Results and Discussion

A total of nine species of fungi namely *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Curvularia brachyspora*, *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride* was isolated from the fresh fruits of two varieties of *M. charantia*.

Table 1 shows eight species of fungi *namely Aspergillus flavus, A. niger, A. fumigatus, Curvularia brachyspora*, *Fusarium* sp., *Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride* which were isolated from *M. charantia* (Local variety). Four species of fungi (*Aspergillus niger, A. flavus, Penicillium* sp., *Rhizopus stolonifer*) were isolated from the samples of Ananda bazar where frequency percentage of association of *A. niger* was the highest (44.) followed by *Rhizopus stolonifer* (26.7) and *Penicillium* sp. (24.3). *A. flavus* showed lowest mean frequency (15.7). Four species of fungi were isolated from Hatirpul bazar where frequency percentage of association of *A. niger* was the highest (31) followed by *A. flavus* (22.3) and *Penicillium* sp. (21). Frequency percentage of association of *Fusarium* sp. was lowest (15.7). Five species of fungi were isolated from the samples of Karwan bazar where frequency percentage of association of *A. niger* was the highest (40) followed by *Trichoderma viride* (20), *Penicillium* sp. (17.7) and *Fusarium* sp. (13.3). Frequency percentage of association of *A. fumigatus* was lowest (09). Five species of fungi were isolated from the samples of Palashi bazar where frequency percentage of association of *Aspergillus niger* was the highest (49) followed by *Penicillium* sp. (42), and *A. flavus* (15.7). Lowest count of *C brachyspora* and *T. viride* (11). Five species of fungi were isolated from the samples of Siddique bazar where frequency percentage of association of *Aspergillus niger* was also found the highest (40) followed by *A. fumigatus* (20), *Fusarium* sp. (15.7) and *Penicillium* sp. (13.3%). Frequency percentage of association of *C. brachyspora* was lowest (11%).

Table 1. Mean frequency (%) of association of fungi with fruits of *M. charantia* (Local variety) samples collected from five different markets.

Name of isolates	Frequency (%) of fungi					Total	Mean
	A	H	K	P	S		
Aspergillus flavus	15.7	22.3	0	15.7	0	53.7	10.74
A. fumigatus	0	0	09	0	20	29	5.80
A. niger	44.3	31	40	49	40	204.3	40.86
C. brachyspora	0	0	0	11	11	22	4.40
Fusarium sp.	0	15.7	13.3	0	15.7	44.7	8.94
Penicillium sp.	24.3	21	17.7	42	13.3	118.3	23.66
Rhizopus stolonifer	26.7	0	0	0	0	26.7	5.34
Trichoderma viride	0	0	20	11	0	31	6.20

A = Ananda bazar, H= Hatirpul bazar, K = Karwan bazar, P = Palashi bazaar,S= Siddique bazar.

Nine species of fungi *namely Aspergillus niger, A. flavus, A. fumigatus, Curvularia brachyspora*, *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *R. stolonifer* and *T. viride* were isolated from fruits of *M. charantia* Hybrid variety (Table 2). Five species of fungi were isolated from the samples of Ananda bazar where frequency percentage of association of *Aspergillus niger* was the highest (40) followed by *Penicillium* sp. (26.7), *Fusarium* sp. (15.3), and *A. fumigatus* (13.3). Frequency percentage of association of *Curvularia brachyspora* was lowest (11). Five species of fungi were isolated from Hatirpul bazar where frequency percentage of association of *A. niger* was highest (35.7) followed by *Penicillium* sp. (22.3), *Fusarium* sp. (15.7) and *A. flavus* (13.3). Frequency percentage of association of *R. stolonifer* was lowest (13.3). Five species of fungi were isolated from the samples of Karwan bazar where frequency percentage of association of *A. niger* was highest (40) followed by *Penicillium* sp. (15.7), *Mucor* sp. (13.3), and *Fusarium* sp. (11). Frequency percentage of association of *A. fumigates* was lowest (6.7). Six species of fungi were isolated from the samples of Palashi bazar where frequency percentage of association of *A. niger* was the highest (49) followed by *Penicillium* sp. (46.7), *T. viride* (22.3), *Mucor* sp. (11) and *A. spergillus flavus* (9). Frequency percentage of association of *R. stolonifer* was lowest (6.7). Six species of fungi were isolated from the samples of Siddique bazar where frequency percentage of association of *A. niger* was also found to be highest (46.7) followed by *Fusarium* sp. (20), *Penicillium* sp. (18), *A. fumigatus* (15.7), *C. brachyspora* (15.7) and *Fusarium* sp. (15.7). Frequency percentage of association of *T. viride* was lowest (11).

Table 2. Mean frequency (%) of association of fungi with fruits of *M. charantia* (hybrid variety) samples collected from five different markets.

Name of isolates	Frequency (%) fungi					Total	Mean
	A	H	K	P	S		
Aspergillus flavus	0	13.3	0	9	0	22.3	4.46
A. fumigatus	13.3	0	6.7	0	15.7	35.7	7.14
A. niger	40	35.7	40	49	46.7	211.4	42.28
C. brachyspora	11	0	0	0	15.7	26.7	5.34
Fusarium sp.	15.3	15.7	11	0	20	62	12.4
Mucor sp.	0	0	13.3	11	0	24.3	4.86
Penicillium sp.	26.7	22.3	15.7	46.7	18	129.4	25.88
Rhizopus stolonifer	0	13.3	0	6.7	0	20	4.00
Trichoderma viride	0	0	0	22.3	11	33.3	6.66

A = Ananda bazar, H= Hatirpul bazaar, K = Karwan bazar, P = Palashi bazar, S= Siddique bazar.

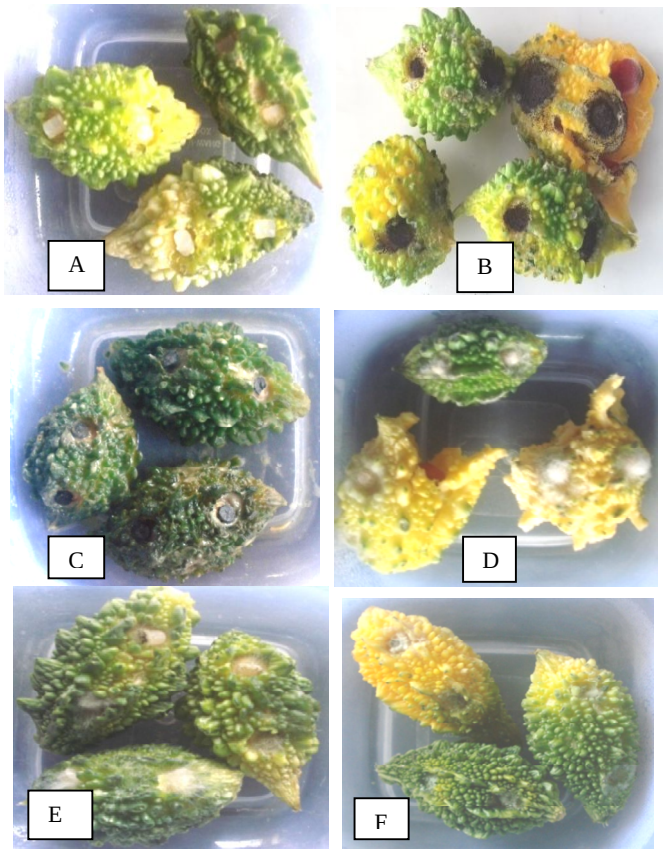

Plate 1. Fruits of *M. charantia* (Local variety) :A. control fruits; B. fruits inoculated by *A. niger, C. brachyspora*; D. *Fusarium* sp.; E. *R. stolonifer* and F. *T. viride.*

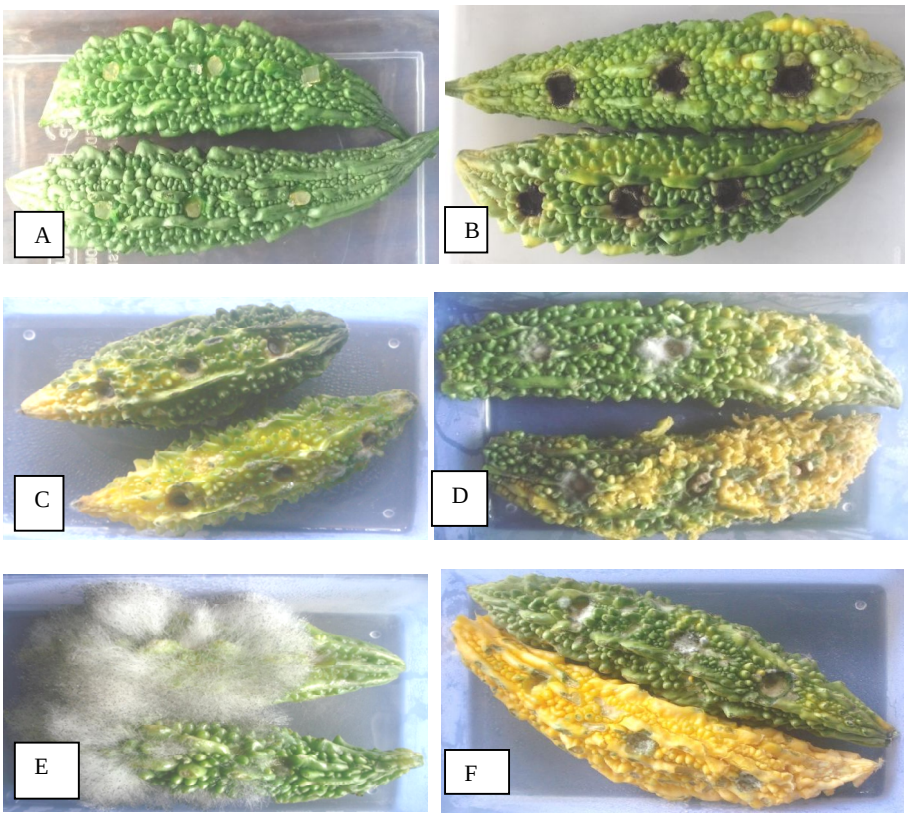

Plate 2. Fruits of *M. charantia* (Hybrid variety): A. control fruits, B. fruits inoculated by *A. niger*, *C. brachyspora* ; D. *Fusarium* sp. E. *R. stolonifer* and F. *T. viride.*

All the isolated fungi from local and hybrid variety of *M. charantia* were tested for their pathogenic potentiality. Five isolated fungi *viz, A. niger*, *C. brachyspora*, *Fusarium* sp., *R. stolonifer* and *T. viride* were found to be pathogenic to both the varieties of *M. charantia*. Control plate remained fresh without any fungal growth (Plates 1 and 2).

From India Mukerji and Bhasin (1986) reported fruit rot of *M. charantia* caused by *Alternaria tenuissima* (Kunze. ex Pers.), *Colletotrichum lagenarium* (Pers.) Ell. & Halst, *Fusarium oxysporum* Schlecht ex fr., *Pythium aphanidermatum* (Exd.) Fitzp. and *Synchytrium wurhii* Rytz.

From Pakistan Sultana and Gaffar (2007*)* reported 29 species of fungi namely *Alternaria alternate* (Fr.) Keisler, *A. tenuissima* Kunze ex Pers. *A. candidus* Link., *A. flavus* Link & Pers., *A. niger* Van Tiegh., *A. tamarii* Kita,Centr., *A. terreus* Thom, *A. wentii* Wehmer, *Chaetomium funicola* Cooke, *C. globosum* Kunze ex Fr., *C. murorum, C. olivaceum* Cook & Ellis, *Cladosporium cladosporioides* (Fr.) de Vries, *C. oxysporum* Schlecht.

Emend. Snyd. & Han., *C. sphaerospermum* Penz., *Drechslera* state of *Cochliobolus spicifer* Nelson, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *F. oxysporum*. *Memnoniella echinata* (Riv.) Gallowany, *M. verrucaria* (Alb. & Schw.) Ditm. *ex* Fr., *Nigrospora oryzae* (Berk & Br.) Petch, *Penicillium purpurogenum* Stoll, *Rizoctonia solani* Kuhn., *Rhizopus* sp., *Stachybotrys atra* Corda, *Stemphylium* sp., and *Trichurus spiralis* as seed borne pathogen of *M. charantia.*

The isolated fungal pathogens reported by Mukerji and Bhasin (1986) are not found in the present investigation in Bangladesh.

Sultana and Gaffar (2007) isolated 29 species of fungi associated with seeds of *M. charantia* among which two species are same as reported in the present investigation.

In the present investigation nine fungi were associated with two varieties of *M. charantia* of which five fungi were found to be pathogenic. This is the first report on association of *C. brachyspora*, *Fusarium* sp., *Mucor* sp., *Rhizopus stolonifer* and *Trichoderma viride* with *M. charantia* in storage. This finding will be helpful for designing effective control measures of *M. charantia* for post harvest handling and storage.

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TAXONOMIC STUDY ON THE ANGIOSPERMS OF CHAR KUKRI MUKRI WILDLIFE SANCTUARY, BHOLA DISTRICT

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Abstract

The paper presents the status of angiospermic flora of Char Kukri Mukri Wildlife Sanctuary, a small Island in the Bay of Bengal close to the Char Fassion Upazila of Bhola district. A total of 277 plant species belonging to 76 families was identified from the Island. For each plant species data on scientific name, local name, family, life form and habitat were provided. Trees of this Island were represented by 91, shrubs by 33, herbs by 118 and climbers by 35 species. The plant species recorded from the island were distributed in different habitats. Among the habitats, maximum species were recorded in homesteads (104) followed by roadsides (79), mangrove areas (47) and cultivated land (47).The study has reported the presence of medicinal plants, wildlife supporting plants, exotics and invasive plants, rare and threatened plants in the Island. The presence of fruit bearing species in the island is very rare because of high salinity. The introduction of exotics and invasive species into the Island has been recognized as the great challenges to the local angiospermic flora in future. This article also highlights the conservation values, management concerns and some measures for conservation of angiosperm diversity in the Island.

Key words: Taxonomic study, Angiosperms, Char Kukri Mukri, Bhola District

Introduction

Char Kukri Mukri Island is located in the southern side of Char Fassion Upazila of Bhola district is isolated from the main land facing the Bay of Bengal. The total area of the island is about 40 km^2. According to local people, human habitation started in the island approximately from 1930 during the British regime. The island was inundated by a big cyclone in 1970 and washed away almost all the people. After the cyclone people again migrated from the main land to the area for fishing and built temporary houses. During the year of 1973/1974, Bangladesh Forest Department started forestation program using the species of *Sonneratia apetala* (Keora), *Avicennia officinalis (*Baine) and *Excoecaria agallocha* (Geoa) in all around the Island. The present planted forest area is about 11307.42 ha (Personal communication with local forest office). Among the forest area, 4973.43 ha is managed by Sadar forest beat and 1360.99 ha by Char Patila beat. The forest is now very dense with many other associated species. Bangladesh Forest Research Institute (BFRI) had introduced plantation trial unit in the island using local mangrove species and some other mesophytic plant species. Such plantation has been performing better in the intertidal zone. The forest bed is muddy and inundated by tidal actions twice

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in a day. The Island is also dissected by 6 small canals and its center part is under huge rice cultivation and human habitation located along the flood protected dams. The soil of the forest is highly alkaline. In 1981, under the Bangladesh Wildlife (Preservation) Amendment Act of 1947, Bangladesh Government has declared the forest area of Char Kukri Mukri as wildlife sanctuary for the protection of its biodiversity. Currently the Island is the dwelling place for 15000 people. The area enjoys a moist tropical maritime climate and rainfall is frequent and heavy during the monsoon season (May to October) ranging between 142 mm to1044 mm. Temperature ranges from 16ºC to 33ºC, whereas humidity ranges from 29% to 99% (BBS 2011).

The diversity of plants is very much essential in shaping human civilization in modern days. Unfortunately, such diversity has been eroding in alarming rate from the nature before evaluation and documentation. At the end of 19th century the head of states from all over the world had realized this burning issue. World leaders met in Rieo De Janerio de Janeiro in 1992 to formulate biodiversity conservation policy including agenda 21 which had also given emphasis on the documentation and sustainable utilization of traditional knowledge of plant diversity. After the convention the assessment works of plant diversity in different countries of the world is in progress. In case of Bangladesh angiosperm diversity assessment of different national parks and wildlife sanctuary has already been started (Khan *et al*. 1994, Rahman and Hassan 1995, Uddin *et al*. 1998, Uddin and Rahman 1999, Khan and Huq 2001, Uddin *et al*. 2011, Uddin and Hassan 2004, 2010, Uddin *et al*. 2013, Uddin *et al.* 2015 and Sajib *et al.* 2015). Literature survey revealed that there is no works on the documentation of the angiosperm flora of Char Kukri Mukri wildlife sanctuary. For the management of the sanctuary, baseline data on the flora are essential. In the present study an attempt was made to attain the following objectives: to document the angiosperms, to identify management concerns of the island and to suggest some conservation measures for Char Kukri Mukri wildlife sanctuary.

Materials and Methods

Extensive floristic survey (Hyland 1972, Balick *et al.* 1982 and Alexiades 1996) was done in different seasons of the year of 2014 and 2015. The survey included mangrove, cultivated land, roadside and homestead area. Special efforts were given to find species of conservation concern including threatened, endemic and rare species. Sample size was determined using species area curve or species time curve (Goldsmith and Harrison 1976). Maximum identification was done in the field sites and in case of confusion in identification, representative plant specimens were collected and processed using standard herbarium techniques (Hyland 1972). Identification was done by consulting different Floras (Uddin and Hassan 2004, Siddiqui *et al.* 2007 and Ahmed *et al.* 2008a, 2008b, 2009a, 2009b, 2009c, 2009d, 2009e). The updated nomenclature of the species are included by following Siddiqui *et al.* 2007 and Ahmed *et al.* 2008a, 2008b, 2009a, 2009b,

2009c, 2009d, 2009e). Threatened categories of plants were confirmed with the help of Khan *et al*. (2001) and Ara *et al*. (2013). Some noxious exotic plant species were also identified comparing with the reports of Hossain and Pasha (2004) and Akter and Zuberi (2009). Families were arranged according to Cronquist (1981). Voucher specimens are preserved at Dhaka University Salar Khan Herbarium (DUSH).

Results and Discussion

A total of 277 plant species belonging to76 families was identified from the Char Kukri Mukri Island. For each plant species scientific name, local name, family, life form and habitat are presented in Table 1. Among the families, Cyperaceae, Poaceae, Fabaceae, Convolvulaceae,Asteraceae, Mimosaceae, Caesalpiniaceae, Euphorbiaceae, Verbenaceae, Amaranthaceae and Acanthanceae were found to be most common. Of 277 species, trees were represented by 91, shrubs by 33, herbs by 118 and climbers by 35 species. The plant species recorded from the island were found to be distributed in different habitats. Among the habitats, maximum species were recorded in the homesteads (104) followed by roadsides (79), mangrove areas (47) and cultivated land(47). Most of the plant species in the homesteads and roadsides were introduced by local people, forest department, forest research institute, enthusiastic people and local government. The number of fruit bearing plants was minimum in the island because of high salinity. During survey much attention was paid in the following habitats:

The mangrove plantations were developed all around the island. Each year the newly accreted lands facing the sea were undertaken by forest department under plantation programs. The top canopy in the mangrove was occupied by *Sonneratia apetala, Sonneratia caselaoris, Avicennia officinalis, Excoecaria agallocha* and *Bruguiera gymnorrhiza.* Besides few representations of *Heritiera fomes, Xylocarpus granatum, Xylocarpus moluccensis, Cerbera mangus, Ceriops decandra, Dolichandronae spathacea, Aegiceros corniculata* were also detected in forest. The forest ground was covered mainly by, the seedlings of *Excoecaria agallocha, Sonneratia apetala,* and*Avicennia officinalis.* In the forest edge the bush forming dominant species were *Acanthus ilicifolius, Dalbergia spinosa, Nipa fruticans, Hibiscus populnea, Thespesia lampus, Sapium indicum* and *Excoecaria agallocha.* The ground near the intertidal zone was mainly dominated by *Porteresia coarctata, Zoysia matrella, Cryptocoryne retrospiralis, Zoysia tenuifolia and Saccharum spontaneum.* Most common climbers in the mangrove forest were *Derris scandens, Derris trifolia, Flagellaria indica, Ipomoea litoralis, and Cercolobus carinatus.* Some members of sedge species including *Cyperus diformis, Cyperus eragrostis, Cyperus imbricatus* and *Cyperus lucidus* were observed in this zone. The banks of the tidal canals were dominated by a good number of tree species such as *Pongamia pinnata, Barringtonia acutangula, Trewia polycarpa, Crataeva nurvala, Heritiera fomes, Nipa fruticans, Tamarindus indica, Sonneratia apetala, Avicennia officinalis, Sonneratia caseolaris, Samanea saman, Albizia procera, Hibiscus populnea, Xylocarpus granatum, Calophylum innophylum, Acacia catechu* and *Albizia recardiana.*

Table 1. Angiosperms flora of Char Kukri Mukri Island.

Scientific name	Local name	Family	Habit	Habitat
Abelmoschus moschatus Mdedic.	Bonderos	Malvaceae	H	Roadside
Ablemoschus esculentus (L.) Moench	bendi	Malvaceae	H	Homestead
Abutilon indicum (L.) Sweet	-	Malvaceae	S	Roadside
Acacia auriculiformis A. Cunn. Ex Benth. & Hook.	Acashmoni	Mimosaceae	T	Roadside
Acacia catechu (L.f.) Willd.	Khiababla	Mimosaceae	T	Roadside
Acacia mangium Willd.	Belgium	Mimosaceae	T	Roadside
Acacia nilotica L.	Babla	Mimosaceae	T	Homestead
Acanthus ilicifolius L.	Hargoza	Acanthaceae	S	Mangrove
Achyranthes aspera L.	Upathlenga	Amaranthaceae	H	Homestead
Adenanthera pavonina L.	Lalchandon	Mimosaceae	T	Homestead
Adhatoda zeylanica Medikus	Bashak	Acanthaceae	S	Homestead
Aegiceras corniculata (L.) Blanco	Khulshi	Myrsinaceae	S	Mangrove
Aegle marmelose (L.) Corr.	Bel	Rutaceae	T	Homestead
*Ageratum conyzoides (*L.) L.	Fulkuri	Asteraceae	H	Roadside
Albizia lebbeck (L.) Benth. & Hook.	Shilkoroi	Mimosaceae	T	Homestead
Albizia procera (Roxb.) Benth.	Sadakoroi	Mimosaceae	T	Homestead
Albizia richardiana (Voigt.) King & Prain.	Shiris	Mimosaceae	T	Homestead
Albizia saman (Jacq.) Merr.	Botkoroi	Mimosaceae	T	Homestead
Allium tuberosum Rottler ex Spreng.	Chinese leek	Liliaceae	H	Homestead
Alocasia macrorrhizos (L.) G. Don	Mankachu	Araceae	H	Homestead
Alternanthera philoxeroides (Mart.) Griseb.	Helencha	Amarnthaceae	H	Cultivated lanc
Alternanthera sessilis (L.) R. Br. *Ex* DC..	Hainchashak	Amaranthaceae	H	Cultivated lanc
Amaranthus gangeticus L.	Lashak	Amaranthaceae	H	Homestead
Amaranthus spinosus L.	Kantanote	Amaranthaceae	H	Roadside
Amaranthus viridis L.	Data shak	Amaranthaceae	H	Homestead
Anacardium occidentale L.	Kajubadam	Anacardiaceae	T	Homestead
Annona squamosa L.	Ata	Annonaceae	T	Homestead
Anodendron paniculatum (Roxb.) A. DC.	-	Asclepiadaceae	C	Mangrove
Anthocephalus cadamba (Roxb.) Miq.	Kadam	Rubiaceae	T	Homestead
Aphanamixis polystachya (Wall.) R. N. Parker	Pitraj	Meliaceae	T	Homestead
Aphania danura(Roxb.) Radlk.	Apin	Sapindaceae	S	Roadside
Areca catechu L.	Supari	Arecaceae	T	Homestead
Argyreia argentea (Roxb.) Choisy	-	Convolvulaceae	C	Roadside
Artocarpus heterophyllus Lamk.	Kathal	Moraceae	T	Roadside
Artocarpus lacucha Buch.-Ham.	Dewa	Moraceae	T	Homestead
Averrhoa carambola L.	Kamranga	Averrhoaceae	T	Homestead
Avicennia officinalis L.	Baine	Verbenaceae	T	Mangrove

Contd.

Scientific name	Local name	Family	Habit	Habitat
Axonopus compressus (Sw.) P. Beauv.	Dhakagass	Poaceae	H	Roadside
Azadirachta indica A. Juss.	Neem	Meliaceae	T	Homestead
Bacopa monnieri (L.) Pennell	Brammi Shak	Scrophulariaceae	H	Mangrove
Bambusa balcooa Roxb.	Baijja Bans	Poaceae	T	Homestead
Barringtonia acutangula (L.) Gaertn.	Hizol	Lecythidaceae	T	Mangrove
Basella rubra L.	Puishak	Basellaceae	C	Homestead
Bauhinia purpurea L.	Kanchan	Caesalpiniaceae	T	Homestead
Benincasa hispida (Thunb.) Cogn.	Chalkumra	Cucurbitaceae	C	Homestead
Blumea lacera (Burm. f.) DC.	Kukurmuta	Asteraceae	H	Roadside
Blumea membranacea Wall.*ex* DC.	Kukurmuta	Asteraceae	H	Roadside
Bombax ceiba L.	Shimultula	Bombacaceae	T	Homestead
Borassus flabellifer L.	Tal	Arecaceae	T	Roadside
Breynia retusa (Dnnst.) Alston.	-	Euphorbiaceae	S	Roadside
Bruguiera gymnorrhiza (L.) Lamk.	Kakra	Rhizophoraceae	T	Mangrove
Bryophyllum pinnatum (Lamk.)Oken	Pathorkusi	Crassulaceae	H	Homestead
Caesalpinia bunduc (L.) Roxb.	Neta	Caesalpiniaceae	C	Roadside
Caesalpinia crista L.	-	Caesalpiniaceae	C	Roadside
Cajanus cajans (L.) Millsp.	Orhor	Fabaceae	S	Roadside
Calamus guruba BUch.-Ham. Ex Martius	Bet	Arecaceae	C	Roadside
*Calophylum innophylum*L.	Kunail	Clusiaceae	T	Roadside
Calotropis gigantea (L.) R. Br.	Akanda	Asclepiadaceae	S	Roadside
Calotropis procera (Aiton) Dryand	Akand	Asclepiadaceae	S	Roadside
Canavalia ensiformis (L.) DC.	Moiseem	Fabaceae	C	Roadside
Canavalia maritime Thou.	-	Fabaceae	C	Roadside
Capsicum frutescens L.	Morich	Solanaceae	H	Cultivated lanc
***Carex caricinus* L.**	Sedge	Cyperaceae	H	Mangrove
Carica papaya L.	Pepe	Caricaceae	S	Homestead
*Cassia alata*L.	Dadmordan	Caesalpiniaceae	S	Homestead
Cassia fistula L.	Sonalu	Caesalpiniaceae	T	Roadside
Cassia occidentalis L.	-	Caesalpiniaceae	H	Roadside
Cassia siamea Lamk.	Minjori	Caesalpiniaceae	T	Roadside
Cassia tora L.	-	Caesalpiniaceae	H	Roadside
Casuarina equisetifolia L.	Jau	Casuarinaceae	T	Roadside
Cayratia japonica (Thunb.) Gagnep.	-	Vitaceae	C	Roadside
*Celosia cristata*L.	Morogful	Amaranthaceae	H	Homestead
Centella asiatica (L.) Urban	Adamoni	Apiaceae	H	Roadside
Cerbera manghas L.	Cerbera	Apocynaceae	T	Mangrove
Ceriops decandra (Griff.) Ding. Hou	Goran	Rhizophoraceae	T	Mangrove
Chrysalidocarpus lutescens (Bory) H. Wendl.	Arecapalm	Arecaceae	T	Homestead
Chrysopogon aciculatus (Retz.) Trin.	Premkanta	Poaceae	H	Roadside
Citrus aurantifolia (Christm.&Panzer) Swingle	Lebu	Rutaceae	S	Homestead

Contd.

Scientific name	Local name	Family	Habit	Habitat
Citrus maxima (Burm. F.) Merr.	Jambura	Rutaceae	T	Homestead
Clerodendrum indicum (L.) Kuntze	Bhat	Verbenaceae	S	Mangrove
Clerodendrum inerme (L.) Gaertn.	-	Verbenaceae	S	Magrove
Clerodendrum Viscosum Vent.	Bhat	Verbenaceae	H	Mangrove
Clitorea turnetea L.	Aparajita	Fabaceae	C	Homestead
Cocos nucifera L.	Narikel	Arecaceae	T	Homestead
Codiaeum variegatum (L.) A. Juss.	Patabahar	Euphorbiaceae	S	Homestead
Coix lacryma-jobi L.	-	Poaceae	H	Roadside
Colocasia esculenta (L.) Schott	Kachu	Araceae	H	Homestead
Cotula hemispherica (Roxb.) Wall.ex CB. Clarke	Cotula	Asteraceae	H	Cultivated land
Crateva nurvala Buch.-Ham.	Borun	Capparadiaceae	T	Mangrove
Crinum amoenum Roxb.	Bonroshun	Liliaceae	H	Mangrove
*Crinum asiaticum*L.	Crinum	Liliaceae	H	Mangrove
Crotalaria juncea L.	Junjuni	Fabaceae	H	Roadside
Croton bonplandianus Baill.	Bankhira	Euphorbiaceae	H	Roadside
Chrozophora plicata (Vahl.) A. Juss. ex. Spreng.	-	Euphorbiaceae	H	Roadside
Cryptocoryne retrospiralis (Roxb.) Fisch.	Kelakachu	Araceae	H	Mangrove
Cucurbita maxima Duchesne	Misti kumra	Cucurbitaceae	C	Homestead
Curcuma domestica Valet.	Halud	Zingiveraceae	H	Homestead
Curcuma gedoaria (Christm.) Rosc.	Shadi	Zingiveraceae	H	Roadside
Cuscuta reflexa Roxb.	Shwarnalata	Cuscutaceae	C	Roadside
Cyclea barbata Miers.	Patalpur	Menispermaceae	C	Roadside
Cynodon dactylon (L.) Pers.	Durbagass	Poaceae	H	Homestead
Cynometra ramiflora L.	Singra	Fabaceae	T	Mangrove
Cyperus diformis L.	Sedge	Cyperaceae	H	Mangrove
Cyperus eragrostis Vahl.	Sedge	Cyperaceae	H	Mangrove
Cyperus imbricatus Retz.	Sedge	Cyperaceae	H	Mangrove
Cyperus lucidus	Sedge	Cyperaceae	H	Mangrove
Cyperus rotundus L.	Muthagass	Cyperaceae	H	Cultivated land
Dalbergia sissoo DC.	Shissu	Fabaceae	T	Roadside
Dalbergia spinosa Roxb.	Tamu	Fabaceae	S	Mangrove
Delonix regia Rafin.	Krishnachura	Caesalpiniaceae	T	Roadside
Dentella repens (L.) J. R. & G. Forst.	Bhuipat	Rubiaceae	H	Cultivated land
Derris scandens (Roxb.) Benth.	Kalilata	Fabaceae	C	Mangrove
Derris trifoliata Lour.	Kalilota	Fabaceae	C	Mangrove
Dillenia indica L.	Chalta	Dilleniaceae	T	Homestead
Dioscorea alata L.	Jora alu	Dioscoriaceae	C	Homestead
Dioscorea bulbifera L.	Matialu	Dioscoriaceae	C	Homestead
Diospyros blancoi A. DC.	Bilatigab	Ebenaceae	T	Homestead
Diospyros malabarica (Desr.) Kostel.	Deshigab	Ebenaceae	T	Homestead
Dolichandrone spathacea (L.f.) K. Schum.	Chamhechandan	Bignoniaceae	T	Mangrove

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Scientific name	Local name	Family	Habit	Habitat
Eclipta prostrata (L.) Mant.	Keshoraj	Asteraceae	H	Cultivated land
Ehretia serrata Roxb.		Boraginaceae	T	Roadside
Eichhornia crassipes (Mart.) Solms	Kachripana	Pontederiaceae	H	Homestead
Elaeocarpus tectorius (Lour.) Poir.	Jolpai	Elaeocarpaceae	T	Cultivated land
Eleocharis geniculata (L.) Roem. & Schult.	Joraghasi	Cyperaceae	H	Cultivated land
Eleusine indica (L.) Gaertn.	Malankuri	Poaceae	H	Cultivated land
Eryngium foetidum L.	Shamadoine	Apiaceae	H	Homestead
Erythrina indica Lamk.	Painnamandar	Fabaceae	T	Homestead
Erythrina ovalifolia Roxb.	Mandar	Fabaceae	T	Homestead
Eucalyptus camaldulensis Dehnhardt	Eucalyptus	Myrtaceae	T	Roadside
Excoecaria agallocha L.	Geoa	Euphorbiaceae	T	Mangrove
Ficus benghalensis L.	Bot	Moraceae	T	Roadside
Ficus hispida L. f.	Dumur	Moraceae	T	Homestead
Ficus infectoria Roxb.	Pakur	Moraceae	T	Roadside
Ficus racemosa L.	Jogdumur	Moraceae	T	Roadside
Ficus rumphii Blume.	Pakur	Moraceae	T	Roadside
Fimbristylis acuminata Vahl	-	Cyperaceae	H	Cultivated land
Fimbristylis dichotoma (L.) Vahl	Fimbristylis	Cyperaceae	H	Roadside
Fimbristylis ferruginea (L.) Vahl	-	Cyperaceae	H	Cultivated land
Flcourtia indica (Burm.f.) Merr.	Paniala	Flacourtiaceae	T	Homestead
Flumeria alba L.	Katgolap	Combretaceae	T	Homestead
Flagellaria indica L.		Flagellariaceae	C	Mangrove
Garcinia cowa Roxb. *ex* DC.	Kao	Clusiaceae	T	Homestead
Gardenia jasminoides J.Ellis	Gandhraj	Rubiaceae	S	Homestead
Gmelina arborea Roxb.	Gamari	Verbenaceae	T	Roadside
Gomphrena globosa L.	Botamphul	Amaranthaceae	H	Roadside
Gosypium herbaceum L.	Karpustula	Malvaceae	H	Roadside
Grangea maderaspatana (L.) Poir.	Nemuti	Asteraceae	H	Cultivated land
Heliotropium curassavicum L.	Nuinna	Boraginaceae	H	Cultivated land
Heliotropium indicum L.	Hatisur	Boraginaceae	H	Cultivated land
Heritiera fomes Buch.-Ham.	Sundari	Sterculiaceae	T	Mangrove
Hibiscus rosa-sinensis L.	Joba	Malvaceae	S	Homestead
Hydrilla verticilata (L.f.) Royle	Jaji	Hydrocaritaceae	H	Cultivated land
Hygrophila phlomoides Nees	-	Acanthaceae	H	Cultivated land
Hygrophila salicifolia (Vahl) Nees	Kakmasha	Acanthaceae	H	Cultivated land
Imperata cylendrica (L.) P.Beauv.	Ulu	Poaceae	H	Roadside
Ipomea batata (L.) Lamk.	Mistialu	Convolvulaceae	C	Homestead
Ipomoea aquatica Forssk.	Kolmi	Convolvulaceae	H	Homestead
Ipomoea fistulosa Mart. *ex* Choisy	Dolkolmi	Convolvulaceae	H	Roadside
Ipomoea littoralis Blume.	-	Convolvulaceae	C	Mangrove
*Ipomoea pes-caprae (*L.) R. Br.	Chagalkhuri	Convolvulaceae	H	Cultivated land
Justicia gendarussa Burm.f.	Justicia	Acanthaceae	H	Roadside
Kyllinga sesquiflora Torr.	Sedge	Cyperaceae	H	Cultivated land

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Scientific name	Local name	Family	Habit	Habitat
Kyllinga nemoralis (J.R.Forst. & G. Forst) Dandy ex Hutchins&Dalziel	Sedge	Cyperaceae	H	Cultivated land
Lablab purpurea (L.) Sweet	Seem	Fabaceae	C	Homestead
Lagenaria siceraria (Molina) Standl.	Lao	Cucurbitaceae	C	Homestead
Lagerstroemia indica L.	Cheri	Lythraceae	T	Homestead
Lagerstroemia speciosa (L.) Pers.	Jarul	Lythraceae	T	Homestead
Lannea coromandelica (Houtt.) Merr.	Bhadi	Anacardiaceae	T	Homestead
Lathyrus sativus L.	Khesari	Fabaceae	H	Cultivated land
Lawsonia inermis L.	Mehendi	Lythraceae	T	Homestead
Leucaena leucocephala (Lamk.) de Wit.	Epilepil	Mimosaceae	T	Roadside
Lindernia indica	-	Scrophulariaceae	H	Cultivated land
Lippia alba (Mill.) N. E. Br. Ex Britt. &Wilson	Bhuiokra	Verbenaceae	H	Roadside
Litchi chinensis Sonn.	Lichu	Sapindaceae	T	Homestead
Ludwigia hyssopifolia G. Don) Excell apud A &R. Fernandes	Panilong	Onagraceae	H	Cultivated land
Ludwigia repens Forst.	Molsi	Onagraceae	H	Cultivatedland
Luffa cylindrical (L.) M. Roem.	Dundul	Cucurbitaceae	C	Homestead
Mangifera indica L.	Aam	Anacardiaceae	T	Homestead
Mariscus squarrosus (L.) C. B. Clarke	Sedge	Cyperaceae	H	Cultivated land
Melia azederach L.	Goraneem	Meliaceae	T	Homestead
Merremia peltata (L.) Hallier f.		Convolvulaceae	C	Roadside
Merremia umbelata(L.) Hallier f.	merrimia	Convolvulaceae	C	Roadside
Mikania micrantha Kunth	Assamilata	Asteraceae	C	Roadside
Momordica cochinchinensis (Lour.) Spreng	Bonkakrol	Cucurbitaceae	C	Roadside
Morinda citrifolia L.	Banach	Rubiaceae	S	Homestead
Moringa oleifera Lamk.	Shajna	Moringaceae	T	Homestead
Mosla dainthera (Buch.-Ham. *ex* Roxb.)Maxim.	-	Lamiaceae	H	Homestead
Mucuna gigantea (Willd.) DC.	Bara-alkuchi	Fabaceae	C	Roadside
Musa paradisiaca L.	Kola	Musaceae	H	Homestead
Nelsonia canescens (Lamk.) Spreng.	-	Acanthaceae	H	Roadside
Nerium indicum Mill.	Korobi	Apocynaceae	S	Roadside
Nymphaea alba L.	Shadashapla	Nymphaeaceae	H	Cultivated land
Nymphaea nauchali Burm.	Shapla	Nymphaeaceae	H	Cultivated land
Nymphaea rubra Roxb. Ex Andr.	Lalshapla	Nymphaeaceae	H	Cultivated land
Nympheae pubescens Willd.	Shapla	Nymphaeaceae	H	Cultivated land
Nypa fruticans Wurmb.	Goalpata	Arecaceae	S	Mangrove
Ocimum sanctum L.	Tulsi	Lamiaceae	H	Homestead
Operculina turpethum (L.) S. Manso.	-	Convolvulaceae	C	Roadside
Oryza sativa L.	Motadhan	Poaceae	H	Cultivated land
Oxalis corniculata L.	Amrul	Oxalidaceae	H	Roadside

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Scientific name	Local name	Family	Habit	Habitat
Paspalum distichum L.	Gitlaghas	Poaceae	H	Cultivated land
Paspalum vaginatum Sw.	-	Poaceae	H	Meadow
Pedilanthus tithymaloides Poit.	Chita	Euphorbiaceae	H	Homestead
Pendanus foetida	Keakanta	Pandanaceae	H	Cultivated land
Phaulopsis imbricata (Forssk.) Sweet	Kantasi	Acanthaceae	H	Roadside
Phoenix paludosa Roxb.	Hetal	Arecaceae	S	Mangrove
Phoenix sylvestris (L.)Roxb.	Khejur	Arecaceae	T	Roadside
Phragmites karka (Retz.) Trin.ex. steud.	Nol	Poaceae	H	Mangrove
Phyla nodiflora (L.) Greene	Kanghas	Verbenaceae	H	Cultivated land
Phyllanthus reticulatus Poir.	Sitki	Euphorbiaceae	S	Roadside
Physalis minima L.	Potpoti	Solanaceae	H	Roadside
Pithecellobium dulce (Roxb.) Benth.	Khoibabla	Mimosaceae	T	Homestead
Polygonum blebeium R. Br.	-	Polygonaceae	H	Cultivated land
Polygonum flaccidum Roxb.	-	Polygonaceae	H	Cultivated land
Pongamia pinnata (L.) Pierre	Koroj	Caesalpiniaceae	T	Homestead
Porteresia coarctata (Roxb.) Tateoka	Urigass	Poaceae	H	Meadow
Portulaca oleracea L.	Nuainashak	Portulacaceae	H	Cultivated land
Potamogeton pectinatus L.	Gechu	Potamogatonaceae	H	Cultivated land
Psidium guajaba L.	Peara	Myrtaceae	T	Homestead
Psilotrichum ferrugineum (Roxb.) Moq.-Tand.	Putishak	Amaranthaceae	H	Cultivated land
Psophocarpus tetragonolobus (L.) DC.	Wingseem	Fabaceae	C	Homestead
Punica granatum L.	Dalim	Punicaceae	S	Homestead
Raphanus sativus L.	Mulashak	Brassicaceae	H	Cultivated land
Ricinus communis L.	Keron	Euphorbiaceae	S	Homestead
Rotala indica (Willd.) Koehne	-	Lythraceae	H	Cultivated land
Ruelia tuberosa L.	Ruelia	Acanthaceae	H	Roadside
*Saccharum officinerum*L.	Akh	Poaceae	H	Cultivated land
Saccharum spontaneum L.	Chan	Poaceae	H	Mangrove
Sapium indicum Willd.	Harua	Euphorbiaceae	T	Roadside
Sarcolobus carinatus Wall.	-	Asclepiadaceae	C	Mangrove
Schumannianthus dichotomus (Roxb.) Ganep.	Patipata	Meratnaceae	H	Homestead
Scirpus articulatus L.	Chesra	Cyperaceae	H	Cultivated land
*Scoparia dulcis*L.	Chinipata	Scropulariaceae	H	Homestead
Sesbania grandiflora (L.) Pers.	Bakul ful	Fabaceae	S	Homestead
Siplanthes acmella (L.) Murray not (L.) L.	Spilanthes	Asteraceae	H	Homestead
Solanum indicum Sensu C.B. Clark	Futki begun	Solanaceae	S	Homestead
Solanum melogena L.	Begun	Solanaceae	H	Homestead
Solanum nigrum L.	Titbegun	Solanaceae	H	Roadside
Solanum virginianum L.	Bonbegun	Solanaceae	H	Roadside
Sonneratia apetala Buch.-Ham.	Keora	Sonneratiaceae	T	Mangrove

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Scientific name	Local name	Family	Habit	Habitat
Sonneratia caseolaris (L.)Engl.	Soilla	Sonneratiaceae	T	Mangrove
Spondias pinnata (L. f.) Kurz.	Deshi amra	Anacardiaceae	T	Homestead
Stephania japonica (Thunb.) Miers	Muchchanilata	Menispermaceae	C	Roadside
Swietenia mahagoni (L.) Jacq.	Mehagoni	Meliaceae	T	Homestead
Syzygium cumini (L.) Skeels	Kalojam	Myrtaceae	T	Homestead
Syzygium fruticosum (Roxb.) DC.	Bhutijam	Myrtaceae	T	Homestead
Syzygium malaccense (L.) Merr. & L. M. Perry	Jamrul	Myrtaceae	T	Homestead
Syzygium samaracens (Blume) Merr. & Perry	Golapjam	Myrtaceae	T	Homestead
Tabarnaemontana recurva Roxb.	Togor	Apocynaceae	S	Homestead
Tagetes patula L.	Gada	Asteraceae	H	Homestead
Tamarindus indica L.	Tentul	Caesalpiniaceae	T	Homestead
Tamarix gallica L.	Nonajau	Tamaricaceae	S	Mangrove
Terminalia arjuna (Roxb. *ex* DC.) Wight & Arn.	Arjun	Combretaceae	T	Roadside
Terminalia bellirica (Gaertn.) Roxb.	Bohera	Combretaceae	T	Homestead
Terminalia catappa L.	Katgolap	Combretaceae	T	Homestead
Terminalia chebula (Gaertn.)Retz.	Bohera	Combretaceae	T	Homestead
*Thespesia lampas*Cav.) Dalz. & Gibs	Boloi	Malvaceae	S	Mangrove
Thespesia populnea (L.) Sol. Ex Corr.	Shon boloi	Malvaceae	S	Mangrove
Tilanthera phyloxeroides (Mart.) Moq.	Tilanthera	Asteraceae	H	Cultivated land
Tinospora cordifolia (Willd.) Hook.f. & Thoms.	Gulancha	Menispermaceae	C	Homestead
Toona ciliate M. Roem.	Toon	Meliaceae	T	Roadside
Trewia nudiflora L.	Pidali	Euphorbiaceae	T	Homestead
Typha elephantina Roxb.	Hogla	Typhaceae	H	Cultivated land
Urena lobata L.	Jogagota	Malvaceae	H	Homestead
Utricularia exoleata R. Br.	Jhaji	Utriculariaceae	H	Cultivated land
Vernonia cinerea (L.) Less.	Kuksim	Asteraceae	H	Roadside
Vigna unguiculata (L.) Walp.	Borboti	Fabaceae	C	Homestead
Vitex negundo L.	Nishinda	Verbenaceae	S	Roadside
Vitex trifolia L.f.	Neelnishinda	Verbenaceae	S	Roadside
Wedelia calendulacea (L.) Less.	Mohabingaraj	Asteraceae	H	Mangrove
Xanthium indicum Koen. *ex* Roxb.	Ghagrashak	Asteraceae	H	Cultivated land
Xanthosoma violaceum Schott	Dudkachu	Araceae	H	Homestead
Xylocarpus granatum Koen.	Dundul	Meliaceae	T	Mangrove
Xylocarpus moluccensis (Lamk.) Roem.	Posur	Meliaceae	T	Mangrove
Ziziphus mauritiana Lamk.	Boroi	Rhamnaceae	T	Homestead
Zoysia matrella (L.) Merr.	Gass	Poaceae	H	Meadow
Zoysia tenuifolia Willd. ex Thiele	Gass	Poaceae	H	Meadow

(T = tree, S = shrub, H = herb, C = climber).

Two tayers of dams were made all around the Island to save it from hurricane and high tidal surges. The dams are criss-cross by many roads made by the local government to facilitate communication among the people living in and around the dams. Such dams and roads were planted by the forest department using a number of both native and exotic species. The noteworthy species are *Samanea saman, Acacia catechu, Borassus flabelifer, Phoenix syvetris, Casuarina litoralis, Acacia auriculiformis, Acasia maengeum, Artocarpus heterophyllus, Calophylum innophylum, Eucalyptus camaldulensis, Dalbergia sissoo, Ehretia serrata, Ficus benghalensis, Sapium indicum, Toona ciliata, Gmelina arborea, Ficus racemosa, Leucaena leucocephala, Terminalia arjuna, Ficus rumphii, Excoecaria agallocha, Cassia siamea* and *Cassia fistula.* Some bushy plants were also found in both sides of the road. The major species are *Ricinus communis, Cajanus cajans, Sapium indicum, Excoecaria agallocha, Cassia alata, Calotropis procera, Calotrpis gigantean, Hibiscus pupolnea* and *Vitex negundo.* Many climber species were also ornamented the road sides. Most common species are *Mikania cordata, Caesalpinia bunduc, Canavalia ensiformis, Cuscuta reflexa, Merremia umbellate, Operculina turpethum, Stephania japonica, Anodendron paniculatum* and *Canavalia maritima.*

Each homestead was planted by a good number of tree species. The appearance of such homestead looks like a segment of mini forest. During our survey *Moringa oleifera, Acacia nilotica, Aegle marmelose, Albizia lebbeck, A. procera, A. richardiana, Samanea saman, Anacardium occidentale, Annona squamosa, Anthocephalus chinensis, Aphanamixis polystachya, Areca catechu, Artocarpus lacucha, Averrhoa carambola, Azadirachta indica, Bambusa balcooa, Bombax ceiba, Citrus maxima, Cocos nucifera, Ziziphus mauritiana, Trewia nudiflora, Terminalia chebula, Terminalia bellirica, Tamarindus indica, Syzygium malaccense, S. S. cumini, Swietenia mahagoni, Spondias pinnata, Psidium guajaba, Pongamia pinnata, Pithecellobium dulce, Melia azederach, Mangifera indica, Litchi chinensis, Lawsonia inermis, Lannea coromandelica, Erythrina indica, Diospyros malabarica* and *Diospyros blancoi* were recorded.

Apart from the dams and homesteads, maximum land of the island is highly fertile. Local people use such land ones in a year for rain feed aman rice and fish production. During winter and summer some of the lands are used for winter crops and summer crops. Winter and summer crops are chili, watermelon, sweetpumpkin, sweetpotato, tomato and legumes. Some aquatic seasonal plants grow in rainy season. The most common plants recorded are *Potamogeton pectinatus, Eichhornia crassipes, Jussiaea repen, Hydrila verticillata, Nymphaea pubescens, Nymphaea nouchali, Nymphaea rubra, Nymphaea capensis, Ipomoea aquatica, Tilanthera phyloxeroides, Alternanthera sessilis, Baccopa monnieri, Commelina benghalensis* and also a good number of sedges and grasses. In summer the land was covered by a number herbaceous plant. Among them the common species are *Baccopa monnieri, Dentella repens, Psilotrichum ferrugineum, Polygonum plebejum, Phyla nodiflora, Grangea madarspatana, Xanthium indicum, Portulaca oleracea, Heliotropium curassavicum, Heliotropium indicum, Eclipta prostrata* and

Alternanthera sessilis. A rare occurrence of *Typha elephantina* (Hogla) and *Phragmitis karka* (Nol) was also recorded in the wetland.

The following five species occurring in the island seem to be rare in the habitat. These are *Sarcolobus carinatus, Tamarix gallica, Calophylum inophylum, Typha elephentanea* and *Phragmitis karka.* To confirm their status further detailed survey is needed. The survey also recorded the occurrence of one species, namely *Dolichandrone spathacea* (Ara *et al.* 2013) in the Island that had already been listed as threatened in Bangladesh. A good number of medicinal plants was identified that plays an important role for the primary healthcare of local people of the island. Priority should be given for their conservation. The recorded species in the Island are *Sonneratia apetala, Sonneratia caseolaris, Nipa fruticans, Centella asiatical, Mangifera indica, Scoparia dulcis, Mikania cordata, Ipomoea fistolusa, Kalanchoe pinnata, Terminalia arjuna, Stephania japonica, Cassia alata, Terminalia belliricha* (Bohera), *Diilenia indica* (Chailta), *Terminalia chebula* (Horitaki), *Terminalia arjuna* (Arjun), *Eupatorium odoratum* (Pisais), *Mikania scandens* (Refugeelata), *Cynodon dactylon* (Durba), *Colocasia esculenta* (Kachu), and *Ficus racemosa* (Jogdumur).

Exotics and invasive species are a part of total floristic composition of the island. Some exotics, such as *Acacia auriculiformis, Acacia mengium, Eucalyptus camaldulensis, leucaena leucocephala,* and *Cassia siamea* were planted in the island area. Invasive species of the island are *Eichhornia crassipes, Mikania cordata* (Refugeelota), *Chromolaena odorata* (Pisais), *Ipomoea fistulosa, Ageratum conyzoides, and Xanthium indicum bonplandianum.* Such species are a challenge to the management of the plant diversity of the Island. A good number of wildlife supporting plant species namely by *Sonneratia apetala, Sonneratia caseolaris, Avicenneia alba, Ficus benghalensis, Ficus racemosa, Ficus rhumphii, Syzygium cummuni, Sizygium fruticosum* and *Tamarindus indica* was recorded from the island. Such species play an important role in conservation of biodiversity.

Char Kukri Mukri Island is very interesting area for eco-tourism. During this survey a number of features of the Island was indentified which has great values for conservation and ecotourism. Such features are to watch the isolated and remote Island facing to the Bay of Bengal; to watch the presence of coastal belt plantations turned into natural ecosystem; to enjoy mangrove forest; to observe the presence of introduced wildlife with their natural population; to meet friendly local people and also can enjoy local hospitality with fresh sea fish; to enjoy serine and virgin environment; to roaming and cruising all around the island by boat; to observe natural succession in the newly accreted Island; to provide huge opportunity for nature photographers; to watch shore and aquatic birds paradise.

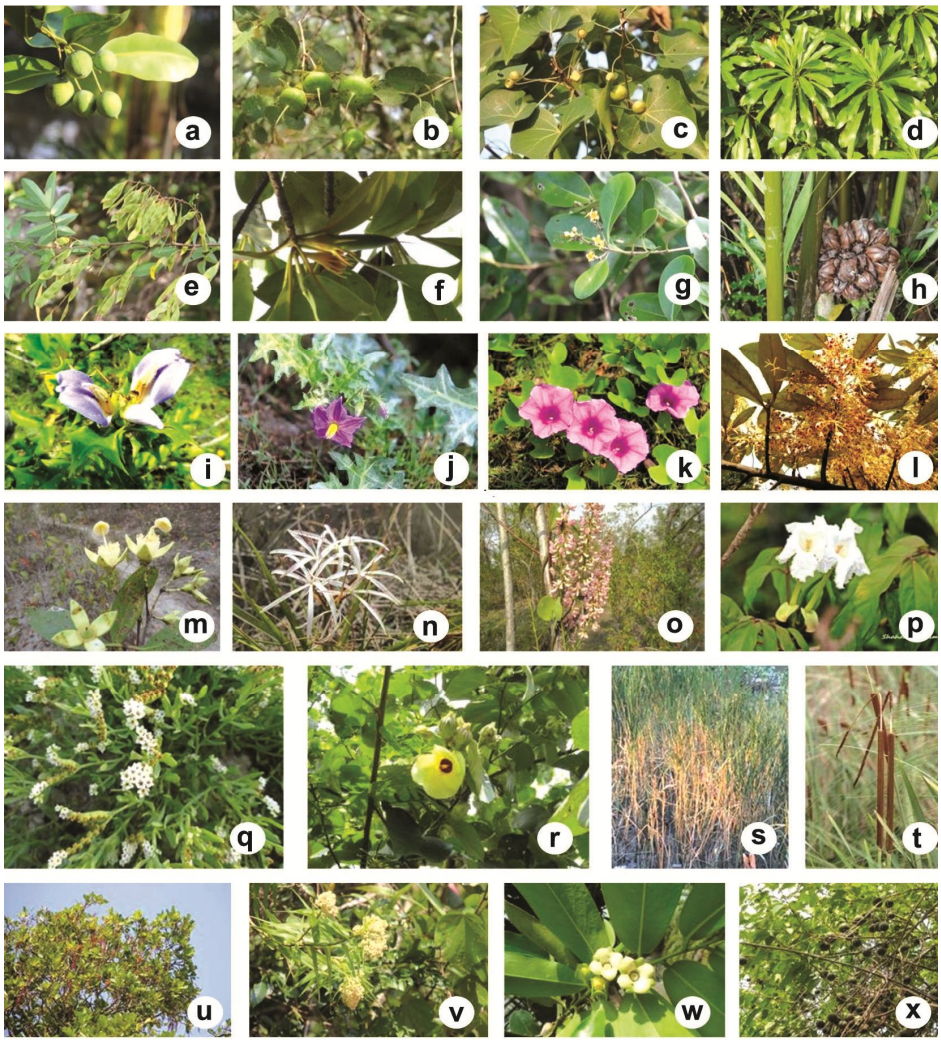

Plate 1. *a. Calophyllum inophyllum,* b. *Sonneratia caseolaris*, c. *Thespesia lampas,* d. *Cerbera manghas,* e. *Derris scandens,* f. *Barringtonia acutangula*, g. *Avicennia officinalis*, h. *Nypa fruticans,* i. *Acanthus ilicifolius,* j. *Solanum virginianum, k. .Ipomoea pes-caprae,* l. *Heritiera fomes, m. Sonneratia apetala,* n. *Crinum amoenum,* o. *Derris trifoliata*, p. *Dolichandrone spathacea*, q. *Heliotropium curassavicum,* r. *Thespesia populnea*, *s. Porteresia coarctata,* t. *Typha elephantine*, u. *Barringtonia acutangula*, *v. Flagillaria indica, w. Diospyros malabarica*, x. *Sapium indicum.*

Based on observations and discussion with local people and foresters it is evident that the island is not yet facing major threats. But the east part of the island is facing erosion

during rainy season. The species planted there are *Pongamia pinnata* (Koroz), *Barringtonia acutangula* (Hizol), *Crateva nurvala* (Baorun), *Trewia nudiflora* (Pidali) *and Acacia catechu* (Babla) all of which are fresh water enduring species. Initially such species were doing better in producing branches and canopy. But their root systems are poorly developed. During high tide period the wave actions made them uprooted easily. Mangrove species like *Sonneratia apetala* (Keora), *Sonneratia caseolaris* (Soila), *Avicennia officinalis* (Baine) and *Excoecaria agallocha* (Geoa) were found to grow well in the intertidal zone. They have strong root systems and can withstand with high wave action during rainy season. Navigation to the island is one of the major constrains. Facilities and man power of local forest department are not much adequate. Introduction of exotics by forest department and BFRI are also noticeable. Grazing by buffalos in the mangrove forest area and newly accreted lands were also observed.

In order to manage the Island local knowledge based policy is very necessary. During the field trips we discussed with local forest personals, local elites and general people to find some clues for formulating recommendations. A number of suggestions which are made based on our visit experiences are: to undertake short term and long term management plans, to develop eco-tourism, to ensure security for tourist, to develop infra-structure for tourism including road construction, guest houses with local food supply, to create the sources of fresh water both for human and wildlife, to create stairs in river station to make easy movement for tourist, to establish watch towers to enjoy the beauty of the bay, to introduce more tourist boats to facilitate movement, to record local knowledge from the elders about nature and adaptation and to record health care knowledge of local people, to introduce tourist police using coast guards, to create awareness programs about environment, biodiversity and wildlife, to increase literacy rate of local people, to accelerate plantation programs using local species, to provide risk allowance for the people who involved in forest management process, to increase capacity of forest and forest personals, to develop modern infrastructures for forest personals, to detect and remove invasive species, to avoid exotics in plantation programs to arrange traditional knowledge based cultural program, to create traditional medicinal knowledge sharing programs, finally to ensure land ownership and forest territory using GIS map.

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DYNAMICS OF ENVIRONMENTAL FACTORS IN RELATION TO PHYTOPLANKTON SPECIES IN A POND OF OLD DHAKA, BANGLADESH

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Abstract

The relationship between different environmental factors and abundance of phytoplankton species was studied for one year in a pond of old Dhaka named Sikkatuli pond. The range of annual mean of different variables in the pond was air and water temperatures 20 - 31.75˚C, secchi depth 21 - 54 cm, pH 7.39 - 8.3, alkalinity 3.9 - 9.2 meq/l, conductivity 484.5 - 2273.34 µS/cm, DO 3.35 - 8.33 mg/l, TDS 224.67 - 380.5 mg/l, SRS 12.03 - 79.93 mg/l, NO_3-N 0.18 - 0.435 mg/l, SRP 0.33 – 4.28 µg/l, chlorophyll *a* 196.08 - 362.76 µg/l and phaeopigment 30.51 - 212.2 µg/l. During the investigation *Cryptomonas erosa* var. *reflexa, Rhodomonas lens, Cyclotella comensis, Merismopedia gluaca, Euglena acus, Lyngbya limnetica, Chlorella vulgaris, Arthrospira platensis, Trachelomonas volvocina, Oscillatoria agardhii, Synechocystis aquatilis, Pelonema aphane* and *Peridinium* sp. were found to be dominant phytoplankton. Pearson correlation showed that only alkalinity correlated with phytoplankton at 5% significant level. RDA orientation showed that air temperature, water temperature, secchi depth, chl *a* and pheopigment concentration are the important environmental factors. RDA ordination plot also showed that phytoplankton species of *Pelonema aphane* and *Peridinium* sp. were negatively correlated with secchi depth. Negative correlation was also observed between *Trachelomonas volvocina* and water temperature.

Key words: Environmental factors, Phytoplankton, Old Dhaka, Bangladesh

Introduction

Mutualistic relationship exists in pond ecosystems between biological communities and environmental factors. The dominance of the algal community promotes the removal of nutrients, organic matter and pathogenic organisms (Curtis and Mara 1994 and Nurdogan and Oswald 1995). In pond ecosystem phytoplankton plays an important role and pond ecosystems play a significant role in the urban life, their existence and water quality. Different environmental factors such as high rainfall, optimum temperature, light help the growth of phytoplankton throughout the year**.**

Two or three decades earlier, Dhaka city was rich in pond ecosystems but because of rapid urbanization many of these are now extinct. The industrial pollution and domestic organic waste pollution have been considered as a great threat (Khan *et al.* 1978 and Maroof *et al*. 1986). High court pond, Dhaka university play ground pond, Press club pond and Shahabag pond are few of those extinct ponds. Recently Govt. of the People's

Republic of Bangladesh has taken a serious action regarding the extinction of ponds and other wetland habitats. Local inhabitants also become conscious day by day regarding a healthy condition of pond situated in dense urban areas. Sikkatuli pond, Bangshal in old Dhaka is one of them. The present work was carried out to study the dynamics of environmental factors of this pond with particular reference to its phytoplankton population.

Materials and Methods

Sikkatuli pond located at Kazi Alauddin Road in Bangshal of old Dhaka is demarcated by the geographical coordinates of 23°47°7° and 90°24°23°° E. According to local people its age is about 150 years. It is square shaped and its total area is about 0.15 hectors. Several sewerage lines come across to the pond from nearby houses which aggravates the water quality of the pond. Thus local inhabitants can't use water for domestic or other purposes. But fish culturing was observed in this pond during the study period.

The investigation was carried out from June 2010 to May 2011 for one year at fortnightly interval and 24 samples were collected. Air temperature, water temperature, secchi depth, pH, total dissolved solids (TDS) and conductivity were measured in *situ* using portable devices. The water samples for chemical analysis were collected from 0.5 meter depth of water. After collection, the water samples were brought to the National Professor A.K.M Nurul Islam laboratory in the Department of Botany, University of Dhaka for further analysis. Alkalinity was determined after Mackereth *et al*. (1978) and dissolved oxygen (DO) and soluble reactive silicate (SRS) after Wetzel and Likens (1979), soluble reactive phosphorus (SRP) and nitrate nitrogen (NO_3-N) after Murphy and Rilley (1962) and Müller and Wiedemann (1955). Chl *a* and phaeophytin were determined after Marker *et al*. (1980). Samples of Phytoplankton were collected by sedimentation technique with Lugol's solution and quantification of plankton was done with the help of a HBCC (Helber bacterial counting chamber, having a fixed volume 1.005 µl) under compound microscope, Nikon (Optiphot, UFX-11A) fitted with a camera (Nikon FX-35 WA, Japan).

Pearson correlation was done (SPSS v20) to find out the relationship between the environmental factors and total phytoplankton density and Redudancy analysis (RDA) was applied (CANOCO v4.5) to show the relationship between individual phytoplankton species and environmental factors. Prior to RDA analysis air temperature, water temperature and pH were standardized while rest of the environmental variables were log (x+1) transformed. Abundance of different phytoplankton species, concentrations of Chl *a* and phaeopigment was also transformed log (x+1) during application of correlation matrix.

Results and Discussion

During the present study, monthly mean air and water temperatures were found to be 20-31.75^{o}C. The fluctuation of air and water temperatures followed almost similar trend throughout the investigation period. The highest air temperature was recorded in June and the lowest was recorded in December (Fig. 1). The effects of water temperature on phytoplankton were directly examined in many aquatic ecosystems and it was found that water temperature strongly regulates the phytoplankton variations (Richardson *et al*. 2000). Secchi depth (Zs) *i.e.* the water transparency varied from 21-54 cm in the pond. Other investigations on different ponds of Dhaka showed a wide variation in secchi depth such as 28.67 - 44.5 cm in Samad Nagar pond (Akter *et al*. 2015), 17.05-26.58 cm in Wapda pond (Naher *et al*. 2015) and 50 to 81 cm in Bangshal pond (Pramanik *et al*. 2016). Transparency of water (Zs) is affected mainly due to the increase in the seston load. Monthly mean alkalinity ranged from 3.9-9.2 meq/l. The highest (9.2 meq/l) and lowest (3.9 meq/l) fortnightly mean alkalinity were recorded in early April and early September, respectively. In some ponds of Dhaka city alkalinity was recorded to range between 0.48 meq/l and 7.95 meq/l (Naher *et al*. 2015, Akter *et al*. 2015 and Pramanik *et al*. 2016). The present observation of fortnightly mean pH value of 7.39 - 8.30 is more or less similar with the results of Nahar *et al*. (2010) in Joysagor and Pramanik *et al*. (2016). The highest (2273.34 µS/cm) monthly mean conductivity was found in April while the lowest (484.5 µS/cm) was recorded in November. The conductivity reported by Nahar *et al*. (2010) was reported to be 4 times lower in Joysagar than the present value. This clearly indicates an increased loading of ions in the pond water. This might be a due to crucial breakdown of organic and inorganic matters in the water body and this was followed by high phytoplankton densities in dry months. DO recorded in the pond showed an overall range of 3.35-8.33 mg/l. The concentration of DO was found to decrease from June and continue till April. There was very low fluctuation in the concentration of DO content in the pond, which might be due to temperature, rainfall and absence of macrophytes in the pond.

The overall range of TDS for the pond was 224.67-380.5 mg/l which was comparable to the result of Pramanik *et al*. (2016) where TDS ranged from 264.66 mg/l to 358.6 mg/l. A study from some pond ecosystems of southern part of Bangladesh indicated that TDS range from 5.13-2165 mg/l (Paul 2008). SRS concentration in the water varied from 12.03-79.93 mg/l. Similar observation was also made by Nahar *et al*. in Joysagar (2010). Silica metabolism is associated with diatom population. According to Welch (1952) much of the soluble silicate is utilized by diatoms which results in the presence of lower silicate in water. The studied pond was very poor in diatom population. Bacillariophyceae was represented by 2% of the total population. Mean concentration of SRS fluctuated in the same order of magnitude as those observed from Dhaka metropolis (Banu 1995 and Sultana 1997). In the present investigation the concentration of NO_3-N

was found to range from 0.18-0.435 mg/l. Some measurements of NO_3-N carried out by using Hach Kit in some ponds ecosystems in various parts of Bangladesh showed a range of 30.0-1050.0 µg/l (Zaman *et al*. 1993). SRP concentration in the pond was found to be high (0.33-4.28mg/l) which exceeded the mandatory phosphate limit (22-300 µg/l) specified in the surface water regulations in the EU. This finding indicates a tremendous pollutant load of organic origin in the pond water (Fig. 1).

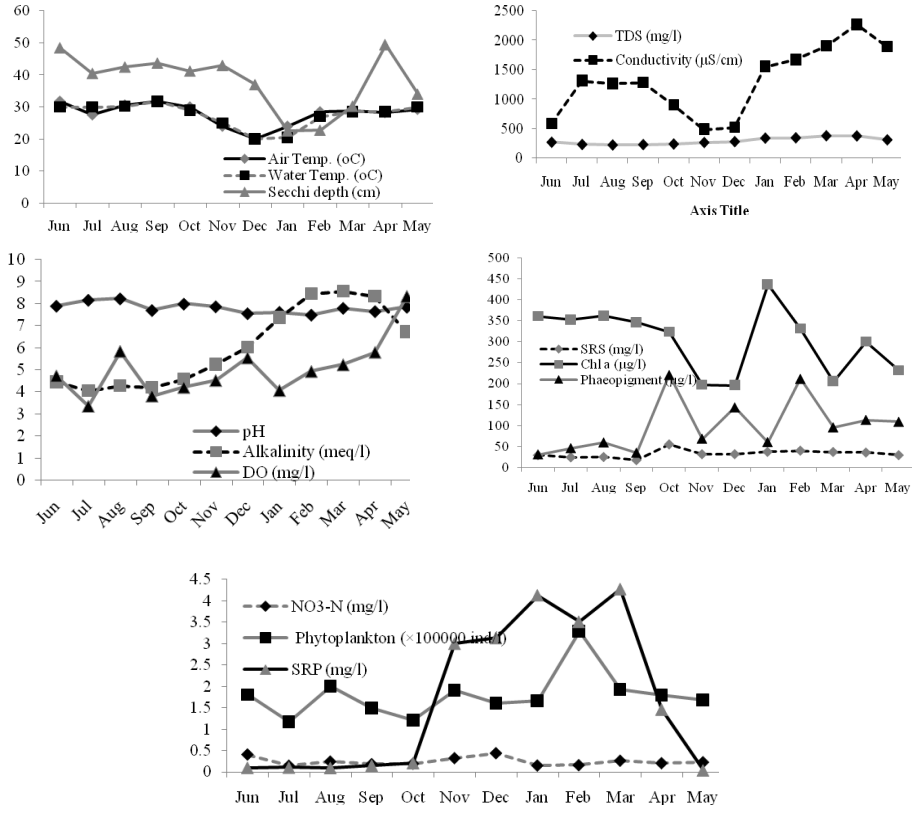

Fig. 1. Monthly variation of different environmental parameters and abundance of phytoplankton observed during the study period.

The biomass of phytoplankton as chl *a* concentration ranged from196.08 to 362.76 µg/l. The recorded higher chl *a* concentration than the previous studies in urban ponds (Begum 2008, Akter *et al*. 2015 and Pramanik *et al*. 2016) indicates an eutrophic nature of the studied pond. In the present investigation the highest phytoplankton abundance coincided with the highest concentration of chl *a*. The degraded product of chl *a i.e.* phaeopigment concentration varied from 30.51-212.2 µg/l (Fig. 1).

Table 1. Total number of phytoplankton (genera and species) which was categorized within different classes of algae during the study period.

Class	No. of genera	No. of species
Chlorophyceae	25 (50%)	53 (40.15%)
Cryptophyceae	08 (16%)	12 (9.09%)
Bacillariophyceae	06 (12%)	13 (9.85%)
Euglenophyceae	06 (12%)	33 (25%)
Cyanophyceae	04 (8%)	20 (15.15%)
Dinophyceae	01 (2%)	01 (0.76%)
Total	50	132

Table 2. List of dominant phytoplankton species with their abundance (monthly mean) recorded during study period.

	Month											
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Phytoplankton species	Density ($\times 10^5$ ind/l)											
Cryptomonas erosa var. *reflexa*	3.35	2.45	2.12	1.71							1.93	
Rhodomonas lens	1.54	2.16		3.84	2.01	1.56				4.76	7.33	
Cyclotella comensis	2.77	2.32	2.94		1.84	5.44	4.22	3.42	1.66	4.35	2.94	
Merismopedia glauca	2.88	1.99	3.1	1.17			3.36	1.36	1.87	2.05	1.93	
Peridinium.sp.		3.7								1.95		1.97
Euglena acus			4.03								5.85	3.17
Lyngbya limnetica			3.2	1.81		2.73		1.36				
Chlorella vulgaris			2.12	1.38	1.56				8.04			
Arthrospira platensis						2.03						
Trachelomonas volvocina								2.24				
Oscillatoria agardhii								1.8				1.56
Synechocystis aquatilis									12.27			2.48
Pelonema aphane										4.1		2.73

In the present study a total of 132 species of phytoplankton belonging to 6 different algal classes was recorded. Highest number of species was recorded from the class Chlorophyceae (40.15%) and the lowest number belonged to Dinophyceae (0.76%) represented by only one species in the pond (Table 1). During the investigation *Cryptomonas erosa* var. *reflexa*, *Rhodomonas lens*, *Cyclotella comensis*, *Merismopedia* glauca, *Euglena acus*, *Lyngbya limnetica*, *Chlorella vulgaris*, *Arthrospira platensis*, *Trachelomonas volvocina*, *Oscillatoria agardhii*, *Synechocystis aquatilis*, *Pelonema aphane* and *Peridinium* sp. were found to be dominant phytoplankton species in different sampling periods. Among them *Cyclotella comensis* was the most frequent occurring phytoplankton observed through the year except September and May. *Synechocystis aquatilis* was the highest abundance during February among other dominant

phytoplankton (Table 2). The abundance of phytoplankton community was found to range from 1.17×10^6 ind /l to 3.25×10^6 ind /l (Fig. 1). Recent studies on different urban ponds in Dhaka city showed different abundance of phytoplankton such as Samad Nagar Pond: 6.16×10^6-26.84×10^6 ind /l; Bangshal Pond: 5.11×10^5 -34.9×10^5 and Wapda Pond: 35.20×10^6-104.72×10^6 ind/l (Naher *et al*. 2015, Akter *et al*. 2015 and Pramanik *et al*. 2016).

Result of Pearson correlation showed that only alkalinity was positively correlated with phytoplankton abundance at 5% significant level. Out of 13 environmental factors five were used in RDA analysis according to the Monte Carlo test and the inflation factor (which had to be ≤ 20). The first two ordination axes explained 69.4% of the variance of the species-environment relation and 21.9% of the variance of species data (Fig. 2).

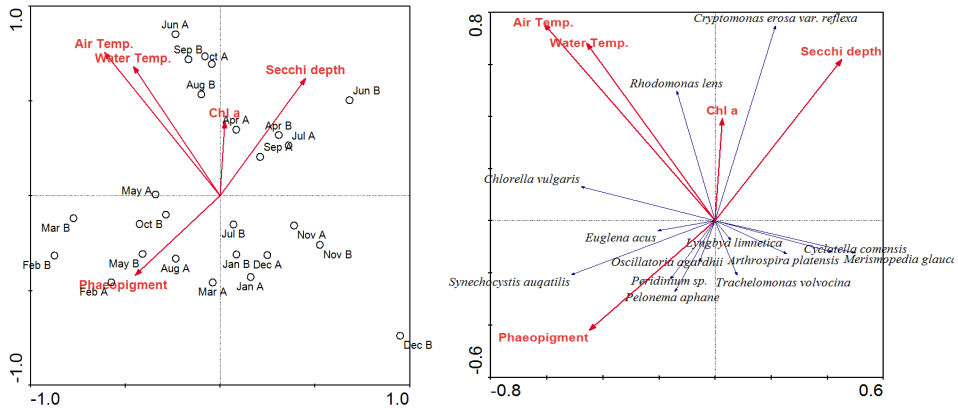

Fig. 2**.** RDA ordination plot: A. Sampling periods and environmental factors (Air temp., Water Temp., Secchi depth, Chl *a* and Phaeopigment). B**.** Phytoplankton species and environmental factors.

Between the two axes in the ordination, axis I1 (eigenvalue = 0.099) showed greater affinities to the environmental variables than axis I (eigenvalue = 0.120). Axis II (eigenvalue = 0.099) was positively correlated with air temperature(r = 0.6377), water temperature (r = 0.5753), secchi depth (r = 0.5221) and Chl *a* (r= 0.3308), while negatively correlated with phaeopigment (r = -0.3545).

RDA ordination plot also showed that phytoplankton species *Pelonema aphane* and *peridinium* sp. were negatively correlated with secchi depth. Negative correlation was also observed between *Trachelomonas volvocina* and water temperature.

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GROUNDWATER VARIABILITY IN BANGLADESH: ASSESSMENT BASED ON RAINFALL VARIATION AND USE OF WATER IN IRRIGATION

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Abstract

This study attempts to portray the scenario of groundwater level with respect to rainfall variability and its use for irrigation purpose for rice production in Bangladesh. Data on groundwater level and irrigation water usage were collected from BWDB and BBS. The changing pattern of groundwater level are presented in maps using Inverse Distance Weighted (IDW) interpolation method in ArcGIS 10.3. Analysis shows the increasing dependency on groundwater than on surface water for irrigation purpose at varied range across the country. The groundwater level is declining at a higher rate in northern parts of the country than the southern parts. In the context of climatic variability, excessive use of groundwater can trigger the lowering of groundwater level which will require more energy to uptake water for irrigation and so the input cost of production of rice will be increased. Therefore, apposite measures are required to ensure sustainable use of groundwater resources.

Key words: Groundwater, Rainfall, Irrigation, Rice, Bangladesh

Introduction

Groundwater is an essential natural resource of our mother earth that constitutes about 95 per cent of the freshwater on our planet, making it fundamental to human life and economic development. The contribution from groundwater is vital; perhaps as many as two billion people depend directly upon aquifers for drinking water, and 40 per cent of the world's food is produced by irrigated agriculture that relies largely on groundwater (Morris *et al.* 2003). Bangladesh, a small country is blessed with plenty of water resources being located in the basins of mighty Ganges, Meghna, Barhmaputra and Karnaphuli rivers. With numerous rivers, Bangladesh is also affluent in groundwater resource. Since last couple of decades, groundwater is being extensively used for drinking, irrigation and several other purposes eventually declining the ground water level. Groundwater is a vital input for sustaining crop production.

Irrigation is the most important water use sector accounting for about 70 percent of the global fresh water withdrawals and 90 percent of consumptive water uses (Siebert *et al.* 2010). Availability of groundwater for irrigation has contributed to manifold increase in

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crop productivity in Bangladesh (Dey *et al.* 2013). About 90 percent of irrigation water in Bangladesh is provided from groundwater (Zahid and Ahmed 2006). As a result of dense population and higher level of rural poverty, Bangladesh is very much in need of higher crop production. As far as the crop production is concerned, groundwater irrigation has contributed significantly to the rice production, mainly *Boro* rice, by supplementing soil moisture in the dry months of November/December to April/May (Kirby *et al.* 2014). The introduction of high yielding varieties (HYV) in 1980s revolutionized rice cultivation in Bangladesh. Increased water availability has encouraged farmers to grow irrigated *Boro* rice during the dry winter season. Thus, cropping pattern is being moved towards HYV rice. In the dry winter months, more than 70 percent of crop production is *Boro* rice, which can use up to 11,500 m^3 per ha of water in the production process (Biswas and Mandal 1993 and Chowdhury *et.al.* 2013). Currently, about 4.2 million ha of land is irrigated by groundwater (both shallow and deep tube wells) whereas only 1.03 million ha is irrigated by surface water using low lift pumps (BADC 2013). Methods that are available to achieve these ends depend mainly on irrigation particularly minor irrigation technologies comprising low lift pumps (LLPs), deep tube wells (DTWs), shallow tube wells (STWs) and manually operated pumps (MOPs), which together are responsible for 85 percent of irrigation coverage in the country (Mondal and Wasim 2004). Qureshi *et al.* (2014) mentioned in a study that the principal supply of groundwater for irrigation is from shallow tube wells, the numbers of which have grown from around 100,000 in the early 1980s to more than 1.5 million in 2010. At present, 35,322 DTWs are working in Bangladesh to provide water for irrigation purposes. Thus groundwater irrigation is of vital importance as an input to the agricultural economy and for food security (Haque *et al.* 2013). With increasing use of groundwater, the issues of sustainable groundwater usage are very much correlated. In several studies, it has been mentioned that falling groundwater levels in some areas have led to concerns about unsustainable groundwater use. Shamsudduha *et al.* (2009), Shahid and Hazarika (2010) and Kirby *et al.* (2016) in separate studies have agreed in the fact that groundwater use is unsustainable in some areas, such as the Barind area of northwest Bangladesh and around Dhaka. The reason behind such instability is reduced flow of river water in these regions and greater dependency on groundwater for irrigation purpose which has led to the excessive withdrawal of groundwater. Ali *et al.* (2012) mentioned that falling water levels in the northeast of Bangladesh are also due to excessive use of groundwater. Dey *et al.* (2013) also found a declining trend of groundwater table over the last 30 years (1981-2011) in their study, which implies groundwater use is not sustainable in northwest region. They identified the severely depleted district as Rajshahi followed by Pabna, Bogra, Dinajpur and Rangpur. They also mentioned about the increase of irrigation cost if the rate of dependence on groundwater for irrigation purpose increases. Therefore, it is clear that groundwater has been used extensively since before and this increasing rate of groundwater use may threaten the existing groundwater resource. Along with irrigation use, rainfall has also impact on groundwater recharge and so

reduction in rainfall may affect lowering of groundwater level. Excessive lowering of groundwater level may results in scarcity of water, deteriorated water quality, increase of pumping cost and land subsidence. In the context of Bangladesh, rice production is largely dependent on groundwater irrigation alongside its overuse for drinking purpose. Therefore, it is important for everyone to know the consequences of unsustainable groundwater extraction and thus use groundwater optimally to maintain groundwater sustainability.

Several studies had been conducted regarding the water balance across the country, but each study claimed their limitations for being unable to assess the regional water balance precisely. Besides, study on the changing pattern of groundwater level has focused only on the northwester part of the country rather the rest of the regions. Despite being a small country, there lies spatial variability in the context of climate as well as in terms of groundwater fluctuation. So, at this state it is of utmost importance to explore the spatial variability of changing groundwater level to adopt effective measures to ensure sustainable use of groundwater. Therefore, this study is an effort to explore the changing pattern of groundwater level at spatio-temporal scale with response to rainfall variability and its use for irrigation purpose.

The aim of this study is to explore the spatial variability of groundwater level from 1985 to 2010 in the context of its use in irrigation purpose along with rainfall variation and to explore the impacts of variability on rice production in Bangladesh.

Materials and Methods

The spatio-temporal change of groundwater level was assessed based on the two-major rice growing seasons in Bangladesh. *Aus*, *Aman* are the traditional varieties of rice which grow in the months of wet season (March-April to October-November) whereas *Boro* rice grows in dry season (December to March-April). As both the growing period coincides the distinct seasons (wet and dry) thus analysis was made focusing on this seasonal variation along with spatio-temporal variation. Both quantitative and qualitative data were used to sketch the scenario of groundwater level fluctuation in response to its use for irrigation purpose and variability in seasonal rainfall. Rigorous reviewing of literature was conducted to get a strong background for this study. To explore the variability of groundwater level, data on groundwater level of 1097 wells distributed within the study area were collected from Bangladesh Water Development Board (BWDB) from 1985-2010. To investigate the contribution of groundwater resources for rice production through irrigation, historical data of irrigation usage for rice was collected from Yearbook of Agricultural Statistics of 1985-2011 published by Bangladesh Bureau of Statistics (BBS). Rainfall variability has been analyzed using the historical data (1981-2010) of rainfall from Bangladesh Meteorological Department (BMD).

Collected data on groundwater level was sorted and categorized using MS Excel 2016. To portray the change of groundwater level at two different rice growing seasons, change of groundwater level was assessed separately both for *Aus -Aman* (wet) and *Boro* (dry) growing season for the year of 1985 and 2010. As groundwater level data are available from 1985 to 2010 comparison was made within this time period. The change in groundwater level was measured in terms of the groundwater level of 1985 from the water levels of 1990, 2000 and 2010. Thus, the negative values in change indicates an increase of groundwater level than the previous year level and the positive values reveal the decrease of water level which is presented in the maps. The maps, showing the changing pattern of groundwater level were prepared using Inverse Distance Weighted (IDW) interpolation method in ArcGIS 10.3. Besides, the usage of ground water for three major types of rice (*Aus, Aman* and *Boro*) was analyzed using MS Excel 2016. Change of average rainfall pattern of dry and wet season was assessed by calculating the collected rainfall data using MS Excel 2016. The changing scenario was presented in maps using Kriging Interpolation method in ArcGIS 10.3. Based on these analyzed data, groundwater variability was assessed.

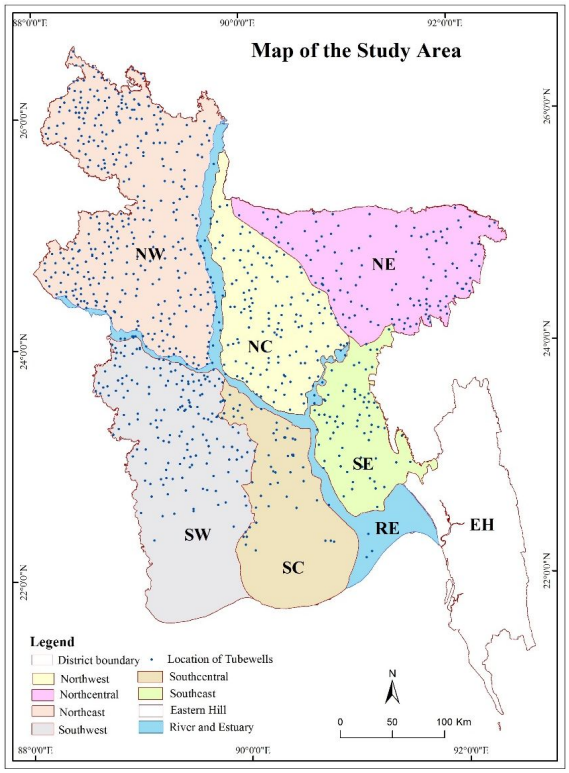

Fig. 1. Map of the study area showing hydrological regions.
(Source: Compiled from WARPO, 2004)

Study Regions: This study is an attempt to reveal the spatial variation of groundwater level across the country and to correlate this variation along with its use for irrigation purpose. As the prime focus is to portray the variation at spatio-temporal scale, entire country is needed to categorize in several homogenous regions. Thus, the categorization of the study area was done on the basis of the hydrological regions of Bangladesh. In National Water Management Plan of 2004, Bangladesh had been classified in eight hydrological regions, based on appropriate natural features, for planning the development of their water resources (WARPO, 2004). These are Northwest (NW), Northcentral (NC), Northeast (NE), Southwest (SW), Southcentral (SC), Southeast (SE), Eastern hill (EH) regions and the active floodplains and *char*lands of rivers and estuaries (RE) which are presented in Fig. 1.

It is to mention that, the hydro-geological settings of the eastern hilly region are far different from the rest of the regions and it is also complex to explore the variability of groundwater level in this region. Besides, the rivers and estuaries have also excluded from this study. That is why, this study incorporates the Northwest, Northcentral, Northeast, Southwest, Southcentral, Southeast regions excluding the Eastern Hill region and rivers and estuaries.

Spatial Variability of Groundwater level: The spatio-temporal changes of groundwater level were analyzed by comparing the ground water level of 1985 and 2010. The comparison of average groundwater depth of 1985 and 2010 for both *Aus-Aman* (wet) and *Boro* (dry) growing season clearly indicates a variation in groundwater level which is not uniform across the study area. Fig. 2 represents the average groundwater level of 1985 in *Aus-Aman* and *Boro* growing seasons. It reveals that there lies marked seasonal variation in terms of water level across the country. The average groundwater depth is higher in northern regions than the southern regions.

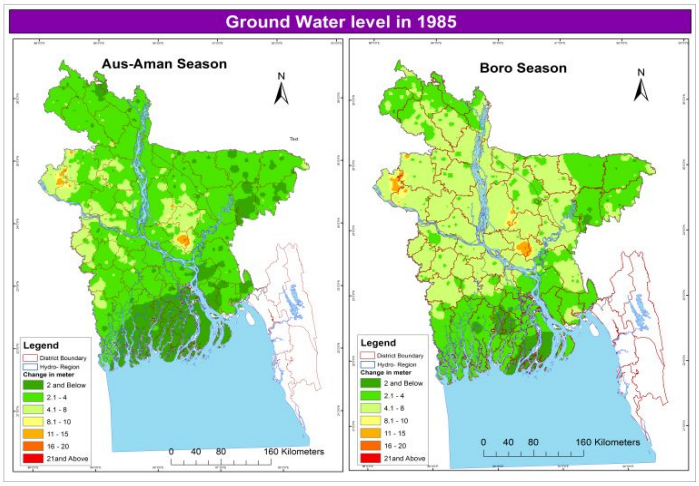

Fig. 2. Comparative scenario of groundwater level in *Aus-Aman* and *Boro* seasons in 1985. (Source: Prepared from collected data of 1985from BWDB using IDW method)

The average depth of groundwater level in wet season has found about 3.4 m and in dry season about 4.67 m. About 1.8 percent of the total area had a water depth of more than 10 m in wet season whereas it was about 2.7 percent in dry season in 1985 (Fig. 2). It is also noticeable that the areas where water level is at a depth of more than 10 m, are located in the northern regions. Similarly, the average groundwater level of 2010 (Fig. 3) shows continuous lowering of groundwater level in most cases.

Analysis showed that the average groundwater depth of wet season was 5.33 m while in dry season it was about 6.5 m 2010. To quantify the change, the average depth of ground water level has declined about 7.8 percent in wet season and about 7.2 percent in dry season in 2010 than that of 1985. In Fig.4 the eventual change of groundwater level from 1985 to 2010 for both the seasons is presented. Analyzed data suggest varied declining trend of groundwater level except in some parts of the southern region. The average decline in groundwater level from 1985 to 2010 was found to be about 1.65 m in wet (*Aus-Aman*) season and 1.59 m in dry (*Boro*) season. For both the seasons, maximum decrease of groundwater level was found in northern regions. About 10 percent areas of the northern region have experienced a decline of more than 5 m within the last 25 years for both the seasons. In northern regions (NW, NC and NE), more than about 30 percent of the total study area have shown a decline of groundwater level more than 2 m. In case of the rise of groundwater level, about 7-8 percent area have shown a rise of not more than 2 m in 2010 than in 1985 in wet season.

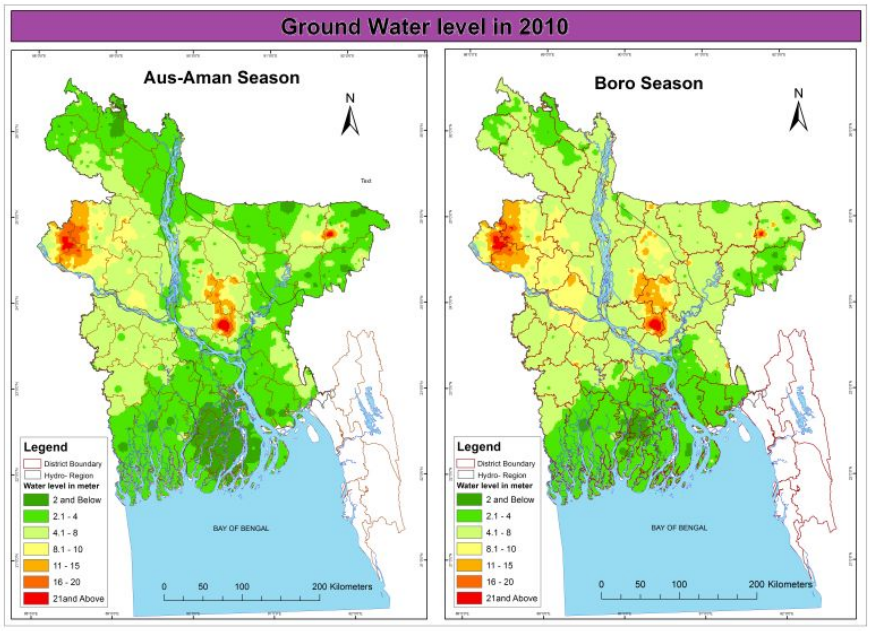

Fig. 3. Comparative scenario of groundwater level in *Aus-Aman* and *Boro* seasons in 2010. (Source: Prepared from collected data of 2010 from BWDB using IDW method)

On the contrary, in about 12-15 percent area, water level rose not more than 2 m. The rest of the parts of the study area showed a decline in groundwater level ranging from 0.01 to 2 m.

Comparative scenario of groundwater level of 1985 and 2010 indicates overall decline in groundwater level. In 2010, the areas having groundwater depth more than 10 m from the surface has expanded to about 7.2 percent and 10 percent in wet and dry season from 1.8 percent and 2.7 percent in 1985 accordingly. In areas of northwest and northcentral region, water depth has declined more than 15 m that comprises almost 10 percent of the total study area. Another 10 percent area, surrounding the above zones are found at risk of depletion of water level to an extent of more than 10 m.

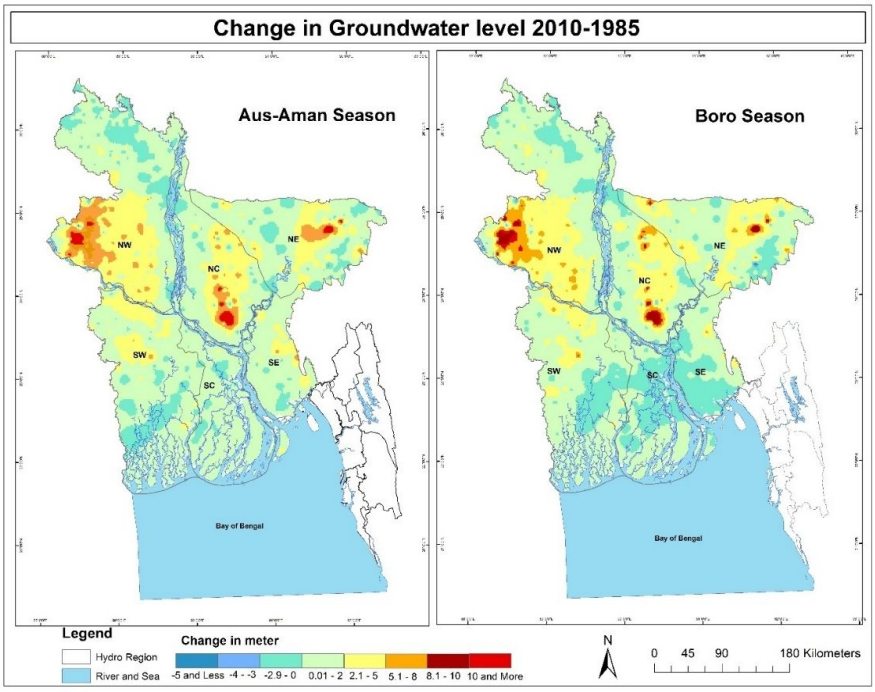

Fig. 4. Comparative changes in groundwater level from 1985 to 2010.

(Source: Prepared from collected data of 1985 to 2010 from BWDB using IDW method)

Rainfall Variability and Groundwater: In natural system, groundwater recharge largely depends on rainfall as it contributes to recharge directly the underlying aquifers. Jahan *et al*. (2010) has mentioned rainfall as the major source of groundwater recharge in Bangladesh and found that with the cessation of rainfall the major source for recharging groundwater also gradually stops. Adham *et al*. (2010) found that declining trend of rainfall with increased Potential Evapotranspiration, Crop Evapotranspiration and Net

Irrigation, consequently depending more on groundwater results in a depleted trend in the groundwater table levels. In this study, along with the variability of groundwater level, variability of average annual rainfall was assessed which is presented in Fig. 5. The classification of wet and dry season rainfall was made based on the rice growing seasons as mentioned earlier. Variability in dry season rainfall affects the groundwater level of next wet (*Aus-Aman*) seasons and wet season rainfall affects its following dry (*Boro*) season water level.

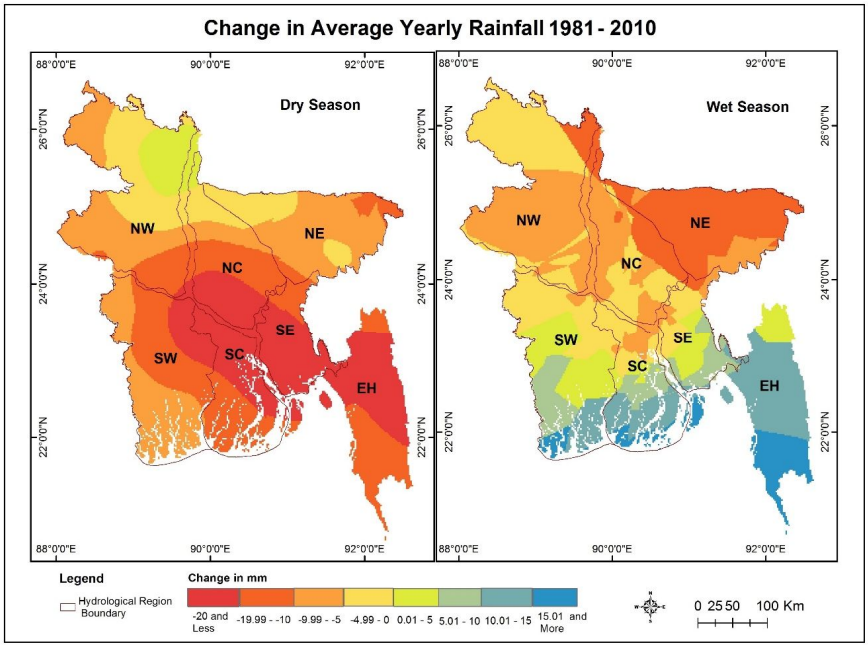

Fig. 5. Comparative changes in average rainfall from 1981 to 2010.
(Source: Prepared from collected data of 1981-2010 from BMD using Kriging method)

Comparative assessment of Fig. 4 and 5 reveals that the fluctuation of groundwater level is significantly related to the changing scenario of average seasonal rainfall. Evident decrease in rainfall of dry season particularly in southcentral, southeast, southwest, northcentral and northeast parts has consequently triggered the lowering of groundwater level in *Aus-Aman* growing season. A little increase in average rainfall in the northern part of northwest region may have resulted in the rise of water level in this part which is evident in Fig. 4.

Similarly, the increase of average rainfall in southern regions have resulted in the rise of average groundwater level in some parts of southwest, southcentral and southeast region in *Boro* season (Fig. 4). Despite known as the *haor* region, there has been a visible decline in groundwater level which may have accelerated by the decrease of rainfall in

this region (Fig. 5). In several studies, it has come out that the rivers of the northwest region have dried up severely that has put an excessive pressure on groundwater resource in this region and so, in spite of minimum decrease in average rainfall, the water level is declining at an alarming rate in northwest and northcentral region.

Groundwater Variability and its Use in Irrigation: Rice is the largest irrigation water user, accounting for about 77 percent of the total irrigated area (BBS 2014) and Bari and Anwar (2000) found that about 75 percent water for irrigation comes from groundwater. Traditionally Bangladesh is known as riverine country and it has an enriched history of using river water for various purposes. Historical evidences indicate that, river or canal water was the prime source of irrigation water earlier. With the advent of time as well as introduction of high yield varieties especially after 1980s, the dependency on groundwater for irrigation increased significantly across the country. Rasheed (2016) claimed in his study that in mid -1970s, surface water was the potential source of irrigation accounting for 92 percent of the total irrigated area. Since 1986-87, phenomenal expansion of groundwater utilization for irrigation has noticed (Rasheed 2016). In several researches, *Boro* rice has been considered as the most irrigation intensive rice varieties. In this study, use of groundwater for irrigation of rice was considered as one of the factors that affect the decline of groundwater level. Analyzed data show that, among the three varieties of rice, *Boro* rice is the most irrigation intensive rice whereas *Aus* and *Aman* rice require less irrigation (Fig. 6). The irrigated area under *Boro* rice production is 31 times higher than *Aus* growing area and 10 times of *Aman* growing irrigated areas.

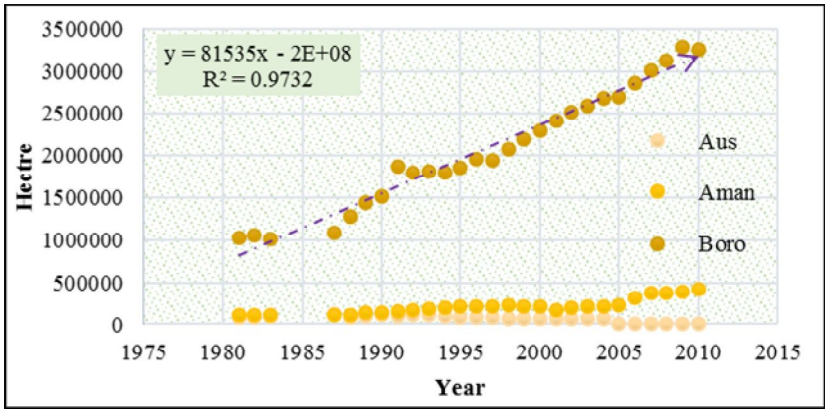

Fig. 6. Use of irrigation water for the production rice varieties in Bangladesh.

(Source: Analyzed from collected data 0f 1981-2010 from BBS)

Likewise, the spatial variation of groundwater and rainfall, the use of groundwater for irrigation are not uniform across the study area. The maximum use of groundwater for irrigation was observed in southwest region, followed by northwest and north central

region, while the least use has seen in northeast region. The southwest region is like the west, affected by low water availability in the dry season and salinity problems has caused dependency on groundwater for irrigation development (Kirby *et al.* 2014). Accordingly, with the variability of rainfall in southern regions, increase of groundwater use for irrigation than surface water was evident (Fig. 7).

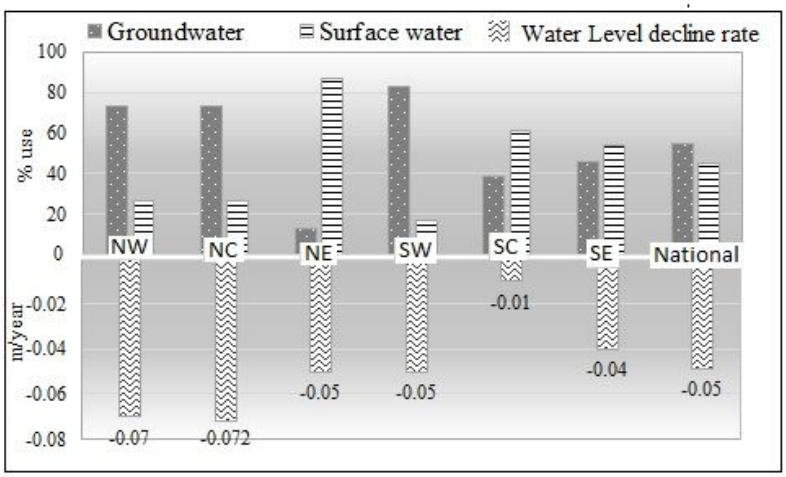

Fig. 7. Use of groundwater for irrigation and groundwater level decline rate 1985-2010. (Source: Author's own calculation based on the data of 1985-2010 from BWDB and BBS)

It is known that ground water is largely used for drinking and other purposes together with its use for irrigation purpose. Keeping aside the use for drinking purpose, only irrigation usage and associated change has observed in this study. Based on the analysis, it was found that the declining rate of groundwater is highest in northcentral region (0.072m/year) and minimum in southcentral region (0.01 m/year) whereas the average rate of groundwater decline was found to be 0.049 m/year (Fig. 7). The average groundwater level decline rate from 1985 to 2010 was found to be 0.05m/year across the study area whereas Zahid and Ahmed (2006) estimated the lowering trend of groundwater level during the last 32 years is 20 to 30 m with an average decline of more than 1.0 m/year. Undoubtedly, extraction of groundwater for irrigation has adversely affected the lowering process of groundwater level regionally.

Climate Variability, Irrigation and Groundwater Sustainability: Climatie variability (change) has been the global concern since last couple of decades. In the context of climate change, the average temperature and rainfall pattern of this country have not remained the same as before. Studies showed that, the average temperature is increasing countrywide along with variation in rainfall pattern (Sumiya 2016). Assumptions can be made that the variation of temperature and rainfall will affect the groundwater recharge while more dependency on groundwater for irrigation may occur to ensure food security. In the present study, it was found that with the decrease of rainfall, decline of

groundwater level has occurred. Despite decrease of rainfall regionally, the production of rice has shown manifold increase across the country. Sumiya (2016) mentioned that the seasonal rainfall pattern is changing and the adverse impact of such changes in rainfall distribution on rice production has been minimized through extensive irrigation using ground water. As a result, in the context of future climate change, the overuse of groundwater may threaten the sustainability of groundwater resource of the country.

Apart from these, it was found that greater areas of northern regions have a water level at a depth of more than 10 m. It is said that, if the groundwater level is at a depth of more than 10 m from the surface, additional force is required to withdraw water from the ground which ultimately increases pumping cost and the input cost of rice production. Indirectly, the rice production may face instability in response to its dependency on groundwater for irrigation.

In Bangladesh, along with drinking purpose, groundwater is widely used for irrigation. Among the three varieties of rice, irrigation is largely required for *Boro* production. Variability of rainfall has also increased the use of irrigation for *Aus* and *Aman* rice production too. The contribution of groundwater in irrigation has increased from 41 percent in 1982/1983 to 75 percent in 2001/2002 and surface water has declined accordingly. This study reveals that the contribution of groundwater in irrigation has increased from 38 percent in 1980/1981 to 78 percent in 2011/12 and surface water has declined from 62 percent to 22 percent accordingly. Thus, there has been a distinct decline in ground water level across the country with some exception. The declining rate of groundwater is higher in northern regions in comparison with the southern region. Though, rice production has increased dramatically in recent years than the past, it is time to ensure its progression by ensuring the availability of all the external inputs like groundwater. In this context, proper measures should be taken to ensure sustainable use of groundwater. New rice varieties which require less irrigation should be invented so that it can minimize the use of groundwater for irrigation purpose. Moreover, groundwater has proved one of the major inputs of rice production and so proper attention should be given on sustainable use of groundwater that compliments the development of rice production.

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OPTIMIZING CHLORELLA VULGARIS AND ANABAENA VARIABILIS GROWTH CONDITIONS FOR USE AS BIOFUEL FEEDSTOCK

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Abstract

Isolation and characterization of *Chlorella vulgaris* (green alga) and *Anabaena variabilis* (cyanobacterium) were made from natural and artificial water bodies of Dhaka University and Khulna, Bangladesh from March through December 2014 using modified Chu-10D medium to determine their potential as feedstock for biofuel production. Optimum growth measured as total chlorophyll and optical density under varying physical and chemical environments was determined. The optimum growth for *C. vulgaris* was obtained at pH 6.5 under light intensity of 110 µE m^{-2} s^{-1} and one and a half times the concentration of the Chu-10D. Compared to this, the optimum growth for *A. variabilis* was obtained at 7.0 pH, 90 µE m^{-2} s^{-1} light intensity and normal Chu 10D. Both organisms were grown at 25^{o} C temperature. Aeration of medium showed a significant positive growth for both the isolates. Supplementation of medium with vitamin B_1, B_6, B_7 and B_{12} would yield higher biomass of *C. vulgaris* as biofuel feedstock. Vitamins were not required for growing *A. variabilis.*

Key words: Microalgae, *Chlorella vulgaris*, *Anabaena variabilis*, Feedstock, Biofuel, Growth optimization

Introduction

Rising concern over depleting fossil fuel and greenhouse gas emissions has resulted in high level of interest in non-conventional fuel like biodiesel and bioethanol originating from bio-renewable sources including sugars, starches and ligno-cellulosic materials from solid wastes and plant biomass including algal biomass. Microalgae in particular have been reported to have several advantages which include high productivity, no competition with conventional agricultural land, utilization of waste water, brackish water and sea water, recycling of carbon dioxide, and compatibility with integrated production of fuels and co-products within bio-refineries such as agar, dye stuff, protein rich animal feed etc. (Sahoo *et al.* 2012 and Kumar *et al.* 2013).

Although the first-generation bioethanol production from food crops such as corn, grain, or sugar cane is well established and the industry is growing throughout the world, the use of these staple food crops as feedstock is not ideal because of the high price of raw

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materials, which account for almost 40–75% of total ethanol production cost (Jang *et al*. 2012). It has raised doubts about its possible impact on food supply and security, which is mainly reduced if its residues are used for bioethanol production (Song *et al*. 2013). In contrast, the second-generation bioethanol is derived from ligno-cellulosic feedstock. Currently, no commercial-scale cellulosic ethanol plants are in operation largely because of the high price of production, which is almost twice that of corn ethanol (Jones and Mayfield 2012). In view of the aforementioned issues, microalgae are gaining wide attention as an alternative renewable source of biomass for the production of biofuel, which is grouped under 'third-generation bioethanol' (Nigam and Singh 2011).

Certain species of microalgae have the ability to produce high levels of carbohydrates as reserve polymers instead of lipids. These species are ideal for the production of bioethanol as carbohydrates produce fermentative sugars. It has been estimated that approximately 46760-140290 L/ha ethanol can be produced from microalgae (Nguyen and Vu 2012). This yield is several orders of magnitude larger than the yields obtained from corn, soybean, etc. (Lombardi and Maldonado 2011). Green algae including *Spirogyra* and *Chlorococcum* were reported to accumulate high levels of polysaccharides both in their complex cell walls and as starch (Nigam and Singh 2011). This starch accumulation can be used in the production of bioethanol (Harun *et al.* 2010). Bioethanol is used in fuel mixtures such as E85 (a blended fuel of 85% ethanol and 15% gasoline) in Brazil and USA (Davis *et al*. 2000).

In Bangladesh, a large number of algal species were reported to occur in freshwater, brackish water and marine habitats (Web 1, Ahmed *et al*. 2008) which could be potential sources of biofuel feedstock, but the potential of algal biomass production for biofuel has not been properly addressed. Therefore, the present research was initiated to identify probable potential microalgae of Bangladesh for using as biofuel feedstock by optimizing their growth conditions.

Materials and Methods

Isolation of microalgae: Water samples with algal boom were taken in to plastic bottles as well as in glass vials containing sterilized modified Chu-10D medium (Aziz and Whitton 1987 adapted from Chu-10D of Sinclair and Whitton (1977) by a dropper from different fountains, ponds, ditches, etc. located at Dhaka University campus and Khulna for obtaining fast growing and frequently occurring microalgae. The liquid medium used in this study is not an absolute inorganic medium as two organic compounds, the EDTA as a chelating agent and HEPES as a buffer were used. Four vitamins i.e. B_1, B_6, B_7, B_{12} in six combinations i.e. B_1+B_6, B_1+B_7, B_1+B_{12}, B_7+B_{12}, B_1+B_7+B_{12} and B_1+B_6+B_7+B_{12} with a control in each case were used to find out their effects.

Unialgal culture was obtained by repeated subculturing in liquid and solid agar media using platinum wire loop or sterile Pasture pipette. In some cases, series of dilutions were

made in sterile medium using homogenized suspension of algae. Unialgal condition was confirmed by compound microscope.

Green algae and cyanobacteria of 8 to 30 days old cultures were used for microscopic study, described, photographed and identified following Ahmed *et al*. (2008), Starmach (1966) and Desikachary (1959) and Siddiqui *et al*. (2007).

Maintenance and subculture: The present experiments were conducted in the controlled growth room of National Professor KM Nurul Islam laboratory, Department of Botany, DU. Stock cultures were maintained in 30 ml liquid medium in the controlled growth room with an average temperature of 25^{o} C under continuous low light of *ca.* 40 µE m^{-2} s^{-1} provided by cool white fluorescent light from glass bottom. Subculture was made after about every three months. Stock cultures for experimentation were inoculated under a continuous average light flux of 71 µE m^{-2} s^{-1} and 4-7 days old culture was used as inoculum. Each treatment had four replicates and were randomized after every 24 hr.

Estimation of growth: Growth was estimated by measuring chlorophyll(s) and Optical density (O.D.). Chlorophyll *a* and *b* for *Chlorella vulgaris* were estimated following APHA (American Public Health Association 1985), only chl *a* for *Anabaena variabilis* following Marker *et al*. (1980) and O.D. in both cases by measuring absorbance at 750 nm wave length in spectrophotometer following Rodolfi *et al.* (2009).

pH was measured using Hanna pocket model and average light flux by Li-Cor, USA, using aerial probe. Temperature was measured by maximum-minimum wall thermometer.

Aeration: Aeration of the culture-flasks was done to optimize the effect of bubbling on algal growth (for CO_2 utilization) at a pressure of 0.0067 M Pa using an aquarium air pump with a pumping capacity of 8 L min^{-1} at 25 ± 1^{o} C temperature and at an average light flux of 71 µE m^{-2} s^{-1}.

The standard deviations were done to measure the sample variations, analysis of variance (ANOVA) of the data was computed to determine the F-value and test of significance was computed by Duncan's New Multiple Range Test (DMRT) in IBM SPSS statistics version 22.

Results and Discussion

Chlorella vulgaris (Class: Chlorophyceae, Order: Chlorococcales, Family: Chlorellaceae, Genus: *Chlorella*) and *Anabaena variabilis* (Class: Cyanobacteria, Order: Nostocales, Family: Nostocaceae, Genus: *Anabaena*) were isolated from freshwater bodies and characterized as follows:

***Chlorella vulgaris* Beyer (Figs. 1a-c)** (Ahmed *et al.* 2008)

Cell solitary or in small colony of indefinite shape; Individual cells spherical to broadly oval; Cell wall thin; Chloroplast massive cup-shaped, parietal with indistinct pyrenoid; Cell 6.5-7.5 µm long, 6.0-8.0 µm broad. Collected from TSC fountain, Dhaka University, planktonic

***Anabaena variabilis* Kütz. *ex* Born. *et* Flah (Figs. 1d-f)** (Siddiqui *et al.* 2007)

Thallus gelatinous; cells barrel-shaped, constricted at the cross walls**,** 4.5-6.0 µm broad, 5.4-6.5 µm long; end cells conical; heterocysts intercalary, oval, 7.5-12.0 µm broad, 7.8-10.8 µm long; akinete not differentiated in the present observation with 30 days old culture. Collected from a pond, Khulna University.

Optimization of growth conditions

Chlorella vulgaris grew better, measured as chlorophylls at pH 6.5 followed by 6.0. Growth at neutral and alkaline pH were significantly lower (Fig. 1). Mayo (1997)

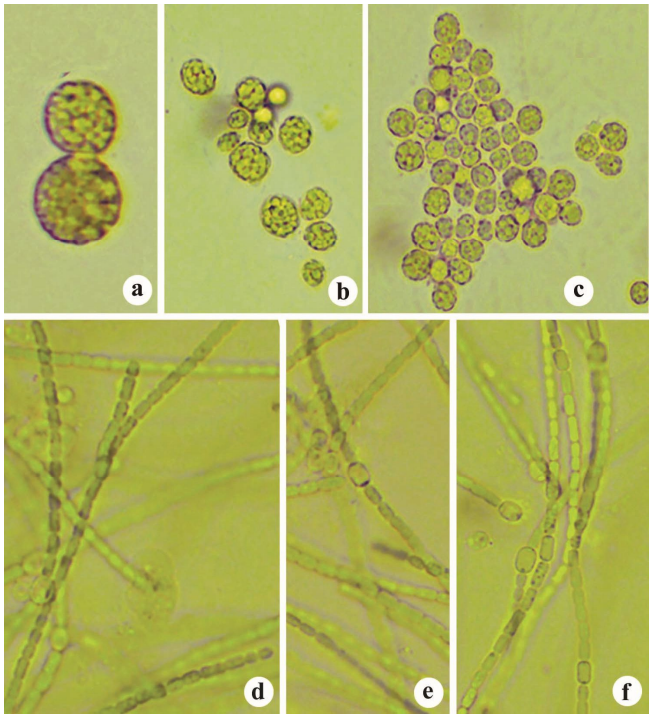

Plate 1. *Chlorella vulgaris*: a) Two associated cells enlarged showing nemerous starch granules around a pyrenoid; b) cells of various shape and size at lower magnification, smaller ones are released autospores; c) mature cells many having one pyrenoid in each cell and surrounding starch grains. d-f. *Anabaena variabilis*: d) young filaments as hormogonia, e) hormogonia and a mature filament with heterocyst, f) only mature filaments.

observed maximum growth more or less at 6.5 pH in *Chlorella* sp. In *C. vulgaris* a complete inactivation at acidic pH was found (Carberry and Brunner 1991). However, *Anabarna variabilis* had best growth at 7.0 pH (Fig. 3) which was similar to the findings of Yoon *et al.* (2008). In the same species Nagle *et al.* (2010) observed growth inhibition at <5.0 and >10.5 pH. The present organism also was severely affected at lower and much higher pH (Fig. 2).

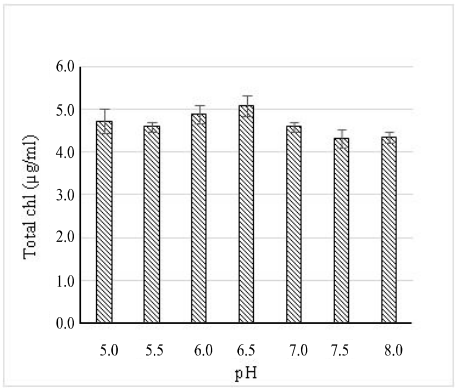

Fig. 1. Effect of pH on the growth as total chlorophyll (*chl a* and *b*) of *Chlorella vulgaris*. Significant at 5% level.

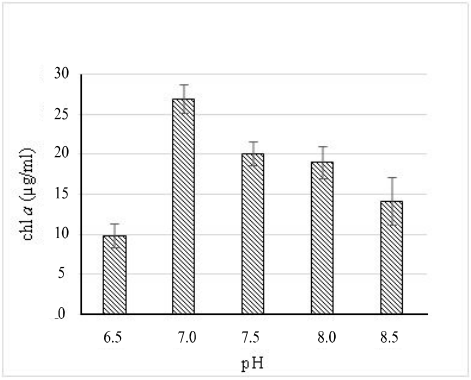

Fig. 2. Effect of pH on the growth as chlorophyll (*chl a*) of *Anabaena variabilis*. Significant at 5% level.

Chlorella vulgaris had best growth as optical density at 110 µE m^{-2} s^{-1} (Fig. 3). It is possible that continuous exposure of the cells under adequate light energy, in particular, during cell division process, *C. vulgaris* is able to grow faster. Similar observations were reported by Wijanarko *et al.* (2004) and Sharma *et al.* (2012). Best growth was in *Anabaena variabilis* observed at a light flux of 90 µE m^{-2} s^{-1} (Fig. 4).

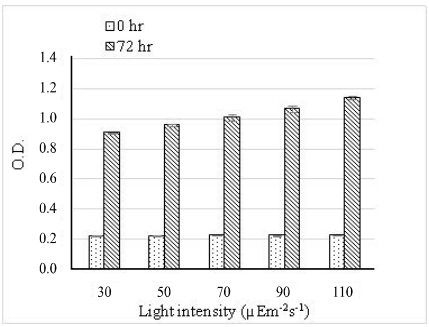

Fig. 3. Effect of light intensity on the growth of *Chlorella vulgaris*. Significant at 5% level.

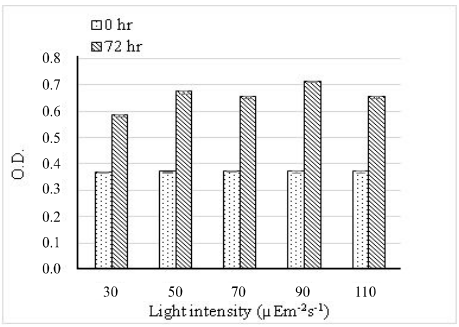

Fig. 4. Effect of light intensity on the growth of *Anabaena variabilis*. Significant at 5% level.

Temperature: Both *C. vulgaris* and *A. variabilis* responded similarly to temperature variations, highest growth as O.D. was found at 25° C and lowest at 35° C after 72 hr of incubation (Figs 6-7). Temperature ranging from 25 to 30° C was suggested to be favourable for the overall growth of *C. vulgaris* (Sharma *et al*. 2012). Specific growth rate of *C. pyrenoidosa*, increased uniformly with enhanced temperature, in the range 22° C to 30° C but dropped at higher temperature and cells were unable to grow at above 33° C (Ong *et al.* 2010). In *A. variabilis* and *A. nidulans*, Sato *et al.* (1979) obtained about double the cell growth at 25° C temperature.

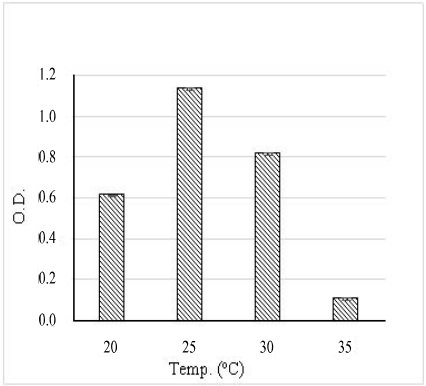

Fig. 5. Effect of temperature on the growth of *Chlorella vulgaris.* Significant at 5% level.

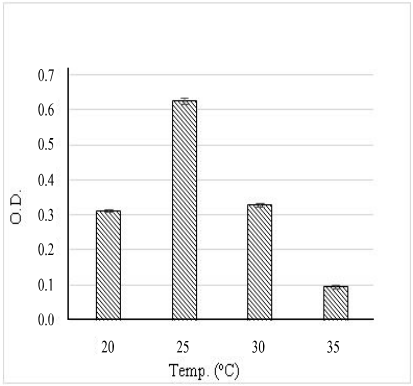

Fig. 6. Effect of temperature on the growth of *Anabaena variabilis.* Significant at 5% level.

Aeration: Effects of aeration for 72 hr on growth of *C. vulgaris* and *A. variabilis* are presented in Fig. 7. O.D. of the two algae increased by 81% and 90%, respectively due to bubbling for 72 hr. The positive effect was due to the utilization of CO_2 and continuous contact of organisms with the medium thereby helping nutrient absorption. These findings are similar to the works reported by Pirt and Pirt (1980) and Berberoğlu *et al.* (2008) on *C. vulgaris* and *A. variabilis*, respectively.

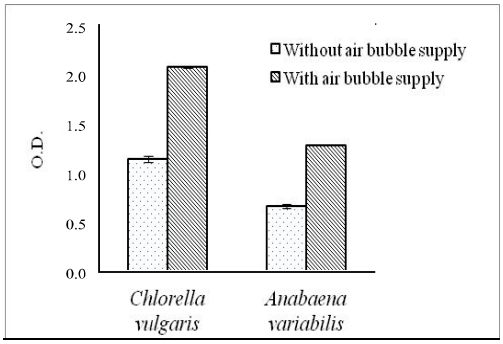

Fig. 7. Effects of air bubbling on the growth of two microalgae. Significant at 5% level.

Nutrient element concentration of Chu 10D: Chu 10D medium has relatively low concentration of elements compared to Bold's Basal Medium, in some cases less than half. Therefore, Chu 10D medium concentration was increased by one and one half and doubled. Increased nutrient status had significantly different effects on the growth as O.D. of both the organisms after 48 to 72 hr growth (Figs 8-9). Both the organisms were affected at double the strength of Chu 10D. Of the three concentrations *C. vulgaris* showed maximum growth at one and one half strength (Fig. 8) whereas *A. variabilis* showed maximum at normal Chu 10D strength (Fig. 9). The maximum in *A. variabilis* might be due to optimum concentration of nutrient elements in normal Chu 10D except nitrogen and the cyanobacterium supplemented it by fixing atmospheric N_2 (Stewart and Gallon 1980). At normal Chu 10D strength on the other hand *C. vulgaris* showed lowest growth and was most likely due to low nitrogen medium and inability of the organism to fix atmospheric N_2. However, Chia *et al.* (2013) showed that the growth obtained in *C. vulgaris* grown in the Chu-10D medium was the highest. Elser *et al.* (1990) suggested that N (also P) potentially limits algal growth where N-fixing cyanobacterium *Anabaena* has the ability to fix atmospheric N_2 when the water becomes N-depleted.

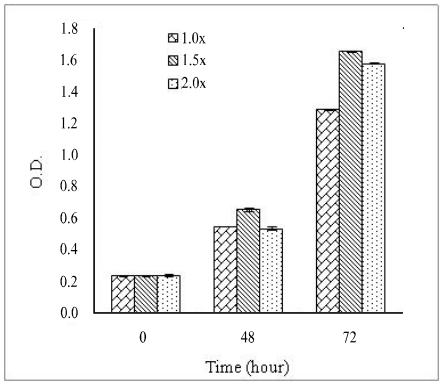

Fig. 8. Effect of nutrient concentration in medium on the growth of *chlorella vulgaris.*

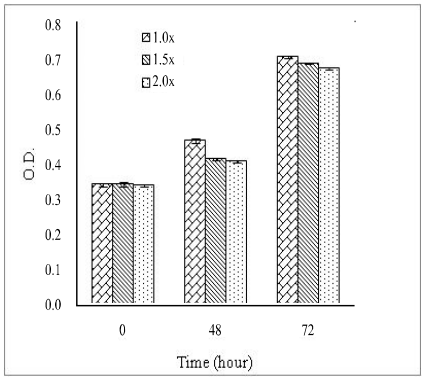

Fig. 9. Effect of nutrient concentration in medium on the growth of *A. variabilis.*

Vitamin supplement: Effect of vitamin B_6 alone or in combination with B_1, B_7 and B_{12} for *Chlorella vulgaris* is (Fig. 10). Therefore, supplementation of medium with vitamin B_1, B_6, B_7, B_{12} would yield higher biomass as feed stock for producing biofuel. In marine diatom *Chaetoceros calcitrans* vitamin B_6 addition also increased growth (Krichnavaruk *et al.* 2005). *Anabaena variabilis* does not require any vitamins and produced higher biomass after 72 hr growth than *Chlorella vulgaris* (Fig. 11).

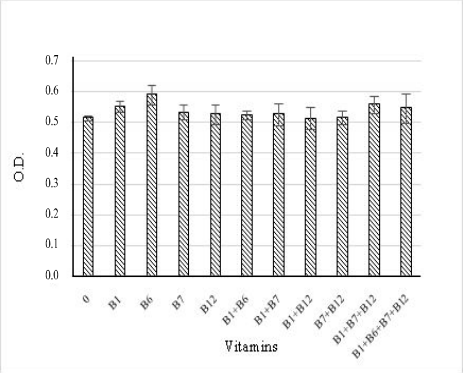

Fig. 10. Effect of vitamins on the growth of *Chlorella vulgaris*.
Significant at 5% level.

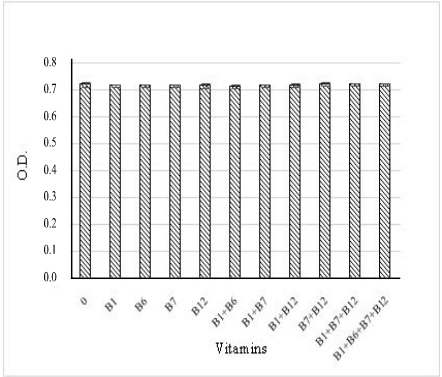

Fig. 11. Effect of vitamins on the growth of *Anabaena variabilis*.
Significant at 5% level.

Acknowledgments

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ELECTROPHORETIC BANDING PATTERN OF ESTERASE ISOZYME IN DIFFERENT TISSUES OF PUNTIUS SOPHORE (CYPRINIDAE : CYPRINIFORMES)

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Abstract

Esterase isozyme of different tissues of *Puntius sophore* was analyzed using 7.5 % polyacrylamide gel electrophoresis (PAGE). Fish specimens were collected from local market. The tissues used were taken from different muscles, stomach, fore-, mid- and hind-gut, liver, eyes, gill, heart, kidney, fore-, mid- and hind- brain, eggs and ovary. Six different esterase bands were detected, named Est-1, Est-2, Est-3, Est-4, Est-5 and Est-6 and their relative mobility were 1.0, 0.84, 0.62, 0.33, 0.26 and 0.13 respectively, each of them representing a single allele. The highest esterase activity was found in liver, followed by gill, kidney, heart, brain, intestine, stomach, eye, reproductive organ and skeletal muscles as detected in the staining intensity. Staining intensity of Est-4 and Est-5 was higher and Est-6 was the least stained in all the tissues.

Key words: Esterase, Isozymes, PAGE, *Puntius sophore*

Introduction

Puntius sophore, belonging to the family Cyprinidae, is the most common among captured fishes (Rahman 2005) in Bangladesh. It is easily cultivable (Kohinoor 2000), highly demanding, cheap and considered to be one of the main protein source for low income group. It has high nutritional value, containing 19.0 g of protein, 37 µg of vitamin-A, 1,059 mg of calcium, and other micro nutrients per 100 g of fish (Thilsted *et al.* 1997). The residual effect of pesticides on fish is high and they reduce the survival, growth and reproduction of fish (McKim *et al.* 1975) even cause a significant mortality (Nishat and Choudhury 1985). Besides, pesticides can be accumulated in the fish muscles which are consumed by human causing serious diseases ultimately.

In Bangladesh, industries are situated on the bank of the rivers and dispose organic, inorganic wastes and biohazards on to them in an untreated form. Moreover, pesticides used in the agriculture wash out into these rivers polluting the aquatic environment. Esterases are reported to be associated with pesticide resistance. So, it may be hypothesized that those fishes which are alive in the polluted environment might have

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higher resistance to pesticides and higher accumulation of the chemicals treated as pesticides. Thus, resistance status, as well as, nature of pollution in the aquatic body, could have relation to amount of esterases produced in the body of fish and other aquatic organisms.

Several toxicity tests on different agro-chemicals were done on *P. sophore* (Prabhuji *et al.* 1983) and other species of *Puntius* (Gill *et al.* 1990). Esterases are multifunctional, lipid hydrolyzing enzymes occurring in multiple forms and capable of separation by electrophoresis (King 1974). Isozyme analysis was used to estimate the genetic distance between different populations and the variability of isozyme was also used to devise the genetic sexing system (Shahjahan 1988). Esterases are reported to be involved in regulation of juvenile hormone levels (Kort and Granger 1981), reproduction (Richmond *et al.* 1980), functioning of nervous system and development of resistance to insecticides (Karunaratne *et al.* 1999). It may be used as bio-indicators to monitor pollutants in the environment (Vanda *et al.* 2003). So, in the present study, different forms of esterases found in different tissues of *Puntius sophore* were analyzed.

Materials and Methods

Sample collection: *P. sophore* (*punti*) were brought from Fokirapool and Jatrabari fish market, Dhaka, during January to April 2006. Procurement was made from selected fishermen who directly catch fish from the river Meghna. Collections were made early in the morning in polythene bags with sufficient ice, with no preservatives and transported to Laboratory of Genetics and Molecular Biology, Department of Zoology, University of Dhaka. These were identified following relevant literature (Rahman 2005) and stored in refrigerator at -80˚C.

Sample preparation: Female *P. sophore* was dissected and around 15 mg of twenty different tissues were collected: 1. Anterior neck muscle (white), 2. Mid-body muscle (white), 3. Tail muscle$_1$ (anal region, white), 4. Tail muscle$_2$ (Tip-spotted portion, red), 5. Buccal muscle (white), 6. Stomach, 7. Intestine$_1$ (fore-gut), 8. Intestine$_2$ (mid-gut), 9. Intestine$_3$ (hind-gut), 10. Liver, 11. Eye (lence), 12. Eye ball (entire, except- lence), 13. Gills, (3rd) 14. Heart, 15. Kidney, 16. Fore-brain, 17. Mid-brain, 18. Hind-brain, 19. Eggs and 20. Ovary. Each of the above tissues was squashed in a labeled eppendorf tube in 40 µl of TBE buffer, vortexed for 1 min. 40 µl of bromophenol blue (marker) was added and finally centrifuged at 13,000 rpm for 12 min. The samples were then subjected to electrophoresis and were always put on ice to ensure cold temperature.

The extraction was repeated with selected tissue samples (viz. muscle, stomach, intestine, liver, eye, gill, heart, kidney, brain and reproductive organ) and aliquots of extracts from each of the tissues (without marker) were subjected to Lowry test to quantify the amount of total protein in each of the extract. Rest of the aliquots from each tissue extract was mixed with an equal amount of bromophenol blue and the samples

were loaded to gel slot such a way that each slot receive an equal amount of protein i.e. each sample contained an equal amount of protein extract and these were subjected to electrophoresis.

Polyacrylamide Gel Electrophoresis: Electrophoresis was conducted on 7.5% PAGE prepared following Standard method (Hames 1986). An initial pre-run was made at 100 V for 30 min. which followed another ~1 hour and 45 min. run after loading 10 µl of samples on to the gel. The run was stopped when the markers came to the bottom and the gel was put onto freshly prepared staining mixture (0.2 M Monobasic sodium phosphate (NaH_2PO_4) 1.32 g, 0.2 M Dibasic sodium phosphate (Na_2HPO_4) 0.5362 g, α-napthyl acetate, dissolved in acetone at room temperature. Afterwards the solution was out poured and the gel was incubated in the Fast blue RR solution (29/ 120 ml) at 37˚C for 25 minutes.

Photography and scoring of bands: The gel was photographed using a digital zoom lense camera (Sony Cybershot, DSC-F88), putting the gel on a white background. Positive and negative poles were marked and bands were numbered as per recommendations of the Standard Committee for Enzyme (Webb 1964). Relative mobility was calculated considering the value of the band with highest mobility as 1.

Results and Discussion

Altogether six esterase bands were detected in the 20 different tissues of *P. sophore*, depending on their relative mobility (Fig. 1). These were numbered: Est-1, Est-2, Est-3, Est-4, Est-5 and Est-6. All six esterase bands were not present in each tissue. Since the differentiation of the esterases of various tissues of *P. sophore* was done according to their substrate preference, as they hydrolyze the substrate α-naphthyl acetate and produced black colouration. Therefore, all of the above six esterases could be commonly named as α-esterase (Vanda *et al.* 2003). The highest relative mobility value of esterase was 1.0 (± 0.04), possessed by Est-1, located near the (+) pole and it runs 4.5 cm. The relative mobility of Est-2, Est-3, Est- 4 and Est-5 was 0.84 (± 0.04), 0.62 (± 0.04), 0.33 (± 0.02), and 0.26 (± 0.02), respectively. Est-6 with the slowest relative mobility, valued 0.13 (± 0.02), was found near the (-) pole (Fig. 1). Presence of more than two bands is not artifactual (Raymond *et al.* 1996) and each band corresponds to one allele as per Mendelian inheritance studies (Stordeur 1976). The esterase activity of the different bands obtained from different tissues was arbitrarily measured based on eye estimation of staining intensity, categorized into deep stained (DS), medium deep stained (MDS) and faint stained (FS). The results made for the various esterase bands from twenty different tissues were as follows:

Muscles: Anterior neck muscle (white), mid-body muscle (white), anal region of tail muscle and spotted portion of tail muscle were examined and all of the muscle tissues showed two faint esterase bands (Est-4 and Est-5) (Fig. 1).

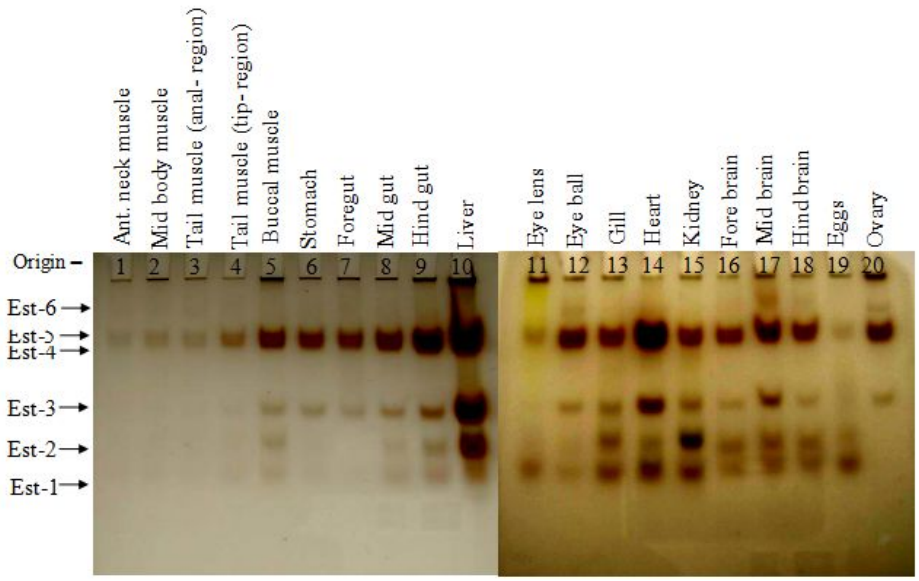

Fig. 1. Electrophoretic banding pattern of esterases of different tissues of *P. sophore* [Lane 1-20): 1. Anterior neck muscle (white), 2. Mid-body muscle (white), 3. Tail muscle$_1$ (anal region, white) 4. Tail muscle$_2$ (Tip-spotted portion)(red) 5. Buccal muscle (white), 6. Stomach 7. Intestine$_1$ (foregut), 8. Intestine$_2$ (midgut), 9. Intestine$_3$ (hindgut), 10. Liver, 11. Eye (lence), 12. Eye ball (entire, except- lence), 13. Gills, (3rd) 14. Heart, 15. Kidney 16. Fore brain, 17. Mid brain, 18. Hind brain, 19. Eggs, 20. Ovary]. Arrows indicate position of esterase and the relative mobility (RM).

Buccal region: Est-1, Est-2, Est-3, Est-4 and Est-5 were the five bands observed in the muscle of buccal region. Among the five esterase bands Est-4 and Est-5 were deeply stained and the rest of the bands were faintly stained.

Stomach: Three esterase bands (Est-3, Est-4 and Est-5) were found in the stomach.

Intestine (gut): In the foregut three esterase bands viz. Est-3, Est-4 and Est-5 were observed where Est-3 was faintly and Est-4 and Est-5 were deeply stained. Both mid-and hind-gut showed five esterase bands. In both midgut and hindgut Est-1 and Est-2 were faintly stained and Est-3 was medium deep stained and Est-4 and Est-5 were deeply stained.

Liver: Altogether five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were found and all of the bands were deeply stained except Est-1.

Eye: Three faintly stained esterase bands (Est-1, Est-3 and Est-4) were observed in the lens of eye. However, in the ball of eye five esterase bands namely Est-1, Est-3, Est-4, Est-5 and Est-6 were found in the eye ball where Est-1 and Est-6 showed faintly stained band. Est-3 band was medium deep.

Gill: Est-1, Est-2, Est-3, Est-4 and Est-5 were the five esterase bands found in the 3rd gill and all the bands were deeply stained.

Heart: Five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were recorded in the heart where all the bands were deeply stained except Est-2.

Kidney: Altogether five deeply stained esterase bands (viz. Est-1, Est-2, Est-3, Est-4 and Est-5) were found in the kidney.

Brain: Five esterase bands (Est-1, Est-2, Est-3, Est-4 and Est-5) were found in both fore- and hind-brain where Est-1, Est-2 and Est-3 were faintly stained. In the mid brain all mentioned six esterase bands were observed and Est-6 was faintly stained. Est-1, Est-2 and Est-3 were faintly and Est-4 and Est-5 were deeply stained in all of the three parts of the brain.

Eggs: Est-1, Est-2, Est-4 and Est-5 were the four faintly stained esterase bands found in eggs.

Ovary: Altogether four esterase bands (viz. Est-3, Est-4, Est-5 and Est-6) were found in the ovary .

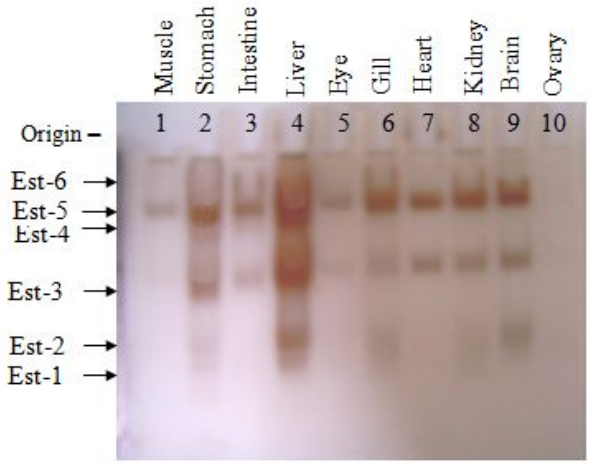

Fig. 2. Proportionately loaded after protein test. Lane 1-10: 1-Muscle, 2-Stomach, 3-Intestine, 4-Liver, 5-Eye, 6-Gill, 7-Heart, 8-Kidney, 9-Brain and 10-Reproductive organ.

However, according to staining intensity esterase activity in different tissues could be arranged as ascending order: Liver > gill > kidney > heart > brain > Intestine > stomach > eye > reproductive organ > skeletal muscles. Frequency of occurance of various esterase bands was compared in all the tissues and it was revealed that, the maximum frequency of esterase was observed for Est-4 and Est-5 and the minimum frequency was found for Est-6 (Figs. 1-3). Loading equal amount of total protein extracted from different tissues also supported the fact that expression of esterase in *P. shophore* was tissue specific in general (Fig. 2), because the bands maintained the similar order of variable staining intensity (viz. Liver > stomach > gill > kidney > heart > brain > Intestine > eye > reproductive organ > skeletal muscles) except stomach and those were not influenced by the procedure of extraction.

As regards the occurance of number of esterase bands order of organs are as follows: six esterase bands in mid brain; five bands in buccal region, midgut, hindgut, liver, gill, eye ball, heart, kidney, fore brain and hind brain; four bands in egg, ovary; three bands in stomach, foregut, lens of eye; two esterase bands in anterior neck muscle, mid-body muscle and tail muscle.

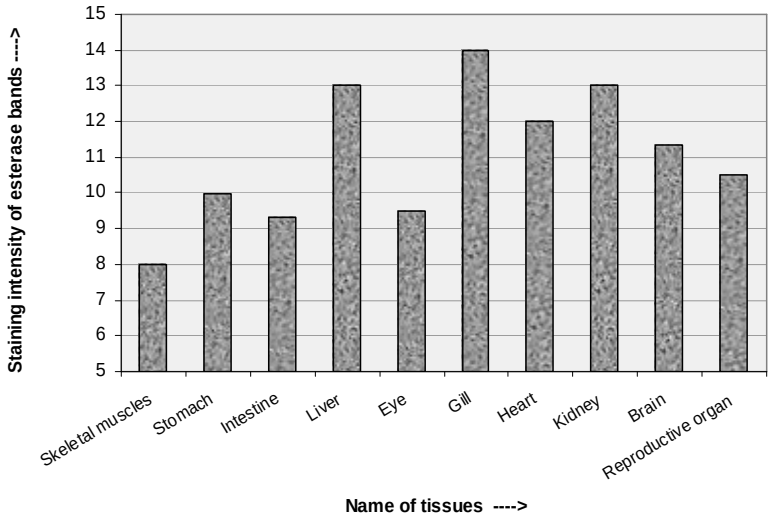

Fig.3. Comparative analysis of staining intensity of esterase bands of various tissues of *Puntius sophore*.

During the present study higher band intensity (deep stained bands) was observed in the tissues of digestive system more specifically liver and intestine. In 1997 Li and Fan made similar observation in the tissues of different body parts of several fishes viz- topmouth

gudgeon (*Pseudorasbora parva*), goldfish (*Carassius auratus*), nile tilapia (*Tilapia nilotica*), mosquitofish (*Gambusia affinis*) and rainbow trout (*Salmo gairdneri*). But the absence of enzyme activity in the gill of the mentioned species was quite different from the present study.

Eight esterase bands were found in different tissues (brain, eye, heart, muscle and liver) of *Orechromis aureus* (Hongtuo *et al.* 1993). Six esterase zones were found in the brain of channel catfish *Ictalurus punctatus* (Knowles *et al.* 1968). Seven esterase isozymes were observed in four populations of blunt snout from three lakes and one river (Sifa *et al.* 1993). In the present study, six esterase isozymes were recorded in *punti* (Fig. 1).

As previously reported (Gunning *et al.* 1998, Manwell *et al.* 1968 and Vanda *et al.* 2003), tissue specific distribution and intensity correlates pesticides residue and pollutants in the dwelling environment; an intensive study of different esterase band index of different species of *Puntius* and related fish in different cultural area would provide a potential input in the management tactics.

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EFFECT OF TOXICITY OF NEEM (AZADIRACHTA INDICA A. JUSS) AND MOHANEEM (MELIA AZEDARACH LINNAEUS) ON THE LARVAE OF MOSQUITO CULEX QUINQUEFASCIATUS (SAY) (DIPTERA: CULICIDAE)

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Abstract

The larvicidal activities of three solvent extracts, viz. ethanol, chloroform and water of two plants neem *Azadirachta indica* (A. Juss) and mohaneem *Melia azedarach* (Linn.) against the fourth instar larvae of mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae) were studied in the laboratory at 27 ± 2 °C and 75-85% RH. No larval mortality was observed in control treatment. The larval mortalities by the ethanol extracts of *A. indica* at the five dose concentrations were 37.33, 64.00, 64.00, 76.00 and 97.33%, respectively; by the chloroform extracts were 24.00, 54.66, 80.00, 96.00 and 100%, respectively; by the water extracts were 32.00, 56.00, 62.66, 68.00 and 81.33%, respectively. The larval mortalities by the ethanol extracts of *M. azedarach* at the five dose concentrations were 29.33, 58.66, 64.00, 74.66 and 89.33%, respectively; by the chloroform extracts were 40.00, 49.33, 61.33, 73.33 and 84.00%, respectively; by the water extracts were 29.33, 58.66, 64.00, 74.66 and 89.33%, respectively. In case of *A. indica*, LC_{50}, LC_{90} and LC_{99} values for the ethanol extracts were 1.805, 3.581 and 6.261 mg/ml, respectively; for the chloroform extracts were 0.686, 1.112, and 1.648 mg/ml, respectively; and for the water extracts were 3.002, 5.584 and 9.262 mg/ml, respectively. In case of *M. azedarach*, LC_{50}, LC_{90} and LC_{99} values for the ethanol extracts were 1.949, 3.89 and 6.835 mg/ml, respectively; for the chloroform extracts were 0.695, 2.256, and 5.886 mg/ml, respectively; and for the water extracts were 3.536, 6.662 and 10.866 mg/ml, respectively.

Key words: Azadirachta indic, Melia azedarach, Cx. Quinquefasciatus, Toxicity, Lethal concentration

Introduction

Mosquitoes transmit more diseases than any other group of arthropods affecting millions of people throughout the world (Ghosh *et al*. 2012) and causing millions of deaths every year (Kamaraj *et al*. 2011). The World Health Organization (WHO) has declared mosquito as "*public enemy number one*" (Ghosh *et al*. 2012) because it is the principal vector of many of the "vector-borne" diseases affecting human beings and other animals (Ravichandran *et al.* 2014). Several mosquito species of the genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogen of various diseases like malaria, yellow fever, dengue, chikungunya, Zika, West Nile, Japanese encephalitis and filariasis (Kazembe and Makusha 2012).

In Bangladesh Ahmed (1987) reported 25 species of *Culex* and recorded 22 species of mosquitoes related to medico-veterinary importance among which eight species belonged to

Culex. In Dhaka city, the population of *Cx. quinquefasciatus* is peaked during the dry season from November to December (Khan *et al*. 2015). Begum *et al*. (1996) studied the larval population of *Cx. quinquefasciatus* in Dhaka city and its suburbs. Recently, Khan *et al*. (2014 and 2015) reported 13 species of mosquito in five wards of Dhaka city of which *Culex quinquefasciatus* was the predominant one.

Botanicals as potential insecticides were studied for the first time in the country by Ameen *et al*. (1985) and Ameen *et al*. (1983a and b); they bioassayed the solvent based root extracts of *Derris elliptica* plant on the larvae of two mosquito species of the genera *Aedes* and *Culex*. The principal toxicant of *D. elliptica* as insecticide is rotenone. Neem, *Azadirachta indica,* is a member of the family Meliaceae, which has been reported to contain several biologically active constituents, such as azadirachtin, meliantriol, salanin, nimbin and nimbidin (Naganishi 1975 and Aliero 2003). Mohaneem, *M. azedarach,* is another member of the family Meliaceae which contains azadirachtin, meliantriol steroids, tarpenoids, saponins and tannins (Azam *et al.* 2013 and Ahmed *et al*. 2012). Among the chemicals, azadirachtin showed maximum biological activity (95%) against the larvae, pupae and adult of *A. stephensi* (Nathan *et al*.2005). Nour *et al.* (2012) tested acetone, chloroform and ethanolic extracts of the bark, root, leaf and seeds of *A. indica* against the larvae of *Ae. Aegypti*. Batabyal *et al*. (2007) tested petroleum ether, carbon tetra-chloride and methanol extracts from the seeds of *A. indica* against the larvae of *An. stephensi*. The methanolic leaf and seed extracts of *M. azedarach* were tested against *An. stephensi* for its larvicidal, pupicidal, adulticidal, oviposition deterrent and repellent activities by Nathan *et al*. (2006). The fruit extracts of *M. azedarach* and *A. indica* elicit a variety of effects in insects, such as antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects and changes of behavior (Wandscheer *et al*. 2004). Alouani *et al*. (2009) studied the effects of azadirachtin of neem on the fourth instar larvae of *Cx. pipens* at different concentrations and reported that *A. indica* was potentially more effective to mosquito control than that of *M. azedarach*.

Since neem and mohaneem plants are easily available in the country and since they have immense potentiality as the source of botanical insecticides, the objective of the present paper was, therefore, to assess the three solvent extracts of each of the two plant species *A. indica* and *M. azedarach* on the larvae of *Cx. quinquefasciatus* (Say).

Materials and Methods

After the collection of larvae of *Cx. quinquefasciatus,* rearing of the larvae into adults, collection of the leaves of neem and mohaneem, extraction process of the leaves, and bioassay tests were conducted from September 2015 to March 2016 at the Entomology Research Laboratory of the Department of Zoology, University of Dhaka and also in the Center for Advanced Research in Sciences (CARS), University of Dhaka.

Collection and rearing of larvae and adults in the laboratory: The larvae of the mosquito *Cx. quinquefasciatus* were collected from some potential breeding places in Dhaka city, such as the drains of Curzon Hall area, Dhaka University. These were then reared in the laboratory at 27±2 °C and 75-85% RH. The procedure of rearing of *Cx. quinquefasciatus* was done following Bilkis (1997). During rearing the larvae were served with yeast powder while the emerged adults were provided with 10% glucose solutions as their food. After pupation, the

pupae were transferred to small plastic containers and then to an adult rearing cage (30 cm x 30 cm x 30 cm) for emergence. After 3-4 days of emergence, the adult female mosquitoes were given a blood meal from a pigeon, *Columba livia* for egg maturation. A petridish containing tap water was placed inside the cage for the females to oviposit. After couple of days, the females laid egg rafts on the surface of water of the petridish. Then the egg masses were allowed to hatch into the first instar larvae which were subsequently moulted into 2nd, 3rd and 4th instar larvae and pupae, and finally emerged into adults. In this way the rearing process continued for several generations for obtaining adequate number of larvae for bioassay tests with plant extracts of neem and mohaneem.

Preparation of plant extract: The leaves of neem *A. indica* and mohaneem *M. azedarach* were collected from these trees located in the premises of Curzon Hall, Dhaka University. Both the plant species were authenticated from the Botany Department, Dhaka University. The collected leaves were washed with tap water, sun-dried for seven days, and then powdered by using an electrical blender. Each 50g of the leaf powder of neem and mohaneem was dissolved separately in 300 ml of ethanol, chloroform and water, and kept for 24 hours with periodic shaking in a Shaking Orbital Machine at 100 rpm and 30°C, then filtered and the sample solutions were collected. This procedure was repeated three times with fresh volume of respective solvents. The total volumes of the samples were concentrated separately in a Rotary Vacuum Evaporator machine. The water in the samples was completely evaporated and dried at 200 rpm and 60^0C, and the ethanol and chloroform samples were at 100 rpm and 40 ^{0}C; these dried extracts were then stored at 4°C in an air tight white glass bottle for future use in dose preparation.

Dose preparation and bioassay test: The fourth instar larvae of *Cx. quinquefasciatus* were exposed to test doses of 150, 200, 250, 300 and 350 mgs each of the ethanol based extracts, 50, 75, 100, 125 and 150 mgs each of the chloroform based extracts, and finally 250, 300, 350, 400 and 450 mgs each of the water based extracts of the leaves of both *A. indica* and *M. azedirach.* The concentrations of the above doses calculated were: 1.5, 2.0. 2.5, 3.0 and 3.5 mg/ml, respectively for the ethanol extracts; 0.5, 0.75, 1.0, 1.25 and 1.50 mg/ml, respectively for the chloroform extracts; and 2.5, 3.0, 3.5, 4.0 and 4.5 mg/ml, respectively for the water extracts. For each of the dose concentrations, 25 fourth instar larvae of *Cx. quinquefasciatus* were exposed and three replicates were maintained for each case. The measured amount of the extracts was dissolved in 2 ml of dimethyl sulfoxide (DMSO) which was used to solubilize the plant extracts in water as suggested by Nour *et al.* (2012), but the water extracts were dissolved directly in water and no DMSO was required to add into it. Each of the dissolved plant extracts in DMSO was added to 100 ml water in a beaker. A set of control, using 2.0% DMSO as Control 1 and an untreated set of larvae in water (tap) as Control 2, were also used for comparison. The larvae were fed with dry yeast powder sprinkled on the surface of water at the rate of 50 mg/ml.

The mortality of the larvae was recorded after 36 hours of exposure and moribund larvae were counted as dead. The toxicity of the plant extracts was calculated in the form of LC_{50}, LC_{90} and LC_{99} values, which indicate 50, 90 and 99 per cents death of test larvae, respectively. The recorded mortality percentage values were calculated by using the formula-

$$\text{Percentage mortality} = \frac{\text{Number of larvae died}}{\text{Number of test larvae}} \times 100$$

When mortality in control treatment was more than 5%, the percentage mortality was corrected by using Abbott's (1925) formula-

$$\text{Corrected mortality} = \frac{\text{Larval mortality in the treatment - Larval mortality in control}}{\text{100-control mortality}} \times 100$$

Statistical analysis: $LC_{50,}$ LC_{90} and LC_{99} values at 95% confidence intervals of lower and upper confidence limits were calculated by following the probit analysis method suggested by Finney (1971). Other statistics like chi-square values, regression at 95% confidence intervals of upper and lower confidence limits and t-tests were calculated using the IBM SPSS statistics 20 (Statistical Package of Social Science) software; here significance levels were set at $p < 0.05$.

Results and Discussion

After 36 hours of exposure, the larval mortalities at five different concentrations of three solvent extracts of *A. indica* and *M. azedarach* are presented in Table 1 and no mortality was observed in control treatments.

LC_{50}, LC_{90} and LC_{99} values in relation to the larval mortalities of *Cx. quinquefasciatus* due to the effects of solvent extracts of neem and mohaneem leaves and their lower and higher confidence limits at 95%, and chi-square test values were calculated and the results are presented in Table 2.

Comparison of toxicity of different solvents based extracts of neem and mohaneem: The values of $LC_{50,}$ LC_{90} and LC_{99} show that the water extracts of neem (*A. indica*) and mohaneem (*M. azedarach*) were least toxic to the mosquito larvae followed by the ethanol extracts which was again followed by Chloroform extracts (Fig. 1). For neem and mohaneem leaf extracts, the grading of LC_{50} for these three solvent extracts on the basis of their toxicity is as follows: neem, Chloroform (0.686) > ethanol (1.805) > water (3.002); mohaneem, Chloroform (0.695) > ethanol (1.949) > water (3.536). The grading

Table 1. Mean percentage mortality of larvae of *Cx. quiquefasciatus* exposed to different concentrations of three solvent leaf crude extracts of *A. indica* and *M. azedarach.*

Solvens		*Azadirachta indica*		*Melia azedarach*	
	Concentration (mg/ml)	Mean no. of larvae died (Mean±SD)	Mean % of larvae died	Mean no. of larvae died (Mean±SD)	Mean % of larvae died (Mean±SD)
Ethanol	Control	0.00±0.00	0.00	0.00±0.00	0.00
	1.50	9.33±1.33	37.33	7.33±1.33	29.33
	2.00	16.00±1.00	64.00	14.66±0.33	58.66

	2.50	16.00±0.00	64.00	16.00±1.00	64.00
	3.00	19.00±1.00	76.00	18.66±0.33	74.66
	3.50	24.33±1.33	97.33	22.33±0.33	89.33
Chloroform	Control	0.00±0.00	0.00	0.00±0.00	0.00
	0.5	6.00±1.00	24.00	10.00±1.00	40.00
	0.75	13.66±2.33	54.66	12.33±0.33	49.33
	1.00	20.00±1.00	80.00	15.33±0.33	61.33
	1.25	24.00±1.00	96.00	18.33±0.33	73.33
	1.50	25.00±0.00	100	21.00±1.00	84.00
Water	Control	0.00±0.00	0.00	0.00±0.00	0.00
	2.50	8.00±1.00	32.00	6.00±1.00	29.33
	3.00	14.00±1.00	56.00	9.00±1.00	58.66
	3.50	15.66±0.33	62.66	12.66±2.33	64.00
	4.00	17.00±1.00	68.00	14.33±1.33	74.66
	4.50	23.00±2.33	81.33	17.66±0.33	89.33

SD standard deviation.

Table 2. LC_{50}, LC_{90}, LC_{99} and chi square values of the larvicidal activities of leaf extracts of *A. indica* and *M. azaderach* on the larvae of *Cx. quinquefasciatus*.

Plant and Solvents	LC_{50} (mg/ml) (LCL-UCL)	LC_{90} (mg/ml)(LCL-UCL	LC_{99} (mg/ml) (LCL-UCL)	x^2(df13) (Significant value)
Neem:	(LCL-UCL)	(LCL-UCL)	(LCL-UCL)	nt value)
Ethanol	1.805(1.606-1.962)	3.581(3.188-4.299)	6.261(5.024-9.042)	14.384 (0.347[a])
Chloroform	0.686(0.638-0.731)	1.112(1.029-1.229)	1.648(1.454-1.962)	7.564 (0.871[a])
Water	3.002(2.736-3.208)	5.584(4.888-7.130)	9.262(7.224-14.852)	4.605(0.983[a])
Mohaneem:				
Ethanol	1.949(1.761-2.107)	3.890(3.433- 4.740)	6.835(5.43010.006)	5.149(0.972[a])

Chloroform	0.695(0.576-0.792)	2.256(1.1763-3.551)	5.886(3.694-14.356)	3.553(0.995[a])
Water	3.536(3.315-3.792)	6.662(5.574-8.891)	10.866(8.216-18.459)	2.513(0.999[a])

LCL lower confidence limits; UCL upper confidence limits; x^2 chi-square; df degrees of freedom, [a]Since the significance level is greater than 150, no heterogeneity factor is used in the calculation of confidence limits.

of LC_{90} for these three solvent extracts as follows: neem, Chloroform (1.112) > ethanol (3.581) > water (5.584); mohaneem, Chloroform (2.256) > ethanol (3.890) > water (6.662). The grading of LC_{99} for these three solvent extracts as follows: neem, Chloroform (1.648) > ethanol (6.261) > water (9.262); mohaneem, Chloroform (5.886) > ethanol (6.835) > water (LC_{99} = 10.866). So, for three solvents, chloroform extract of both plants showed highest mortality rate when compared with two other solvents.

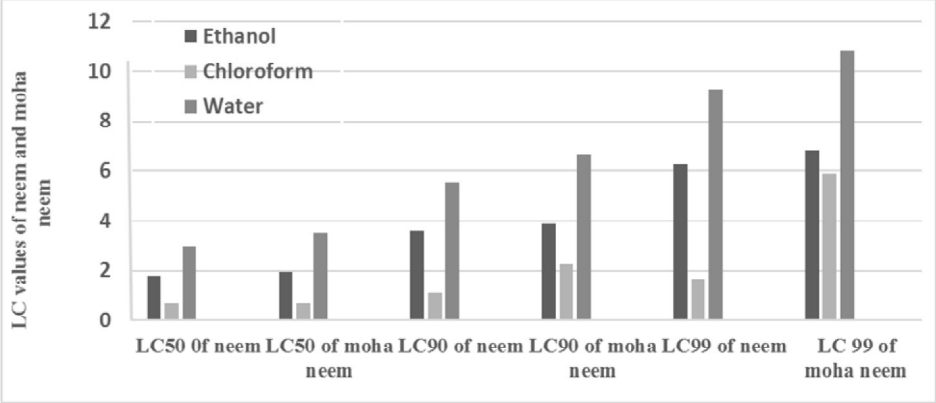

Fig 1. Comparision of lethal concentrations (LC) at three solvent extracts of *A. indica* and *M. azedarach.*

*Comparison of toxicity following paired t-test for neem and mohaneem***:** From the above discussion it is apparent that the extracts of the mohaneem (*M. azedarach*) is less effective than the extracts of the neem (*A. indica*). To compare between neem and mohaneem solvent extracts, significant paired t-test was followed (Table 3). For the ethanol, chloroform and water extracts of neem and mohaneem, the mean difference was 0.944, 1.944 and 2.556 for which the calculated t-test value is 3.183, 2.331 and 5.569 which were significant (Table 3).

Table 3. Paired t-test with the ethanol, chloroform and water extracts of *A. indica* and *M. azedarach* leaves bioassayed on the fourth instar larvae of *Cx. quinquefasciatus.*

Solvents	Dead at each level		Paired samples				
	Mean of neem	Mean of Mohaneem	Mean difference	SD (df17)	Standard error mean	t	Significant (2tailed)
Ethanol	14.11	13.17	0.944	1.259	0.297	3.183	0.005
Chloroform	14.78	12.83	1.944	3.539	0.834	2.331	0.032

Water	12.5	9.94	2.556	1.947	0.459	5.569	0

SD standard deviation, df degrees of freedom, t test.

The ethanol extracts of neem *A. indica* at 3.5 mg/ml dose concentration caused 97.33% 4th instar larval mortality (Table 1). Maragathavalli *et al.* (2012) reported that the ethanol extracts of *A. indica* leaves at 200 mg/ml dose concentration caused 100% mortality of the 3rd and 4th instar larvae of *Cx. quinquefasciatus* and 90% mortality of *Ae. aegypti.* The ethanol extracts of mohaneem *M. azedarach* at 0.5 mg/ml (500 ppm) caused 45% larval mortalities of *Cx. quinquefasciatus* in 24 hours (Ravichandran *et al.* 2014). After 36 hours of exposure, the larval mortalities of the mosquito at 2.0 mg/ml and 3.5 mg/ml dose concentrations of ethanol leaf extracts of *M. azedarach* were 58.66 and 89.33%, respectively (Table 1). The LC_{50} and LC_{90} values of the ethanol extracts of neem leaves bioassayed on the 4th instar larvae of *Cx quinquefasciatus* mosquito were 1.805 and 3.581 mg/ml, respectively (Table 2) while LC_{50} and LC_{90} values of the same extracts of neem bioassayed on the same mosquito species were found to be 0.565 mg/ml and 2.39 mg/ml, respectively (Ravichandran *et al.* 2014). The above findings indicate that the toxicity of the neem leaves used in the present study seem to have less toxic potentiality than the neem leaves used by Ravichandran *et al.* (2014), but seem to have higher toxic potential than the neem leaves used by Maragathavalli *et al.* (2012).

The chloroform extracts of *A. indica* leaves at 1.50 mg/ml dose concentration caused 100% of 4th instar larval mortality of *Cx quinquefasciatus* (Table 1.). Chakaravarthy *et al.* (2011) reported that the chloroform extracts of the same plant *A. indica* produced maximum 87% mortality of *Cx. quinquefasciatus* larvae at 1 mg/ml (1000 ppm) in 24 hours; LC_{50} and LC_{90} were 0.198 mg/ml (198.32 ppm) and 1.15 mg/ml (1147.5 ppm), respectively. In the present study the chloroform extracts of mohaneem *M. azedarach* at 1.50 mg/ml caused 84% mortality of the 4th instar larvae of *Cx quinquefasciatus*.in 36 hours (Table 1). The chloroform extracts of *M. azedarach* caused 35% mortality of *Cx. quinquefasciatus* larvae at 0.5 mg/ml (l500 ppm) in 24 hours (Ravichandran *et al.* 2014).

The LC_{50} and LC_{90} values of chloroform extracts of mohaneem *M. azedarach* leaves bioassayed on the 4th instar larvae of *Cx quinquefasciatus* mosquito were 0.695 mg/ml and 2.256 mg/ml, respectively (Table 2) while LC_{50} and LC_{90} values of the same extracts of mohaneem bioassayed on the same mosquito species were found to be 0.93 mg/ml mg/ml and 5.65 mg/ml mg/ml, respectively (Ravichandran *et al.* 2014).

The water extracts of neem *A. indica* at. 2.50, 3.0, 3.50, 4.0 and 4.50 mg/ml dose concentrations caused 32.00, 56.00, 62.66, 68.00 and 81.33% larval mortalities of *Cx. quinquefasciatus*, respectively (Table 1). Aliero (2003) reported that the aqueous leaf extract of *A. indica* was 83% mortality of *Anopheles* mosquito when the larvae were treated with 20 ml extract while 75% and 68% mortality were recorded with 10 and 5 ml extracts, respectively after 12 hours.

Kubmarawa *et al.* (2008) reported that the most important substance present in *A. indica* include alkaloids, glucocides, sterids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement and body building. Among the limnoids of *M. azedarach*, azadirachtin has been found to be the main ingredient for fighting insects

and pests, being upto 90% effective in most instances (Azam *et al*. 2013). It can also be suggested that the two plants have a number of chemical components, which may be responsible for the many pharmacological actions. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Maurya *et al*. 2007). Finally, it may be suggested that the leaf extracts of neem (*A. indica)* and mohaneem (*M. azedarach)* act as a natural larvicidal for controlling mosquitoes. Both these plants are easily available, environmentaly friendly and less expensive for controlling mosquitoes. More research is needed to develop an easy, economically viable and sustainable method to isolate the main toxic ingredients particularly azadirachtin from both neem and mohaneem in order that these insecticidal ingredients may be commercially produced and effectively applied in the country for controlling the insect pests.

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ASSESSMENT OF THE DYNAMICS OF COASTAL ISLAND IN BANGLADESH USING GEOSPATIAL TECHNIQUES: DOMAR CHAR

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Abstract

Erosion and accretion rate are found very high in the estuary of an active delta which can be observed by satellite imageries. According to the multispectral satellite imageries it is found that accretion of Domar Char (a little island belongs to Meghna estuary) was more than 1192 hectares in last 25 years. The study was conducted in five years interval from 1990 to 2015 using spatial analyst extension of ArcGIS. It is revealed that accretion rate is about 208 hectares/year and erosion is about 160 hectares/year, which indicates the dynamic nature of the island. According to Landsat imageries about 2500 hectares of land rose in five years (1990 to 1995) and again lost 2300 hectares of land in next five years (1995 to 2000). Though the total area of intertidal zone has decreased, the area of barren land, vegetation and sand dune has increased over the time.

Key words: Shifting, Dynamic, Estuary, Coastline, Intertidal zone, Remote sensing

Introduction

Shoreline or coastline indicates a dynamic area that changes through short and long term process. Shoreline changes due to sediment shifting are known as morpho-dynamics (Schwartz 2005). Coastal zones are the most complex systems in the world with a large number of both living and non-living organisms (Aedla and Reddy 2015). Climate change and sea-level rising are the current issue in all over the world, especially in Bangladesh which is the worst affected and is facing early impacts of climate change (Sikder 2010). The physiography of coastal area of Bangladesh is more diverse and dynamic than is generally recognized. Failure to recognize this could lead to serious misconceptions about the potential impacts of a rising sea-level on Bangladesh due to global warming (Brammer 2014). Tidal bores are devastating in Chittagong, Cox's Bazar, Barisal, Noakhali, Patuakhali, Barguna and Khulna (Joseph 2006). The recent air temperature is increasing twice faster than the twentieth centuries (IPCC 2001) and average sea level has risen by 3.2 mm/year (IPCC 2013). Bangladesh is at extreme risk of floods, tropical cyclones, sea level rise and drought, all of which could bound millions of people to migrate (IPCC 2014). A report by UN scientists has projected that rising sea

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levels will inundate 17% of Bangladesh by 2050, making about 30 million people homeless (Dummett 2008). During years of severe flooding in 1987, 1988 and 1998 the country was transformed by intermittent inland seas that occupied as much as 60% of its land surface (Werle *et. al* 2000). If the sea-level rises by 45 cm, it may dislocate about 35 million people from 20 coastal districts of Bangladesh by 2050. In an extreme-case scenario, Bangladesh could lose almost 25 percent of its 1989 land area by around 2100 (Alam and Uddin 2013). According to the vulnerability index (Islam *et al.* 2015), about 57 km of the entire coast is under very high-risk and another more than 75 km is under high risk, about 67 km shoreline is at moderate risk and 63 km shoreline is at low risk. The most vulnerable coastal regions are found mainly along the western coast of CharFasson and northern and southwestern coast of BholaSadar of Bhola Island (Islam *et al.* 2015). Islands in the Meghna estuary were especially dynamic; Hatiya Island accreted along some of its shoreline by 50 km between 1989 and 2009, but has lost 65 km^2 through erosion elsewhere, resulting in the island is moving southward (Sarwar and Woodroffe 2013). The areal extent of Urir Char Island gets larger during the monsoon compared to the post monsoon and winter which is expanding at a very high rate of about 3.4 km^2 per year (Taguchi *et al* 2013). The Sandwip Island has gained 25 km^2 and lost about 64 km^2 through 1980-2014-time period and the net shoreline has shifted by 3.1 km in this time period (Emran*et. al* 2016). The coastal zone has countless importance in human life because different studies have revealed that the bulk of humanity is concerted along or near the coasts on just 10% of the earth's land surface. As of 1998, over half of the population lives and works in a coastal strip with width of just 200 km. Being a low laying deltaic country, the morphology of Bangladesh coastal zone is quite unstable and is changing with the time due to erosion and accretion. Natural threats, such as erosion, water logging and increase in water and soil salinity, risks from climate change like sea-level rise, and cyclone have adversely affected the morphology of coastal zone and reduced the pace of social, economic and infrastructure developments in this region (Shibly and Takewaka 2012). The impacts of this rise are wide-ranging encompassing coastal biological, physical and socio-economic realms such as loss of coastal wetlands, coastal flooding, coastal erosion, salinization of water resources, destroy of usual coastline shield, alteration and loss of coastal biodiversity, decline in fishing stocks, decrease in coastal land area and the migration of human population from the coastline are just some of these example (Blankespoor *et al.* 2014). Coastal wetlands are also highly exposed to sea level rise (SLR) because of their low elevation and their dependence on the active coastal physico-chemical regime for their unique habitats and species diversity (Nicholls 2004). Change detection can be defined as the process of identifying differences in the state of an object or spectacle by observing it at different times. This process is usually practiced to earth surface changes at two or more times period (Singh 1989). Timely and accurate change detection of earth's surface geographies provides the foundation for better understanding of relationships and interactions between human and natural phenomena towards better management and use

of resources. The accurate shoreline change extraction and change detection analysis is an important task that has applications in different fields such as development of setback planning, hazard zoning, erosion-accretion studies, regional sediment budgets, adopting different conservation measures e.g. protection of human life, protection of biodiversity, poverty, natural environment and conceptual or predictive modeling of coastal morpho-dynamics (Aedla and Reddy 2015). Thus, coastal change detection is critical in coastal zone application and is important for the future coastal dynamic studies (Mausel *et. al* 2004). Therefore, coastal morpho-dynamic studies have greater economic value to the socio-economic development of non-land locked nations (Adegoke *et. al* 2010). In recent years, satellite remote sensing data is widely used (Lipakis *et. al* 2008) for different change detection studies and change mapping because of its low cost and reliable information source with high frequency and repeatable observations (Emran *et. al* 2016). GIS technology has been used by many researchers in measuring, quantifying, calculating and monitoring shoreline rate-of-change statistics from multiple historic shoreline positions and sources (Oyedotun 2014). The repetitive acquisition and synoptic capabilities of remote sensing systems exploited to provide timely spatial data for coastal geographical information systems (GIS) which enables detection and monitoring the shifting of coastline (White and Asmar 1999). As no study on the changes of this coastal island in temporal scale is available, in the present investigation an attempt was taken to measure and compare the erosion and accretion from the year 1990 to 2015 to identify the intertidal zone and their shifting andto analyze the trend of shoreline shifting.

Materials and Methods

Study area:Domar Char (the wave island) one of two detached limbs of Hatiya Island has stepped into secondary succession, and offer gigantic salt marshes encircled by mudflats and centered by planted mangroves. It is a small island and is extending from 22°1’30” N to 22°5’0” N latitudes and 91°2’30” E to 91°6’30” E longitudes where few peoples lives only for fishing and rearing. It is located at south-east part of Hatiya Island and in the eastern portion of Nijhum Dwip (Fig. 1). Recently the forest department started mangrove afforestation along the coast of Domar Char where plantation is possible (Chowdhury 2006). Fig. 1 shows the location of study area.

Materials

Optical images are simple to interpret and easily obtainable; such images are TM (Thematic Mapper), ETM+ (Enhanced Thematic Mapper) and OLI-TIRS (Operational Land Imager & Thermal Infrared Sensor) imagery (Niya *et. al* 2013). In this present study, mainly Landsat 5 TM and Landsat 8 OLI-TIRS sensor images were used. From 1990 to 2015 in five years’ interval images were analyzed to fullfil the objectives. The images were downloaded from Global Visualization (Glovis) of United States Geological Survey (USGS). ArcGIS 10.3.1 and Erdas Imagine 2014 were used to analyze the images and MS Excel also used for making Chart and diagram.

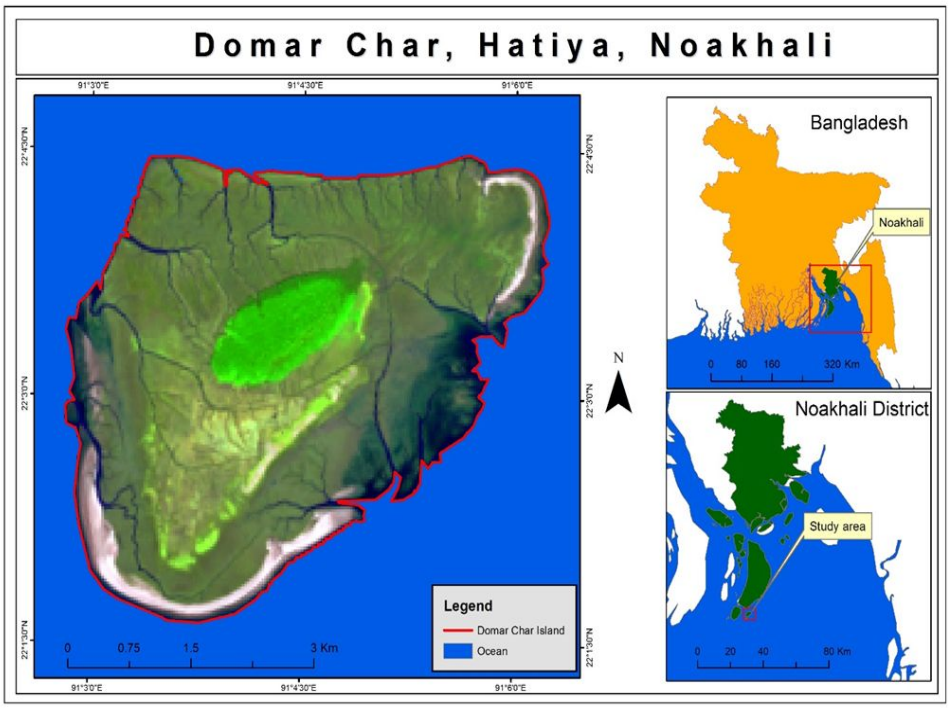

Fig. 1. Study area map.

Methods

Analysis of coastline changes are generally carried out using survey maps (Kadib 1969), historical coastline mapping, and comparison of beach profiles over a long period of time (Inman and Jenkins 1985). Some other more recent methods including simulation of coastline changes using numerical models; combination of coastline survey using Global Positioning System (GPS) receivers, long-shore sediment transport using numerical modeling packages such as MIKE21 and LITPACK (Pandian *et. al* 2004) and airborne Light Detection and Ranging. Monitoring shoreline change needs a long-term observation based on the temporal change modelling using remote sensing (RS) and geographic information system (GIS) (Bouchahma and Yan 2012). The use of satellite remote sensing techniques and geographic information systems (GIS) for the identification, mapping and analyses of coastline changes have gained prominence in recent years as high resolution satellite data have become more readily accessible (Adegoke *et al.* 2010). Data from remote sensors allow users for analyzing of a region with sufficient accuracy in an efficient, rapid and low-cost way (Berlanga-Robles and Ruiz-Luna 2002). In these instances remote sensing is the most beneficial method for different change detection studies. Since the reflection of water in NIR and MIR bands

are almost zero and most of vegetation has a higher reflection against water, coastline can be extracted using one NIR/MIR band (Ahmadi *et. al* 2014). The most used water indices are normalized difference water index (NDWI) (McFeeters 1996) and modified normalized difference water index (MNDWI) (Xu 2006). The change detection was assessed by processing of multi-temporal images (1990-2015), by image differencing, post-classification image overlaying, image visual interpretation and onscreen digitizing (Ahmadi *et. al* 2014). Ground truthing has been conducted using a hand-held GPS (spatial accuracy +/- 5m) through the filed visit to verify the satellite images (Emran *et. al* 2016). Kappa co-efficient which is an error matrix (Foody 2002) as accuracy assessment was calculated to verify the consistency of the classified images. Overall accuracy is found as 87% and Kappa co-efficient is found as 81% indicating high accuracy (Emran *et al.* 2016).

Results and Discussion

Erosion-Accretion: Erosion and accretion are common in coastal area since it is dynamic in nature. Domar Char is very dynamic as the large volume of sediment from three great river systems Ganges-Brahmaputra-Meghna (GBM) deposited here. Erosion and accretion rate is very high along the coast of Domar Char. From 1990 to 1995, accretion occurred significantly of about 2582 hectares and erosion only about 0.34 hectares, whereas unchanged area remains about 979 hectares. From 1995 to 2000, the

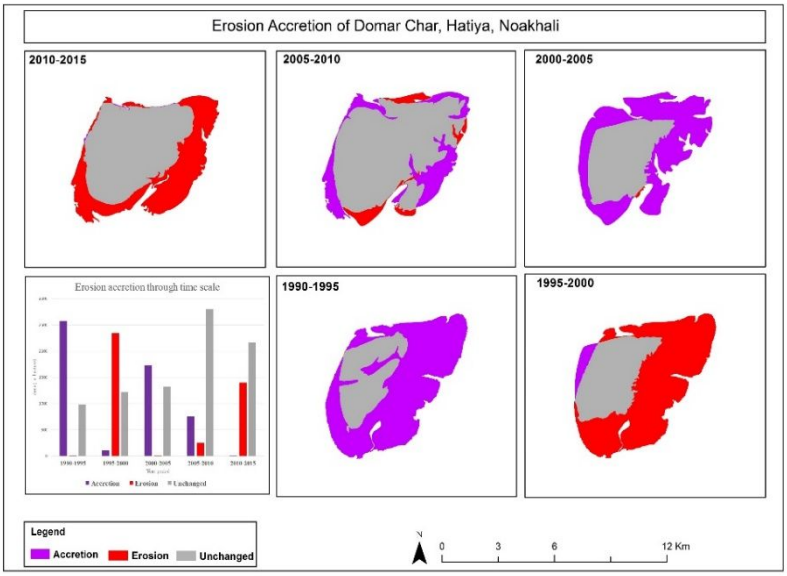

Fig. 2. Erosion-Accretion map of Domar Char Island.

accretion rate was very slow while 113 hectares of land upraised and about 2344 hectares of land was found to be unchanged, as it is a dynamic place erosion and accretion

rate/trend drastically varies from time to time. In next five years (2000-2005) again the accretion rate was very high (app. 1734 hectares) and stable/unchanged land was about 1321 hectares whereas erosion was insignificant (app. 8 hectares). Unchanged area was doubled in 2005-2010 period, whereas accretion was 758 hectares and erosion was only about 249 hectares. In last five years (2010-2015) the volume of newly deposited land was insignificant and unchanged land decreased while erosion increased about 1398 hectares (Fig. 2). Erosion rate was very high in both second and fifth time period and accretion rate was very high in both first and fifth time periods. Erosion and accretion rate was similar in fourth time period.

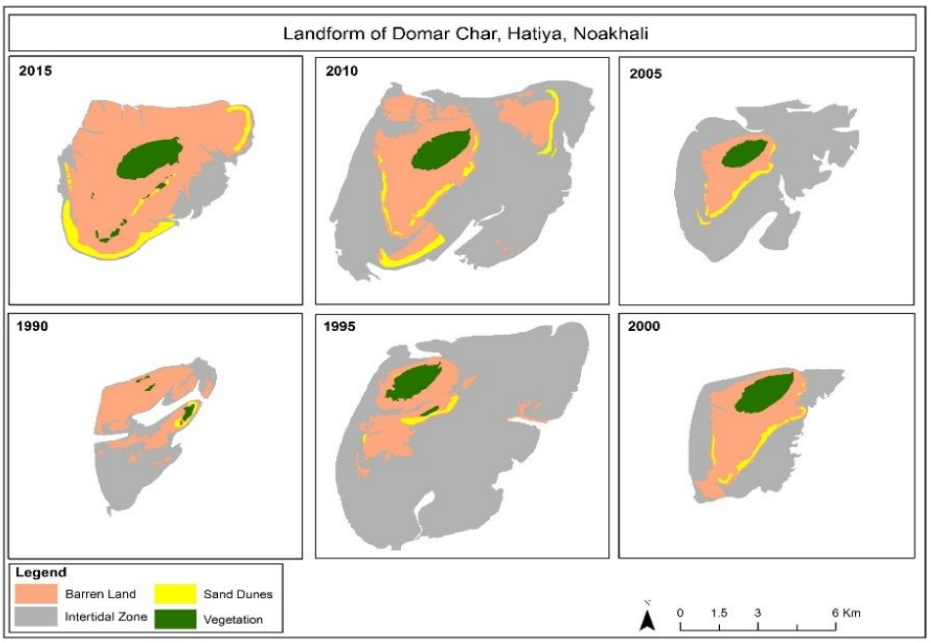

Fig. 3. Landform change map of Domar Char Island.

Intertidal zone: The area between the land and sea that is covered by water at high tide and uncovered at low tide is called intertidal zone or mud flat. Intertidal zone is a very important zone for the coast development. According to the satellite imageries, there was an extreme shifting observed in intertidal zone. In 1990, a very little amount of land was found in the intertidal zone that increased to 3000 hectares in 1995 (Figs. 4 and 5). All other zones (except intertidal) increased in 2000. The intertidal zone increased in 2005 and remain unchanged till 2010 and other zones increased a lot during this time period. The intertidal zone reduced in 2015 but other zones increased (Figs. 4 and 5). From Figs 4 and 5 it is apparent that the amount of high land (vegetation, barren land and sand dunes) over the intertidal zone has increased in the last 25 years.

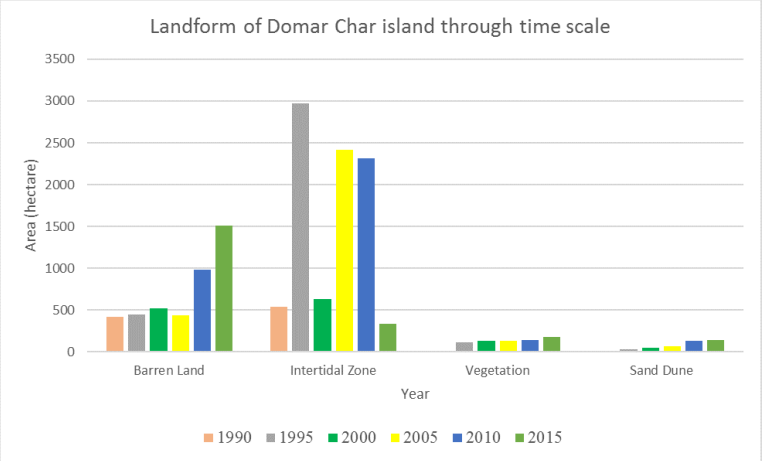

Fig. 4. Landform change graph of Domar Char Island.

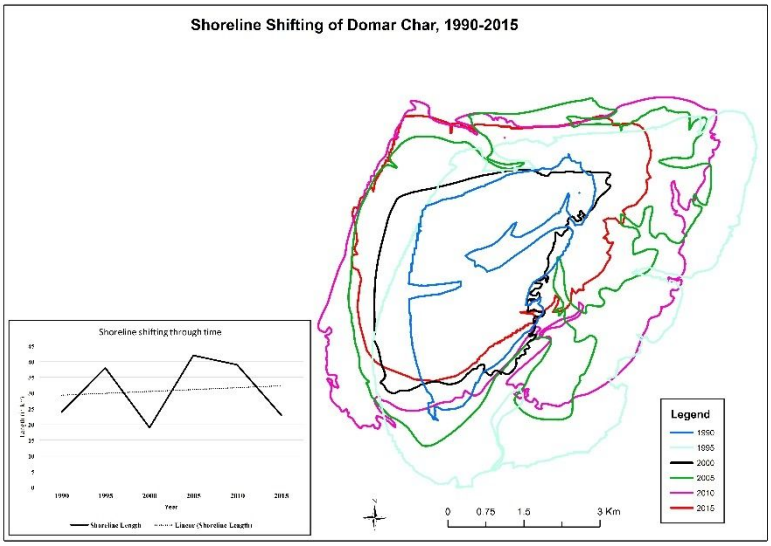

Fig. 5. Shoreline shifting of Domar Char Island.

Shoreline shifting: Shoreline shifting is common in coastal area. The shoreline in the study area has never been constant and is experiencing a dynamic pattern. Both the spatial and temporal variations in the deposition and accretion were observed in the study area. The temporal intervals (being 1990 to 1995, 1995 to 2000, 2000 to 2005, 2005 to 2010 and 2010 to 2015) were used in the study for assessing the changes. The changing pattern is not uniform. However, the erosion and accretion patterns clearly showed a

continuous geomorphic sculpturing over the coastal tract in each temporal interval. Net shoreline has just shifted only -1 km from 1990 to 2015 which indicates that the island is eroded as well as accreted over the time. In 1995 the shoreline extended to the northern to eastern part whereas in 2000 the shoreline eroded and shifted to the western part. In 2005 Shorelines approached in all parts of the island. In 2010 and 2015 the shoreline shifted only in the western portion of the island. Fig.5 shows shoreline shifting of Domar Char.

In the context of the sea level rise or tidal bores a proper management plan must be taken to save the coastal areas of Bangladesh from submerging. Despite the dynamic (erosion and accretion) nature of coast, the total land mass of this island has increased in last 25 years. Though the island is expanding over time, the shoreline length has reduced. The Domar char is located at the estuary of Meghna river which is one of the biggest river of Bangladesh. Regular monitoring is required for change detection analysis in coastal morphology.

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COMPARISON OF GROWTH PERFORMANCE THROUGH DIFFERENT LEVELS OF SUPPLEMENTARY FEED IN FISH POLYCULTURE SYSTEM

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Abstract

An experiment was carried out to evaluate the growth performance of carp polyculture system supplemented with different levels of supplementary feed. To undertake this investigation, two earthen ponds were stocked with 25% *Labeo rohita*, 25% *Catla catla*, 20% *Labeo calbasu*, 20% *Ctenopharyngodon idella* and 10% *Hypopthal-michthys molitrix* with a total stocking density of 10000/ha. The net fish production was found to be 2,166 and 3,874 kg/ha/yr in pond-01 and pond-02, respectively. The total cost of fish farming of pond-01 and pond-02 were 64,268 BDT and 88,568 BDT per ha. The total return of pond-01and pond-02 were BDT 1,04,280 BDT and 1,69,250 BDT per ha. Net benefit from pond-01 and pond-02 was 40,312 BDT and 80,682 BDT respectively. Net profit margin of pond-01 and pond-02 was 62.73% and 91.10%. And finally the benefit cost ratios (CBR) were found to be 0.62:1 and 0.91:1 in pond-01 and pond-02, respectively.

Key words: Carp polyculture, Supplementary feed, Cost-benefit ratio

Introduction

Aquaculture plays an important role in food production as well as creates an employment opportunities in the world. Bangladesh is blessed with water resources and aquaculture is one of the fast growing sectors in this country. Fishes serves as valuable ingredient to a healthy diet because of its easily digestible high protein and unsaturated fat contents. It is often recommended for heart patients by doctors, since it is an excellent source of Omega-3 fatty acid. Fish are also rich in vitamins (fat-soluble vitamins A, D and E, and water-soluble vitamins, B complex) and minerals (especially calcium, phosphorus, iron, selenium and iodine in marine fishes) (Choo and Williams 2003, Sandhu 2005, Razvi 2006, Salim 2006 and Yildrim 2008). Therefore, fish can provide an important source of nutrients, particularly for those whose diets are lacking these nutritional constituents (World Aquaculture 2010). The demand for fish as main protein source increases every year due to increase of population in the country. But scientists, fish farmers and fishers face various constraints and vulnerabilities as they are main triggers for technology generation, production enhancement and sustainable fisheries development. The basic principle of this polyculture system is that the fish species with different feeding habits

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are cultured together to increase productivity by a more efficient utilization of the ecological resources in the aquatic environment (Lutz 2003).

Supplementary feeding is a management protocol to enhance the fish production in a pond culture system within the shortest possible time. Supplementary feeding increases the carrying capacity of culture systems and can enhance fish production by manifold (Hepher 1975 and Devaraj *et al*. 1986). Supplementary feed is found to be a useful tool for providing nutrient components and energy required for better fish growth and production (Abdelghany *et al*. 2002). According to Azim *et al*. (2002), specific growth rate of major carps were higher in fertilized pond supplemented with supplemental feed than in control (fertilization alone). Use of supplementary feed is also recommended along with the organic manure and chemical fertilizers in order to get maximum fish production from limited water bodies within the shortest possible time (Mahboob *et al*. 1995). Ali *et al*. (2003) also observed prominent increment in weight gain, feed conversion ratio (FCR) and net production in major carps supplemented with supplementary feed at the rate of 6% of body weight. These feed can be utilized in different combinations to provide optimum source of dietary nutrients. Combination of fish meal, sesame oil cake and mustard oil cakes proved to be cost effective and significantly affect on the growth performance of fish (Stickney 2000). Keeping in view the significance of supplementary feeding, the present study was conducted to assess the growth performance of carps in semi-intensive culture system.

Materials and Methods

The experiment was carried out in two earthen ponds (pond-01 and pond-02), measuring 890 and 2200 m^2, respectively, located at Sagar para of Boalia thana under Rajshahi district of Bangladesh. Before starting the experiment, all aquatic weeds and unwanted biota were removed. Aquatic weeds were removed manually and unwanted fish was removed by using Phostoxin tablet at the rate of 4-5 pieces/decimal in pond-01 and 6-7 piece/decimal in pond-02. Then agricultural lime (calcium carbonate, $CaCO_3$) was applied at the rate of 230 kg/ha. After primary preparation, both ponds were fertilized with cowdung and triple super phosphate (TSP) at the rate of 1720 and 20 kg/ha, respectively. Each pond was stocked with 25% *Labeo rohita* (individual weight 5-6 g), 25% *Catla catla* (6-7 g), 20% *Labeo calbasu* (4-6 g), 20% *Ctenopharyngodon idella* (4-5 g), 10% *Hypophthalmichthys molitrix* (4-5 g) with a total stocking density 1000/ha. Both ponds were fertilized with above mentioned rate at 10 days intervals at. Two ponds were supplemented with mustard oil cake, rice bran, maize bran at the rate of 1.09 kg/ha and 1.336 kg/ha, respectively for a period of six months as daily basis. After that period, fish were caught and measured in terms of body weight.

Fish survival rate (S) was calculated as the number of fish harvested as percentage of the number of fish stocked, S (%) = (Number of fish /Number in stocked) × 100

Fish yield (kg/ha/month)/Total production = Fish biomass at harvest – Fish biomass at stock

Finally, fish were harvested and counted.

Economics of two ponds : An economic analysis of two ponds was performed on the basis of the expenditure incurred and the total estimated return from the sale price (BDT) of the harvested fish. At the end of the experiment, all fish were sold locally and the total return was estimated. The following factors were used to the economics of different treatments of two ponds.

Net benefit (Tk.) = total return (sale) – total cost (investment)

$$\text{Net profit margin (\%)} = \frac{\text{Net benefit}}{\text{Total investment}} \times 100$$

$$\text{CBR} = \frac{\text{Net benefit}}{\text{Total investment}}$$

Data and statistical analyses were done by using Microsoft Excel-add-in-DDxl. All data were checked for homogeneity of variance.

Results and Discussion

Production : The initial average body weight, the average body weight at the time of harvest, net body weight gain, survival rate and total yield of *Labeo rohita, Catla catla, Labeo calbasu, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* of pond-01 and pond 02 are presented in Table 1.

Table 1. Growth performance and survival rate of carps in pond-01 and pond-02.

Ponds	Carp variety	Initial average Weight (g)	Final average Weight (g)	Average Weight (g)	Survival rate (%)	Total Production (kg/ha/yr)
Pond-01	*L. rohita*	5.5	400.6	395.1	96	1976
	C. catla	6.5	550.7	544.2	96	
	L. calbasu	5	360.8	355.8	95	
	C. idella	4.5	500.6	496.1	95	
	H. molitrix	4.5	380.2	375.7	94	
Pond-02	*L. rohita*	5.5	600.8	595.3	97	2791
	C. catla	6.5	800.7	793.8	98	
	L. calbasu	5	550.6	545.6	97	
	C. idella	4.5	700.5	696	94	
	H. molitrix	4.5	650.2	654.7	93	

Economics analysis: A simple economic analysis was performed to estimate the net profit (total returns from harvest - total cost of production) and cost benefit-ratio (CBR = total benefit – total cost) from polyculture of carps of two ponds separately which is shown in Table 2.

Table 2. Economics of two cultural ponds.

Parameters \ Pond	Pond-01	Pond-02
Total cost (BDT/ha)	61628	104158
Total return (BDT/ha)	118190	169250
Net benefit (BDT/ha)	56562	65092
Net profit margin (%)	91.779	62.494
CBR	1:0.91	1:0.62

The result of the present study shows that net body weight of all fishes in pond-02 was higher than in pond-01. The use of supplementary feed caused a significant increased yield in pond-02. The net fish production in pond-02 was found to be 3874 kg/ha/yr while it was 2166 kg/ha/yr in pond-01. So feed based on semi-intensive culture system gave 1.41 times greater fish production than the simple extensive one. The yield of this semi-intensive polyculture system is similar to several production levels obtained in other semi-intensive polyculture in the South Asian region, e.g, Shahabuddin *et al.* (1994) obtained yields of 2000-3400 kg/ha/year. The result found to be similar with pond-02. Mahboob *et al.* (1995) suggested that application of supplementary feed along with chemical fertilizers and organic manure is the best mean to obtain maximum production in fish culture practices from confined water bodies within the limited possible time in semi intensive culture system.

The production cost was higher in pond-02 but the highest net revenue was obtained from this pond. The feeding rate was relatively higher in pond-02 that's why the total production might be higher than pond-01. However, the simple economic analysis showed that the cost benefit-ratio (CBR) was higher in pond-02. Net benefit in polyculture of carps ranged from Tk. 88,745 to 93,805/ha/l0 months (Miah *et al*. 1993) which is found similar with pond-02. In another study cost-benefit ratio (CBR) revealed significantly higher ratio (1:0.69) where formulated feed was used with fertilizer (Samad *et al*. 2014).

A simple cost and return analysis were done on the basis of both cost and full cost to determine the profitability (Zaher and Mazid 1993). Use of higher level of inputs usually results in higher outputs, consequently higher investments produces higher gross and net return on per unit water body of ponds (Rahman1995 and Biswas1990). Higher net return from the pond fish production is influenced by the price of outputs and economic use of both material inputs and labor (Rahman 1995).

This study indicated that the higher level of supplementary feeding increased cost of polyculture system of carps but it is more profitable and economically feasible than application of lower rate of supplementary feeding. The enhanced production in second

pond can be justified by the increase of supplementary feeding which contributes to increase fish yield and finally total aquaculture production. This research could be helpful for sustainable aquaculture with supplementary feeding in North West of Bangladesh as well as also other parts of the country.

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BACTERIOLOGICAL QUALITY OF FROZEN AND UNFROZEN PABDA (OMPOK PABDA: SILURIFORMES) IN A FISH PROCESSING PLANT

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Abstract

The present study investigated the bacteriological quality of frozen and unfrozen pabda in a Fish Processing Plant during the period from January 2015 to October 2015. The bacteriological parameters, such as, total viable count (TVC), total coliform (TC), and the occurrence of *Escherichia coli*, *Salmonella. spp.* and *Vibrio spp.* were studied. The TVC of frozen samples were 2.9×10^5, 1.8×10^5, 1.5×10^5, 2.5×10^5 and 3.5×10^5 CFU/g and the TVC of unfrozen samples were 5×10^7, 3×10^6, 6×10^5, 4×10^6 and 4×10^7 CFU/g respectively. The mean bacterial loads of frozen and unfrozen pabda were log 5.37 ± 0.15 and log 7.02 ± 0.59. The above results were statistically significant ($p < 0.05$) between frozen and unfrozen samples of pabda.The frozen samples contained lower bacterial load than unfrozen samples. The bacterial loads of frozen pabda complied with ICMSF standard but the same of unfrozen pabda did not comply with this standard. The TC values of frozen samples were 20, 15, 20, 21 and 27 MPN/g but the same values of unfrozen were 160, 120, 120, 120 and 150 MPN/g respectively. The mean TC values for both frozen and unfrozen samples were 20.6 ± 4.28 and 134 ± 19.49. It reveals that the TC of frozen pabda complied with ICMSF standard but the same of unfrozen pabda did not comply with this standard. The detected pathogenic bacteria were *Esherichia coli*, *Salmonella spp.* and *Vibrio cholerae*. In frozen Pabda all the identified pathogenic bacteria were absent. All of the unfrozen samples were contaminated with *Esherichia coli*, but two of the samples were contaminated with *Salmonella spp.* and one sample was polluted with *Vibrio cholerae*. So, the findings of bacteriological quality of frozen pabda complied with ICMSF standard but the same quality of unfrozen samples did not comply with ICMSF standard.

Key words: Bacteriological quality, Frozen and unfrozen pabda, Fish processing plant, ICMSF standard

Introduction

Bangladesh is blessed with rich and extensive fishery resources as well as wide variety of indigenous and exotic aquatic fauna. The soil, water and climate of Bangladesh are

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favourable for inland fisheries production in both open and closed waters. Fish and fisheries play a dynamic role in the meeting up nutritional demand of people, generating employment and earning foreign currency (Alam 2002). There are 100 fish processing plants in Bangladesh of which 75 plants are approved by European Commission (DoF 2014).

The rivers and several haors of Sylhet district are renowned for producing fish around the year. Due to presence of haor, baor, lake and other open water bodies indigenous natural fishes are available in this region. Some cultured fishes are also available there. There are three fish processing plants in which lot of fish products are processed for export. In one plant several fish products like block frozen, IQF of fresh water fishes and other frozen white fishes are produced. Fish and fishery products are regarded as high risk commodity in respect of pathogen contents, natural toxin and other possible contaminations (Saeed *et al*. 2003). Microorganisms are found on outer surfaces like skin and the intestine of live newly caught fishes. Total number of bacteria found in fish skin is about 10^2-10^7cfu/cm^2 (Liston 1980) and availability of bacterial flora in gill and intestine is 10^3-10^9cfu/gm (Shewan1977). Population densities of bacteria in water ranged from $10^3$to 10^6cells in 1 ml of water depending on the environmental conditions. Microbial actions play an important role in the spoilage of fish (Eyo 2001). Micro flora of fish and shellfish are closely connected to these of water and sediment (Kadota 1990).

It is necessary to assess the microbial load of the processed fish products during raw and frozen state because of the presence of pathogenic bacteria like *Vibrio cholerae*, *Salmonella* spp. etc. in processed products causing serious health complexities to the consumers (Noor 2013). *Ompokpabda* is a tasty fish and also highly demandable for export. But no detailed research was conducted on the bacteriological quality of frozen and unfrozen pabda. Thus in the present study an attempt was taken to know the bacteriological quality of frozen and unfrozen pabda for ensuring the safety measures for the consumers.

Materials and Methods

Collection of Samples: Total five frozen samples were taken from different lots of a Fish Processing Plant and five (5) unfrozen samples taken from raw fish. All of the collected samples were transferred to Microbiological Laboratory of the plant to determine total bacterial load and total coliform and also to the Laboratory of Microbiology and Immunology Dept. of Veterinary and Animal Science Faculty of Sylhet Agricultural University, for isolating and identifying the bacteria from frozen and unfrozen samples.

Preparation of sample: Firstly 25 g of fish sample was taken aseptically from fish muscle, gill and intestine and was mixed homogeneously with 225 ml distilled water in a stomacher lab blander. Each sample was mixed aseptically with sterile distilled water at the ratio of 1:10. Then the sample was shaken properly to make a homogenous

suspension. Later on 10 fold serial dilutions (1:10) were prepared ranging from 10^{-2}-10^{-9} according to the recommendation of International Standard Organization (ISO 1995).

Calculation of total viable count (TVC): 1 ml of each tenfold diluted sample was transferred and spreaded to plate count (PCA) agar using a sterile pipette and a sterile glass spreader. The incubated plates were then kept in an incubator at 37°C for 24-48 hours. Only plates having 30 to 300 colonies were considered for counting to get their acceptable values. Number of bacteria per gram of the sample (CFU/g) was calculated by using the following formula:

$$\text{CFU/g} = \frac{\text{No. of colonies on petridish} \times 10 \times \text{dilution factor} \times \text{Volume of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Total coliform count (TCC): MPN (Most Probable Number) method was used to count total coliform of samples. MPN is a method to estimate the population density of viable microorganisms in a test sample. It is based upon the application of the theory of probability to the numbers of observed positive growth responses to a standard dilution series of sample inoculums placed into a set of number of culture media tubes. Positive growth response after incubation is indicated by observation of gas production in fermentation tubes. The sample was diluted in such a manner that higher dilutions of the sample would result in fewer positive culture tubes in the series. The number of sample dilutions to be prepared is generally based on the expected population contained within the sample.

Isolation and identification of pathogenic bacteria: For the isolation and identification of bacteria characters the morphological (size, shape, arrangement, and motility) were considered and study was made by Gram's staining reaction, colony characteristics, biochemical reaction, catalase test, motility test. Firstly TVC (Total Viable Count) and TCC (Total Coliform Count) were determined by using plate count agar and lactose broth. The suspected colony from these media was subcultured in Nutrient agar, VRB, EMB, MacConkey, SS, and BGA to promote the growth of a particular type of bacterium. Finally the pure culture was obtained from the selective media. Staining with "Gram's staining" method along with other tests were done. Strict aseptic measures were maintained during the period of study. Striking on different solid agar was done under laminar air flow. After performing the above mentioned tests, the results were analyzed and the isolated bacteria present in samples were identified.

Detection of Escherichia coli: For the isolation and identification of *Escherichia coli*, the samples were first inoculated on Violet red bile agar then the colonies from VRB agar were sub cultured on EMB, MacConkey, and BGA. Colonies on EMB agar with metallic sheen and colonies on MacConkey with pink color were suspected as positive for E. coli. The *Escherichia coli* were characterized by positive to indole test, catalase test, motility test and MR tests and *negative* to VP.

Detection of *Salmonella spp: Salmonella* were grown on nutrient agar, MacConkey agar, Brilliant Green agar, Salmonella-Shigella (SS) agar and Violet Red Bile agar. On nutrient agar the *Salmonella* colonies were translucent, opaque, and smooth. On MacConkey agar colonies were pale or colorless. On Brilliant Green agar media was pink color and colonies were cream color. On SS agar colonies were black color with dark centre. On VRB agar colonies were pale cream color. *Salmonella* produced Hydrogen sulphide (H_2S) which is black color on TSI (Triple Sugar Iron) slant and SS-agar. So the slant became black in color and black colonies were grown on SS-agar. *Salmonella* were positive to MR test, motility test, catalase test, TSI test and negative to Indole test, VP test.

Detection of *Vibrio cholerae:Vibrio cholerae* was grown on nutrient agar, Thiosulfate Citrate Bile Salts Sucrose (TCBSS) agar. On nutrient agar the *Vibrio spp.* colonies were translucent, opaque, and smooth. On Thiosulfate Citrate Bile Salts Sucrose (TCBSS) agar *Vibrio cholerae* colonies appeared yellow and also green. *Vibrio spp* produced Hydrogen sulphide (H_2S) which is yellow in color on TSI (Triple Sugar Iron) slant. *Vibrio cholerae* was positive to MR test, motility test, catalase test, and TSI test and negative to VP test.

Statistical analysis: For preliminary processing of raw data obtained from both unfrozen and frozen pabda, the mean, standard deviation was calculated first. These values were useful and other statistical analysis and interpretations thereafter were done by using the computer software like Microsoft Excel.

Results and Discussion

The results of bacteriological quality of frozen and unfrozen Pabda (*Ompokpabda*) from the fish processing plant are presented in Tables 1 and 2. In this study selected bacteriological qualities were TVC, TC, *E.coli*, *Salmonella* and *Vibrio cholerae*. From both type of samples all the findings were verified with ICMSF standard 1986 for the acceptable or unacceptable in terms of export and as well as food safety for the consumer.

Table 1. Total results for unfrozen pabda collected from Processing Plant before processing.

Raw samples of specific lot	Date	TVC (CFU/g)	log TVC (CFU/g)	TC	*E. coli*	*Salmonella*	*Vibrio cholerae*	Remarks
1	05/01/15	5×10^7	7.698	160	P	P	P	Results did not comply with ICMSF standard
2	01/03/15	3×10^6	6.477	120	P	A	A	
3	20/04/15	6×10^5	6.698	120	P	P	A	
4	14/05/15	4×10^6	6.602	120	P	A	A	
5	29/07/15	4×10^7	7.602	150	P	A	P	

Table 2. Total results for frozen pabda collected from Processing Plant after processing.

Lot no.	Date	TVC (CFU/g)	log TVC (CFU/g)	TC	*Escherichia coli*	*Salmonella*	*Vibrio cholerae*	Remarks
1	26/02/15	2.9×10^5	5.462	20	A	A	A	Results
2	02/04/15	1.8×10^5	5.255	15	A	A	A	complied
3	04/05/15	1.5×10^5	5.176	20	A	A	A	with
4	07/06/15	2.5×10^5	5.397	21	A	A	A	ICMSF
5	20/08/15	3.5×10^5	5.544	27	A	A	A	standard

Total Viable Count of bacteria: The concentration of TVC was calculated both for frozen and unfrozen pabda. The collected samples of the frozen pabda contained lower bacterial load than unfrozen pabda of this plant. The TVC of frozen samples was 2.9×10^5, 1.8×10^5, 1.5×10^5, 2.5×10^5 and 3.5×10^5 CFU/g; on the other hand, TVC of unfrozen samples was 5×10^7, 3×10^6, 6×10^5, 4×10^6 and 4×10^7 CFU/g respectively The bacterial load of frozen pabda complied with ICMSF standard but failed in case of unfrozen pabda.

Comparative Analysis of Bacterial Load (TVC) : The mean of bacterial load was calculated as Log CFU ± SD. The mean bacterial load determined for both frozen and unfrozen samples were log 5.3668 ± 0.150232 and log 7.0154 ± 0.585568 (Fig. 1). The above results were statistically significant ($p < 0.05$) between frozen and unfrozen samples of pabda.

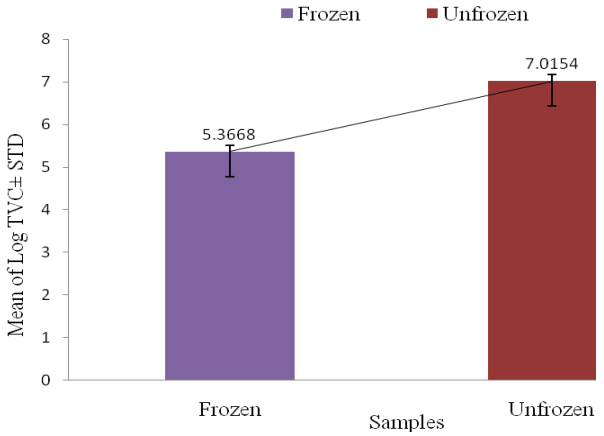

Fig. 1.Comparison of total bacterial load (TVC) of frozen and unfrozen pabda.

Total Coliform (TCC) Count: Total Coliform (TC) was calculated by using Most Probable Number (MPN) index. The frozen Pabda contained lower total coliform than unfrozen pabda of the collected samples of this plant. The TC of frozen samples was 20, 15, 20, 21 and 27 MPN/g whereas the same of unfrozen samples was 160, 120, 120, 120

and 150 MPN/g. The total coliform of frozen pabda complied with ICMSF standard but in case of unfrozen pabda did not comply with this standard.

Comparative Analysis of Total Coliform (TC): The mean of total coliform was calculated as log MPN/g ± SD. The mean total Coliform determined for both frozen and unfrozen samples were 20.6 ± 4.27785 and 134 ± 19.49359 (Fig. 2).

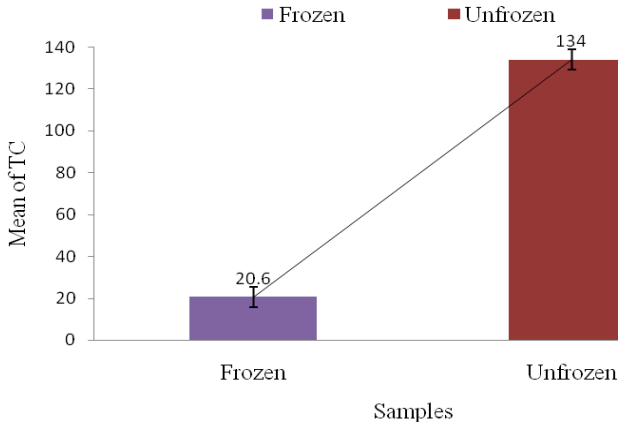

Fig. 2. Comparison of Total Coliform (TC) of frozen and unfrozen pabda.

Pathogenic bacteria in frozen and unfrozen pabda : In frozen pabda pathogenic bacteria was absent. All of the frozen samples were safe due to the absence of pathogenic bacteria but in unfrozen samples pathogenic bacteria were present. The identified bacteria were *Esherichia coli, Salmonella spp.* and *Vibrio cholerae*.

During this experiment frozen and unfrozen pabda were taken from a plant and studied to find the bacterial load of the sesamples. The TVC of frozen samples were 2.9×10^5, 1.8×10^5, 1.5×10^5, 2.5×10^5 and 3.5×10^5 CFU/g , whereas TVC of unfrozen samples were 5×10^7, 3×10^6, 6×10^5, 4×10^6 and 4×10^7 CFU/g respectively.The mean bacterial load of frozen and unfrozen pabda was log 5.3668 ± 0.150232 andlog7.0154 ± 0.585568. The frozen samples contained lower bacterial load than unfrozen samples. The bacterial load of frozen pabda complied with ICMSF standard but unfrozen pabda did not comply with this standard. The findings of this experiment were almost similar to the previous report Saeed *et.al* (2003) who found that the bacterial load of frozen Hilsha (*Tenualosa ilisha*) in four lots were 1.33×10^5, 1.05×10^5, 0.63×10^5 and 0.49×10^5cfu/gm and also in the same species of unfrozen Hilsha were 9.75×10^5, 7.50×10^5, $4.40\times10^5$and 3.71×10^5cfu/gm. SPC of frozen Hilsha was lower than that of the unfrozen Hilsha fish collected from different markets and habitats. Khan (2012) compared the microbial analysis between frozen and raw shrimp in “ARK Sea Foods Ltd.” in Chittagong, Bangladesh. During this study the mean TVC ± SEM of raw and frozen shrimp were 4.37 ± 0.328×10^5 and 1.42×10^5 ± 0.187×10^5 respectively. The TC of frozen samples of the present study was 20, 15, 20,

21 and 27 MPN/g, where the TC of unfrozen samples was 160, 120, 120, 120 and 150 MPN/g. The mean Total Coliform determined for both frozen and unfrozen samples were 20.6 ± 4.27785 and 134 ± 19.49359. The Total Coliform of frozen Pabda complied with ICMSF standard but unfrozen pabda did not comply with this standard. According to ICMSF (1986) the acceptable limit of total coliform is <100 MPN/g. The present result wasfound with the findings of Saeed *et al.* (2003) in frozen and unfrozen Hilsha. Ali *et al.* (2012) observed that the mean total coliform count of cooked IQF shrimp was < 3 ± 0.00 MPN/g, while it was 23.50 ± 13.72 MPN/g in Raw Block Frozen Shrimp which support the present study. The pathogenic bacteria isolated and identified during this experiment were *Esherichia coli, Salmonella spp.* and *Vibrio cholerae*. In frozen pabda all of the identified pathogenic bacteria were absent. So, it may be mentioned that frozen samples complied with ICMSF standard. In case of all frozen Hilsha, *Salmonella spp*. and *Vibrio cholerae* were absent (Saeed *et al*. 2003). The study was conducted to determine the microbial quality of *Tenualosa ilisha* at different stages of processing. There was no evidence of presence of *Salmonella* and *Vibrio cholerae* at any stages of processing (Shamsuzzaman *et al*. 2011).

In unfrozen samples pathogenic bacteria were present. All the unfrozen samples were contaminated with *Esherichia coli*, two of the samples contaminated with *Salmonella spp.* and one sample polluted with *Vibrio cholerae*. The unfrozen samples did not comply with ICMSF standard. According to ICMSF (1986), zero tolerance of *Salmonella spp.* and *Vibrio cholerae* is in fish and fishery products. *Salmonella* spp. in aquaculture shrimp products mainly originates from the environment rather than from poor standards of hygiene and sanitation. But sometimes, incidence of this bacterium in fish, shrimp or similar foods of aquatic habitats may happened due to external contamination (Huss 1993). *Salmonella* was isolated from fresh, frozen, canned and sun dried marine fish products (Natarajan *et al*. 1985).

The findings of the bacteriological quality of frozen pabda were quite satisfactory and also complied with ICMSF standard. It was due to the proper processing of raw fish. After complied with this standard it is considered for export having health certificate from FIQC, DoF for export to EU and other countries. On the other hand, bacteriological quality of unfrozen pabda was not satisfactory and it did not comply with ICMSF standard due to the presence of higher bacterial load and pathogenic bacteria. So, the study reveals that frozen pabda was better than unfrozen pabda considering food safety as well as for export.

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AN ANALYSIS OF BIRTH INTERVALS IN BANGLADESH USING FRAILTY MODELS

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Abstract

This research dealt with the extension of the Cox's proportional hazards model that allows for heterogeneity among the responses due to random effects of covariates using frailty (random effect) approach. The results of this study showed that the Gaussian frailty model is better for the data than gamma frailty model and the unobserved cluster effect has a sizeable impact on the second birth spacing in Bangladesh. This research also showed the current pattern of the second birth spacing in Bangladesh and different demographic and socio-economic factors which affect the second birth spacing. It was found that mother's education, survival status of 1st birth, region, place of residence and mother's age of marriage have great influence for the variation of the second birth spacing.

Key words: Frailty model, Birth space, Cox proportional hazards model

Introduction

Birth spacing (interval) refers to the time interval from one child's birth until the next child's birth i.e; length of the time between two successive births. Identification of the factors causing variation in the length of birth interval could have of great importance for it's direct relation to fertility. The covariates may play some key role to the second birth spacing. Determination and identification of the factors causing variation in the length of birth interval are of great importance for its direct relation to fertility.

Conventional Cox's model assumes that the investigated subjects under given experimental conditions are independent and identically distributed and hence homogeneous by nature. There may be situations where there may exist some factors other than the measured covariates which can significantly influence the parameters and hence modify the distribution of the survival time. There may be various reasons for such unmeasured or neglected covariates. If there are too many covariates to consider, it is nearly impossible for the researchers in practice to include all the relevant covariates. Then they are tempted to overlook some of the relevant covariates. Another common reason may be that researchers are not aware of the influence of the potential covariate that might exist. For example, if there is a genetic risk factor responsible for potential occurrence of some diseases, which may be unknown to us, it is not possible for the researcher to include that

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as a covariate. Such covariates are said to be the unobserved covariates. Therefore, for practical reasons such unobservable covariates are ignored by considering them as a part of the error component and not controlled in conventional survival analysis. This may greatly simplify the calculation, but this advantage comes at a great price.

Therefore, as individuals in any group are dissimilar in their own rights, the model has to be improved to account such hidden heterogeneity and modification of homogeneity assumption is necessary. Keyfitz (1978) and Vaupel *et al.* (1979) suggested for the mixture of individuals with different hazards in the same population. Experiences in their studies suggested that there exists a considerable variation in the risk of developing various diseases and thus, individuals in a biological population differ substantially in susceptibility for various mortality and morbidity events. Individuals have different frailties, and that those who are most frail will die earlier than the others. The reason can be different patterns of gene they carry, the distinct life style they follow etc (Zahan 2010). To deal with such problem in the survival analysis, frailty models have been suggested by various researchers. This model corrects the bias of the regression coefficients in the Cox's Proportional Hazard model (Chamberlain 1979). The frailty models also plays role in describing the non-proportionality of the conditional hazards, which in turn improves the fit. The frailty model was historically first introduced by Clayton (1978) as a bivariate model.

Materials and Methods

Secondary data extracted from the Bangladesh Demographic and Health Survey (NIPORT 2011) conducted under the authority of the National Institute for Population Research and Training (NIPORT) of the Ministry of Health and Family Welfare, Government of the People's Republic of Bangladesh have been used for this study. The sample for the 2011 BDHS is nationally representative and covers the entire population residing in no institutional dwelling units in the country (NIPORT 2011). The second birth interval was considered because if second birth interval is higher then the chance of getting more children is lower. At first the gamma frailty model and Gaussian frailty model were fitted with the help of Bangladesh Demographic and Health Survey data to estimate the corresponding standard errors of the regression coefficients. Then they were compared by using Akaike Information Criterion (AIC) value.

Birth space was taken as dependent variable and defined as continuous variable. Birth spacing for 1st two births was considered. The 2nd birth after 2005 was considered for showing the current pattern of 2nd birth space, 1st birth was considered at any time.

The important factors of demographic and socio-economic had been identified as explanatory variables on the basis of the previous studies. They were mother's age of marriage, mother's education, mother's working status, wealth index, region, place of residence, religion, gender of 1st child and survival status of 1st child.

Estimation Procedures of Frailty Model: There are several procedures for the estimation of frailty model. In this study the penalized approach was used. The penalized approach is based on a modification of the Cox's partial likelihood so that both the regression coefficients and frailties are included and optimized over. Specifically the likelihood is described as a product where the first term is the partial likelihood including the frailty terms as parameters. The second term is a penalty introduced to avoid large differences between the frailties for the different groups. In practice it is fitted by first setting the frailty values to 1. Then an iterative procedure was used with a first step of optimizing the partial likelihood, treating the frailties as fixed and known parameters. In the second step the frailties were evaluated as the conditional means giving their observations, using the formulas, like the EM-algorithm. The experiment was repeated until convergence.

Assuming that the data for subject *i* , who is member of the j^{th} family, follows proportional hazards shared frailty model is given by,

$$h_{if} = h_o(t) \exp (x^p{}_i\beta + z_i w_j)$$

where x is the vector of covariate for subject *i* and β is a vector of regression coefficients, W_j is the frailty for family, *j* with independently and identically distributed from some positive scale family with density function $f(w; \theta)$, having mean 1 and variance θ. Z is matrix of indicator variables such that $z_{ij} = 1$ when subject *i* is a member of family j and 0 otherwise, and each individual belongs to only one family.
Estimation under this model is done by maximizing the penalized partial log-likelihood,
$PPL = PL(\beta, W\text{i } data) - g(w; \theta)$,
over both β and w. Here PL is the log of the usual Cox's partial likelihood function,

$$PL(\beta, w) = \sum_{i=1}^{n} \int_0^{\infty} \left[Y_i(t)(x_i\beta + z_i\ w) - \log\left(\sum_k Y_k(t) \exp(x_k\beta + z_k\ w) \right) \right] d N_i\ (t);$$

Where $Y_i(t)$ is an observed process taking the value 1 or 0 according to whether or not subject *i* is observed at time *t* and *g* is a penalty function chosen by the investigator to restrict the values of *w*. Typically, one would choose the penalty function to shrink *w* toward zero and use to control the amount of shrinkage. A penalized Cox model with penalty function $g(w;\theta) = \frac{1}{\theta}\sum [w_i - \exp(w_i)]$ is equivalent to the gamma frailty model discussed in Klein (1992) and Nielson *et al.* (1992). The w_is are distributed as the logs of independently and identically distributed gamma random variables and the tuning parameter θ is their variance. A penalized Cox model with penalty function $g(w;\theta) = \frac{1}{2\theta}\sum_i w_i^2$ is equivalent to the Gaussian random effects model.

Results and Discussion

Different choices of distributions for the unobserved covariates are possible. In this study gamma and Gaussian frailty model were chosen. The variance of frailty distribution determines the degree of heterogeneity in the study population. It is apparent from Tables 1

and 2 that for Gaussian frailty model unobserved cluster variance was more than for gamma frailty model (for gamma 0.004 and for Gaussian 0.041). Thus Gaussian frailty model was observed to be more heterogeneous than gamma frailty model.

Table 1. Parameter estimates, standard errors (SE), p-values and hazards ratio (HR) obtained from Gaussian frailty model.

Covariates	Estimate	SE	p-value	HR
Child's sex				
Female	---			
Male	-0.022	0.038	0.552	0.978
Region				
Dhaka	---			
Barisal	-0.016	0.075	0.827	0.984
Chittagong	0.266	0.065	0.000	1.302
Khulna	-0.040	0.071	0.571	0.961
Rajshahi	-0.012	0.070	0.866	0.988
Rangpur	0.034	0.072	0.640	1.034
Sylhet	0.405	0.072	0.000	1.497
Place				
Rural	---			
Urban	-0.140	0.044	0.002	0.870
Mother's Education				
No education	---			
Primary education	-0.101	0.063	0.108	0.904
Secondary education	-0.380	0.063	0.000	0.684
Higher education	-0.378	0.095	0.000	0.685
Wealth Index				
Poor	---			
Middle	0.003	0.054	0.960	1.002
Rich	0.040	0.055	0.485	1.041
Working women				
No	---			
Yes	0.112	0.056	0.057	1.121
Survival status of 1st child				
Death	---			
Alive	-1.026	0.071	0.000	0.358
Religion				
Other	---			
Islam	0.040	0.062	0.512	1.041
Mother's age of marriage				
Linear Effect	-0.113	0.044	0.010	0.893
Squared Effect	0.002	0.001	0.156	0.002
Cluster Variance	0.041		0.000	

SE$_{Standard\ Error}$, *HR*$_{Hazard\ Ratio.}$

Table 1 shows the hazard ratio of different variables. When the 2nd birth interval was compared with different divisions', it was found that the birth interval was more in Chittagong and Sylhet than in Dhaka. It was observed that in urban areas 2nd birth interval

Table 2. Parameter estimates, standard errors (SE), p-values and hazards ratio (HR) obtained from gamma frailty model.

Covariates	Estimate	SE	p-value	HR
Child's sex				
Female	---			
Male	-0.022	0.038	0.552	0.978
Region				
Dhaka	---			
Barisal	-0.016	0.075	0.827	0.984
Chittagong	0.266	0.065	0.000	1.302
Khulna	-0.040	0.071	0.571	0.961
Rajshahi	-0.012	0.070	0.866	0.988
Rangpur	0.034	0.072	0.640	1.034
Sylhet	0.405	0.072	0.000	1.497
Place				
Rural	---			
Urban	-0.140	0.044	0.002	0.870
Mother's Education				
No education	---			
Primary education	-0.101	0.063	0.108	0.904
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Wealth Index				
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Survival status of 1st child				
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Islam	0.040	0.062	0.512	1.041
Mother's age of marriage				
Linear Effect	-0.113	0.044	0.010	0.893
Squared Effect	0.002	0.001	0.156	0.002
Cluster Variance	0.004		0.000	

SE_{Standard} Error, $_{\text{HR}}$Hazard Ratio.

was 0.870 times lower than in rural areas. It was also observed that if previous child is alive then birth space has 0.358 times lower compared to survival status of 1st child is dead. From the Table 1 it is apparent that 2nd birth space was 0.684 and 0.685 times lower in secondary and higher educated mother respectively compared to non educated mother. Mother's age of marriage was found statistically significant at 1% level of significance (Table 1). It was observed from this findings the linear effect and square effect for mother's age of marriage were -0.113 < 0 and 0.002 > 0 respectively. These results indicate that with the increase of mother's age of marriage birth spacing decreases up to a particular level of age of marriage, after that birth spacing increases with the increase of mother's age of marriage. Gender of 1st child, religion and wealth index were found statistically insignificant. The results are in agreement with the work of Rabbi *et al*. 2012.

For the data for a given set of candidate model, the preferred model is that have minimum Akaike Information Criterion (AIC) value. Results presented in Table 3 show that the AIC value for Gaussian frailty model was minimum indicating that among the frailty models Gaussian fit well for the data. The AIC value for this study for gamma frailty model was higher than Gaussian frailty model but for gamma model the study gave the significant result also, so gamma frailty model was moderately well for the data.

Table 3. Comparison of frailty models.

Frailty Model	Log likelihood value	AIC	Cluster Variance
Gamma	-23370.01	46776.2	0.004
Gaussian	-23278.85	46593.7	0.041

$AIC_{\text{Akaike Information Criterion.}}$

This study evaluated the current effect of some selected demographic and socio-economic variables on subsequent birth interval using 2011 BDHS data. Among the nine explanatory variables examined mother’s education, survival status of 1st child, region and place of residence were found to have strong impact on 2nd birth interval.

Normally educated women always have longer birth interval than non-educated women but this result shows that secondary and higher educated women 2nd birth interval is shorter than non-educated women. Urban mothers’ have smaller birth interval than that of their rural counterparts which indicates that the lack of development in fertility behavior among the rural families, who are not aware of high parity progression. Chittagong and Sylhet divisions have larger 2nd birth interval compared to Dhaka which indicates that they are aware about fertility behavior. Other divisions differ insignificantly with Dhaka. The result also shows that if 1st birth was dead then 2nd birth interval increases. It might be due to cause of mothers' physical complication of pregnancy for which mother’s are bound to wait for recovery and having the next child. Thus 2nd birth interval increased.

The rate of fertility is started to decline in Bangladesh but in near future a rapid reduction of current fertility trends is needed for achieving replacement level of fertility. In this

study it was found that mother's education, survival status of 1st birth, region, place of residence and mother's age of marriage have much influence for the variation of the birth spacing. For getting replacement level of fertility these factors should be considered.

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