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Vol. 38 **No. 1** **June 2012**

CONTENTS

1 ION TRANSPORT AND ACCUMULATION OF TOTAL SUGAR, SOLUBLE PROTEIN AND AMINO ACID IN *LENS CULINARIS* MEDIK VAR. BARIMASUR-4 (LENTIL) UNDER PHOSPHORUS DEFICIENCY–Bimal Chandra Sarker and J .L. Karmoker

7 MINERAL CONTENT OF DIFFERENT BOTTLED WATER AVAILABLE IN BANGLADESH: ASSESSMENT OF THEIR COMPLIANCE WITH CURRENT REGULATIONS–Mohammad Arifur Rahman, Md. Abdus Salam, Abdus Salam, Madhusudan Roy, Nargis Jahan Ara and A. M. Shafiqul Alam

17 EFFECT OF SUBSTITUENTS ON REACTIVITY AND REACTION MECHANISM OBSERVED IN S_N REACTION OF SOME ORGANOPHOSPHORUS COMPOUNDS: BASED ON PHYSICAL ORGANIC METHODOLOGIES–Bilkis Jahan Lumbiny

29 GROUNDWATER IRRIGATION AND CROP ECONOMY IN THE LOWER GANGETIC PLAIN AT MATBARER CHAR, MADARIPUR, SOUTH-CENTRAL BANGLADESH–M. Aminul Haque, M. Saiful Islam and Anwar Zahid

41 FARMERS' PERCEPTION ON WATER RELATED ISSUES: A STUDY OF BHERAMARA *UPAZILA*, KUSHTIA–Md. Humayun Kabir and Md. Faruk Hossain

53 A PRELIMINARY CHECKLIST OF THE ANGIOSPERMIC FLORA OF DAULATPUR UPAZILA IN KUSHTIA DISTRICT, BANGLADESH–Md. Moniruzzaman, Md. Abul Hassan, Md. Miganur Rahman, Sheheley Layla and Md. Rafiqul Islam

67 EFFECT OF INDUSTRIAL SLUDGE ON CONCENTRATION AND DISTRIBUTION PATTERN OF LEAD, CADMIUM AND NICKEL IN ROOT, STEM AND LEAF OF RED AMARANTH (*AMARANTHUS GANGETICUS* L.) IN SILTY CLAY LOAM SOIL – M.A. Rauf, P. Parkpian and M.T.I. Miah

75 HOST PREFERENCE OF PUMPKIN BEETLE TO CUCURBITS UNDER FIELD CONDITIONS–M. M. H. Khan

83 HAEMOLYTIC EFFECTS OF *SAMANEA SAMAN* (JACQ.), *TECTONA GRANDIS* (L.) AND *DIPTEROCARPUS TURBINATUS* (GAERTN) SEED EXTRACTS ON SINGHI FISH, *HETEROPNEUSTES FOSSILIS* (BLOCH) –Munira Nasiruddin, Mohammad Ali Azadi and Shabnam Chowdhury

93 GASTROINTESTINAL HELMINTHS IN PIGEON *COLUMBA LIVIA* (GMELIN, 1789) –A. Begum and S. Sehrin

99 ECOLOGY AND BREEDING BIOLOGY OF THE POND HERON, *ARDEOLA GRAYII* (SYKES, 1832) AND ITS CONSERVATION ASPECTS–M. Firoj Jaman, Md. Nazmul Hoque, Noor Jahan Sarker and Md. Saidur Rahman

111 SPECIES COMPOSITION OF LIMNETIC ZOOPLANKTON FROM THE SOUTHERN COASTAL AREAS (MATHBARIA AND BAKERGANJ) IN BANGLADESH–Pronob K. Mozumder, Samsun Nahar, M. Niamul Naser, Munirul Alam, Anwar Huq, R. Bradley Sack and Rita R. Colwell

Short Communication

119 AIRBORNE FUNGAL AND PTERIDOPHYTIC SPORES IN CHITTAGONG UNIVERSITY CAMPUS, CHITTAGONG–Mohammad Sohrab Hossain and M. K. Pasha

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ION TRANSPORT AND ACCUMULATION OF TOTAL SUGAR, SOLUBLE PROTEIN AND AMINO ACID IN *LENS CULINARIS* MEDIK VAR. BARIMASUR-4 (LENTIL) UNDER PHOSPHORUS DEFICIENCY

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Abstract

Some ionic and biochemical responses of lentil (*Lens culinaris* Medik.) under phosphorus deficient condition were studied. Seedlings were raised in solution culture containing phosphorus and without phosphorus (P-deficiency). Phosphorus deficiency decreased Ca^{2+} and Fe^{2+} accumulation and slightly decreased Mg^{2+} accumulation in root and shoot of lentil. Phosphorus deficiency caused a decrease in accumulation of total sugars in leaf and stem but increased in root. Accumulation of total soluble proteins was depressed in lentil following P-deficiency treatment. Phosphorus deficiency increased total amino acid contents in both root and shoot.

Key words: Phosphorus deficiency, Sugar, Amino acid, Protein, Lentil , Ca^{2+}, Mg^{2+} , Fe^{2+}, SO_4^{2-}.

Introduction

Phosphorus (P) is one of the major elements required by all living species for growth and development (Hammond *et al.* 2004). Phosphate is intimately involved with cellular bioenergetics and metabolic regulation. Phosphorylated compounds like ATP are involved in the transfer and storage of energy within plant. Despite its ubiquitous importance to plant metabolism, Pi is one of the least available nutrients in many natural ecosystem (Barber 1980).

Availability of phosphorus to plant roots is limited both in acidic and alkaline soils, mainly due to formation of sparingly soluble phosphate compounds with Al and Fe in acidic and Ca in alkaline soil (Marschner 1995). The highly mobile P in sodic soils is thought to be associated with sodium. Thus, soils become deficient in phosphorus. Low phosphorus availability strongly limits plant productivity.

The relationship between P, Ca^{2+}, Mg^{2+} and Fe^{2+} concentration in plants grown in P-deficient soil is noteworthy. An optimum P nutrition was found to increase the leaf concentration of both Ca^{2+} and Mg^{2+} in wheat seedlings (Reinbott and Blevins 1991). Phosphorus deficiency in soil markedly reduced the grain Mg^{2+} content in rice (Saleque *et al.* 2001). When phosphorus supply in the growth medium was limited, accumulation of Fe^{2+} increased in the tolerant cultivar of rice but decreased in the sensitive cultivar (Li *et al.* 2004).

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As major nutrient ions, S and P are intimately involved in plant metabolism and growth having various interactions with S- and P- dependent metabolic processes. It would, therefore, be expected that in plants deprived of optimal P supply, assimilation of S may be altered significantly.

Tissue carbohydrate was found to increase in *Gracilaria cornopifolia* after 8-day of phosphorus deficiency (Lee 2004). Rufty *et al.* (1993) reported that phosphorus deficiency increased accumulation of asparagines in the root and stem of nitrate-fed soybean plants. Usuda (1995) reported that in maize, soluble and insoluble protein contents decreased as compared to that of control plants in low phosphorus condition.

The information on the effect of phosphorus deficiency on transport of some ion and biochemical changes may help to understand the mechanism of retarding growth and development of plants. Therefore, the present research was undertaken to study on transport of Ca^{2+}, Mg^{2+} , Fe^{2+}, SO_4^{2-} and accumulation of total sugar, amino acid and soluble protein in *Lens culinaris* Medik. var. Barimasur-4 (Lentil) under phosphorus deficiency.

Materials and Methods

Lens culinaris Medik (2n=14) var. Barimasur-4 was used as plant material. The seeds were surface sterilized by soaking the seeds in 4% sodium hypochlorite solution for one minute, followed by washing 7 to 8 times in tap water running and three times in distilled water.

Plants were grown in solution culture for ion transport and biochemical changes study. Phosphorus–containing solution (+P) was used as control and phosphorus free solution was used as treatment (-P). Plants were subjected to phosphorus deficiency treatment for 7, 14, 21 and 24 days prior to collection of samples and three replicates were taken for each treatment. Iron (Fe^{2+}), Ca^{2+}, Mg^{2+} were extracted in a mixture of nitric acid and perchloric acid (HCl_3O_4) at 4: 1 ratio. The amount of Fe^{2+} Ca^{2+}, Mg^{2+} in the extract was measured by an Atomic Absorption Spectrophotometer following ASI method. Sulphate (SO_4^{2-}) in plant material was determined through turbidimetric method as described by Hunt (1980).Total sugar was determined by phenol-H_2SO_4 method of Dubois *et al.* (1956). Soluble protein estimation was done by Lowry *et al.* (1951) and total amino acid was measured by the method of Lee and Takahasi (1966)

Results and Discussion

The Effect of phosphorus deficiency on transport of divalent cation: In the root of lentil, Ca^{2+} accumulation was decreased by 19.0 to 45% during the treatment period (Fig.1a). Similarly, Ca^{2+} content decreased from 6.7 to 42.7% in shoot (Fig.1b). A decrease in Ca^{2+} level may have impaired the cell membrane permeability of the root which in turn may alter its uptake properties of P-deficient triticale (Quartin *et al*. 2001).

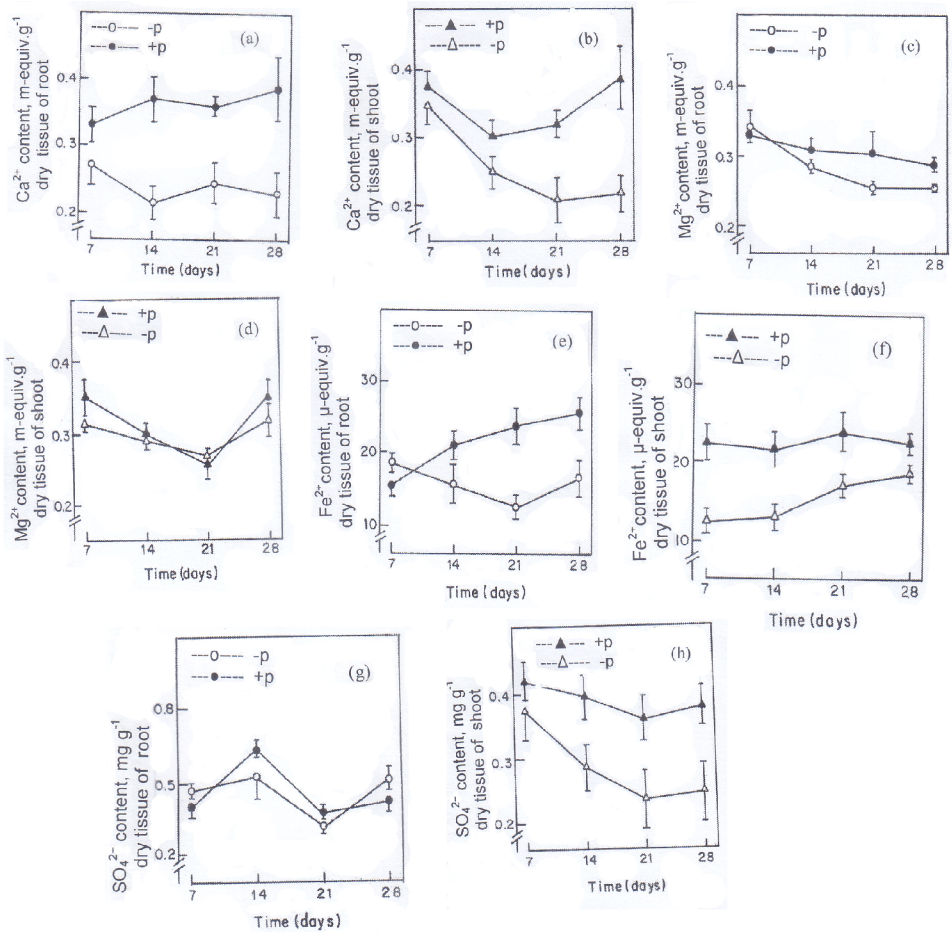

Fig. 1. The effect of phosphorus deficiency on accumulation of Ca^{2+} in (a) root and (b) shoot; Mg^{2+} in (c) root and (d) shoot; Fe^{2+} in (e) root and (f) shoot; SO_4^{2-} in (g) root and (h) shoot of lentil plants grown in solution culture. Solid symbols..... + P and Open symbols ... - P. O root, Δ shoot. Each value is the mean of three replicates and vertical bar represents ± standard error of mean.

Phosphorus deficiency caused A 5.9 and 12.0% decrease in accumulation of Mg^{2+} in root at 14 and 28-day of phosphorus deficiency treatment (Fig. 1c) and slightly decreased in shoot of lentil (Fig. 1d). Phosphorus deficiency-induced decrease in Mg^{2+} accumulation was found in the leaves of triticale (Quartin *et al.* 2001), and tolerant and sensitive cultivars of rice (Li *et al.* 2004).

Accumulation of Fe^{2+} in root decreased by 27.7 to 35.0% during the treatment period except an initial increase of 10% at the 7-day of treatment (Fig.1e). Similarly, Fe^{2+} accumulation in shoot was decreased by 44.5% at the 7-day of treatment and this

inhibitory effect was continued up to 28-day of phosphorus deficiency treatment (Fig.1f). Similar results were found due to phosphorus-stress in Fe^{2+} accumulation in phosphorus sensitive rice cultivars (Li *et al.* 2004).

The Effect of phosphorus deficiency on transport of divalent anion: Phosphorus deficiency had no effect in the accumulation of SO_4^{2-} in root (Fig. 1g) but decreased in shoot (Fig.1h). Jayalalitha and Naryanan (1996) also reported similar result in cotton.

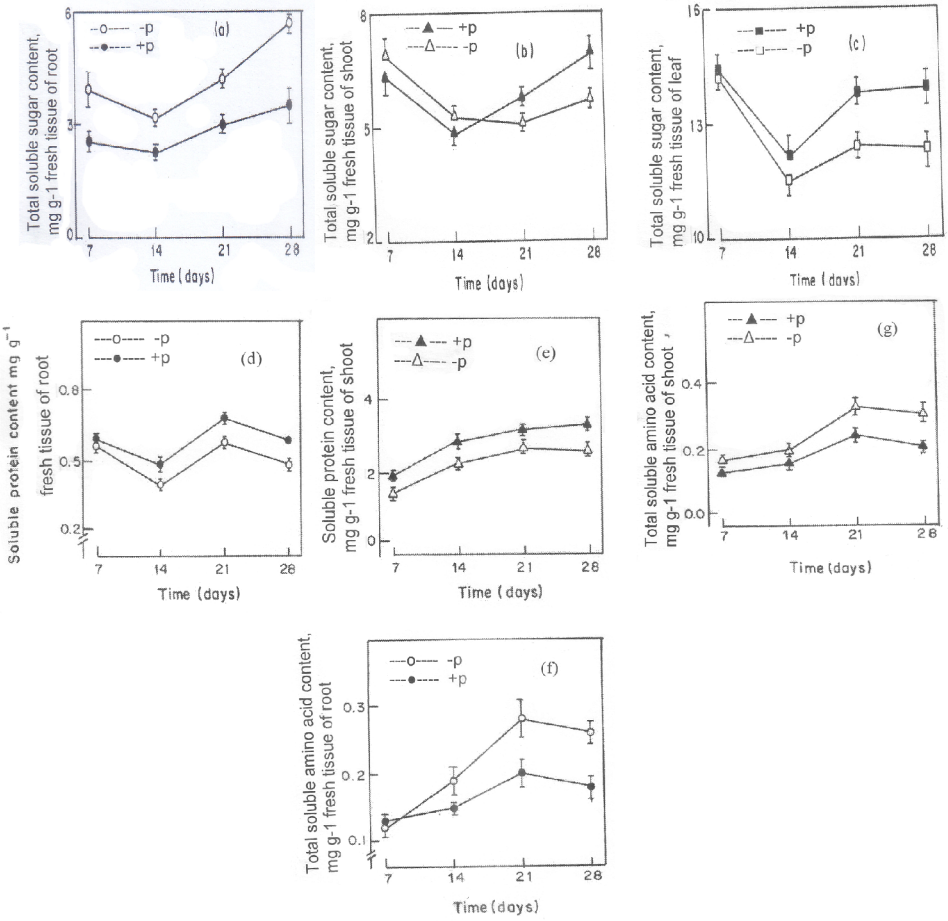

Fig. 2. The effect of phosphorus deficiency on accumulation of total soluble sugar in (a) root , (b) shoot and (c) leaf ; protein in (d) root and (e) shoot , amino acid in (f) root and (g) shoot of lentil plants at different developmental stages.

The Effect of phosphorus deficiency on transport of total sugar: Phosphorus deficiency increased accumulation of total sugar from 55.3 to 70.1% in root of lentil during the treatment period (Fig. 2a). Phosphorus deficiency increased total sugar content in the stem by 9.35 and 7.07% at 7 and 14-day of treatment respectively followed by an

inhibition of that by 9.9 and 17.5% at 21 and 28-day of treatment respectively (Fig. 2b). On the other hand, total sugar accumulation in leaf was decreased by 5.5 to 11.0% from 14 to 28-day during phosphorus deficiency treatment (Fig. 2c).In lentil, phosphorus deficiency increased total sugar accumulation in root whereas it decreased that in leaves (Fig. 2). The increase in total sugar in root might also be due to translocation of that from the leaves to root. This result was concomitant with Li *et al*. (2001) who found that soluble sugar content increased under phosphorus deficient conditions in root of rice due to its translocation from the shoot to root.

The Effect of phosphorus deficiency on transport of soluble protein and total amino acid: Phosphorus deficiency inhibited the accumulation of soluble protein in root by 20.5 to 14.0% from 14 to 28-day of treatment period (Fig. 2d). Phosphorus deficiency caused a decrease in soluble protein content of shoot by 23 to 18.3% during the treatment period (Fig. 2e).Similar inhibition of soluble protein content under phosphorus deficiency stress was reported in Larch (Guo *et al.* 2005) and Maize (Usuda and Shimogwara 1992).

Accumulation of total soluble amino acids increased from 26.4 to 44.6% in root of lentil from 14 to 28-day of treatment except an initial decrease at 7-day of phosphorus deficiency treatment (Fig. 2f) and the same increased from 21 to 47.6% in shoot (Fig.2g). Results obtained are similar to that of Singh and Pandey (2003) and Rabe and Lovatt. (1984, 1986). An increase in amino acid with concomitant decrease in protein synthesis may be due to inhibition of amino acid incorporation into protein, because phosphorus is an important structural component in DNA and RNA needed for protein synthesis.

Phosphorus deficiency-induced decrease protein synthesis as well as decrease carbohydrate synthesis might lead to decrease energy supply in plant which may affect transport of ions. The decrease of transport of ions may lead to decrease in growth of lentil plants.

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MINERAL CONTENT OF DIFFERENT BOTTLED WATER AVAILABLE IN BANGLADESH: ASSESSMENT OF THEIR COMPLIANCE WITH CURRENT REGULATIONS

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Abstract

Nine bottled water brands collected from retail shops and food shops beside the University of Dhaka were analyzed for their major and trace element constituents to ascertain their suitability for human consumption. The results obtained were compared with parametric values (PVs) set by European Community Council Directive 98/83/EC and the guideline values (GVs) recommended by WHO. It was found that the majority of the brands tested were low in mineral contents. Moreover, lower values of hardness, TDS and conductivity than the prescribed limits of WHO showed that water was deficient in essential minerals. Minerals like magnesium, potassium, calcium were present in some cases in such a low concentration that water seemed to be as good as distilled water**.** In case of heavy metals, only lead and cadmium were found to be below the detection limit (0.001 mg/L) which indicates bottled water brands are better for drinking.

Key words: Mineral, Guide line, Bottled water, WHO.

Introduction

Freshwater is scarce and resources are unevenly distributed over the world, with much of the water located far from human populations. The total amount of usable freshwater supply is around 4×106 km^3, which is only 0.2% of all the water on Earth. It is estimated that three billion people will be in the "water scarcity" category (having <1000 m^3 of renewable water per capita per year) by 2025 (UNEP 2002).

In Bangladesh, groundwater abstracted from drilled wells is the sources utilized by the bottled water industry. Despite the continued expansion of the bottled water industry and positive trend on bottled water consumption in Bangladesh, reports on bottled water analysis and their mineral contents are scattered and not well documented. Mineral water in Western countries is obtained from natural springs and is, generally, named after those springs. Most of the bottled water passed off as mineral water in Bangladesh, however, is filtered, boiled or purified by other means such as chlorination, deionization and reverse osmosis. A better description of bottled drinking water sold in Bangladesh therefore, would be ''purified bottled water''. Given the extensive consumption of bottled water, the question naturally arises of the long-term impact of water of various chemical compositions on human health.

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Elements such as magnesium and calcium have been linked to the reduced frequency of sudden death and Osteoporosis respectively and both may exert protective effects against gastric cancer (Garzon and Eisenberg 1998). WHO advocated the consumption of brands that are high in magnesium and calcium and low in sodium. However, individuals with stones in the upper urinary tract are ill-advised to consume bottled drinking water with high calcium content (Mayne and Edwards 1990). Nitrate is a common contaminant in ground water and has been implicated in gastric cancer mortality and other disorders (Schuber *et al.* 1999 and Lauwerys 1979). Through conversion to nitrite, nitrate is the causative agent of Methaemoglobinemia in infants. Cadmium may have a half-life in bones of 38 years and has carcinogenic properties (Yang *et al.* 1998). Lead builds up in the body over many years and can cause damage to the brain, red blood cells and kidneys. Children and pregnant women are open to greatest risks. Amounts of lead that won't hurt adults can slow down normal mental and physical development of growing bodies (Needleman 1993). Lead and cadmium are unusual among drinking water contaminants in that they seldom occur naturally in water supplies (Krishnamurti and Viswanathan 1991). Copper is an essential element in human nutrition but it may reach high levels in tap water through contact with copper fittings. It has been found that short-period exposure to copper may lead to stomach and intestinal distress, liver and kidney damage and anemia. Persons with Wilson disease may be more sensitive than others to the effects of copper contamination (WHO 1996). Zinc is also known to be a beneficial element when present in low amounts, but may produce various health hazards ranging from anemia to gastric and kidney disorders at higher levels of occurrence.

The main purpose of this study is to investigate the physical and chemical characteristics of some of the most widely distributed domestic brands of bottled water in Dhaka market. Additionally, this paper aims to assess the compliance of Bangladesh bottled water brands to several standards around the world including European Community Council Directive 98/83/EC (EC 1998) and World Health Organization (WHO) guideline values (WHO 2006).

Materials and Methods

Sampling: Commercially-available nine brands of domestically-produced bottled water were purchased randomly from nearby shops of Dhaka University area in November 2009. The samples were collected from different nearby retail shops of Dhaka university such as (a) Chankarpool (b) In front of Dhaka Medical College **(**c) New Market area on the 14th October 2009. As indicated on their labels, all the bottled water had a shelf life of one year and were certified by Bangladesh Standard Testing Institute (BSTI). To keep the brand names anonymous, the waters were given a numerical code from 1 to 9 and this convention was used throughout the study. All bottled water were in polyethylene terephthalate (PET) containers with plastic screw caps. The holding capacities of bottled water containers ranged from 0.5 to 2.0 L. After the collection of samples, they were

brought to the laboratory, where they were stored in the refrigeration at 4°C and in the dark until analysis. Prior to analysis, manufacturer seals were broken in the laboratory and then two 50 mL aliquots were aseptically removed from each bottle for anion and cation analyses. These samples were placed in sterile high density polyethene (HDPE) containers, which were carefully rinsed several times with the sample water. Water samples were not acidified or filtered, but they were analyzed in the normal condition. Collected bottled water samples did not contain any particulates. Manufacturer labels on the bottles were used as a source of basic information on a particular water sample.

Physical and chemical analysis of samples: Color, taste, pH, specific conductance, total dissolved solids (TDS), total hardness, chlorides, nitrates, sulphates, phosphates, copper, iron, magnesium, calcium, manganese, lead, zinc, cadmium, chromium and nickel were analyzed. All the samples were taken in different 100 mL transparent beaker, then the colors were observed, pH, and specific conductance were measured and the taste was taken. TDS were calculated by the following formula:

$$\% \text{ of dissolved solids} = K \times 0.062, \text{ Where K is the specific conductance.}$$

Methods of Analysis: Total hardness of the samples was determined by complexometrically where Na_2EDTA was used as complexing agent and Erichrome black T was used as indicator. Chlorides content in a sample was determined by mercuric nitrate method (Vogel 2002), it is a titrimetric method, in which a mixed indicator is used. The nitrate, sulphate and phosphates were determined by using UV spectrophotometer (UV-160A, Shimadju, Japan) at 410, 380 and 358 nm wavelength respectively (Horwitz 2001). For this technique calibration curve was prepared using standard solutions. Copper, iron, magnesium, calcium, manganese, lead, zinc, cadmium, chromium and nickel were determined by Atomic Absorption Spectrometer (A Analyst 800, Perkin Elmer, USA) (Vogel 2002). Before analysis the samples were concentrated 10 times by evaporation and the reading was taken with AAS.

Results and Discussion

According to WHO guidelines, the maximum desirable limit of pH is 7.0 – 8.5. EC established pH limits from 6.5 – 9.5. Water with pH lower than 4.0 have a sour taste and above 8.5 an alkaline bitter taste (EC 1998). High pH induces the formation of tri-halomethanes, which are toxic. pH below 6.5 starts corrosion in pipes, thereby releasing toxic metals such as Zn, Pb, Cd and Cu, etc. Out of total nine samples analyzed, eight had pH values between 7 and 7.5. One sample was just slightly acidic with pH values of 6.98, thus all the commercially available drinking water samples analyzed had pH within the prescribed limits recommended by WHO/EC. All the samples analyzed had conductance well within the prescribed limit of 2500µS/cm by EC. The conductance is a reflective of dissolved mineral solids (Trivedi and Goel 1986). The presence of dissolved solids in water does affect its taste. As per the ISI (Indian Standard Specification for

Drinking Water 1983) rating about palatability of drinking water, in relation to its TDS, water may be considered as TDS< 300 mg/L –excellent; 300–600 mg/L – good; 600–900mg/L–fair; 900–1200mg/L–poor and >1200mg/L–unacceptable. All the samples fall in the category of 'excellent'. This is the general impression of all Bangladeshi people that (genuine) bottled water has a very good taste. But water with extremely low concentration of TDS may also be unacceptable and may be flat and insipid in taste and lacking minerals. It was observed that all the samples had TDS values lower than the prescribed limit of 500 mg/L. Both conductance and TDS point out that demineralization had been carried out to more than the desirable limit. The results are presented in the Tables 1- 3. The recommended guideline values for drinking water are presented in Table 4.

Table 1. Analysis results of physical parameters of nine Bangladeshi bottled water.

Code	Name	pH	Conductance (µS/cm)	TDS (mg/L)	Total hardness(mg/L)
1	Mum	7.19±0.05	300±2.0	186±2.5	30.5±0.5
2	Jibon	7.31±0.04	100±0.8	62±1.0	125.6±1.2
3	Libra	7.06±0.05	200±1.3	124±1.5	96.2±0.8
4	Santi	7.55±0.06	100±0.5	62±1.0	11.5±0.5
5	Pran	7.24±0.06	0±0.05	0±0.5	102.6±1.2
6	Fresh	7.73±0.08	100±0.6	62±1.1	160.5±1.6
7	Spa	6.98±.02	400±3.2	248±2.5	120.6±1.0
8	Nayagra	7.02±.03	200±1.3	124±1.2	112.0±0.9
9	Acme	7.50±.05	100±0.6	62±0.5	56.2±0.3

n=3 (no. of analysis)

The limits of WHO for hardness for drinking water are between 100 – 500mg/L. Hardness of water which is due to the presence of calcium and magnesium salts in water, does contribute towards total calcium and magnesium human dietary needs, which has a beneficial effect on bone structure. Studies on water hardness and cardiovascular disease mortality have suggested a lower incidence of heart disease in communities drinking of hard water. Extremely hard water (hardness 4500 mg/L) is also unfit for consumption because the constituent minerals such as calcium can deposit inside the body if present in high amounts leading to kidney or gall bladder stones. Consumption of very soft water (less than 50 mg/L) lacking in essential minerals like calcium, magnesium and other trace minerals is also harmful for the body because water low in mineral content would rob off the body's minerals. People drinking such treated water excrete huge amounts of calcium, magnesium and other trace minerals in urine (Consumer Research 1991). The more the mineral loss, the greater the risk for osteoporosis, osteoarthritis, hypothyroidism, coronary artery disease, high blood pressure and a long list of degenerative diseases generally associated with premature aging. Also, cooking food in soft water pulls the minerals out of them and lowers their natural value. All the nine

samples presently analyzed had hardness higher than the lower limit of 100 mg/L. Most of the samples had calcium and magnesium higher than the respective lower limit of 75 and 30 mg/L as prescribed by the WHO. Even some samples had zero calcium content. Thus 100% of the samples were of the lower limit of the WHO for calcium and magnesium.

Chloride content of the nine samples was in the range from 4.08 – 71.57 mg/L whereas the EC prescribed a limit of 250 mg/L in drinking water. Thus, all the bottled water samples were safe for drinking with respect to chloride content. Seven out of nine samples analyzed had nitrate content below the WHO recommended limit of 50 mg/L and hence do not pose health concern. And two out of nine had extremely low values than the WHO limit for nitrate. Nitrate levels above the WHO Maximum Contaminant Level of 45 mg/L NO_3^- may cause methemoglobinemia in infants. Sulfate in drinking water currently has a maximum contaminant level (SMCL) of 250 mg/L, based on aesthetic effects (i.e., taste and odor) (Indian Standard Specification for Drinking Water 1983). This regulation is not a federally enforceable standard, but is provided as a guideline for states and public water systems. EPA estimates that about 3% of the public drinking water systems may have sulfate levels of 250 mg/L or greater. Two out of nine samples analyzed have sulphate above the prescribed limit. Phosphates obtained in the samples are from 4.33 to 32.85 mg/L. Though there is no standard value of phosphate in drinking water but when the concentration of phosphates rises above 100 mg/L the coagulation processes in drinking water treatment plants may be adversely affected.

Table 2. Analysis results of major anionic constituents of nine Bangladeshi bottled water.

Code	Name	Cl^-(mg/L)	NO_3^- (mg/L)	SO_4^{2-} (mg/L)	PO_4^{3-}mg/L
1	Mum	34.08±0.80	72.93±0.80	17.99±1.20	9.69±0.30
2	Jibon	10.22±0.30	18.03±0.60	5.56±0.20	11.33±0.20
3	Libra	10.22±0.50	110.66±0.90	0.14±0.02	10.32±0.10
4	Santi	8.860±0.40	20.74±0.40	12.97±0.10	11.74±0.40
5	Pran	4.089±0.60	8.322±0.20	9.45±0.30	5.87±0.20
6	Fresh	25.21±0.50	12.84±0.40	20.47±0.20	7.26±0.20
7	Spa	71.56±0.60	21.87±0.30	72.37±2.10	32.85±0.50
8	Nayagra	17.04±0.20	17.50±0.20	49.57±0.50	4.33±0.20
9	Acme	4.08±0.20	14.34±0.50	6.28±0.20	6.04±0.20

n=3 (no. of analysis)

Copper is an essential element for living organisms, including humans, and-in small amounts-necessary in our diet to ensure good health. However, too much copper can cause adverse health effects, including vomiting, diarrhoea, stomach cramps and nausea. It has also been associated with liver damage and kidney disease. The human body has a natural mechanism for maintaining the proper level of copper in it. However, children under one year old have not yet developed this mechanism and, as a result, are more

Table 3. Concentrations of trace elements of nine Bangladeshi bottled water.

	Name of the water brand								
Metal (mg/L)	**Mum**	**Jibon**	**Libra**	**Santi**	**Pran**	**Fresh**	**Spa**	**Nayagra**	**Acme**
Mg	0.82± 0.02	1.91 ± 0.05	2.34 ± 0.80	0.19 ± 0.02	0.06 ±0.01	0.08 ± 0.01	7.62± 0.50	3.95± 0.60	0.05± 0.01
Ca	14.76± 0.30	2.47± 0.20	3.70± 0.30	2.44±0.40	0.34± 0.05	0.13± 0.01	14.21± 0.50	5.52± 0.30	0.10±0.01
Cu	BDL	BDL	BDL	BDL	BDL	BDL	0.003±0.001	0.002±0.001	0.002±0.001
Fe	0.10±.002	0.23±.02	0.52±.03	0.67±.03	0.70±.04	0.91±.05	1.13±0.70	1.25±0.80	1.48±0.60
Mn	BDL	BDL	0.01± 0.001	0.01± 0.002	BDL	BDL	BDL	BDL	BDL
Pb	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Zn	0.01±0.001	0.01 ±0.002	0.01±0.001	BDL	0.005±0.001	0.002±0.001	0.005±0.001	0.002±0.001	0.005±0.001
Cd	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Cr	BDL	0.002±0.001	0.003±0.001	0.003±0.001	BDL	BDL	0.002±0.001	0.002±0.001	0.003±0.001
Ni	0.003±0.001	0.003±0.001	0.002±0.001	0.003±0.001	0.002±0.001	0.005±0.002	0.005±0.002	0.005±0.001	0.005±0.002

n=3 (no. of analysis); BDL: Below Detection Limit

vulnerable to the toxic effects of copper. People with Wilson's disease also have a problem with maintaining the proper balance and should also exercise particular care in limiting exposure to copper. Water brands analyzed contain 0.002 to 0.003 mg/L and most of the brand has below the detection limit of the instrument (0.001 mg/L) for Flame Atomic Absorption Spectrophotometer). These results are presented in Table 3. When the level of iron in water exceeds 0.3 mg/L limit, we experience red, brown, or yellow staining of laundry, glassware, dishes and household fixtures such as bathtubs and sinks. The water may also have a metallic taste and an offensive odor. Water system piping and fixtures can also become restricted or clogged. Water analyzed here contains 0.10 to 1.48 mg/L. EC has a guideline about 2.0 mg/L iron may contain in drinking water. Here eight out of nine did not follow the guideline value. So the water brands are not good for health with respect to iron except MUM. All the samples analyzed contain manganese which varied from 0.001 to 0.002 mg/L below the upper limit 0.05 mg/L prescribed by WHO and EC and few contains below the detection limit of the instrument. All the samples are good for drinking with respect to manganese. All the brands analyzed had lead below the detection limit of the instrument and also below the maximum guideline value of WHO and EC. All the brands are good under the guideline of EC and WHO with respect to lead.

Acute toxicity arises from the ingestion of excessive amounts of zinc salts, either accidentally or deliberately as an emetic or dietary supplement. Vomiting usually occurs after the consumption of more than 500 mg of zinc sulfate. Mass poisoning has been reported following the drinking of acidic beverages kept in galvanized containers; fever, nausea, vomiting and stomach cramps occurred 3–12 h after ingestion. Food poisoning attributable to the use of galvanized zinc containers in food preparation has also been reported; symptoms occurred within 24 h and included nausea and vomiting, sometimes accompanied by bleeding and abdominal cramps. Impairment of the copper status of volunteers by dietary intake of 18.5 mg of zinc per day has been reported. Gastric erosion is another reported complication of a daily dosage of 440 mg of zinc sulfate. Daily supplements of 80–150 mg of zinc caused a decline in high-density lipoprotein cholesterol levels in serum after several weeks. The brands analyzed contain 0.002 to 0.01 mg/L Zinc. The sample contains a little Zinc which is not so bad for health. All the samples analyzed contain cadmium below the detection limit of the instrument that means it is very low in concentration. The guideline value of WHO and EC are 3.0 and 5.0 mg/L respectively (Table 4). All the samples are good for health with respect to cadmium.

Chromium has the potential to cause the following effects from a lifetime exposure at levels above the MCL: damage to liver, kidney circulatory and nerve tissues; skin irritation. The daily chromium requirement for adults is estimated to be 0.5–2 µg of absorbable chromium (III) If a fractional absorption value of 25% for “biologically incorporated” chromium (III) in food is assumed, this is provided by a daily dietary

intake of 2–8 µg of chromium (III) equivalent to 0.03–0.13 µg of chromium (III) per kg of body weight per day for a 60-kg adult. Ingestion of (1–5)g of "chromate" (not further specified) results in severe acute effects such as gastrointestinal disorders, haemorrhagic diathesis and convulsions. Death may occur following cardiovascular shock. In some

Table 4. Drinking water standards recommended by WHO and EC.

Character	**pH**	**Conductance** (µS/cm)	**TDS**	**Total hardness** (mg/L)	**Cl^-** (mg/L)	**SO_4^{2-}** (mg/L)	**NO_3^-** (mg/L)	**PO_4^{3-}** (mg/L)
WHO limit	7-9.2	-	500-1500	300	200-600	200	50	-
EC limit	6.5-9.5	2500	0-500	-	250	-	50	-

Character	**Ca** mg/L	**Mg** mg/L	**Mn** mg/L	**Fe** mg/L	**Pb** mg/L	**Cu** mg/L	**Zn** mg/L	**Cd** mg/L	**Cr** mg/L	**Ni** mg/L
WHO limit	75-200	30-150	0.4	-	0.01	2.0	3.0	0.003	0.05	0.02
EC limit	0-200	-	0.5	2.0	0.01	2.0	0.0-5.0	0.005	0.05	0.02

occupational studies, increased incidences of genotoxic effects such as chromosomal aberrations and sister chromatid exchanges have been found in workers exposed to chromium (VI) compounds (Garzon and Eisenberg 1998). In epidemiological studies, an association has been found between occupational exposure to chromium (VI) compounds and mortality due to lung cancer. All the brands analyzed here contain BDL (Below detection level) to 0.003 mg/L chromium which is below the guideline value of WHO and EC. Therefore, the brands are free from toxic effects of chromium in drinking. Nickel is an important metal that occurs widely in the environment and is found naturally in odd and drinking water. It is known to be toxic at high intakes but toxicity in humans, other than those exposed in particular industrial circumstances, is very unusual. The World Health Organization considered nickel in their drinking water guidelines and developed a guideline value of 0.02 mg/L based on effects in laboratory animals. The EC has established a lifetime drinking water health advisory level of 0.02 mg/L based on the same studies as those used to develop WHO guidelines. The brands analyzed contain nickel below the WHO and EC limit. Under the guideline of both WHO and EC all the brands are safe for drinking. The analytical quality control included daily analysis of standards and triplicate analysis of samples and blanks. The accuracy and precision of the analytical technique was evaluated by analyzing a certified standard reference material, CWW-TM-B, Certified Waste Water Trace Metals Solution (B) (High-Purity Standards, Charleston, SC, USA). The relative error (RE, %) is less than ±5% for all analyzed elements.

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EFFECT OF SUBSTITUENTS ON REACTIVITY AND REACTION MECHANISM OBSERVED IN S_N REACTION OF SOME ORGANOPHOSPHORUS COMPOUNDS: BASED ON PHYSICAL ORGANIC METHODOLOGIES

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Abstract

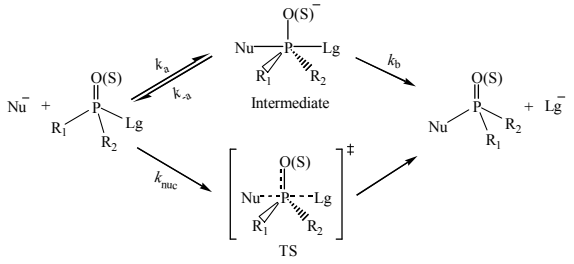
The aminolyses of tetracoordinated organophosphorus compounds were investigated by varying substituents around phosphorus center or in nucleophile. The reactivity is expressed in terms of second-order rate constant, k_2 and measured conductometrically. Physical organic chemistry tools; Hammett (ρ), Brönsted (β) LFER, CICs and heavy atom KIE have been used in quest for the mechanistic information. The pyridinolysis of *O*-aryl phenyl phosphonochloridothioates [PhP(=S)(OPh-Y)Cl, 1, and *O,O*-diphenyl Z-*S*-phenyl phosphorothiolates [$(PhO)_2P(=O)(S\text{-}Ph\text{-}Z)$], 2, in acetonitrile at 35.0 ^oC, were observed by varying substituents around phosphorus centre (Y in 1, Z in 2) or in nucleophile (X) and extended to pyridinolysis of 4-Chlorophenyl phenyl Chlorophosphate [4–ClPhOP(=O)(OPh)Cl], 3, in acetonitrile at 5.0 ^oC (present study). The variation in X and Y in system 1 shows LFER with negative value of the Hammett coefficients, ρ_X, −(4.35 ~ 4.75), CICs, ρ_{XY} = −0.46, which is in favour of concerted S_N2 mechanism. The LFER plots obtained for 2 with the variation in X, with negative value of the ρ_X, −(4.43 ~ 4.76) indicating same mechanism as the system 1, substituent (Z) variations (log k_2 *vs*. Z) are biphasic concave downwards with breaks at Z = H, ρ_{XZ} = −0.70 for Z = electron donating group, ρ_{XZ} = + 0.76 for Z = electron withdrawing group interpreting as the change in mechanism at Z = H from concerted to stepwise. In the light of the above reported results the LFER obtained for 3 with negative value of the ρ_X , −5.66 can be interpreted as S_N2 process, with greater extent of bond formation in TS than that of 1, 2.

Key Words: Organophosphorus compounds, pyridinolysis, reactivity, Hammett (ρ), CICs, KIE, TS structure

Introduction

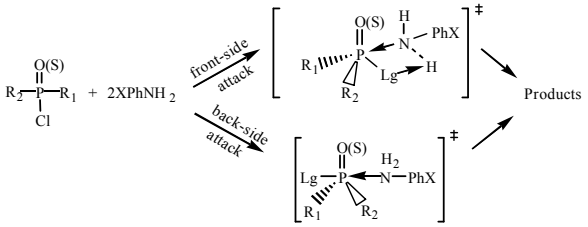
Phosphoryl transfer and related reactions are ubiquitous in environment as well as biological chemistry with many opportunities for research or applications development especially due to its relevance to biological chemistry as well as its usefulness as agricultural chemicals like pesticides, oil and gasoline additives, etc. (Lumbiny 2009). Despite many researches that have been carried out there are still many facets of the aminolyses of organophosphorus and their thio analogues compounds that are not well understood. A series of kinetics and mechanism of nucleophilic substitution reactions on phosphoryl group (P=O) and thiophosphoryl group (P=S) have been reported earlier (Guha *et al*. 2000, Lee *et al*. 2000, Hoque *et al.* 2007, Lumbiny *et al*. 2008, Adhikary *et*

al. 2011). The above studies are relied on Physical organic chemistry methods. Hammett and Brönsted linear free energy relationship (LFER), cross interaction constants (CICs), heavy atom kinetic isotope effects (KIE), activation enthalpy and activation entropy have provided unambiguous evidence for the interpretation of the mechanistic pathway. It has been reported that nucleophilic substitution at a (thio)phosphoryl (P=S/O) center generally proceeds either through stepwise mechanism with a trigonal bipyramidal pentacoordinated (TBP-5C) intermediate (upper route) or an S_N2 mechanism with TBP-5C transition state (TS) (lower route), Scheme 1. (Skoog and Jencks 1984, Thatcher and Kluger 1989, Hosfield *et al.* 1999, Guha *et al*. 2000, Lee *et al*. 2000, Williams 2000, Chapados *et al*. 2001, Harger 2002, Hengge 2002, 2005, Humphry *et al.* 2004**,** Swamy and Kumar 2006, Um and Kumar. 2006, Hoque *et al.* 2007, Adhikary *et al*. 2008, Lumbiny *et al*. 2008, Adhikary *et al*. 2011)



Scheme 1. Schematic representation on nucloephilic substitution at P centre on (P=S/O) group transfer reaction.

Our very recent studies showed that in case of concerted mechanism the nucleophile can approach towards reaction centre in two different ways. A hydrogen-bonded, four-center type TS is suggested for a frontside attack while the TBP-5C TS is suggested for a back-side attack based on the deuterium kinetic isotope effects. as shown in Scheme 2 (Guha and Kumar. 2000, Lee *et al*. 2000, Hoque *et al*. 2007, Lumbiny *et al*. 2008, Adhikary *et al*. 2011).



Scheme 2. Schematic representation of nucleophilic attack on P centre in concerted mechanism.

This is also very strongly claimed from previous studies as the substituents around the P centre varies in leaving or nonleaving group, the reactivity, selectivity, finally mechanistic pathway for S_N reaction varies a lot. The variation can be rationalized from our recent investigation on the pyridinolysis of *O*-aryl phenyl phosphonochloridothioates

[PhP(=S)(OPh-Y)Cl, 1**,** and *O,O*-diphenyl Z-*S*-phenyl phosphorothiolates [$(PhO)_2$ P(=O)(S-Ph-Z)], 2 in acetonitrile at 35.0 ^oC, being observed by varying substituents around P centre (Y in 1, Z in 2) or in nucleophile (X). The reported mechanism according to LFER is S_N2 without any change in mechanism for the system 1, but for 2 a change in mechanism is interpreted at Z = H from concerted to stepwise with rate-limiting expulsion of the leaving group(Lumbiny *et al*. 2008, Adhikary *et al*. 2011).

To gain further evidence in support of the above mechanism, the aminolyses of 4-Chlorophenyl phenyl Chlorophosphate [4–ClPhOP(=O)(OPh)Cl], 3**,** with tertiary amine, substituted pyridines have been carried out at 5.0 ^oC in acetonitrile (present study). It is also very instructive to compare the behavior of 3, [4–ClPhOP(=O)(OPh)Cl], with that of 1, [PhP(=S)(OPhY)Cl] and 2, [$(PhO)_2P(=O)(SPhZ)$] comparing the reactivities, the selectivity parameters, and the magnitudes of the CICs with those obtained in our previous studies to assess their influence on TS and the reaction mechanism as well.

Materials and Methods

GR grade starting materials, substitued pyridines, deuterated pyridine (C_5D_5N; 99 atom% D) were purchased and used. HPLC grade acetonitrile (less than 0.005% H_2O content) were used to prepare nucleophile solution to study kinetics (Guha *et al*. 2000, Lee *et al*. 2000, Hoque *et al*. 2007, Lumbiny *et al*. 2008, Adhikary *et al*. 2011).

Kinetics (Guha *et al*. 2000, Lee *et al*. 2000, Hoque *et al*. 2007, Lumbiny *et al*. 2008, Adhikary *et al*. 2011): The kinetic study was performed with a computer controlled conductivity bridge, equipped with a constant temperature circulating bath to keep the reaction mixture at 35.0 ± 0.2 ^oC (for 1, 2), 5.0 ± 0.2 ^oC (for 3). Reactions were carried out under pseudo first-order conditions in which amine concentrations were at least 20 times greater than the substrate concentration. Thus the pseudo first- order rate (k_{obsd}) was obtained experimentally by using Guggenheim equation (equation 1), and nonlinear curve fitting method in ORIGIN program,

$$\lambda_t = \lambda_\infty - (\lambda_\infty - \lambda_0)e^{(-k_{obsd} \times t)} \ldots (1)$$

[where, λ_0 = initial conductivity, λ_t = conductivity at any time, λ_∞ = conductivity at infinity]

which will ultimately produce second-order rate constant (k_2) from the slope of the plots of k_{obsd} vs [Nu], in equation 2, which gave very good linearity in all cases. For these plots at least five different amine concentrations were employed and replicate values of k_{obsd} were determined to obtain the second-order rate constants (k_2) reproducible to within ± 3%.

$$k_{obsd} = k_0 + k_2 [Nu] \ldots (2)$$

Similarly deuterated pyridine were treated to obtain k_2, values for deuterium effect. In this study k_H is expressed as average of second-order rate constants with pyridine and k_D indicates average of second-order rate constant with deuterated pyridine.

Theory : Free Energy Relationship (Issacs 1995, Willams 2003). The mechanism of a chemical reaction can be interpreted using LFER; an empirical observations which can be derived when the shapes of the potential energy surfaces (PES) of a reaction are not substantially altered by varying the substituent (Willams 2003).

Hammett Equation (Hansch *et al*. 1991, Issacs 1995, Pross 1995, Mihai *et al*. 2003, Willams 2003): Hammett's success is in treating the electronic effect of substituents on the rate of equilibria of organic reactions, can be expressed as follows equation 3(a) and 3(b)

$$\log (k_X/k_H) = \sigma\rho....[3(a)] \quad \text{or} \quad \log (K_X/K_H) = \sigma\rho...[3(b)]$$

applied to the influence of *m*- or *p*-substituents X on the reactivity of the functional group Y in the benzene derivative *m*- or *p*-XC_6H_4Y. k_X or K_X is the rate or equilibrium constant, respectively, for the given reaction of *m*- or *p*-XC_6H_4COOH(4), k_H or K_H refers to the reaction of C_6H_5COOH, i.e., X = H, σ is the substituent constant, ρ is the reaction constant.

Significance of Sign and Magnitude of ρ: the susceptibility of the reaction to substituents, (+ve) value; a reaction favored by EWS, (–ve) value; reverse. (a) $\rho = 1$, for benzoic acid (4) ionization. (b) $\rho > 1$, the reaction is more sensitive to substituents than (4) and negative charge is built during the reaction. (c) $0 < \rho < 1$, less sensitive to substituents than (4) and negative charge is built. (d) $\rho = 0$, no sensitivity to substituents, and no charge is built. (e) $\rho < 0$, the reaction builds positive charge.

The Brönsted equation (Zuman and Patel 1984): The Gibbs free energy for proton dissociation is proportional to the activation energy (equation 4a, 4b) for the catalytic step. When the relationship is not linear, the chosen group of catalysts do not operate through the same reaction mechanism.

$$k_b = G_b K_b^{\beta} = G_b (K_W/K_a)^{\beta} = G_b K_a^{-\beta} \qquad (4a)$$

$$\log k_b = \beta pK_a + \text{constant} \qquad (4b)$$

The Brönsted correlation of rate constants with nucleophile pK_a (β_X) is one measure of the degree of nucleophile bond formation in the rate determining TS. Reactions that have low values for proportionality constants (β_X) are considered to have a transition state closely resembling the reactant with little proton transfer, with a high value, resembles product .

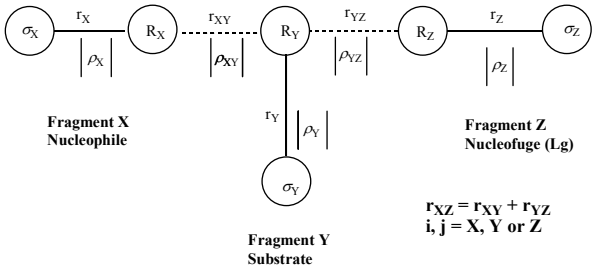
Cross-Interaction Constant Theory (Miller 1959, Cordes and Jencks 1962, Jencks and Jencks 1977, Dubois *et al*. 1984, Jencks 1985, Lee 1990, 1992, 1995, Lee and Lee 1999, Williams 2000, Lee and Lee 2001,).

Definition: The multiple substituents effect can also be analyzed quantitatively by extending these classical equations to include second derivative parameter, termed as Cross-interaction constants (CICs), shown in equation 5a and 5b. The CICs, ρ_{ij} (Hammett type constant) and β_{ij} (Brönsted type constant) represent the intensity of interaction

between the two interacting molecules i (e.g., a nucleophile) and j (an electrophile) in the adduct (Scheme 3)

$$\log (k_{ij}/k_{HH}) = \rho_i\sigma_i + \rho_j\sigma_j + \rho_{ij}\sigma_i\sigma_j \ldots (5a)$$

$$\log (k_{ij}/k_{HH}) = \beta_i \Delta pK_i + \beta_j \Delta pK_j + \beta_{ij} \Delta pK_i \Delta pK_j \ldots (5b)$$



Scheme 3. Typical S_N2 TS. . (Lee 1990, 1992, 1995, Lee and Lee 1999, Williams 2000, Lee and Lee 2001).

Table 1. Summarization of significance of the sign and magnitude of the CICs in explaining quantitative mechanistic criteria.

Mechanism	Sign
S_N1	$\rho_{XY} = 0, \rho_{YZ} > 0, \rho_{XZ} = 0$
Concerted S_N2	$\rho_{XY} < 0, \rho_{YZ} > 0, \rho_{XZ} > < 0$
Addition-Elimination	
formation of $T^{\pm}$	$\rho_{XY} < 0, \rho_{YZ} \equiv 0, \rho_{XZ} > < 0$
breakdown of $T^{\pm}$	$\rho_{XY} > 0 , \rho_{YZ} < 0, \rho_{XZ} > 0$

Kinetic Isotope Effects (Paneth and O'Leary 1991, Issacs 1995, Olaf *et al*. 1995, Carrol 1998)

The measurement of the KIEs tells about bonding changes in the rate limiting step of a reaction as the rate of reaction varies when an atom is replaced by an isotope (usually comparison of H with D, k_H/k_D); provide details of the TS structures. The following types of isotope effect are distinguished:

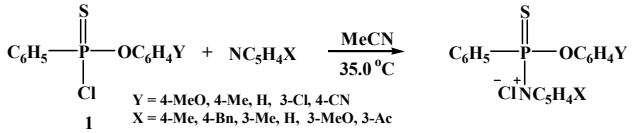
(a) Primary kinetic isotope effect (PKIE): in which the bond is broken in the rate determining step, favors front side nucleophilic attack.; $k_H/k_D >> 1$.

(b) Secondary kinetic isotope effect (SKIE), in which the bond to the isotopic atom (s) remains intact throughout the reaction, $k_H/k_D << 1$ or k_H/k_D, around, 1; favors back side nucleophilic attack.

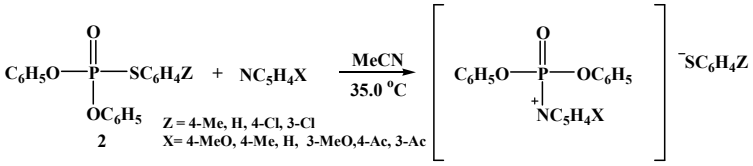
(c) Solvent isotopic effects, which result from isotopic differences in the medium, e.g., if the solvent is changed from H_2O to D_2O, then $k_{(H2O)} / k_{(D2O)}$ is obtained as solvent isotope effect.

Results and Discussion

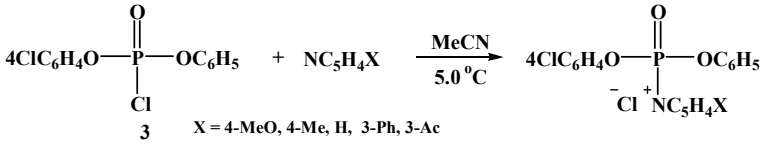
All the reactions in this study pyridinolysis of 1, 2 in acetonitrile at 35.0 ºC (Schemes 4 and 5) and 3, in acetonitrile at 5.0 ºC (Scheme 6) follow pseudo first-order kinetics. The second-order rate constants, (k_2), obtained from pseudo first-order rate constants (k_{obsd}), are summarized in Tables 2, 3 and 4 respectively together with the selectivity parameters, Hammett (ρ_X, ρ_Y), Brönsted (β_X) coefficients and CIC's (ρ_{XY}).



Scheme 4. Reaction systems for the pyridinolysis of 1, in acetonitrile at 35.0 °C.



Scheme 5. Reaction systems for the pyridinolysis of 2, in acetonitrile at 35.0 °C.



Scheme 6. Reaction systems for the pyridinolysis of 3, in acetonitrile at 5.0 °C (present study).

Table 2. k_2 (× 10^3/M^{-1} s^{-1}) and selectivity parameters[a] for the pyridinolysis of compounds 1.

X\Y	4-MeO	4-Me	H	3-Cl	4-CN	ρ_Y[d]
4-Me	85.5	99.6	106	152.0	195.0	0.38
4-Bn	40.6	43.2	46.8	61.9	80.6	0.32
3-Me	24.7	25.7	27.5	36.8	47.8	0.31
H	9.28	10.4	11.2	14.4	17.5	0.29
3-MeO	3.82	4.31	4.64	5.77	6.57	0.24
3-Ac	0.33	0.35	0.36	0.39	0.43	0.11
$-\rho_X$[b]	4.35	4.39	4.40	4.62	4.75	ρ_{XY}[e] =
β_X[c]	0.87	0.88	0.88	0.93	0.95	−0.46

[a] σ (Hansch *et al*. 1991) and pK_a (Albert and Serjeant 1984), [b] Correlation coefficients, r, were better than 0.994. [cd] r ≥ 0.988. [e] r = 0.990.

Table 3. k_2 (× 10^3/M^{-1} s^{-1}) and selectivity parameters for the pyridinolysis of compounds 2.

X\Z	4-Me	H	4-Cl	3-Cl	ρ_Z[d]	ρ_Z[e,f]
4-MeO	590.0	1090	513.0	467.0	1.57	–1.04
4-Me	183.0	284.0	77.8	68.3	1.12	–1.75
H	28.3	44.2	11.0	8.70	1.14	–1.98
3-MeO	17.5	34.8	5.89	3.85	1.76	–2.66
4-Ac	2.63	3.42	2.08	1.60	0.67	–0.90
3-Ac	0.24	0.37	0.23	0.20	1.	–0.75
$-\rho_X$[b]	4.64	4.76	4.43	4.51	ρ_{XZ}[d,g]= –0.70	ρ_{XZ}[d,h] = +0.76
β_X[c]	0.93	0.95	0.88	0.89		

[a] σ (Hansch *et al*. 1991) and pK_a (Albert and Serjeant 1984), [b]Correlation coefficients, r, were better than 0.974. [c]r ≥ 0.963 [d]Z = 4-Me and H. [e]Z = H, 4-Cl, and 3-Cl. [f]r ≥ 0.955. [g]r = 0.956. [h]r = 0.951.

Table 4. k_2 (× 10^2/M^{-1} s^{-1}) and selectivity parameters[a] for the pyridinolysis of compounds 3.

X	4-MeO	4-Me	H	3-Ph	3-Ac	$-\rho_X$	β_X[c]
4-Me	121	27.8	3.16	0.918	0. 026	5.66	1.14

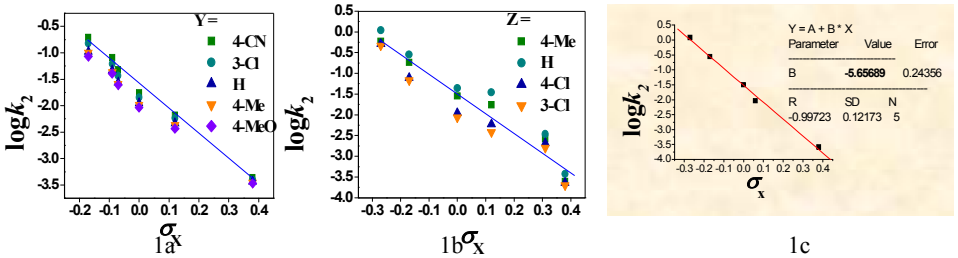
[a] σ (Hansch *et al*. 1991) and pK_a (Albert and Serjeant 1984) [b]Correlation coefficients, r, were better than 0.997. [c]r = 0.997

The second-order rate constants (k_2 × 10^3/$M^{-1}s^{-1}$) of the pyridinolysis 1 with Y = H: (PhO)PhP(=S)Cl, 2 with Y = H: $(PhO)_2P(=O)SPh$, 3: (4–ClPhO)PhOP(=O)Cl obey the following order of reactivity respectively (Tables 2 - 4)

$$2\ (44.2) > 3\ (31.6) > 1\ (11.2)$$

At a glance, the reaction rates seem to be proportional to the positive charge on reaction center P. However, it is well known that P=O substrates are more reactive than P=S because of electronegativity difference between O and S, favoring O over S (Gregersen *et al.* 2003, Hengge 2005).

The Hammett plots for substituent (X) variations in the nucleophile (log k_2 vs. σ_X, Fig. 1) and Bronsted plots (log k_2 vs. pK_a(X), Fig. 3) show linearity for 1, 2, 3, suggesting no change in mechanism. It differs from 2 for the Hammett plots for substituent (Z) variations in the leaving group (log k_2 vs. σ_Z) are biphasic concave downwards with breaks at Z = H as shown in Fig. 2 is interpreted as the change in mechanism with variation in Z.



1a 1b 1c

Fig. 1. The Hammett plots for the determination of ρ_X for the reactions of 1 (1a), 2(1b), 3(1c).

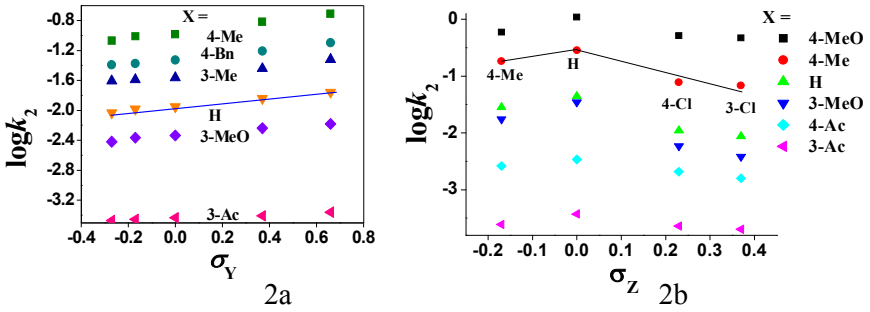


Fig. 2. The Hammett plots for the determination of ρ_Y for the reactions of 1 (2a), 2(2b).

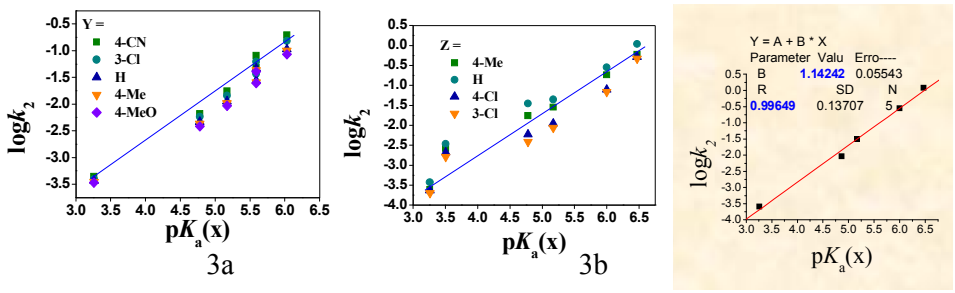


Fig. 3. The Brönsted plots for the determination of β_X for the reactions 1 (3a), 2(3b), 3(3c).

The selectivity parameters, the negative CIC's, ρ_{XY} (−0.46, Table 2) of 1 implies that the reaction proceeds through a concerted S_N2 mechanism. The sign of CIC's for 2, reversal of ρ_{XZ} from positive in $\sigma_Z \leq 0$ (ρ_{XZ} = −0.70) to negative in $\sigma_Z \geq 0$ (ρ_{XZ} = +0.76) may indicate the mechanism change from concerted to stepwise with rate-limiting expulsion of the leaving group. The obtained ρ_X = −4.35 to −4.75, and β_X = 0.87 to 0.95, of 1, ρ_X (= −4.43 to −4.76) and β_X (= 0.88-0.95) in the present work, 2, are somewhat smaller than those in 3, ρ_X = −5.66, and β_X = 1.14. The comparable ρ_X and β_X of 3 with those of 1, 2 indicates concerted mechanism for 3, with a later TS i.e., a greater extent of bond-formation than that of 1 as well as 2.

The observed k_H/k_D values of 1 are greater than unity (1.05-1.11, for 1, 1.06 for 3, Table 5) implies the primary KIE favors front side nucleohilic attack in TS. The larger magnitude of selectivity parameters also observed for this.

Table 5. Deuterium kinetic isotope effects (k_H/k_D) for the reactions of 1 and 3 with *d*-5 pyridine(C_5D_5N) in acetonitrile at 35.0 °C and 5.0 °C respectively.

Y	4-MeO	4-Me	H	3-Cl	4-CN
Y(1) k_H (× 10^3/M^{-1} s^{-1})	9.28 ± 0.05	10.4 ± 0.09	11.2 ± 0.2	14.4 ± 0.2	17.5 ± 0.1
Y(1) k_D (× 10^3/M^{-1} s^{-1})	8.50 ± 0.07	9.88 ± 0.17	10.1 ± 0.1	13.6 ± 0.1	16.5 ± 0.1
Y(3) k_H (× 10^3/M^{-1} s^{-1})			31.6 ± 0.2		
Y(3) k_D (× 10^3/M^{-1} s^{-1})			30.6 ± 0.1		
1,$(k_H/k_D)_{obsd}$	1.09 ± 0.01[a]	1.05 ± 0.02	1.11 ± 0.02	1.06 ± 0.02	1.06 ± 0.01
3,$(k_H/k_D)_{obsd}$			1. 04 ± 0.08		

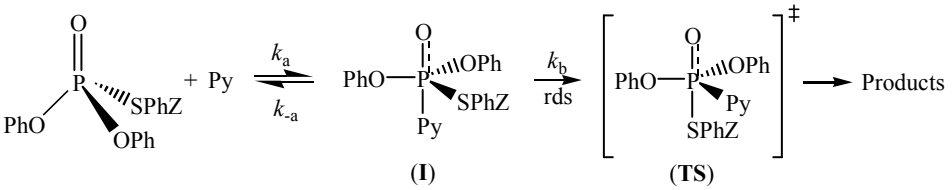
[a]Standard error {= $1/k_D[(\Delta k_H)^2 + (k_H/k_D)^2 \times (\Delta k_D)^2]^{1/2}$} (Crumpler and Yoh 1940).

We can suggest possible TS structures of the pyridinolysis of 1 (TS 1a), 3 (TS 1b), in Fig. 4, as these structure are in line with the frontside nucleophilic attack which we have proposed in a earlier paper (Guha *et al*. 2000, Lee *et al*. 2000, Hoque *et al*. 2007, Lumbiny *et al*. 2008, Adhikary *et al*. 2011) and with the primary KIE, due to the hydrogen bond between the leaving group Cl and the H(D) atom in the C−H(D).

TS 1a **TS 1b**

Fig. 4. The TS structure for 1 (1a), 3(1b).

In the case of the frontside nucleophile attack [ap(Nu)-eq(Lg)] is observed rather than eq(Nu)-ap(Lg) in a TBP-5C intermediate. Since pyridine is less bulky than PhO and/or ZPhS it can undergo an intramolecular ligand exchange process by Berry-type pseudorotation (or turnstile rotation) and thus [eq(Nu)-ap(Lg)] can be observed in the TS (Scheme 7).



(**I**) (**TS**)

Scheme 7. Schematic representation for Berry-type pseudorotation (or turnstile rotation) for the present system.

Finally it can be concluded that according to the reported mechanism is S_N2 without any change in mechanism for the system 1, but for 2 a change in mechanism is interpreted at Z = H from concerted to stepwise with rate-limiting expulsion of the leaving group. In the light of the system 1, 2 the pyridinolysis of 3 with ρ_X , −5.66 should proceed through S_N2 indicating no change in mechanism with greater extent of bond formation in TS than that of 1, 2 having TS structure 1b.

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GROUNDWATER IRRIGATION AND CROP ECONOMY IN THE LOWER GANGETIC PLAIN AT MATBARER CHAR, MADARIPUR, SOUTH-CENTRAL BANGLADESH

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Abstract

Attempts have been made in this paper to overview the present agricultural inputs for crop production and crop economy in the lower Gangetic plain. Results of the field survey show that the average labour requirement of Boro rice production is 35 person-days/acre. The fertilizer application by the farmers is not balanced and is well below the recommended doses. N, P_2O_5, K_2O ratio used by the farmers was 7:3:1 against the appropriate ratio of 5:4:3 for HYV Boro rice. The cost of Boro rice cultivation has been estimated on the basis of input use. The rice cultivation costs were Tk.14,335, Tk 13,875 and Tk. 11,271 per acre for the water buyer, water seller with diesel pump and water seller with electric pump respectively. Labour and irrigation costs together normally account for two- thirds of the total cost of production - increases in the prices of these inputs can greatly depress Boro rice profitability. Moreover, Gross income of the water sellers from major crops is about 32% higher than that of the water buyers. The study also shows that the total cost for a Shallow Tube Well (STW) run by diesel motive power is almost twice that for electric motive power. If groundwater irrigated agriculture system is framed in a comprehensive manner, this could play an important role in poverty alleviation.

Key words: Groundwater, irrigation, crop economy, lower Gangetic Plain.

Introduction

Bangladesh is a lower riparian country in the flood plains of three great rivers- the Ganges, the Bhramaputra and the Meghna, and their tributaries and distributaries. Water scarcity in the long dry season and sometimes drought even in the monsoon affects its agriculture, domestic and industrial water supply. This is primarily responsible for the shortage of water for agriculture. Moreover rural areas of Bangladesh suffer from lack of quality drinking water as surface water supplies are generally polluted and, therefore, have to depend on groundwater. Heavy withdrawals of groundwater for irrigation have also lowered the water table in many areas below the effective reach of suction mode tube wells including traditional hand tube wells. About 90% of irrigation water in Bangladesh is provided from groundwater (Zahid *et al.* 2008, 2009).

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Bangladesh is predominantly an agricultural country and its economy depends on agriculture. The natural catastrophe like floods, droughts etc. hinder the agricultural development. Flood and drainage congestion generally destroy the Boro crop at the ripening stage. The dominant food crop of Bangladesh is rice which accounts for about 75 percent of agricultural land use (and 28 percent of GDP). Rice production increased every year since 1980s (through 1987) except Fiscal Year (FY) 1981, but the annual increases have generally been modest, barely keeping pace with the population. Rice production exceeded 15 million tons for the first time in FY 1986-87. In the mid-1980s, Bangladesh was the fourth largest rice producer in the world, but its productivity was low compared with other Asian countries, such as Malaysia and Indonesia (Mandal 2006). High yielding varieties of seed, application of fertilizer and irrigation have increased yields, although these inputs also raise the cost of production and chiefly benefit the richer farmers.

The cultivation of rice in Bangladesh varies according to seasonal changes in the water supply. With the increasing use of irrigation, there has been a growing focus on another rice-growing season extending during the dry season from October to March. The production of this *Boro* rice, including high yielding varieties, expanded rapidly until the mid-1980s, when production leveled off at just below 4 million tons. Where irrigation is feasible, it is normal for fields throughout Bangladesh to produce rice for two harvests annually. Between rice-growing seasons, farmers do everything possible to prevent the land from lying fallow and grow vegetables, peanuts, pulses or oilseeds if water and fertilizer are available.

Groundwater irrigation plays a crucial role in agriculture of Bangladesh and thus in the national economy. Given its dense population and level of rural poverty, Bangladesh is very much in need of enhanced crop production. As far as the crop production is concerned, groundwater irrigation has contributed significantly to the cereal production, mainly *Boro* rice and wheat, by supplementing soil moisture in the dry months of November/December to April/May. Thus cropping pattern is being moved towards HYV rice. Almost all of its 9.1 million hectares of net cultivable area, however, is already in use and any additional crop output can only come from increasing yield and/or cropping intensity. Methods that are available to achieve these ends depend he mainly on irrigation – particularly minor irrigation technologies comprising low lift pumps (LLPs), deep tubewells (DTWs), shallow tubewells (STWs) and manually operated pumps (MOPs), which together are responsible for 85% of irrigation coverage in the country (Mandal 2006, Economic Review 2006). Thus groundwater irrigation is of vital importance as an input to the agricultural economy and for food security. Therefore, it is attempted in this paper to examine present agricultural development and assess the irrigated crop economy in the lower Gangetic plain as a case study.

Materials and Methods

Study Site: The study area was selected considering the poor agricultural activities and crop production, vulnerable soil fertility and less development of groundwater use compared to other floodplain areas of the country. Five consecutive villages, namely, Naktikandi, Kharakandi, Natunkandi (Latif Matbarerkandi), Purankandi and South Bakharerkandi (Sikderkandi) under Shibchar thana were selected as these villages have more involvement in groundwater irrigation, though these are surrounded by many river branches. The location of the study villages is shown in Fig. 1. The area is characterized by the erosion and accretion game of the Ganges which give rise to emergence of medium to large size shoals/sand bars (locally known as *char*). Study shows that the development of groundwater irrigation in this part of the country is not as old as the evolution of these villages (Islam *et al.* 2007).

In the study area, crop year is mainly divided into two seasons- Rabi and Kharif; again Kharif season has been subdivided into Kharif-I and Kharif-II. Rabi season starts from mid-October and ends in mid-March, while Kharif-I season spreads from mid-March to mid-July and Kharif-II within the span of mid-July to mid-October. Crop production is greatly influenced by the seasons. Farmers select their crops based on the land type, availability of irrigation water and on the local market demand. Rice, wheat, mustard, jute, lentil, spices, vegetables, sugarcane are the major crops cultivated in the area. The dominant irrigated crop in the study area is HYV Boro rice. With the increasing demand for municipal and rural supplies, agricultural, industrial and other uses, and understanding the natural distribution for long-term sustainability of groundwater irrigation is very important.

Sample Collection: The study area covered five adjacent villages of Naktikandi, Kharakandi, Natunkandi, Purankandi and Sikderkandi under Matbarer Char Union of Shibchar Upazila, Madaripur district of southwest Bangladesh. The area was selected considering the low agricultural growth and groundwater development. This study was based on the field survey data collected during the survey period (January-March 2007) through direct interviews of 83 respondents using structured questionnaires. The respondents were selected randomly covering all the study villages. Among the respondents, there were 49 water buyers (WBs) and 34 water sellers (WSs) which have been given in Table 1. The study also covered as many as 44 wells out of which 3 were of electric centrifugal pump and the rests were of diesel centrifugal pump.

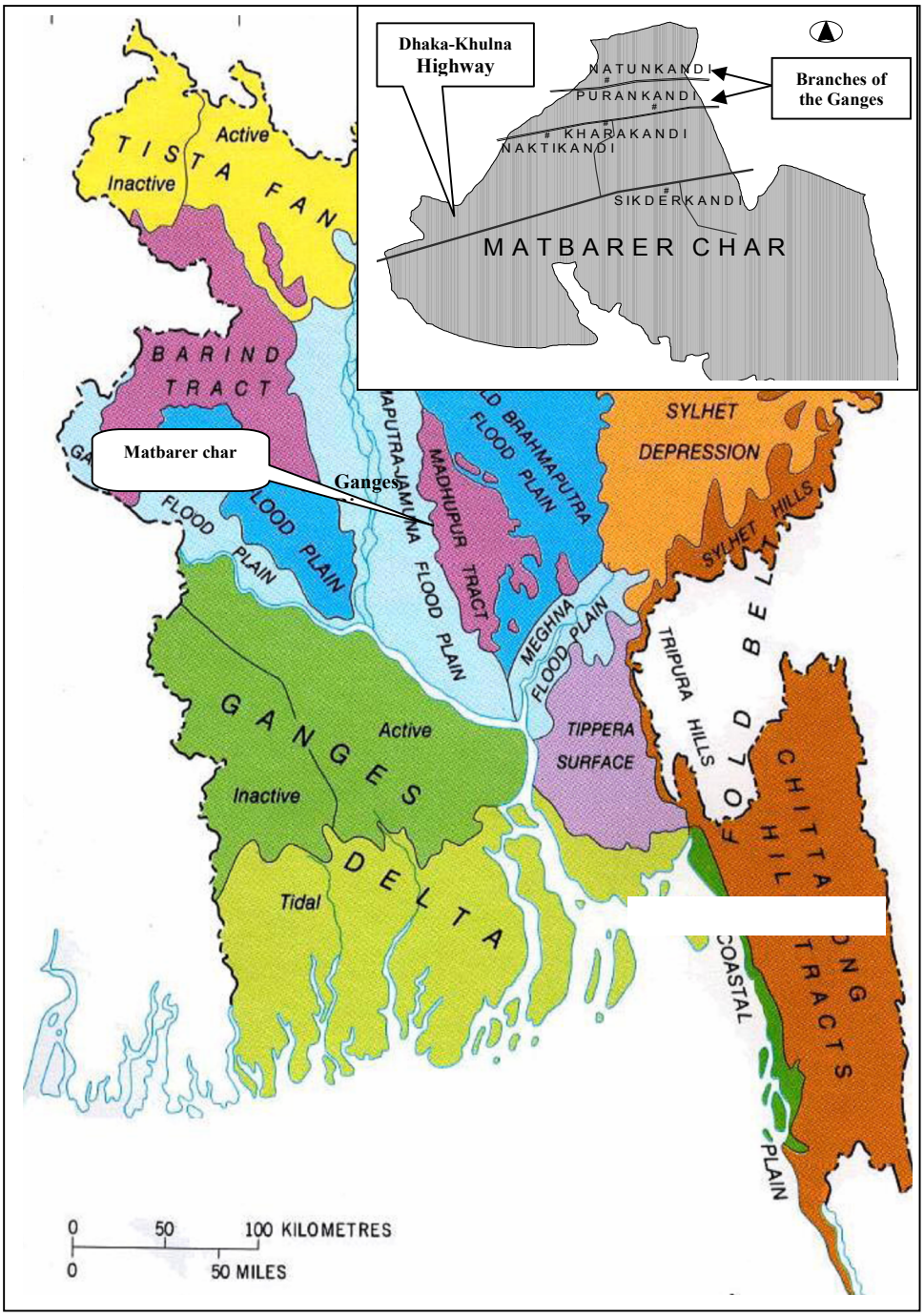

Fig. 1. Location of study villages under Matbarer char Union, Shibchar, Madaripur (modified after Alam *et al.* 1990).

Table 1. Summary of the respondents' count.

Water Transaction Code	Villages with number of respondents					Total
	Naktikandi	Kharakandi	Purankandi	Natunkandi	Sikderkandi	
WB	9	12	10	15	3	49
WS	5	4	8	11	6	34
Total	14	16	18	26	9	83

Results and Discussion

Economics of Groundwater Extraction **:** details of economics of groundwater extraction presented in Table 2 show that capital costs of well installation per well including pumps are approximately Tk 1,000 for Diesel Centrifugal (DC) and 900 for Electric Centrifugal (EC) power motive. Costs have been indexed at current prices using wholesale price indices. Repair and maintenance cost of running the well/season in 2005-06 is estimated to be about Tk 1,380 and Tk 300 for DC and EC power motive, respectively. The average supervision, diesel and lubricant cost per well per season is about Tk 11,364, Tk 34,976 and Tk 1,351, respectively while the supervision and electricity cost for EC motive power is Tk 8,000 and Tk 15,000, respectively. The study shows that the total cost for a STW run by DC motive power is almost twice that for EC motive power. This is why the farmers in the study villages are strongly in favor of EC motive power.

Table 2. Comparative economic analysis for DC and EC motive power.

Item	DC Cost (Tk)	EC Cost (Tk)
Well construction cost	1,00	900
Distribution line cost	566	566
Repair and maintenance cost	1,380	300
Supervision cost	11,36	8,00
Diesel/electricity cost	34,97	15,00
Lubricant (Mobil) cost	1,351	-
Total cost per STW	50,63	24,76

Cropping Pattern, Cropping Intensity and Yield of Major Crops : Cropping pattern for each village under present situation is furnished in Table 3. A wide range of cropping patterns was found in the study area, but the major patterns are rice based. Pulses, oilseeds, vegetables, jute, wheat, sugarcane are the major non-rice crops. With the availability of ensured irrigation water, farmers shifted towards growing more productive Boro-Fallow-Aman pattern. Previously the cropping pattern in the study area was Aus-Jute-Fallow. Rice is the most dominant crop being grown almost everywhere in the study villages throughout the year in each of the Kharif-I, Kharif-II and Rabi seasons. The cropping pattern was covered by 79.22% with rice and mustard, and the remaining 20.78% by jute, sugarcane, onion, pulses, oilseeds and vegetables etc.

Table 3. Present cropping patterns in the study area.

Village Name	Cropping Patterns
Naktikandi	Rabi Crops-Boro Rice-Fallow/ Sugarcane
Kharakandi	Rabi Crops-Boro Rice-Fallow/ Wheat-Jute/Mesta-Fallow
Purankandi	Boro Rice-Fallow-T Aman/ Wheat-Jute-Fallow
Natunkandi	Boro Rice-Fallow-T Aman/ Rabi Crops-T Aman-Fallow
Sikderkandi	Rabi Crops-Boro Rice-Fallow

The cropping intensity is determined as the ratio of total cropped area to net cultivated area, which is expressed in terms of percentage. Farmers grow one or two crops and left their lands fallow during the rest of the time. The highest cropping intensity was found to be 173% in Purankandi village while the lowest was 124% in Sikderkandi village. The average cropping intensity in the study area was 146%. The country has achieved an average cropping intensity of about 185% (NWMP 2001).

The yield of different crops in the study area presented in Table 4 shows that the survey result produced mixed evidence about the change in yields of different crops. For irrigated Boro rice, only about a quarter of the respondent farmers reported an increase, whereas 65% reported decreases and the remainder had no change in yield in the year 2006 compared to 5 years ago. For other crops there were mixed responses as well. Main causes of yield decline/stagnation of HYV Boro rice as per farmers' opinions were intensive cropping, unbalanced fertilizer application with more use of nitrogenous fertilizer and/or no fertilizer use over mining soil nutrients compared to crop removal etc.

Table 4. Yield of major crops in the study area.

Crop Name	Average Yield (Kg per acre)
HYV Boro	1945 (for Water Buyer) 2086 (for Water Seller)
T. Aman	371
Wheat	720
Mustard	148
Sugarcane	12160
Onion	2304
Jute	640

Groundwater Productivity on Boro Rice : The rice variety widely adopted in the study area is BR-29. This variety has grown in duration of 140 to 150 days. The preferred planting method is transplanting of seedling using wet bed technique. After land is prepared, 35-40 days old seedlings are transplanted to the field and then the irrigation water supply period commences. The 3-day rotation method is practiced for water distribution in the study area. The water sellers maintain the rotational system. On an average, water is applied 35-40 times in the field during rice crop season. Due to seasonal

river flood in the area, deposition of new finer sediments allows more percolation of irrigated water causing absorption of more water. It was observed that continuous shallow ponding is needed to obtain a good rice yield. Most of the farmers are familiar with the rotational irrigation schedule and have accepted it as an equitable program for water sharing. The applied groundwater productivity and applied irrigation rate for water sellers and buyers are presented in Table 5. The survey result shows that water sellers applied more water than the water buyers.

Table 5. Groundwater productivity and applied irrigation rate for Boro rice in the study area.

Village Name	Water Productivity		Applied Irrigation Rate (m^3 per acre)	
	Water Seller	Water Buyer	Water Seller	Water Buyer
Naktikandi	0.27	0.36	7720	5340
Kharakandi	0.57	0.58	3643	3357
Purankandi	0.31	0.35	6665	5554
Natunkandi	0.42	0.46	4895	4200
Sikderkandi	0.35	0.41	8053	2739
Average	0.38	0.43	6195	4238

There is a common understanding among the farmers that the more the water depth in the field within limits, the better the yield of irrigated rice. In fact, this is not true. Even under irrigated conditions, occasional drainage is necessary for aeration. A series of water management studies conducted by BRRI indicated that a range of water depths ranging from soil saturation only to 10 cm standing water gave statistically insignificant differences in rice yields, provided other management practices were uniform and equal (Islam 1986, 1987). It was observed that the average groundwater productivities were 0.38 and 0.43 kg m^{-3} for water sellers and water buyers, respectively. National Water Management Plan (NWMP 2001) recommended total required water depth for HYV Boro rice is between 1200 and 1500 mm per season, depending on soil condition. Considering the maximum value the average water used by farmers was 34 % more than the recommended limit, which has no contribution to the growth of crops (Islam 1989, 1991).

Crop Economy and Cost of Boro Rice Cultivation : Crop production inputs such as human labor, draft power and most seeds are generally supplied by farmers in Bangladesh. However, with the shift to HYV technologies, there great importance of purchased inputs such as chemical fertilizer, pesticides, irrigation and power tiller services etc., which are marketed through private-sector traders.

The input use and cost of Boro rice cultivation at present situation are presented in Table 6. The cultivation costs were Tk 1,4335, Tk 1,3875 and Tk 1,1271 per acre for the water buyer, water seller with diesel pump and water seller with electric pump respectively.

Water buyers' cultivation cost was slightly higher than that of the water sellers. This is mainly due to the difference in irrigation cost (Table 6).

Table 6. Cost of cultivation of Boro rice in the study area.

Line Items	Qty. (Kg)/No	Rate (Tk kg^{-1} or Tk Day^{-1})	Total Tk per acre
Seed	20	30	600
Seed bed preparation	2 (man-day)	125	250
Seed bed Management	2 (man-day)	125	250
Land Preparation (Power Tiller)	8	125	1,000
Transplanting, Weeding and Harvesting	35 (man-day)	125	4,375
Pesticide	2		300
Fertilizer			1,400
Irrigation (Water Buyer)			6,160
Irrigation (Water Seller with DC motive power)			5,700
Irrigation (Water Seller with EC motive power)			3,096
Total cost for water buyer			14,335
Total cost for water seller with DC motive power			13,875
Total cost for water seller with EC motive power			11,271

The gross income analysis from major crops both for water sellers and water buyers is presented in Table 7. Two major crops were considered for income analysis in the study area. The gross income for water buyers was estimated from crop yield per acre multiplied by its unit price. The income from water selling makes the difference between water buyers' and water sellers' gross income per year. Gross income of water sellers is about 32% higher than that of water buyers.

Table 7. Gross income analysis from major crops for water buyers and water sellers.

Income	WBs (Tk per acre)	WSs (Tk per acre)
Boro rice	21,250	22,789
Mustard	2,812	2,812
Water selling	-	6,160
Gross income	24,062	31,761

Impact of Groundwater Irrigation and Other Inputs on Crop Economy*:* Besides irrigation, crop production needs various agricultural practices- land preparation, seedling or plantation, application of fertilizers, intercultural operations, plant protection etc. for a good harvest as those have impacts on crop economy. A timely cultural operation increases the yield, but in the study area cultural practices are not upto the mark. As a result, yield obtained for different crops are not satisfactory. Human labor and animal power are required in various stages of crop production. Peak period for human labor is during transplantation and harvesting while animal labor in land preparation. There is a

high demand for labor during the *Boro* rice transplantation and harvest. Power tiller is used for land preparation in the study area. Farmers started using power tillers to meet up the shortage of draft power. Field survey shows that the average labor requirement of Boro rice production is 35 person-days/acre. Vegetable production is more labor-intensive than rice crops, requiring an average of 70 person-days.

Chemical fertilizers are important inputs for HYV rice production. In the study area, all farmers apply chemical fertilizers and only a few are reported to use organic manures. Nitrogen is the most common and widely used nutrient and urea is the major source of nitrogen. Phosphate is next only to nitrogen in total volume of use as fertilizer. The most widely used phosphate fertilizer is TSP. Potash is the third most widely used nutrient, and MP is the only source of potash. In some areas the farmers have reported zinc and sulpher deficiency and they used gypsum or zinc sulphate to overcome that deficiency. In the area, farmers use Urea, TSP and MP at the rate of 110-150, 60-90 and 20-30 kg/acre respectively. The fertilizer application by the farmers is not balanced and is well below the recommended doses. N, P_2O_5, K_2O ratio used by the farmers was 7:3:1 against the appropriate ratio of 5:4:3 for HYV Boro rice (NWMP 2001). Previously, organic manure was applied but now-a-days cow dung is mostly used as fuel. Though the application of organic manure as cow dung would enrich the soil structure and increase the water holding capacity of soil but it is used mostly for household fuel consumption instead of field application.

Agricultural Constraints **:** Over the recent years, because of land and technological constraints, growth of crop agriculture has been less than expected. Bangladesh has a very little scope to increase agricultural production through expansion of land, as the cultivated land has remained constant and even decreased in many cases. The introduction of modern varieties of rice, wheat, oil seeds, potato and other crops has shortened the production cycles and increased cropping intensities and yield. With the expansion of boro cultivation, diesel for operating pumps is becoming a major agricultural input, and cost on this account will increase due to the high price of petroleum fuels in the world market. The higher cost of engines, pumps and installation cost of the tube well along with higher operation cost will make it less economical. The allocation for subsidies has been kept at a minimum level for allowing Government intervention at times of scarcity, which has been taken as an advantage by the private sectors to make undue profits. Lack of electric supply and/or diesel outlet is big problems being faced by the farmers in the study area. Due to very small size of holdings and large number of individual holdings, the financial position of individual farmers to own individual tube wells is very difficult and of uneconomical proposition. Only cooperatives, association or share of farmers can have the full benefits from the shallow tube wells if they are suitably supported and encouraged by the concerned authority.

In the study area, the water sellers install their wells or pumps in their own plots or on other farmers' plot to irrigate their own plots and sell excess water to other farmers under

varied contractual arrangements. There are very few water sellers who do not have any land in their tubewell command areas and run their pumps absolutely with business interests. Small farmers dominated the ownership of STWs. Entry or exit of pump owners in the irrigation business depends on profits or losses, pump capacity, size of the command area, operating capital, managerial efficiency, condition of machine, reputation, social relationship etc. The labor engaged in agriculture has also been declining. Apart from the rural to urban migration of households, there has also been a movement from farm to non-farm occupations within the rural areas supported by the opportunities of employment created in the rural trade and transport sectors with the expansion of rural roads, the increased marketed surplus of agricultural product, improvement of literacy rates, migration to foreign countries etc. However, there is huge potentiality of agricultural expansion applying and increasing suitable technology and enhancing knowledge and efficiency of local farmers. Moreover, the provision of electricity for irrigation pumps should be given top priority, as the cost of irrigation by electric pumps is about 50% lower than that for diesel run pumps. There should have diesel supply at subsidized rate until and unless the electricity reaches the irrigation pump motors in the study area.

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FARMERS' PERCEPTION ON WATER RELATED ISSUES: A STUDY OF BHERAMARA UPAZILA, KUSHTIA

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Abstract

Bheramara *Upazila* of Kushtia District, located in the southwestern part of Bangladesh is one of the most affected areas due to severe water flow reduction in the lower part of the Ganges (the Padma) which is seriously hampering agricultural production. The local people/farmers are tremendously suffering due to water related problems for their domestic as well as agricultural activities. The present study based on the primary field investigation (including open ended questionnaire interview with a total of 100 farmers), key informant interviews and direct field observation attempts to identify farmers' perception on water related issues and to understand the process, of how these problems are mitigated both in domestic and agricultural sectors.

Keywords: Water Issues, Farmer's Perception, Mitigation Measures, Bheramara *Upazila*

Introduction

Water is one of the most important natural resources being used in a variety of ways at many different levels (McDonald and Kay 1988). It is among the important requisites that nature provides to sustain life for plants, animals and humans. At the same time, water related problems are also becoming increasingly more and more interconnected with other development related issues and also with socioeconomic, environmental, legal and political factors, at local, national and sometimes at regional and even international levels (Biswas 2005). Bangladesh is, however, endowed with enormous water resources as it is crisscrossed by a large number of intricate (more than 200 rivers along with 54 common rivers with India and 3 with Myanmar) systems (Rasheed 2011). Although the country is recognized as the land of rivers on the globe, it has already experienced severe problems related to water scarcity in one of its major rivers, the Padma especially in the drier months. The country being located at the mouth of the lower end of the three great rivers (the Ganges, the Brahmaputra and the Meghna) has a deltaic fertile landmass. Among the three river systems the moribund part (southwestern region of Bangladesh) of the delta is totally dependent on the Ganges water (Rob *et al.* 2003). The Ganges, the most sacred of all the Hindus, rises west to the Nanda Devi Range in the Himalayas (Rashid 1991). The traditional source of the Ganges, however, is known as the Bhagirathi river that rises in India from the Gangotri glacier in the Himalayas at an elevation of more than 7,000 meters (Rasheed 2008). In 1975, after the commissioning of the Farrakka Barrage, the case of Indo-Bangladesh relations over the Ganges water sharing typically calls validity

of this argument into question (Crow *et al*. 1995). Since the barrage was built by the Indian Government to divert the Ganges river water into the Hoogly river and to make the Kolkata port alive, it suddenly ceased significant water flow into the downstream in Bangladesh. Before the initiation of the project, it was largely felt by the Pakistani political leaders that inadequate water flow in the river channels would enhance siltation at river beds leading to the reduction of depth and navigability and increase proneness due to the rivers' retreating conveyance capacity. About 2.5 billion tons of sediments are annually carried to the Bay of Bengal through the Meghna estuary (Uddin *et al.* 2011). A part of the huge sediments is accumulated in the beds of major rivers resulting in the decrease of water level. Since water is crucial in the formation of so many phenomena, both living and non-living, it is not surprising that its diversion and use by humans would often cause environmental effects (Simmons 1993). However, the construction of Farrakka Barrage is basically responsible for water flow reduction in the lower part of the Ganges causing severe threats to a large number of people of the Ganges dependent areas (e.g., south and southwestern parts of Bangladesh). The gradual reduction of water flow in the river Padma has already caused severe social, economic and environmental problems in the downstream river basin especially in the moribund part of Bangladesh (Dewan and Nizamuddin 1999). It is reported that various environmental problems are often related to inadequate water quality that include sedimentation, saline intrusion and wetland and biodiversity loss (Ahmad 2000). After the construction of the Farrakka barrage, water flow in the Padma reduced remarkably and the lower part of the Ganges and its dependent area especially the southwestern region has been largely suffering (Crow *et al.* 1995). On the other hand, after the independence in 1971, the population of Bangladesh has been more than doubled. So, the competition over water use in fishery, agriculture, navigation etc. has enormously increased. The case of water related issues therefore, increases the conflict over trans-boundary river water. It then suggests that the conflict needs to be solved not only internationally on the Governmental level but also domestically within the particular local context of the affected areas. The local farmers of the present study area have already experienced many problems related to water use especially for agricultural production along with domestic use.

The present study attempts to understand local people's (areas those are located close to the Padma river) perception (especially the farmers' perception) regarding water related issues and the process of how the farmers are mitigating these problems both in household level and in agriculture. The present study can help us to understand and address of how and to what extent governance is functioning at a grass root level in rural life of Bangladesh. It can also be assessed with a particular reference to the role of local Government. Apart from the intervention by the central Government and bilateral negotiation about water sharing agreement, the roles played by the local Government are of great importance in addressing and mitigating the problems of the local people. The study on micro-level socio-political process can possibly explore the possibilities and limitations of the local Government in achieving more equitable resource allocation and

forming the local basis of democratic governance over water. Another relevance of the study is related with the debate on the role of Non-Governmental Organizations (NGOs) and civil society in forming people centered-governance over resources and in consolidating democracy at a grass root level in Bangladesh.

In the context of Bangladesh, it is often argued that the active role of NGOs in this country reflects the weakness of the state and limited capacity of the Government to meet people's needs. It is also reported that NGOs activities are mostly limited in the private sphere of people's daily life and they hardly contribute to strengthen the state-society relations and thus to enhance democratic governance in this country. The study may also contribute to this debate on the role of NGOs and local Government in Bangladesh by exploring the scope of the role played by NGOs concerning water issues.

Materials and Methods

For the purposes mentioned above, a Participatory Rural Appraisal (PRA) technique was conducted in randomly selected two villages of the study area (Fig. 1) that are affected with reduced water flow from the Ganges. The PRA approach was helpful in identifying issues on water faced by the local farmers, and in cross-checking their opinions raised in the informal meetings. First of all, in order to understand the water crisis situation of the study area, a rigorous field observation was made by the study team. Informal group discussions with the farmers in the villages were also conducted, while various issues related to water scarcity were widely raised by the farmers. In the group discussions with the farmers, the local representatives and other key informants (for example, *imams* of mosques) also participated. The group discussions with farmers of different category have provided necessary and important information. Alongside, in order to have individual farmer's perception on water related problems, an open ended interview method was applied. A total of 100 randomly selected local farmers of several different categories (based on their level of income, education and age) of the study site were interviewed. Questions were mainly concerned with the present nature of the water-related problems, background and factors behind their emergence response taken by each category of people and changes in the nature and extent of the problems in course of social interaction. For the information, interviews were conducted among the farmers of different levels (elite/land owners, middle class farmers, small farmers, landless workers or tenant/share croppers and women as household heads). At the same time, local Union Parishad (UP) chairmen and members, elderly people/village *matbors, imams,* teachers of primary and high schools were interviewed with a view to cross-checking the water related issues as perceived by the farmers.

Study Site: Bheramara *Upazila* of Kushtia District is one of the most affected areas due to the reduction of water flow in the Ganges hampering serious agricultural production. Juniadaha Union of this *Upazila* has been purposively selected for the present study.

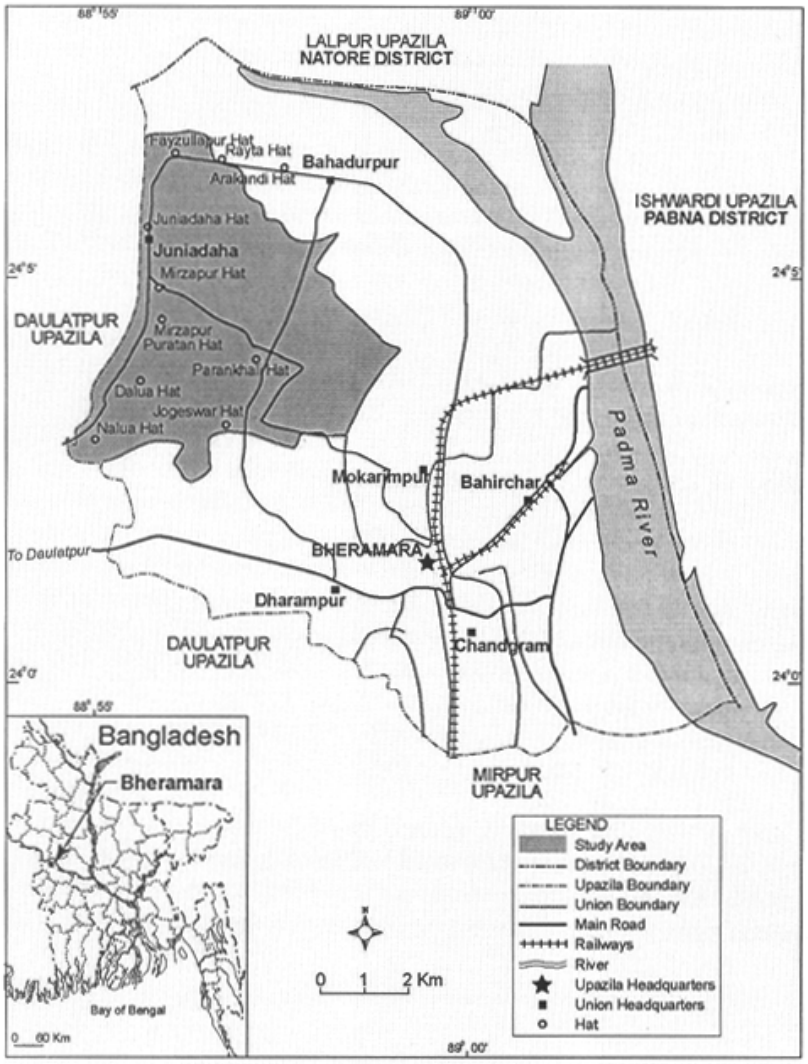

Fig. 1 Location of the Study Area

Geographically, the area is located in the south-western part of Bangladesh between 24°0' and 24°10' north latitudes and 88°55' and 89°0' east longitudes. The study site has an area of 7,544 acres (BBS 2007), and is very closely located to the river Padma (lower stream of the Ganges). One of the major distributaries (the Gorai) of the Padma is also very close to this region (Fig. 1).

Although the location of the union is very near to the big river, the farmers and the people of all levels are immensely suffering due to water related problems. Chemical fertilizers

are often used in agriculture in the study area and lead to the deterioration of water quality. A large number of shallow tube-wells and deep tube-wells of Kushtia district are directly affected due to over withdrawal of upstream Ganges water (BBS 2005). Arsenic contamination of water has already been a threat to the domestic-level water users. The highest level of arsenic contamination is found in the large basin of the Padma and high concentration of arsenic in the southern and southwestern part of the country (Khuda 2001). Local people are also gradually being dependent on polluted pond water for domestic uses due to the decline of ground water table. The present study however, is an attempt to analyze the farmers' perception regarding water related problems and the mitigation strategies taken by the locals.

Results and Discussion

Emergence of Water Related Problems within the Study Area: The area started facing water related problems mainly after the Farakka Barrage was constructed in 1970 (Rasheed 2011). The severity of water scarcity in the study area started a few years later through the gradual rise of water demand especially for agriculture. In contrast, India continued to withdraw water at Farakka to the full capacity of the feeder canal in the dry season without any further understanding or agreement with Bangladesh. The Padma of Bangladesh experiences severe water flow problem during the lean season. In April 1976, the Ganges flows came down to only 23,200 cusec at Hardinge Bridge from the pre-Farakka flows of around 65,000 cusec (Rasheed 2008). It is evident that the 2,246 meter long Farakka Barrage, located 18 km upstream of Bangladesh near Mohonpur, has been a long lingering source of conflict and tension between Bangladesh and India (Rasheed 2011). Primarily, the barrage had two objectives, a) to maintain navigability of the Hoogly river and flush out the silt deposited in the Kolkata Port and b) to ensure saline free water supply for Kolkata City (Crow *et al*. 1995). However, the Bangladeshi Government tried to negotiate in different forums to ensure adequate flows of water in the Padma river but the situation could not be developed since that period. In the earlier period, monsoon water could enter in the study area due to the construction of embankments along the bank of the Padma river for which water sources declined. In the dry season, most of the ponds, canals and small rivers become dried up and ground water becomes the only source during this period.

Farmers' Problems over Water: Availability of water has been a valuable and scarce resource to people as well as to the farmers. Agricultural sector is severely affected due to water shortage in the dry season in the study area. In the present study, it has been observed that the farmers have to suffer both for irrigation and domestic usages (Table 1). In contrast, those who are relatively well-off have to invest a lot to withdraw ground water in the dry season through shallow-pumps. It has also been observed that the irrigation system of the study location is not well developed. Besides, many of the farmers are incapable of purchasing irrigated water and therefore, have to wait until the

monsoon starts. Alongside, due to over-exploitation of the lands, their lands get infertile and require external inputs. In parallel with water scarcity for the agricultural crops, the farmers of the study area are also suffering from water for drinking and other domestic usage. As a result they often depend on the polluted pond water for the domestic purposes that eventually lead to high health risks. In the area, those who afford tube-wells for drinking water have also to encounter arsenic contamination.

Table 1. Water Related Problems Faced by the Farmers.

Problems (Agriculture)	Number of Responses (%)
Water shortage for irrigation	42(12.6)
Dependence on rain water	36(10.8)
Incapable of purchasing water for irrigation	31(9.3)
Irrigation system is not available	29(8.7)
Rice is not cultivated timely due to lack of water	26(7.8)
Do not get water for irrigation timely	25(7.5)
The water level decreases	22(6.6)
Soil fertility decreases	18(5.4)
Increase of using chemical fertilizers	28(8.4)
Less profit from agriculture due to unavailability of water	20(6.0)
Problems (Domestic)	
Do not have tube-well water for drinking	17(5.1)
Arsenic contamination	12(3.6)
Water level decreases	10(3.0)
Need to depend on polluted pond water	8(2.4)
Do not get water timely from the shared tube-wells	6(1.8)
No domestic problems	4(1.2)
Total (N=100)	334*(100.0)

**Multiple answers were considered*

Factors Causing the Water Issues: Various factors are responsible for creating water related problems in the study area. The water flow in the Padma has tremendously declined in the recent years, whereas water usage has rapidly increased with population growth. The inadequate supply of fresh water in the Ganges system due to upstream diversion threatened on irreversible environmental and agro-ecological damages in the study area. Within a few decade after the barrage was commissioned, water related issues have been identified as a national issue of Bangladesh. Climate change impacts such as fluctuations of rainfall are observed in the study area. In 1974 a total population of the study area was 17,230 (BBS 1977) and this figure has reached to 31,377 in 2001 (BBS

2007). The rapid rise of population enormously increased the demand of water in the study location (Table 2).

Table 2. Factors Causing Water Related Problems.

Factors	Number of Responses (%)
The Padma river does not have enough water	36 (25.4)
The water usage has enormously increased	32 (22.5)
It does not rain timely in the rainy season	24 (16.8)
Farrakka barrage	20 (14.1)
Population have increased	18 (12.7)
Not aware of the factors	12 (8.5)
Total (N=100)	142* (100.0)

**Multiple answers were considered*

Farmers' Responses on Water Scarcity: The people of the study area are mostly involved in agriculture (31%) (BBS 2007). The local farmers think differently when water related problems arise in the study area. The farmers usually change the cropping pattern when they face shortage of water. They try to create new sources of water and water usage techniques (Table 3). They cultivate those crops which require less water and sometimes they introduce new technology to fulfill the immediate demands of water. Local farmers try their best to make the availability of water to their fields. In some cases, they change their occupation and invest money in non-agricultural activities. The marginal farmers who used to cultivate for subsistence agriculture sometimes have to work as the day-laborers.

Table 3. Immediate Response of Farmers in Mitigating Water Issues.

Action/Responses by farmers	Number of Responses (%)
Changes in crops to grow	28 (24.6)
Changes in cultivation	25 (21.9)
Changes in source of water and water use	18 (15.8)
Introduction of new technology	16 (14.0)
Changes in occupation and source of income	15 (13.6)
Investment in non-agricultural activities	10 (8.8)
Others	2 (1.8)
Total (N=100)	114* (100.0)

**Multiple answers were considered*

Seasonal Migration due to Water Related Problems: Due to water related problems the farmers have to migrate seasonally from the study area to other places such as- Bheramara Sadar, Khustia, Dhaka City etc. in order to get employments. This situation

happens almost in every year leading them to go outside their area for works. The farmers fail to manage their family needs from the agriculture. The hopeless farmers try to involve into other activities as they find difficulties to sustain their livelihoods in farming. It has been observed from the field that some of the farmers have to migrate seasonally due to crop failure. Besides, the agricultural production costs have remarkably increased in the recent years leading them to remain away from farming.

Further Problems Due to Water Shortage: The water shortage problems in the study area are increasing over time. Ground water is an important component of the water ecosystem in Bangladesh especially for domestic and irrigation requirements. Dependencies on ground water are rising at an alarming rate. Some surveyed areas are gradually becoming dependent on rain water. Farmers invest more money for fertilizer when they observe that the water supply is inadequate. Siltation could not occur regularly as the area is surrounded by dams which influence in declining soil fertility. In some areas soil colour has changed due to shortage of water. Ultimately, the production capacity of lands of the study villages has been dropping over time (Table 4).

Table 4. Further Problems Due to Water Shortage.

Further Problems	Number of Responses (%)
Dependence on irrigation/ground water increases	42 (23.2)
Totally dependent on rain water	35 (19.3)
Use of chemical fertilizers increases	34 (18.9)
Soil fertility decreases due to lack of siltation	28 (15.5)
The soil colour has changed	20 (11.0)
Productivity of land decreases	12 (6.6)
Not aware of the problems	10 (5.5)
Total	181* (100.0)

**Multiple answers were considered*

Role of Local Government: Local Government plays very little role to solve the water related problems in the study area. But it is very important to involve the representatives of the local Government for any kind of intervention in the community. In the farmers' opinions, no remarkable measures were taken by the local Government, while the local Government authority occasionally sets up new deep tube-wells to meet the demand (Table 5). The Department of Public Health and Engineering (DPHE) with the assistance of local Government distributes tube-wells among the inhabitants of the area. In parallel, they identify the arsenic contaminated tube-wells and encourage people to avoid using water from those tube-wells. The authority seldom dredges the surrounding canals to make the proper flow of water. Given the situation, the local Government has a pivotal role in maintaining supportive activities for the local farmers especially through

providing tube-wells, making them aware of water usage, risk of using pesticides and fertilizers etc.

Table 5. Measures Taken by the Local Govt. to Mitigate Water Issues.

Measures taken	N (%)
No remarkable measures taken	40 (27.3)
Set up new deep tube-wells	35 (24.0)
Trying to dredge the canal surrounded by the union	32 (21.9)
Provide tube-wells for each *mahalla* for drinking water	23 (15.8)
Trying to remove arsenic contaminated tube-wells	16 11.0)
Total (N=100)	146* (100.0)

**Multiple answers were considered*

The Role of Central Government: The central Government does not provide any assistance to the farmers directly. It has been known from the farmers that they hardly receive agricultural goods (seeds, fertilizers, pesticides, equipments etc.) from the UP chairman. In this study, it has been known from the chairman that the central Government has a particular allocation for each union. Sometimes, the Union Parishad receives crop seeds, agricultural equipments etc. The UP chairman opined that the amount of goods they receive is not sufficient enough to satisfy all the farmers. The UP chairman also informed that the central Government is trying to provide sanitation equipments and set up arsenic free tube-wells at every household through the local elected representatives. On the other hand, the farmers opined that the central Government has seldom taken any initiative to solve the water related problems in domestic and agricultural sectors.

Improvement of Water Issues through Power Structure Change: Water resources of Bangladesh are characterized by a number of challenges and achievements. In ancient time, water was collected from dig wells and natural springs or artesian wells (where ground water in confined aquifers flows out on to the surface under hydrostatic pressure). In modern time, diesel and electric pumps withdraw water in larger quantities to supply the farmers as well as to whole population. In the study area, ground water is currently exploited for nearly 80% of the total cultivated land. It is extracted through both the shallow tube-wells (STWs) and deep tube-wells (DTWs) -the latter with depths of more than 100 meters and having a much larger command area. STWs are very popular among the farmers in Bangladesh, and the total number of STWs is now more than 450 and while there are about 40 DTWs in the area. The unreliable availability and fluctuating quality of surface water resources have prompted the authorities to start developing a groundwater supply system in the 1970s, installing wells in an effort to provide safe drinking water. Since arsenic was discovered in Chapai Nawabganj of Bangladesh, the local authority tried to mark the arsenic contaminated STWs and DTWs in the area and made some efforts to provide arsenic free water to the people. Government initiatives

regarding water issues has also solved a considerable problems of water in the study area. Water supplies for agriculture will have to be increased by an additional 15 to 20% over the next 25 years, even after favourable assumptions regarding improvements in irrigation efficiency and crop yield performances.

Measures for Improving Water Related Issues: Farmers of the study area opined that water related problems should be solved without making any delay otherwise the whole community will be seriously affected. In certain cases, dredging of the rivers or *khals* may be beneficial but there is a tendency for dredged section to silt up. Dredging canal to connect with the Padma river was suggested by 25% of the respondents. Some of them (22%) suggested digging new canals throughout the year to enhance the normal flow of water (Table 6). Huge number of new shallow and deep tube-well set up may be another solution. Hand tube-well (HTW) provide about 85% of rural population with potable water supplies (UNDP 1993). Some commented on opening the Farakka Barrage for a certain period of a year. The sitting Government should take effective measures to solve the water disputes between the neighbouring countries. A few of the farmers seems to be frustrated about the water issues. They think that the water problem is deteriorating gradually and situation will not be improved.

Table 6. Necessary Actions/Measures for Improving the Situation.

Actions/Measures	Number of Responses (%)
Dredging canal to connect with the Padma River	48(24.9)
Digging new canals to supply water through out the year	42(21.8)
To set up new shallow and deep tube-wells	38(19.7)
Farrakka Barrage should be opened	30(15.6)
Government should take initiatives	15(7.8)
This situation can not be improved	12(6.2)
Do not know how to improve	8(4.1)
Total (N=100)	193*(100.0)

**Multiple answers were considered*

The Role of NGOs: One way to contact local people and provide various types of services to the people is through NGOs. These organizations may also be of great assistance at solving the water related issues. Different NGOs are working in the study area to improve the water issue. The role of Grameen Bank, Bangladesh Rural Advancement Committee (BRAC), ASA, Prosika etc. are remarkable and they are providing loan facility to the local people for buying tube-wells, supplying and installing shallow and deep tube-wells. As the rain water is available in the monsoon period, BRAC recently has donated money and technical support in five places in the study area for rain

water harvesting. These NGOs are trying to ensure the quality of water to the local people with the Government authority.

Farmers' Perception on Water Agreement Treaty of 1996: Since the partition in 1947, India and Pakistan (later Bangladesh) have been in conflict over their rights to use the water of the Ganges. Limited agreements between the two countries have reached, and some progress has been made toward effective and equitable utilization of the river (Crow *et al*. 1995). Bangladesh and India signed an agreement on the Ganges water sharing on 12 December 1996 for a period of 30 years. According to the Ganges Treaty of 1996, as a downstream country Bangladesh is supposed to receive a balance of flow in the case of maximum availability of water (75,000 cusec or more) (Rasheed 2008). Alongside, in case of the availability of 70,000 cusec or less, both Bangladesh and India should receive 50% each. On the other hand, the farmers of the Ganges Dependent Area (GDA) are directly affected by the fluctuating flow of the Ganges water.

Table 7. Improvement of Water Related Issues after the Treaty in 1996.

Types of Change	N (%)
No improvement has taken place	56 (56)
We get enough water in rainy season	17 (17)
Not aware of the treaty	27 (27)
Total (N=100)	100 (100.0)

In the present study, it has been found that a remarkable number of farmers (56%) opined that the agreement did not bring any improvement of water related issues after 1996. The second highest proportion of respondents (27%) is not aware of the treaty and the least proportion of farmers (17%) reported that they receive enough water in rainy season (Table 7). It can, however, be mentioned that the farmers severely suffer for water for various purposes whether the issues are well-perceived by most of them or not. The scarcity of water in the study area has also led significant number of farmers to migrate to different areas for works and to change their occupations. In contrast, there is hardly any assistance from the Government (local Government) and the NGOs towards the mitigation of the water related issues in the study area. In such a situation, the local farmers and villagers have to be provided with arsenic free tube-wells for safe drinking water and technologies to support agricultural practices. At the same time, the best solution will be to maintain normal water flow in the lower part of the Ganges. The regional cooperation of water sharing (e.g., existing water sharing treaty) therefore, has to be enhanced through bi-lateral negotiation, if the entire southwestern part of the country has to survive.

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A PRELIMINARY CHECKLIST OF THE ANGIOSPERMIC FLORA OF DAULATPUR UPAZILA IN KUSHTIA DISTRICT, BANGLADESH

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Abstract

A total of 125 angiosperms under 103 genera and 52 families have been recorded from Daulatpur Upazila in Kushtia. Magnoliopsida is represented by 41 families, 73 genera and 86 species, whereas Liliopsida is represented by 11 families, 30 genera and 39 species. In Liliopsida, Poaceae appears to be the largest family comprising 15 genera and 20 species. In Magnoliopsida, Euphorbiaceae appears to be the largest family comprising 9 genera and 14 species.

Key words: Angiospermic flora, Daulatpur Upazila, Kushtia

Introduction

Daulatpur Upazila is located in Kushtia district and lies between 23º52′ and 24º12′ N latitudes and 88º42′ and 88º58′ E longitudes. It is bounded on the north by Bagha upazila of Rajshahi district and Lalpur upazila of Natore district, on the east by Mirpur upazila, on the south by Mirpur upazila and Gangni upazila of Meherpur district. The soil mainly calcareous brown floodpain and composed of alluvium and sandy clay with a good percentage of potash and phosphate (Mondal 2003). The area enjoys a tropical climate characterized by a period of high precipitation from June to August and six months of relatively dry period from November to April. The mean annual rainfall is about 1080 mm. Temperature of the area ranges from 21.0-33.7°C. The maximum temperature was recorded in July and the minimum was recorded in January during the study period (Source: Bangladesh Meteorological Department). This upazila is mainly cultivated area and covers an area of about 468.76 square kilometres. There are fallow land, crop fields, ponds, ditches and beels in the study area.

Daulatpur Upazila consists of medium grade plant diversity. A good number of floristic studies have so far been done in Bangladesh including (Khan and Alam 1996, Uddin and Rahman 1999, Uddin *et al*. 2003, Tutul *et al*. 2009, Rahman *et al.* 2010 and Arefin *et al.* 2011). No floristic studies are available in Daulatpur Upazila of Kushtia district. Moreover, the area supports many angiospemic species including herbs, shrubs, trees, climbers, epiphytes, parasites and also plenty of hydrophytes. Like other parts of the country, the floristic elements of this area are in risk because of various anthropogenic activities including irrigation, modern agriculture, population settlements, firewood collection and also habitat degradation. In order to make a documentation of the

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angiospermic vegetation of the area, an attempt has been made to prepare a preliminary checklist of the angiospermic plant species occurring in Daulatpur Upazila of Kushtia district.

Materialas and Methods

The work is based on the fresh materials collected through four field trips to Daulatpur area during 2004 and 2005. Botanical specimens were collected and identified at the department of Botany, University of Dhaka. Identifications were confirmed comparing with herbarium specimens at Dhaka University Salar Khan Herbarium (DUSH) and Bangladesh National Herbarium. In some cases, standard literatures, such as Hooker (1872-1897), Prain (1903), Khan (1977, 1984, 1985) and Uddin and Hassan (2004) were consulted for identification purpose. The families have been arranged according to Cronquist (1981). The specimens were deposited in the Dhaka University Salar Khan Herbarium (DUSH) for future reference.

Results and Discussion

In the present survey, a total of 125 angiospermic species under 103 genera and 52 families were recorded from Daulatpur Upazila. Magnoliopsida is represented by 41 families, 73 genera and 86 species, while Liliopsida is represented by 11 families, 30 genera and 39 species. Habit wise itemization of plant speices shows that 58.4% of the total species represented by herbs, 13.6% by shrubs, 18.4% by trees, 8.8% by climbers and 0.8% by parasites. The genera under each family and the species under each genus are arranged in an alphabetic order. For each species, nomenclature has been brought up to date. Local name (wherever available) and a short annotation are provided.

Magnoliopsida (Dicots)

1. Annonaceae: **Annona reticularia** L., Sp. Pl.: 537 (1753). *Local names*: *Nona ata, Nona, Ata.* A small tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 54 (DUSH).

Annona squamosa L., Sp. Pl.: 537 (1753). *Local names*: *Sharifa, Sitaphal, Maoya*. A small tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 59 (DUSH).

2. Lauraceae: **Litsea glutinosa** (Lour.) C.B. Robinson in Philip. J. Sci. Bot. 6: 321 (1911). *Sebifera glutinosa* Lour., Fl. Cochinch.: 638 (1990). *Local names: Chapaitta*, *Menda, Papoulti*. A medium-sized, evergreen tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 44 (DUSH).

3. Piperaceae : **Peperomia pellucida** (L.) H.B.K., Nov. Gen. *et*. Sp. 1: 64 (1815). *Piper pellucidum* L., Sp. Pl. 1: 30 (1753). *Local names*: *Peperomia*, *Luchipata*. A small, annual herb. *Representative specimen*: Udoynagar, 17.11.2004, Md. Moniruzzaman 96 (DUSH).

Piper longum L., Sp. Pl. 1: 28 (1753). *Local names*: *Pipul, Pipla, Pipla-mul, Pipul-morich.* A perennial herb, branched with swollen nodes. *Representative specimen*: Udoynagar, 17.11.2004, Md. Moniruzzaman 92 (DUSH).

***4. Menispermaceae*: Stephania japonica** (Thunb.) Miers, Ann. Mag. Nat. Hist. Ser. 3, 18: 14 (1866). *Menispermum japonicum* Thunb., Fl. Jap.: 193 (1784). *Local names*: *Maknadi, Aknadi, Bommokopat*. A slender climber. *Representative specimen*: Udoynagar, 25.09.2004, Md. Moniruzzaman 13 (DUSH).

***5. Papaveraceae :* Argemone mexicana** L., Sp. Pl.: 508 (1753). *Local names*: *Sialkanta, Barosial-kanta*. An annual herb. *Representative specimen*: Udoynagar, 24.01.2005, Md. Moniruzzaman 156 (DUSH).

***6. Ulmaceae:* Trema orientalis** (L.) Bl., Mus. Bot. Lugd.-Bat. 2: 63 (1856). *Celtis orientalis* L., Sp. Pl.: 120044 (1753). *Local names*: *Jilan, Narsa, Gobra*. An evergreen small tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 66 (DUSH).

***7. Moraceae:* Artocarpus chaplasha** Roxb., Fl. Ind. 3: 525 (1832). *Local name*: *Chapalish*. A lofty deciduous tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 39 (DUSH).

Ficus benghalensis L., Sp. Pl.: 1059 (1753). *Local name*: *Bot*. A large, spreading tree. *Representative specimen*: Shampur, 10.06.2005, Md. Moniruzzaman 219 (DUSH).

Ficus heterophylla L. f., Suppl.: 442 (1781). *Local name*: *Bhuidumur.* A hispid, scandent shrub. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 97 (DUSH).

Ficus hispida L. f., Suppl. Pl.: 442 (1781). *Local names*: *Kakdumur, Dumur, Khukshidumur*. A small tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 49 (DUSH).

Ficus religiosa L., Sp. Pl.: 1059 (1753). *Local names*: *Ashathwa*, *Pipal*. A large tree. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 100 (DUSH).

***8. Urticaceae*: Laportea interrupta** L., Sp. Pl.: 985 (1753). *Local name*: *Lal Bichuti*. An erect annual herb. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 11 (DUSH).

***9. Nyctaginaceae:* Boerhavia diffusa** L., Sp. Pl. 1: 3 (1753). *Local names*: *Punarnava, Gondhapurna*. A perennial herb. *Representative specimen*: Daulatpur, 27.09.2004, Md. Moniruzzaman 88 (DUSH).

***10. Chenopodiaceae :* Chenopodium ambrosioides** L., Sp. Pl.: 219 (1753). An annual, erect herb. *Representative specimen*: Barogandia, 24.01.2005, Md. Moniruzzaman 155 (DUSH).

11. Amaranthaceae : **Achyranthes aspera** L., Sp. Pl. 1: 204 (1753). *Local name*: *Apang*. A perennial herb. *Representative specimen*: Barogandia, 26.09.2004, Md. Moniruzzaman 58 (DUSH).

Alternanthera philoxeroides (Mart.) Griseb., Abh. Ges. Goett. Wiss 24: 36 (1879). *Bucholzia phyloxeroides* Mart., Beitr. Amar.: 107 (1825). *Local name*: *Helencha.* An annual herb. *Representative specimen*: Barogandia, 26.09.2004, Md. Moniruzzaman 65 (DUSH).

Alternanthera sessilis (L.) DC., Cat. Pl. Hort. Monspel.: 77 (1813). *Gomphrena sessilis* L., Sp. Pl.: 225 (1753). *Local names*: *Chanchi, Haicha, Sachisak.* A herb, usually decumbent or prostrate. *Representative specimen*: Boalia, 26.09.2004, Md. Moniruzzaman 21 (DUSH).

Amaranthus spinosus L., Sp. Pl. 1: 991 (1753). *Local names*: *Kanta-note, Kantadenga, Katamiris*. An annual, erect, spinescent herb. *Representative specimen*: Daulatpur, 25.09.2004, Md. Moniruzzaman 19 (DUSH).

Amaranthus viridis L., Sp. Pl. ed. 2: 1405 (1753). *Local names*: *Note sak, Marissag*. An annual, erect or decumbent, small, slender herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 186 (DUSH).

12. Polygonaceae : **Polygonum plebejum** R. Br., Prodr. Fl. Nov. Holl.: 420 (1810). A prostrate or diffuse herb. *Representative specimen*: Malipara, 25.01.2005, Md. Moniruzzaman 165 (DUSH).

Rumex maritimus L., Sp. Pl.: 335 (1753). *Local name: Ban palang*. An annual herb. *Representative specimen*: Barogandia, 24.01.2005, Md. Moniruzzaman 145 (DUSH).

13. Bombacaceae : **Bombax ceiba** L., Sp. Pl.: 511 (1753). *Local names*: *Shimul tula, Pakra, Tula gachh*. A large tree with buttress base. *Representative specimen*: Malipara, 26.09.2004, Md. Moniruzzaman 43 (DUSH).

14. Malvaceae : **Urena lobata** L., Sp. Pl.: 692 (1753). *Local name: Banokra*. An undershrub. *Representative specimen*: Malipara, 26.09.2004, Md. Moniruzzaman 16 (DUSH).

15. Passifloraceae : **Passiflora foetida** L., Sp. Pl.: 959 (1753). *Local names: Jhumkolata, Gorakphul*. A climbing slender shrub. *Representative specimen*: Malipara, 27.09.2004, Md. Moniruzzaman 82 (DUSH).

16. Cucurbitaceae : **Coccinia grandis** (L.) Voit., Hort. Suburb. Calcut.: 59 (1845). *Bryonia grandis* L., Mant. Pl. 1: 126 (1767). *Local names: Kawajhinga, Telakucha*. A much branched, climbing or prostrate herb. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 101 (DUSH).

17. Moringgaceae : **Moringa oleifera** Lam., Encycl. 1 (2): 398 (1785). *Local names*: *Sajna, Sojne*. A small tree. Repres*entative specimen*: Goalgram, 17.11.2004, Md. Moniruzzaman 94 (DUSH).

18. Primulaceae : **Anagallis arvensis** L., Sp. Pl.: 148 (1753). Erect or decumbent-ascending; glabrous-dotted herbs. *Representative specimen*: Goalgram, 25.01.2005, Md. Moniruzzaman 159 (DUSH).

19. Mimosaceae: **Acacia nilotica** (L.) Delile subsp. **indica** (Benth.) Brenan in Kew Bull. 12: 84 (1957). *Mimosa nilotica* L., Sp. Pl.: 152 (1753). *Local names*: *Babla, Babul*. A tree. *Representative specimen*: Khalishakundi, 09.06.2005, Md. Moniruzzaman 194 (DUSH).

20. Caesalpiniaceae : **Senna tora** (L.) Roxb., Fl. Ind. 2: 340 (1832). *Cassia tora* L., Sp. Pl: 376 (1753). *Local name*: *Chakunda*. A perennial, erect, foetid, often profusely branched herb or undershrub. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 51 (DUSH).

21. Fabaceae : **Desmodium heterophyllum** (Willd.) DC., Prodr. 2: 334 (1825). *Desmodium triflorum* Wight. & Arn., Prodr.: 229 (1834). A procumbent herb. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 42 (DUSH).

Erythrina variegata L., in stickm. Herb. Amboin.10 (1754). *Local names*: *Mandar, Madar, Plitamadar, Paniamadar*. A deciduous, prickly tree. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 45 (DUSH).

22. Onagraceae: **Ludwigia hyssopifolia** (G. Don) Exell., Garica de Orta 5: 471 (1957). *Jussiaea hyssopifolia* G. Don, Gen. Syst. 2: 693 (1832). A branched herb. *Representative specimen*: Udoynagar, 17.11.2004, Md. Moniruzzaman 104 (DUSH).

23. Euphorbiaceae: **Acalypha indica** L., Sp. Pl.: 1003 (1753). *Local name*: *Muktajhuri*. A small robust or woody herb. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 60 (DUSH).

Chrozophora plicata Hook. f., Fl. Br. Ind. 5: 409 (1887). An annual herb. *Representative specimen*: Daulatpur, 09.06.2005, Md. Moniruzzaman 200 (DUSH).

Croton bonplandianus Bill., Adansonia 4: 339 (1864). *Local name*: *Moricha*. An annual herb. *Representative specimen*: Boalia, 25.01.2005, Md. Moniruzzaman 184 (DUSH).

Euphorbia antiquorum L., Sp. Pl.: 42005 (1753). An erect, trunk straight tree. *Representative specimen*: Boalia, 25.01.2005, Md. Moniruzzaman 84 (DUSH).

Euphorbia heyneana Sprengel, Syst. Veg. 3: 791 (1826) *Local name*: *Chhota Kerui*. An annual herb. *Representative specimen*: Udoynagar, 25.09.2004, Md. Moniruzzaman 10 (DUSH).

Euphorbia hirta L., Sp. Pl.: 454 (1753). *Local names*: *Dudhia, Ghaopata, Baradudhi.* An annual, erect or ascending herb. *Representative specimen*: Boalia, 26.09.2004, Md. Moniruzzaman 33 (DUSH).

Euphorbia tithymaloides L., Sp. Pl.: 453 (1753). *Local name*: *Rangchita*. A subsucculent, shrub. *Representative specimen*: Daulatpur, 22.09.2004, Md. Moniruzzaman 91 (DUSH).

Jatropha curcas L., Sp. Pl.: 1006 (1753). *Local name: Veron*. A large, glabrous shrub or rarely small tree. *Representative specimen*: Daulatpur, 27.09.2004, Md. Moniruzzaman 85 (DUSH).

Jatropha gossypifolia L., Sp. Pl.: 1006 (1753). *Local names: Lalbherenda, Laljeol*. A small shrub. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 31 (DUSH).

Phyllanthus fraternus Webster, Contr. Gray Herb. 176: 53 (1955). *Phyllanthus niruri* senu Hook. f., Fl. Brit. Ind. 5: 298 (1887). *Local name*: *Bhui amla*. A herb. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 108 (DUSH).

Phyllanthus reticulatus Poir. in Lamk., Encycl. Meth. B. 5: 298 (1804). *Local name*: *Panseuli*. A large, scandent shrub. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 184 (DUSH).

Phyllanthus urinaria L., Sp. Pl.: 982 (1753). An erect, glabrous, annual herb. *Representative specimen*: Daulatpur, 25.09.2004, Md. Moniruzzaman 5 (DUSH).

Ricinus communis L., Sp. Pl.: 1007 (1753). *Local name*: *Rerhi*. An evergreen shrub. *Representative specimen*: Daulatpur, 24.01.2005, Md. Moniruzzaman 124 (DUSH).

Trewia nudiflora L., Sp. Pl. ed. 3: 166 (1753). *Local names: Lattu, Pitali, Latim*. A deciduous tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 48 (DUSH).

24. Vitaceae: **Cayratia trifolia** (L.) Domin, Biblioth. Bot. 89: 371 (1927). *Vitis trifolia* L., Sp. Pl.: 203 (1753). *Local name*: *Amal-lata*. A slender, herbaceous climber with swollen rootstock. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 61 (DUSH).

25. Anacardiaceae : **Spondias pinnata** (L. f.) Kurz, Pegu Rep. A. 44 (1875). *Local name*: *Piyala*. A medium-sized to tall tree. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 68 (DUSH).

26. Meliaceae: **Melia azedarach** L., Sp. Pl.: 384 (1753). *Local name*: *Gora nim*. A medium-sized tree. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 67 (DUSH).

27. Rutaceae : **Aegle marmelos** (L.) Corr., Trans. Linn. Soc. 5: 222 (1800). *Crateva marmelos* L., Sp. Pl.: 444 (1753). *Local name*: *Bel*. A small, deciduous tree. *Representative specimen*: Daulatpur, 27.09.2004, Md. Moniruzzaman 81 (DUSH).

Glycosmis pentaphylla (Retz.) A. DC., Prodr. 1: 538 (1824). *Limonia pentaphylla* Retz., Obs. Bot. 5: 24 (1788). *Local names: Aisshara, Matmati, Datmajon*. A shrub or small tree. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 50 (DUSH).

28. Oxalidaceae : **Oxalis corniculata** L., Sp. Pl.: 435 (1753). *Local name*: *Ambuli*. An annual herb. *Representative specimen*: Goalgram, 26.09.2004, Md. Moniruzzaman 32 (DUSH).

29. Apiaceae: **Centella asiatica** (L.) Urban in Mart., Fl. Bras. 11: 287 (1879). *Hydrocotyle asiatica* L., Sp. Pl. 1: 234 (1753). *Local names*: *Thankuni, Adamoni*. A perennial herb. *Representative specimen*: Goalgram, 24.01.2005, Md. Moniruzzaman 146 (DUSH).

30. Asclepiadaceae: **Asclepias gigantea** L., Sp. Pl.: 214 (1753). *Local name*: *Akanda*. A small shrub. *Representative specimen*: Goalgram, 26.09.2004, Md. Moniruzzaman 26 (DUSH).

31. Solanaceae: **Datura metel** L., Sp. Pl.: 179 (1753). *Local names*: *Dutra, Dutura*. A stout herb. *Representative specimen*: Malipara, 27.09.2004, Md. Moniruzzaman 90 (DUSH).

Nicotiana plumbaginifolia Viv., Elench. Pl. Hort. Dinergo: 26. t. 5 (1802). *Local name*: *Tamak*. A slender, erect, annual herb. *Representative specimen*: Malipara, 09.06.2005, Md. Moniruzzaman 195 (DUSH).

Physalis minima L., Sp. Pl.: 183 (1753). Annual glabrous herb. *Representative specimen*: Daulatkhali, 17.11.2004, Md. Moniruzzaman 115 (DUSH).

Solanum nigrum L., Sp. Pl.: 186 (1753). *Local names*: *Tit begun, Puti begun*. An annual erect shrub. *Representative specimen*: Daulatkhali, 17.11.2004, Md. Moniruzzaman 113 (DUSH).

32. Convolvulaceae : **Ipomoea aquatica** Forssk., Fl. Aeg.-Arab.: 44 (1775). *Local name*: *Kalmilata*. A glabrous trailer on water. *Representative specimen*: Daulatkhali, 25.01.2005, Md. Moniruzzaman 176 (DUSH).

Ipomoea fistulosa Mart. *ex* Choisy in DC., Prodr. 9: 349 (1845). *Local names*: *Dholkalmi, Durakalma*. A fistular shrub. *Representative specimen*: Daulatkhali, 25.01.2005, Md. Moniruzzaman 190 (DUSH).

33. Cuscutaceae : **Cuscuta reflexa** Roxb., Pl. Corom. 2: 3, t. 104 (1798). *Local names*: *Swarnalata, Jarbuti, Algusi*. A fleshy parasite, forming dense yellow masses on small tree or shrub. *Representative specimen*: Daulatkhali, 17.11.2004, Md. Moniruzzaman 118 (DUSH).

34. Boraginaceae : **Heliotropium indicum** L., Sp. Pl.: 130 (1753). *Local name: Hatisur.* An annual herb. *Representative specimen*: Daulatkhali, 10.06.2005, Md. Moniruzzaman 215 (DUSH).

Cordia dichotoma Forst. f., Fl. Ins. Auster. Proder, 18: 110 (1876). *Local name*: *Boula*. A shrub or small tree. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 87 (DUSH).

35. Verbenaceae : **Callicarpa longifolia** Lamk., Enc. Meth. 1: 403 (1798). *Local name*: *Bormala*. A shrub. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 87 (DUSH).

Lippia alba (Mill). Briton *et* Wilson. Sci. Surv. Puerto Rico. Vergin 6: 141 (1935). An undershrub. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 20 (DUSH).

36. Lamiaceae : **Anisomeles indica** (L.) O. Kuntze, Rev. Gen.: 512 (1891). *Nepeta indica* L., Sp. Pl.: 596 (1753). *Local name*: *Gobura*. A bushy undershrub. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 117 (DUSH).

Dysophylla crassicaulis Benth. in Wall., Pl. As. Rar. 1: 30 (1830). An annual herb. *Representative specimen*: Malipara, 27.09.2004, Md. Moniruzzaman 91 (DUSH).

Hyptis suaveolens (L.) Poit., Ann. Mus. Par. 7: 472, t. 29 (1806). *Ballota suaveolens* L., Syst. Nat. ed. 10: 1100 (1759). *Local name*: *Tokma*. An annual herb. *Representative specimen*: Malipara, 25.01.2005, Md. Moniruzzaman 188 (DUSH).

37. Scrophulariaceae : **Lindernia crustacea** (L.) F. Muell., Cens. Austr. Pl.: 97 (1882). *Capraria crustacea* L. Mant. Pl. 1: 87 (1767). A dichotomously branched, prostrate herb. *Representative specimen*: Khalishakundi, 25.09.2004, Md. Moniruzzaman 6 (DUSH).

Scoparia dulcis L., Sp. Pl.: 166 (1753). *Local name*: *Bondhuna*. A herb. *Representative specimen*: Daulatpur, 25.09.2004, Md. Moniruzzaman 8 (DUSH).

38. Orobanchaceae: **Orobanche aegyptiaca** Pers., Syn. Pl. 2: 181 (1807). *Local names*: *Poramula, Misridana*. A herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 160 (DUSH).

39. Acanthaceae: **Hygrophila salicifolia** (Vahl) Nees in Wall., Pl. As. Rar. 3: 81 (1832). *Ruellia salicifolia* Vahl, Symb. 3: 84 (1794). A prostrate to erect herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 120 (DUSH).

Rungia pectinata (L.) Nees in DC., Prodr. 11: 469 (1847). *Justicia pectinata* L., Amoen. Acad. 4: 293 (1759). *Local name*: *Pindi*. A much branched, prostrate or suberect herb. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 120 (DUSH).

40. Rubiaceae: **Neolamarckia cadamba** (Roxb.) Bosser, Bull. Mus. Hist. Nat. Paris, Ser. 6, Sec. B. Adans. 3: 247 (1984). *Local name*: *Kadam*. A deciduous tree. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 103 (DUSH).

Hedyotis scandens Roxb., Fl. Ind. 1: 369 (1820). A climbing shrub. *Representative specimen*: Daulatpur, 24.01.2005, Md. Moniruzzaman 134 (DUSH).

41. Asteraceae: **Ageratum conyzoides** L., Sp. Pl.: 839 (1753). *Local names*: *Fulkuri, Hialmuti*. An annual herb. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 109 (DUSH).

Eclipta prostrata L., Mant. 2: 286 (1771). *Local name*: *Kalokeshoti*. An erect, annual herb. *Representative specimen*: Udoynagar, 24.01.2005, Md. Moniruzzaman 142 (DUSH).

Enhydra fluctuans Lour., Fl. Cochinch.: 511 (1790). *Local name*: *Helencha*. A profusely branched, annual aquatic herb. *Representative specimen*: Udoynagar, 27.09.2004, Md. Moniruzzaman 80 (DUSH).

Gnaphalium luteo-album L., subsp. **affine** (D. Don) Koster, Blumea 4: 484 (1941). An erect, annual herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 170 (DUSH).

Grangea maderaspatana (L.) Poir., Enc. Suppl. 2: 825 (1811). *Artemisia maderaspatana* L., Sp. Pl.: 849 (1753). An annual herb. *Representative specimen*: Goalgram, 24.01.2005, Md. Moniruzzaman 139 (DUSH).

Mikania cordata (Burm. f.) B. L. Robinson, Contrib. Gray Herb. 104: 65 (1934). *Eupatorium cordatum* Burm. f., Fl. Ind.: 176 (1768). *Local names*: *Asamlata, Tarulata*. A perennial herb. *Representative specimen*: Daulatkhali, 26.09.2004, Md. Moniruzzaman 17 (DUSH).

Spilanthes calva DC. in Wight, Contrib. Bot. Ind.: 19 (1834). An annual herb. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 30 (DUSH).

Synedrella nodiflora (L.) Gaertn., Fruct. 2: 456, t. 171 (1791). *Verbesina nodiflora* L., Cent. Pl. 1: 28 (1755). A small woody herb. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 71 (DUSH).

Xanthium indicum Koen. ex Roxb., Fl. Ind. 3: 601 (1832). An erect, annual herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 187 (DUSH).

Liliopsida (Monocots)

42. Alismataceae : **Sagittaria sagittifolia** L., Sp. Pl. 2: 993 (1753). *Local names*: *Chhotokut, Muyamuya*. A scapigerous herb. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 180 (DUSH).

43. Arecaceae : **Phoenix sylvestris** (L.) Roxb., Hort. Beng. 73 (1814). *Local name*: *Khejur*. A tall palm tree. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 24 (DUSH).

44. Araceae : **Alocasia fornicata** (Roxb.) Schott, Oestr. Bot. Wochenbl. 4: 410 (1854). *Arum fornicatum* Roxb., Fl. Ind. 3: 501 (1832). A tuberous, coarse herb. *Representative specimen*: Khalishakundi, 10.06.2005, Md. Moniruzzaman 217 (DUSH).

Colocasia esculenta (L.) Schott in Schott & Endl., Melet. Bot.: 18 (1832). *Arum esculentum* L., Sp. Pl.: 965 (1753). *Local name: Kachu*. A tall coarse herb. *Representative specimen*: Khalishakundi, 27.09.2004, Md. Moniruzzaman 83 (DUSH).

Typhonium trilobatum (L.) Schott., Wien. Zeitschr. 3: 72 (1829). *Arum trilobatum* L., Sp. Pl.: 934 (1753). A tuberous climber. *Representative specimen*: Khalishakundi, 10.06.2005, Md. Moniruzzaman 223 (DUSH).

45. Commelinaceae : **Commelina benghalensis** L., Sp. Pl.: 41 (1753). *Local names*: *Dholpata, Kanchra*. A slender herb. *Representative specimen*: Malipara, 25.09.2004, Md. Moniruzzaman 2 (DUSH).

Murdania nudiflora (L.) Brenan, Kew Bull. 7: 189 (1952). *Commelina nudiflora* L., Sp. Pl.: 41 (1753). An annual, diffuse herb. *Representative specimen*: Malipara, 25.09.2004, Md. Moniruzzaman 3 (DUSH).

46. Cyperaceae : **Cyperus compressus** L., Sp. Pl. ed. 1: 46 (1753). An annual herb, tufted root. *Representative specimen*: Daulatpur, 25.01.2005, Md. Moniruzzaman 204 (DUSH).

Cyperus difformis L., Cent. Pl. 2: 6 (1756). *Local name: Mutha ghas.* An annual, tufted herb. *Representative specimen*: Taragunia, 10.01.2005, Md. Moniruzzaman 213 (DUSH).

Cyperus diffusus Vahl, Enum. Pl. 2: 321 (1806). A perennial herb. *Representative specimen*: Taragunia, 24.01.2005, Md. Moniruzzaman 140 (DUSH).

Cyperus michelianus (L.) Link., Hort. Bot. Berol. Descr. 1: 303 (1827). *Scirpus michelianus* L., Sp. Pl.: 45 (1753). *Local name: Choto Gotubi.* An annual herb. *Representative specimen*: Taragunia, 25.01.2005, Md. Moniruzzaman 173 (DUSH).

Cyperus rotundus L., Sp. Pl.: 45 (1753). *Local name*: *Motha ghas*. Perennial grass. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 187 (DUSH).

47. Poaceae : **Axonopus compressus** (Sw.) P. Beauv., Ess. Agrost. 12 (154): 167 (1812). *Milium compressum* Sw., Prod.: 24 (1788). A perennial, tufted herb. *Representative specimen*: Moubaria, 17.11.2004, Md. Moniruzzaman 98 (DUSH).

Brachiaria distachya (L.) Stapf. In Prain,Fl. Trop. Afr. 9: 565(1919). An annual or perennial grass. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 18 (DUSH).

Chrysopogon aciculatus (Retz.) Trin., Fund. Agrost.: 188 (1820). *Andropogon aciculatus* Retz., Obs. Bot. 5: 22 (1989). A glabrous herb. *Representative specimen*: Khalishakundi, 26.09.2004, Md. Moniruzzaman 01 (DUSH).

Cynodon dactylon (L.) Pers., Syn. Pl. ed. 1: 85 (182005). *Panicum dactylon* L., Sp. Pl.: 58 (1753). *Local names*: *Durba, Dubla, Dubraghas*. A creeping herb. *Representative specimen*: Khalishakundi, 17.11.2004, Md. Moniruzzaman 114 (DUSH).

Dactyloctenium aegyptium (L.) P. Beauv., Ess. Agrost. Expl. Pl.: 15 (1812). *Cynosurus aegyptius* L., Sp. Pl. ed. 1, 1: 72 (1753). Stoloniferous, annual or short-lived perennial herb. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 39 (DUSH).

Dichanthium annulatum (Forsskal) Stapf. In Prain,Fl. Trop. Afr. 9: 178 (1917). *Local name*: *Loari*. An annual or perennial herb. *Representative specimen*: Daulatpur, 24.01.2005, Md. Moniruzzaman 123 (DUSH).

Digitaria ciliaris (Retz.) Koeler, Dese. Gram. 27. (1802) Annual with weak, branching culms. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 126 (DUSH).

Digitaria ischaemum (Schreb.) Schreb. ex. Muhl., Descr. Gram. Calam. 131 (1817). An annual, erect or geniculately ascending. *Representative specimen*: Daulatpur, 09.06.2005, Md. Moniruzzaman 205 (DUSH).

Digitaria longiflora (Retz.) Pers., Syn. Pl. 1: 85 (182005). An annul with slender. *Representative specimen*: Daulatpur, 10.06.2005, Md. Moniruzzaman 208 (DUSH).

Digitaria sanguinalis (L.) Scop., Flora. Carn. Ed. 2, 1: 52 (1772). An annual or perennial grass. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 29 (DUSH).

Echinochloa colona (L.) Link., Hort. Berol. 2: 209 (1833). *Local name*: *Shyamaghas*. A leafy annual grass. *Representative specimen*: Udoynagar, 26.09.2004, Md. Moniruzzaman 69 (DUSH).

Echinochloa cruss-galli (L.) P. Beauv., Ess. Agrost. 53: 161 (1812). *Panicum crus-galli* L., Sp. Pl. ed. 1, 1: 56 (1753). *Local names*: *Barashyamaghas, Malanga-kuri, Kakligash*. An annual or perennial herb. *Representative specimen*: Udoynagar, 10.06.2005, Md. Moniruzzaman 207 (DUSH).

Eleusine indica (L.) Gaertn., Fruct. 1: 8 (1789). *Cynusurus indicus* L., Sp. Pl. ed.1.: 72 (1753). *Local name*: *Malankuri.* A tufted, annual herb. *Representative specimen*: Khalishakundi, 25.09.2004, Md. Moniruzzaman 14 (DUSH).

Eragrostis tenella (L.) P. Beauv. ex . Rearn. & Schult., Syst. Veg. 2: 576 (1817). *Local name*: *Koni*. A loosely tufted annual or short lived perennial herb. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 46 (DUSH).

Eragrostis unioloides (Retz.) Nees *ex* Steud., Syn. Pl. Glum. 1: 264 (1854). *Poa unioloides* Retz. Obs. Bot. 5: 19 (1789). An annual herb. *Representative specimen*: Daulatpur, 17.11.2004, Md. Moniruzzaman 93 (DUSH).

Hemarthria protensa Steud., Syn. Pl. Glum. 1: 359 (1854). Local name: *Chailla*. An erect to decumbent herb. *Representative specimen*: Khalishakundi, 09.06.2005, Md. Moniruzzaman 199 (DUSH).

Oplismenus compositus (L.) P. Beauv., Ess. Agrost. 54: 168 (1812). *Panicum compositum* L., Sp. Pl. ed. 1: 57 (1753). A perennial grass. *Representative specimen*: Khalishakundi, 25.09.2004, Md. Moniruzzaman 09 (DUSH).

Panicum repens L., Sp. Pl. ed. 2: 87 (1762). A perennial, rhizomatous grass. *Representative specimen*: Daulatpur, 10.06.2005, Md. Moniruzzaman 210 (DUSH).

Paspalum scrobiculatum L., Mant. 1: 29 (1767). *Local name*: *Goicha*. An annual herb. *Representative specimen*: Daulatpur, 10.06.2005, Md. Moniruzzaman 211 (DUSH).

Saccharum spontaneum L., Mant. Pl. 2: 183 (1771). *Local name*: *Kash*. A perennial, tall herb. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 27 (DUSH).

48. Zingiberaceae : **Alpinia nigra** (Gaertn.) Burtt., Notes Roy. Bot. Gard. Edinb. 35: 213 (1977). *Zingiber nigrum* Gaertn. (1788). *Local name*: *Tara*. Stem leafy, leaves sessile or sub-sessile. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 63 (DUSH).

49. Pontederiaceae : **Eichhornia crassipes** (Mart.) Solms in A. DC., Mon. Phan. 4: 527 (1883). *Pontederia crassipes* Mart., Nov. Gen. Sp.: 9, t. 4 (1823). *Local names*: *Kachuripana, Jarmani*. An aquatic, free-floating herb. *Representative specimen*: Goalgram, 10.06.2005, Md. Moniruzzaman 212 (DUSH).

Monochoria hastata (L.) Solms. in A. DC., Mon. Phan. 4: 523 (1883). *Pontederia hastata* L., Sp. Pl.: 288 (1753). An aquatic, emergent herb. *Representative specimen*: Goalgram, 09.06.2005, Md. Moniruzzaman 202 (DUSH).

50. Costaceae : **Costus speciosus** (Koen.) Smith, Trans. Linn. Soc. London 1: 249 (1791). *Banksea speciosa* Koen. in Retz., Obs. Bot. 3: 75 (1783). *Local name*: *Jongliphul.* A rhizomatous herb. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 73 (DUSH).

51. Dioscoreaceae : **Dioscorea alata** L., Sp. Pl.: 1033 (1753). *Local name*: *Chupri alu*. A perennial climber. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 26 (DUSH).

Dioscorea kamoonensis Kunth, Enum. Pl. 5: 395 (1850). *Local names*: *Tepata alu,Erabera lata*. A climber. *Representative specimen*: Daulatpur, 26.09.2004, Md. Moniruzzaman 28 (DUSH).

52. Orchidaceae : **Vanda tessellata** (Roxb.) Hook. f. *ex* G. Don in Loud., Hort. Brit.: 372 (1830). *Epidendrum tessellatum* Roxb., Pl. Corom. 1: 34, t. 42 (1795). An epiphytic herb. *Representative specimen*: Khalishakundi, 09.06.2005, Md. Moniruzzaman 192 (DUSH).

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EFFECT OF INDUSTRIAL SLUDGE ON CONCENTRATION AND DISTRIBUTION PATTERN OF LEAD, CADMIUM AND NICKEL IN ROOT, STEM AND LEAF OF RED AMARANTH (*AMARANTHUS GANGETICUS* L.) IN SILTY CLAY LOAM SOIL

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Abstract

A pot experiment was carried out to evaluate the concentration and distribution of Lead, Cadmium and Nickel in industrial sludge. The test crop was Red Amaranth (*Amaranthus gangeticus* L.). Nitrogen was applied comprising the five combinations of different proportion of urea and industrial sludge and a control. Red Amaranth was harvested at 30 and 42 Days after sowing (DAS). Different physical and chemical analysis of soil, sludge, root, stem and leaf of the plant were carried out as required. Heavy metals concentration indicated the trends of Pb>Ni>Cd and distribution pattern of Pb, Cd and Ni were identified Leaf> root> stem, Root> leaf> stem and Root> leaf> stem respectively, both at 30 and 42 DAS with little exception. Industrial sludge had a significant influence on heavy metal concentration and distribution and contaminant food chain, although the data were below the maximum acceptable level.

Key Words: Industrial sludge, Concentration, Distribution pattern, Lead, Cadmium, Nickel, Red Amaranth

Introduction

In recent time environmental pollution is a global problem and a great threat to the existence of living world, the pollution being continuously aggravated. Heavy metals in sludge and soil are generally present in a variety of forms exhibiting different degrees of bioavailability and mobility (Salim *et al.* 1996). The corollary to the studies on the form of metals in sewage is that the metals coming from raw sludge determine their presence in the first instance. It is known that metals may be present as inorganic forms such as oxides, hydroxides, sulfides (Mosey *et al.* 1971) or as loose organic complex such as with humic acid or polysaccharides (Holtzclaw *et al.* 1970). Most system of controlling sludge application to the land involve the use of total metal concentration in sludge but in developing criteria to evaluate metals in sludge is essential to understand the nature of fraction present in the soil (Matthews 1984). Stover *et al.* (1976) has examined metal fractions in digested sludge by using a sequential extraction procedure for the separation of metals. Nevertheless, the procedure used by Stover *et al.* (1976) to rank the different forms of metal in digested sludge is as follows: Lead, carbonates>organic- bond>sulfides

>adsorbed>exchangeable;Cd,carbonates>sulfides>organic-ond>adsorbed=exchangeable; Ni, carbonates>organic-bond>exchangeable>adsorbed>sulfide.

Heavy metals such as zinc, copper, cadmium, chromium, nickel, mercury and lead can be phytotoxic. They accumulate in aquatic and terrestrial habitats and simultaneously contaminate the animal and human food chains, and hence will also cause diseases in human (Chaney 1983). Plants take up high level of nickel easily which cause toxicity to plants. However, Ni is an essential micronutrient to plant which is required by urease enzyme to hydrolyze urea formed in plants and avoid NH_3 toxicity (Salisbury and Ross 1992). Lead is also micronutrient in plant, but can cause damage to central and peripheral nervous systems, kidney and highly toxic to infants and pregnant women. Lead, Cd, Ni etc. in fertilizers and pesticides are important factors causing pollution. In contrast to most pollutants, these are not biodegradable. The result of pollutants undergoing global ecological cycles causes severe environmental pollution.

Many vegetables are grown in Bangladesh. Red Amaranth is one of them and grown in wide range in the country including industrial effluent contaminated lands too. Farmers use industrial sludge as a nutrient supplement without knowing its nutrient or toxic metal concentration. The crop may contain toxic or heavy metals from the effluent and sludge, which may be harmful to food chain. So, it is important to investigate the concentration and distribution pattern of heavy metals by the different parts of plant collected from sludge and effluent. The information could be helpful for developing techniques to handle such types of wastes and people would be concern about use of waste (sludge/effluent) for the crops and vegetables production. The main objectives of the study was to determine the concentration and distribution pattern of selected heavy metals in different parts of red amaranth, so as to assess the safety and feasibility of consuming red amaranth grown in area contaminated with industrial sludge.

Materials and Methods

The experiment was carried out in pot in a net house of the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. The experimental site was located at latitude 24°05′N, longitude 90°16′E and altitude 8.4 m AMSL. Soil was silty clay loam belonging to Chiaata series in the Bangladesh soil classification system (Brammer and Saheed 1984). Industrial sludge was collected from 'Islam dyeing' and 'Dundy dyeing' industry at Konabari, Gazipur and Badsha, Niki and Combined dyeing industry at Katchpur, Narayanganj. The test crop was Red Amaranth (*Amaranthus gangeticus* L.), variety "BARI Lalshak-1". The experiments were designed to provide nutrient and heavy metals to red amaranth using different ratios of urea and industrial sludge. Six treatment combinations were prepared as T1.UN_{100} + SN_0 (100% urea-N + 0% sludge-N), T2. UN_{75} + SN_{25} (75% urea-N + 25% sludge-N), T3. UN_{50} + SN_{50} (50% urea-N + 50% sludge-N), T4. UN_{25} + SN_{75} (25% urea-N + 75% sludge-N), T5. UN_0 + SN_{100} (0% urea-N +100% sludge-N) and T6. Control (0% urea-N + 0% sludge-N). The

experiment was conducted in completely randomized design (CRD) with four replications. Phosphorous (P) and potassium (K) were applied at the rate of 40 and 112.5 kg/ha, respectively as per fertilizer recommendation guide-2005. Accordingly, each pot (5.0 kg of oven dry soil) was given approximately 0.09 g P and 0.25 g K using triple super phosphate (19.78% P) and muriate of potash (50% K). Nitrogen was applied at the rate of 250 kg/ha and each pot received 0.6 g of N in the combinations of urea and sludge, depending upon the treatments.

Soil samples were collected initially, intermittently and after harvesting the crop. Three soil columns were collected from each pot using a core sampler to the depth of 20 cm. These samples were then mixed to make a composite sample, air-dried, ground, sieved and stored in labeled polythene bags for laboratory analysis. Plant height was measured at 30 and 42 DAS, i.e. just prior to harvesting, in which the height of every plant in each pot from the soil level up to the apical bud was measured and average plant height was calculated and recorded. The plants were then uprooted (when soil was moist), properly washed and leaves, stems and roots were separated. Both fresh and oven dry weights were recorded after drying in an oven for 72 hours at 70 ^{0}C. These samples were separately ground, labelled and stored for laboratory analysis.

The Particle-size analysis of the soil was made by combination of sieving and hydrometer method as described by Day (1965) and textural classes were determined by Marshall's Triangular Coordinate curve. In both soil and industrial sludge samples, pH was determined with distilled water (1:2.5) by Metrohm 691 digital pH meter (Jackson 1973). Organic carbon was determined using the dry combustion method, for which the LECO Model C 200 analyzer was used. The organic matter concentrations in samples were computed by multiplying the organic carbon concentration with 1.72 (Nelson and Sommers 1982). Total N was estimated by Micro-kjeldhal method (Jackson 1973).

Later, soil and industrial sludge extracts were prepared by ammonium acetate extraction method for determination of available K, DTPA extraction method for available Zn, Pb, Cd and Ni and Olsen method for available P (Jackson 1973). Plant extracts were prepared, by nitric (HNO_3) acid (68%) digestion method using a specified weight of ground materials for computing total amount of selected elements including Pb, Cd and Ni. From the above soil, industrial sludge and plant extracts, P was determined colorimetrically (using spectrophotometer UV-Model lamda 11), K flame emission spectrophotometrically (using flame photometer Model Jenway pfp-7), Pb, Cd, Ni and Zn Atomic Absorption Spectrophotometrically.

Results and Discussion

The physical and chemical parameters of soil are tested and shown in Table1. The test results confirm that the soil did not have traceable levels of Pb, Cd and Ni. The total N, labile P and Zn were at low levels; K and organic matter concentrations were at medium

levels while S was at high level. The pH was 6.7, which indicates that the soil was slightly acidic to neutral.

Table 1. Selected parameters of background soil used in the experimental pot.

Parameters	background soil [1/]	Interpretation of Soil test values [2/]
Texture	Silty clay loam	-
pH	6.7 ± 0.091	Slightly alkaline to neutral
Moisture Concentration (%)	20.6 ± 0.072	-
Organic C (%)	1.81 ± 0.063	Medium class
Total N (%)	0.11 ± 0.010	Low
Labile P (ppm)	10.1 ± 0.082	Low
Available K (meq/100g soil)	0.17 ± 0.011	Medium
Available S (ppm)	28.9 ± 1.04	Optimum
Available Zn (ppm)	0.8 ± 0.032	Low
Available Pb (ppm)	1.05 ± 0.012	Below max. Accept. level
Available Cd (ppm)	0.06 ± 0.032	Below max. Accept. level
Available Ni (ppm)	0.46 ± 0.042	-

[1/]Soil used for the experiment. [2/] Source: Anon, 2005.

The physical and chemical parameters of industrial sludge are shown in Table 2. Industrial sludge contained optimum, high and very high levels of K, Organic carbon and Zn, respectively. Labile P concentration was very low. Total N and S were considered to be available at moderate concentrations.

Table 2. Selected parameters of industrial sludge used in the experimental pot.

Parameters	Industrial sludge[1/]	Interpretation of Sludge test values[2/]
pH	7.8 ± 0.08	Slightly alkaline
Moisture Concentration (%)	28.8 ± 0.08	-
Organic C (%)	2.6 ± 0.08	High class
Total N (%)	0.2 ± 0.02	Medium
Labile P (ppm)	14.5 ± 0.09	Low
Available K (meq/100g soil)	0.36 ± 0.01	Optimum
Available S (ppm)	19.9 ± 0.92	Medium
Available Zn (ppm)	47.0 ± 1.28	Very high
Available Pb (ppm)	3.9 ± 0.01	Below max. Accept. level
Available Cd (ppm)	0.2 ± 0.03	Below max. Accept. level
Available Ni (ppm)	1.2 ± 0.04	-

[1/]Industrial sludge used as a proportion of treatment in exp. [2/] Source: Anon, 2005.

Lead concentration and distribution

Root: The highest Pb concentration (3.36mgkg^{-1}) in root was recorded in T_4 receiving 75% N from industrial sludge and 25% N from urea at 30 DAS (Table 3). The effect of this treatment was statistically similar to T_5 but superior to T_1, T_2, T_3 and T_6. The lowest Pb concentration (1.92mgkg^{-1}) in root was observed in T_6 (control). Highest Pb concentration (3.30mgkg^{-1}) in root was recorded in 42 DAS (Treatment T_4). Lead concentration found in treatment T_5 rank second in position. Treatment T_2 and T_3 identical to each other, rank third in position and superior to control.

Stem: The highest Pb concentration (2.73mgkg^{-1}) in stem was recorded in T_5 receiving 100% N from industrial sludge and 0 % N from urea at 30 DAS (Table 3). Lead concentration found in treatment T_4 rank second in position which was followed by T_3. Treatment T_1 and T_2 were identical to each other and superior to control (1.91mgkg^{-1}). The highest Pb concentration (2.47mgkg^{-1}) was also recorded in stem at 42 DAS (Treatment T_5). Lead concentration found in treatment T_4 rank second in position and the effect of this treatment was identical to T_2 and T_3, but superior to control. The lowest Pb concentration (1.32mgkg^{-1}) in stem was observed in T_6 (control).

Leaf: Similar to stem the highest Pb concentration (3.99mgkg^{-1}) in leaf was noted in T_5 at 30 DAS (Table 3). The rank of treatment T_4 was second in position and that was identical to T_2 and T_3 but superior to T_1 and T_6 like as 30 DAS. Treatment T_5 showed highest Pb concentration (3.36mgkg^{-1}) in leaf at 42 DAS. In treatment T_1 and T_2 recorded Pb concentration was statistically similar and inferior from T_3 and T_4, but superior to T_6. The lowest Pb concentration was identified in control (1.89mgkg^{-1}). Increasing sludge concentration in the treatment showed higher Pb concentration in root, stem and leaf of red amaranth.

Table 3. Lead concentration in root, stem and leaf of red amaranth as influenced by urea and sludge-N at 30 and 42 DAS.

Treatment	Pb (mgkg^{-1}dry matter)					
	Root		Stem		Leaf	
	30DAS[1/]	42 DAS	30 DAS	42 DAS	30 DAS	42 DAS
T1. $UN_{100} + SN_0$	2.55d	2.57d	2.00d	1.36c	2.69c	2.66d
T2. $UN_{75} + SN_{25}$	2.70c	2.65c	2.04d	2.00b	3.37b	2.69d
T3. $UN_{50} + SN_{50}$	2.99b	2.69c	2.21c	2.09b	3.33b	2.76c
T4. $UN_{25} + SN_{75}$	3.36a	3.30a	2.59b	2.21b	3.47b	3.31b
T5. $UN_0 + SN_{100}$	3.32a	2.98b	2.73a	2.47a	3.99a	3.36a
T6. Control	1.92e	1.94e	1.91e	1.32c	1.96e	1.89e
LSD (P=0.05)	0.067	0.057	0.055	0.22	0.19	0.033
CV%	1.54	1.37	1.63	7.54	3.81	0.78

U_N – Nitrogen from urea; S_N – Nitrogen from industrial sludge; 1/ DAS – Days after seeding; Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT.

Cadmium concentration and distribution

Root: The highest amount of Cd (0.04mgkg^{-1}) was recorded in T_5 in root at 30 DAS (Table 4). The effect of this treatment was statistically similar to T_2, T_3 and T_4, but superior to T_1, and T_6. The lowest Cd concentration (0.01mgkg^{-1}) in root was observed in T_6 (control). The highest Cd concentration (2.06mgkg^{-1}) was recorded in T_4 in root at 42 DAS. Cadmium concentration found in treatment T_5 rank second in position. Treatment T_2 and T_3 were identical to each other and rank third in position and superior to control.

Stem: Treatment T_5 recoded the highest Cd concentration (0.02mgkg^{-1}) in stem at 30DAS (Table 4). The effect of this treatment was statistically similar to T_3, and T_4, but superior to T_1, T_2 and T_6. The lowest Cd concentration (0.01mgkg^{-1}) in stem was observed in T_6 (control). The highest Cd concentration (1.85mgkg^{-1}) in leaf was recorded in T_5 at 42 DAS. The rank of treatment T_4 was second in position and that was identical to T_2 and T_3, but superior to T_1 and T_6.

Leaf: The highest Cd concentration (0.03mgkg^{-1}) in leaf was recorded in T_5 at 30 DAS (Table 4). The effect of this treatment was statistically similar to T_4, but superior to rest of the treatments. Treatment T_1, T_2, and T_6 were identical in respect of Cd concentration. The lowest Cd concentration (0.01mgkg^{-1}) in leaf was observed in T_6 (control). The highest Cd concentration (1.84mgkg^{-1}) was recorded in T_4 at 42 DAS that was statistically similar to T_5 but superior to T_1, T_2, T_3 and T_6. It was found that cadmium concentration was higher at 42 DAS compared to 30 DAS in all plant parts and higher values were in roots. Higher Cd concentration was also found in the treatment where N was given by 100 % sludge only.

Table 4. Cadmium concentration in root, stem and leaf of red amaranth as influenced by urea and sludge-N at 30 and 42 DAS.

Treatment	Cd (mgkg^{-1}dry matter)					
	Root		Stem		Leaf	
	30 DAS[1/]	42 DAS	30 DAS	42 DAS	30 DAS	42 DAS
T1. $UN_{100} + SN_0$	0.01b	1.70cd	0.01b	1.06c	0.01c	1.08d
T2. $UN_{75} + SN_{25}$	0.02ab	1.84bc	0.01b	1.27b	0.01c	1.28c
T3. $UN_{50} + SN_{50}$	0.03ab	1.88b	0.02ab	1.21b	0.02b	1.54b
T4. $UN_{25} + SN_{75}$	0.04a	2.06a	0.02ab	1.30b	0.02ab	1.84a
T5. $UN_0 + SN_{100}$	0.04a	1.94ab	0.02a	1.85a	0.03a	1.82a
T6. Control	0.01b	1.61d	0.01b	0.05d	0.01c	0.76e
LSD (P=0.05)	0.176	0.176	0.0063	0.136	0.005	0.164
CV%	6.29	6.37	3.04	9.44	21.05	7.86

U_N – Nitrogen from urea; S_N – Nitrogen from industrial sludge; 1/ DAS – Days after seeding; Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT.

Nickel concentration and distribution

Root: The highest Ni concentration (0.53mgkg^{-1}) in root at 30 DAS (Table 5) was recorded in T_5. Treatment T_3 was second in position and that was identical to T_1, T_2 and T_4 but superior to T_6. The lowest Ni concentration (0.26mgkg^{-1}) in root was observed in T_6. The highest Ni concentration (2.48mgkg^{-1}) in root was recorded in T_5 at 42 DAS (Table 5). Treatment T_4 was second in position and that was statistically similar to T_3 but superior to T_1, T_2 and T_6.

Stem: The highest Ni concentration (0.42mgkg^{-1}) in root was noted in T_5 at 30 DAS (Table 5). Treatment T_4 rank second in position and that was identical to T_3 but superior to T_1 T_2 and T_6. The highest Ni concentration (1.72mgkg^{-1}) in stem was recorded in T_5 at 42 DAS (Table 5). Treatment T_4 was second in position and that was statistically similar to T_2 and T_3, but superior to T_1 and T_6.

Leaf: The highest Ni concentration (0.56mgkg^{-1}) in leaf was recorded in T_5 at 30 DAS (Table 5). Treatment T_4 was second in position and that was statistically similar to T_3, but superior to T_1, T_2 and control. The lowest Ni concentration (0.27mgkg^{-1}) in leaf was recorded in T_6 at 30 DAS (Table 5). The highest Ni concentration (2.12mgkg^{-1}) in leaf was observed in T_5 at 42 DAS. Treatment T_4 was second in position. Treatment T_2 rank third in position and statistically similar to T_3 but superior to T_1 and T_6. From above discussion, it is revealed that higher amount of Ni was found at 42 DAS compared to 30 DAS in all parts of plant and higher values were in roots. Nickel is considered as a micronutrient in plants (Salisbury and Ross 1982). This nutrient is considered in many plants to be essential in order to avoid urea toxicity by acting as a co-factor for the urease enzyme to hydrolyze urea formed.

Table 5. Nickel concentration in root, stem and leaf of red amaranth as influenced by urea and sludge-N at 30 and 42 DAS.

Treatment	Ni (mgkg^{-1}dry matter)					
	Root		Stem		Leaf	
	30 DAS[1/]	42 DAS	30 DAS	42 DAS	30 DAS	42 DAS
T1. $UN_{100} + SN_0$	0.31b	2.06d	0.23bc	0.99c	0.31c	1.29d
T2. $UN_{75} + SN_{25}$	0.32b	2.16c	0.24bc	1.02bc	0.32c	1.41c
T3. $UN_{50} + SN_{50}$	0.34b	2.23b	0.25b	1.02bc	0.33bc	1.40c
T4. $UN_{25} + SN_{75}$	0.32b	2.23b	0.26b	1.06b	0.35b	1.51b
T5. $UN_0 + SN_{100}$	0.53a	2.48a	0.42a	1.72a	0.56a	2.12a
T6. Control	0.26c	1.96e	0.21c	0.96c	0.27d	1.10e
LSD (P=0.05)	0.032	0.058	0.031	0.062	0.03	0.074
CV%	6.16	1.77	7.64	3.66	5.6	3.36

U_N – Nitrogen from urea; S_N – Nitrogen from industrial sludge; 1/ DAS – Days after seeding; Means followed by common letter (s) in a column are not significantly different at 5% level by DMRT.

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HOST PREFERENCE OF PUMPKIN BEETLE TO CUCURBITS UNDER FIELD CONDITIONS

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Abstract

To find out preferred cucurbit host(s) of the pumpkin beetle and to determine the susceptibility of ten different cucurbits to the pest, a trial was conducted under field conditions. The results revealed that the most preferred host of the red pumpkin beetle (RPB) was muskmelon, which was followed by khira, cucumber and sweet gourd, and these may be graded as susceptible hosts. Bitter gourd, sponge gourd, ribbed gourd and snake gourd were least or non preferred hosts of RPB and these may be graded as resistant hosts. Other two crops, the bottle gourd and ash gourd were moderately preferred hosts of the insect and these may be graded as moderately susceptible hosts. The order of the most preferred host of the blue pumpkin beetle may be shown as bitter gourd> ribbed gourd> sponge gourd. Of these cucurbits, population of blue pumpkin beetle was much higher than that of red pumpkin beetle. The hosts most preferred to RPB are less preferred or not preferred to the blue pumpkin beetle. The field trials revealed that the order of preference of RPB for ten tested cucurbit hosts was muskmelon> sweet gourd> cucumber > khira > ash gourd > bottle gourd > sponge gourd ≥ ribbed gourd ≥ snake gourd > bitter gourd.

Key words: Host preference, pumpkin beetle, cucurbits

Introduction

The family Cucurbitaceae comprises more than 700 species of plants in about 90 genera (York 1992). Cucurbits are among the most widely grown and important crops in the tropical and subtropical countries of the world. Pumpkin beetle is the major pest and causes considerable damage to almost all cucurbitaceous crops (Butani and Jotwani 1984, Yawalkar 1985). Among different species of pumpkin beetles, incidence of adult stage of the red pumpkin beetle (RPB), *Aulacophora foveicollis* (Lucas) on different cucurbits have been reported by various workers (Nath 1964, Nath and Thakur 1965, Bogawat and Pandey 1967). The pest, however, occurs throughout the year and causes severe damage to the crops, especially at the seedling stage (Alam 1969, Butani and Jotwani 1984). The adult beetles feed voraciously on the cucurbit leaf making irregular holes. They also attack cotyledons and flowers (Butani and Jotwani 1984). The beetles may kill seedlings and sometimes the crops have to be resown 3-4 times (Azim 1966). The grubs feed on roots and underground portions of the host plants as well as fruits touching the soil and thus making such fruits unsuitable for human consumption (Butani and Jotwani 1984). It may cause up to 70 % damage to the leaves and 60 % to the flowers of cucumber plants (Alam 1969).

According to Nath and Thakur (1965), among the gourds, sponge gourd was found to be least preferred by RPB, while the bottle gourd was most preferred and the ridge gourd was intermediate in reaction. Khan and Hajela (1987) found that RPB preferred sweet gourd followed by cucumber, squash melon, sponge gourd and bottle gourd. Dilon and Sharma (1989) observed significant varietal differences for resistance to RPB in summer squash both in the field and in cage experiments.

Report on host preference of pumpkin beetle to different cucurbit hosts is scanty in Bangladesh. If the most preferred cucurbit host plant could be identified, it may be used as a trap or barrier crop to decrease infestation on target cucurbits. In addition, this preferred cucurbit crop may be grown as one of the component crops of mixed cropping to support higher pumpkin beetle load compared to other cucurbits. Therefore, it needs to identify the less and the most preferred cucurbits to pumpkin beetle. The present study was undertaken to find out host preference of the pumpkin beetles and to determine susceptibility of different cucurbit hosts to the pest.

Materials and Methods

The experiment was conducted in the experimental field of the Department of Entomology, Bangabandhu Shiekh Mujibur Rahman Agricultural University (BSMRAU) Salna, Gazipur during March-June 2007. Ten different cucurbit crops viz., sweet gourd (*Cucurbita moschata* L.), bottle gourd (*Lagenaria siceraria* L.), ash gourd (*Benincasa hispida* L.), bitter gourd (*Momordica charantia* L.), sponge gourd (*Luffa cylindrica* L.), ribbed gourd (*Luffa acutangula* L.), snake gourd (*Trichosanthes anguina* L.), cucumber (*Cucumis sativus* L.), khira (*Cucumis sativus* L.), and muskmelon (*Cucurbita melo* L.) were grown in the experimental fields following randomized complete block design with 4 replications. The size of the unit plot was 4 m × 4 m with an inter plot distance of 1 m and row to row distance 1.5 m. Two pits of 30 cm × 30 cm × 20 cm were dug at one side of each plot at a distance of 2 m to facilitate well spreading of cucurbit vines and also for easy inspection and data recording. Cow dung and fertilizer were applied as recommended (Rashid 1993) for cucurbits at the rate of 10,000, 69, 60 and 60 kg of cow dung, N, P and K per hectare, respectively. The half of cow dung, TSP and MP, and one third of urea were applied as basal dose during land preparation. The remaining cow dung, TSP and MP were applied in the pit 15 days before transplanting the seedlings. The rest of urea was top dressed after each flush of flowering and fruiting in three equal splits.

The seeds of different cucurbits were procured from Siddique Bazar, Dhaka. Prior to sowing, the seeds of bitter gourd and bottle gourd were soaked in water for about 12 hours to hasten uniform germination. The seeds were sown on 3rd March 2007 in polythene bag (15 cm × 10 cm) containing 50 % well-decomposed cow dung and 50 % sandy loam soil. Two seedlings of 15 days old were planted in each of the two pits. Irrigation and other recommended agronomic practices were followed as suggested by Rashid (1993). The crop was kept under constant observation from 2 leaf stage to 28 days

for recording data on incidence of pumpkin beetles. The susceptibility of different cucurbits to the pumpkin beetle was observed on the basis of beetle populations and damage caused by them.

Adult pumpkin beetles per plant: The number of the adult pumpkin beetles per plant was recorded through visual observations on upper and lower surfaces of leaves during 7-28 days after transplanting (DAT). The observation was made in the morning at 7:00, 8:00 and 9:00 AM and in the afternoon at 4:00, 5:00 and 6:00 PM. As the beetles are agile, observation was made very carefully without knocking the leaves and touching the plants. Average number of adult beetles per plant at different DAT was computed.

Per cent infestation of leaves: Data on leaf infestation per plant were converted to mean number recorded at 7, 14, 21 and 28 DAT. Per cent leaf infestation per plant was determined using the following simple formula:

$$\text{Infestation of leaves}\,(\%) = \frac{\text{Number of infested leaves}}{\text{Total number of leaves}} \times 100$$

Data collected from the experiment were statistically analyzed using MSTAT-C software. Whenever necessary data were transformed following square root or Arc Sin transformation before analysis. Means were separated by the Duncan's Multiple Range Test (Steel and Torrie 1960).

Results and Discussion

Incidence of pumpkin beetle at different days after transplanting: At 7 days after transplanting, the highest number of the red pumpkin beetle (RPB) was recorded on muskmelon (3.00), which was followed by sweet gourd (2.75), cucumber (2.50) and khira (2.00). Occurrence of the beetle on those four crops was statistically similar but significantly higher as compared to other crops. At this stage, bottle gourd and ash gourd showed statistically similar number of the beetle. It was significantly higher as compared to sponge gourd and ribbed gourd which had less than one beetle per plant. Bitter gourd and snake gourd were free from RPB (Table 1).

At 14 DAT, bitter gourd, sponge gourd, ribbed gourd and snake gourd were found to be free from infestation of RPB. Its occurrence was maximal on muskmelon, which was statistically similar to khira, cucumber and sweet gourd. Number of RPB on bottle gourd and ash gourd was also statistically similar but significantly lower as compared to above four crops and higher as compared to other crops (Table 1).

At 21 DAT, occurrence of RPB on muskmelon, cucumber and sweet gourd was also statistically similar but significantly higher as compared to other crops. The insect was absent from sponge gourd and ribbed gourd. Its occurrence on bitter gourd and snake gourd was less than one per plant. Other three crops showed 1.75-2.25 insects per plant per day, which were statistically similar.

At 28 DAT, number of RPB on muskmelon was significantly higher as compared to other crops except the sweet gourd. Occurrence of the insect on sweet gourd, bitter gourd, ash gourd, cucumber and khira ranged from 1.50 to 3.00 per plant, which was statistically similar. Other three crops had less than one insect per plant (Table 1).

Results of the experiment indicate that the most preferred host of RPB is muskmelon, which was followed by khira, cucumber and sweet gourd. These may be graded as susceptible hosts. On the other hand, bitter gourd, sponge gourd, ribbed gourd and snake gourd are least or non-preferred hosts of RPB. They may be graded as resistant hosts. Other two crops bottle gourd and ash gourd were moderately preferred hosts of the insect and may be graded as moderately susceptible hosts (Table 1).

Host preference of a particular insect pest depends upon the quality of host plants, which influences the growth and development of the pest. Thus the order of preference of different cucurbits as a host of RPB might be attributed to its nutrient contents that enhance the growth and development of the pest. Begum (2002) reported that among the five cucurbit plants (viz., sweet gourd, ash gourd, sponge gourd, snake gourd and cucumber), sweet gourd was identified as the most susceptible and highly preferred host to red pumpkin beetle and cucumber was recognized as less susceptible and less preferred host to this pest. Red pumpkin beetle is a polyphagous pest and prefers cucurbit, vegetables and melons (Butani and Jotwani 1984). This beetle is particularly a severe pest of pumpkins, muskmelons and bottle gourds (Rahman and Annadurai 1985) and attacks cucumber, melon and gourds (Abdullah 1969). In almost all cases, findings of the present studies are in agreement with the findings of other investigators.

Table 1. Incidence of pumpkin beetle per plant on different cucurbit host plants at different days after transplanting during March to June 2007.

Treatment (Cucurbits)	Number of pumpkin beetle per plant					
	7 DAT	14 DAT	21 DAT	28 DAT	Mean of all DATs	Grade
Sweet gourd	2.75a	3.25a	3.50a	3.00ab	3.13	S
Bottle gourd	1.50b	1.50b	2.25b	2.00b	1.81	MS
Ash gourd	1.75b	1.25b	2.00b	1.50bc	1.63	MS
Bitter gourd	0.00c	0.00c	0.50c	0.50c	0.25	R
Sponge gourd	0.25c	0.00c	0.00c	0.75c	0.25	R
Ribbed gourd	0.50c	0.00c	0.00c	0.25c	0.19	R
Snake gourd	0.00c	0.00c	0.75c	0.25c	0.25	R
Cucumber	2.50a	3.00a	3.25a	2.75b	2.88	S
Khira	2.00a	2.75a	1.75b	2.00b	2.12	S
Muskmelon	3.00a	3.50a	3.75a	4.25a	3.63	S

S = Susceptible MS = Moderately susceptible R = Resistant
Means of the same column having common letter (s) do not differ significantly (p=0.05) by DMRT.
Analysis was performed after square root transformation $\{\sqrt{(x + 0.5)}\}$ of the original values.
Values are means of 4 replications.

Population of red and blue pumpkin beetle on cucurbit hosts: The highest number of red pumpkin beetle per plant was recorded on musk melon, which was followed by cucumber, sweet gourd, bottle gourd and khira. Its lowest population was found on ribbed gourd, which was followed by snake gourd and sponge gourd. On the other hand, population of blue pumpkin beetle was highest on bitter gourd, which was followed by ribbed gourd and sponge gourd. Its average population was less than one on sweet gourd, cucumber, ash gourd and khira. Muskmelon, bottle gourd and snake gourd was free from blue pumpkin beetle (Fig. 1).

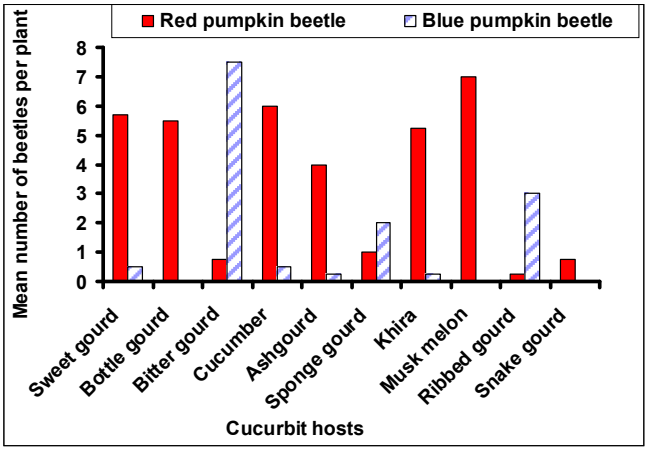

Fig. 1. Relative abundance of red and blue pumpkin beetle per plant on different cucurbit hosts grown at BSMRAU experimental farm during March to June 2007.

Results presented in Fig. 1 clearly indicate that the most preferred host of blue pumpkin beetle is the bitter gourd followed by ribbed gourd and sponge gourd. On those cucurbits population of blue pumpkin beetle was much higher than that of the red pumpkin beetle. The hosts most preferable to RPB are less preferable or not preferable to the blue pumpkin beetle. It indicates that the host choice between two species is not comparable. Similar results have also been reported by other investigators. Pradhan (1969) reported that the RPB has special preference for the leaves of cucurbit plants except those of the bitter gourd on which they do not like to feed. Nayar *et al*. (1995) reported that the beetles were very common on cucurbitaceous plants. They also mentioned that *Rhapidopalpa foveicollis* prefers pumpkin (*Cucurbita maxima*), while *Aulacophora atripennis* prefers sponge gourd (*Luffa cylindrica*), and both the species do not feed on bitter gourd (*Momordica charantia*). In general, both the species feed on *Cucurbita maxima, C. pepo, Cucumis melo* var. *utilissimus, C. melo, C. sativus, Citrullus lanatus, C. vulgaris* var. *fistulosus, Luffa cylindrica, L. acutangula* and *Trichosanthes anguina*. The beetle, *R. foveicollis* also feeds on *Benincasa hispida* and *Lagenaria vulgaris* (David and Ananthakrishnan 2004).

Per cent leaf infestation by red pumpkin beetle (RPB): The per cent leaf infestation by RPB per plant recorded at 7, 14, 21 and 28 DAT are presented in Table 2. At 7 DAT, the highest leaf infestation per plant was recorded on muskmelon (20.70%), which was significantly different from those of all other tested cucurbits. The second highest percentage of leaf infestation was recorded on sweet gourd (5.25%), which was statistically similar to those found on cucumber (4.99%), khira (4.80%) and ash gourd (4.25%) followed by bottle gourd (3.70%). No leaf infestation was recorded on snake gourd.

At 14 DAT, the highest (83.47%) leaf infestation per plant was observed on musk melon which was significantly different from those of all other cucurbits. The second highest (14.30%) infestation was recorded on sweet gourd which was statistically similar to those of cucumber (12.40%). Zero leaf infestation (0.00%) was recorded on snake gourd. Similar trend of leaf infestation was observed at 21 DAT, but at 28 DAT, the highest (95.42%) leaf infestation per plant was observed on musk melon which was significantly different from those obtained from all other cucurbit hosts. The second highest (12.40%) leaf infestation was recorded on sweet gourd which was statistically similar to those of khira (12.08%), cucumber (11.80%) and ash gourd (11.40%). The lowest (3.52%) leaf infestation was recorded on bitter gourd and this was statistically similar to those of snake gourd (4.00%), ribbed gourd (4.19%) and sponge gourd (4.20%).

Results of the present experiments indicated that muskmelon was the most preferred host of the red pumpkin beetle and the highest leaf infestation of this host was caused by the pest at different DAT. The mean of all DATs indicated that the order of preference of RPB for ten tested cucurbit hosts was muskmelon> sweet gourd> cucumber > khira > ash gourd > bottle gourd > sponge gourd ≥ ribbed gourd ≥ snake gourd > bitter gourd (Table 2).

Table 2. Percent leaf infestation per plant by red pumpkin beetle on cucurbit hosts at different days after transplanting during March to June 2007.

Cucurbit hosts	Percent leaf infestation per plant				
	7 DAT	14 DAT	21 DAT	28 DAT	Mean of all DATs
Sweet gourd	5.25b	14.30b	15.20b	12.40b	11.79
Bottle gourd	3.70bc	6.80d	10.95c	8.80c	7.56
Ash gourd	4.25b	8.30c	11.80c	11.40b	8.94
Bitter gourd	0.00d	0.00f	1.50e	3.52d	1.26
Sponge gourd	2.75c	3.65e	6.80d	4.20d	4.35
Ribbed gourd	2.80c	3.75e	5.76d	4.19d	4.13
Snake gourd	0.00d	0.00f	2.00e	4.00d	1.50
Cucumber	4.99b	12.40b	14.30b	11.80b	10.87
Khira	4.80b	9.00c	13.00b	12.08b	9.72
Muskmelon	20.70a	83.47a	89.56a	95.42a	72.29

Means of the same column having a common letter (s) do not differ significantly (p=0.05) by DMRT. Analysis was performed after Arc Sin $\sqrt{x}$ transformation of the original values.
Values are means of 4 replications.

The literature review provided variable results on the host preference of pumpkin beetle to cucurbits. Glimpses of these works related to the present study are discussed below:

Mehta and Sandhu (1989) studied 10 cucurbitaceous vegetables and noted that bitter gourd was highly resistant to the RPB, while sponge gourd and bottle gourd were resistant. The cucumber, muskmelon and water melon were moderately resistant to the pest. Roy and Pande (1990) investigated the preference order of 21 cucurbit vegetables and noted that bitter gourd was highly resistant to the beetle, while the sponge gourd and bottle gourd were moderately resistant; muskmelon and cucumber were susceptible to the pest. Roy and Pande (1991) also observed that banana squash, muskmelon and bottle gourd were the preferred hosts of the adults, while cucumber, white gourd/ash gourd, chinese okra, bitter gourd, snake gourd, watermelon and sponge gourd achieved the second order of preference to the beetle, *Aulacophora foveicollis*.

From the present finding it may be concluded that the most preferred host of RPB was muskmelon and was graded as the most susceptible host. Bitter gourd, sponge gourd, ribbed gourd and snake gourd were least or non preferred hosts of RPB and these may be graded as resistant hosts. The most preferred host of blue pumpkin beetle was found to be the bitter gourd.

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HAEMOLYTIC EFFECTS OF *SAMANEA SAMAN* (JACQ.), *TECTONA GRANDIS* (L.) AND *DIPTEROCARPUS TURBINATUS* (GAERTN) SEED EXTRACTS ON SINGHI FISH, *HETEROPNEUSTES FOSSILIS* (BLOCH)

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Abstract

The haemolytic effects of distilled water, 50% ethyl alcohol and 100% ethyl alcohol extracts of three plant seeds, Fulkoroi (*Samanea saman)*, shegoon (*Tectona grandis)* and Gargan (*Dipterocarpus turbinatus)* were studied on a predatory fish *Heteropneustes fossilis* under laboratoary conditions. Comparative data of control and treated fishes showed that the toxic components induced changes in the blood parameters. Piscicides inducing toxicity were evident from decreased RBC counts, low haemoglobin percentage and decreased PCV, MCV, MCH values but increased WBC counts. Haemolysis included shrunken or swollen RBC, granulated nuclei of RBC and pyknotic and swollen nuclei of WBC, cellular damage resulting in bare nuclei of RBC and WBC, clumping of RBC and syncitium of RBC matrix. Damage of blood cells with 100% ethyl alcohol extract was pronounced with all the seed extracts. The order of toxicity on the blood parameters statistically were *T. grandis* > *S. saman* > *D. turbinatus* seed extracts. On the basis of haemolysis of blood cell the effects were *D. turbinatus* > *S. saman* > *T. grandis* seed extracts.

Key words: Haemolytic effects, Bloo parameters, *Samanea saman*, *Tectona grandis, Dipterocarpus turbinatus*, Seed extracts, *Heteropneustes fossilis*.

Introduction

The haematological assessment is the rising motive to prove any disturbance of environmental influences on fishes. Study of haematological parameters on the toxic extracts of the blood parameters of fishes is an important basic effort which leads to understand the impact of the piscicides on fishes. Haematological parameters are related to the response of the organisms to changing environmental conditions. These parameters of a fish reflect the ecological conditions of its habitat. Blood parameters are probably one of the rapid and detectable variations under stress. Any deviation from the normal values may be due to the alterations in the physiological and pathological states of the fish (Blaxhall 1972, Smith *et al*. 1979). The effect of toxicants on fish can be assessed by the use of haematological indices as it has been reported to be a routine procedure in toxicological research, environmental monitoring and fish health conditions. The changes

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in the characteristics of blood cells are important diagnostic indicators of environmental stress on fish (Blaxhall 1972). These changes can provide evidence of exposure to toxicants (Lester and Budd 1979, Dick and Dixon 1985).

A number of studies has been carried out on the effects of plant pisicicidal compounds on fish haematology at home and abroad. Effect of sub lethal concentrations of *Parkia biglobosa* on haematological parameters of the African catfish, *Clarias gariepinus* has been reported by Fafioye and Adebisi (2000). Agbon *et al*. (2002) studied on the acute toxicity of tobacco (*Nicotiana tobacum*) leaf dust on *Oreochromis niloticus* and haematological changes resulting from sublethal exposure. Adeyemo (2005) observed the haematological and histopathological effects of *Cassava mill* effluent in *Clarias gariepinus*. Haematological and piscicidal effects of *Carica papaya* seeds on catfish, *Clarias gariepinus* were studied by Ayotunde *et al*. (2011). At home, haemolytic effects of *Cassia siamea, Datura metel, Cassia tora* and *Albizzia lebbeck* seed extracts on *Heteropneustes fossilis* and *Channa punctatus* were studied by Nasiruddin *et al*. (2001, 2002). Nasiruddin *et al*. (2010) also studied the haemolytic effects of six plant seed extracts on *H. fossilis* whilst haematological changes in the blood parameters and blood cells of *H. fossilis* treated with *Tabarnaemontana divaricata* plant part extracts were studied recently (Nasiruddin *et al*. 2011).

It is desirable to evaluate the haematological changes in the blood parameters and blood cells of *H. fossilis* (Bloch) resulting from the toxic effects of the extracts of plant seeds of indigenous plants *S. saman* (Jacq.), *T. grandis* (L.), and *D. turbinatus* (Gaertn). The present work thus deals with the differential cell counts of the RBC, WBC and determination of haemoglobin content of *H. fossilis*. From the reading, the packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were calculated. The present study would provide a baseline data on the effect of the plant seed extracts on some haematological parameters and blood cells of the fish.

Materials and Methods

Seeds of *Samanea saman*, *Tectona grandis* and *Dipterocarpus turbinatus* locally called 'Fulkoroi', 'Shegoon' and 'Garjan' respectively were collected from Chittagong University campus. Seeds were collected from ripe fruits and pods, dried and preserved in separate air tight glass jars in the laboratory.

In order to extract the toxicant, well dried seeds were grinded at first in a mortar, and then in an electric blender and sieved through a 0.0025 cm^2 sieve. Ten grams of the grinded powder were dissolved with 100 ml of distilled water, 50% ethyl alcohol or 100% ethyl alcohol separately and thoroughly mixed by a magnetic stirrer. The resultant liquid was filtered through fine cloth to make up the stock solution. The five test concentrations (ppm) were calculated from this stock solution by appropriate dilution (APHA 1976). Healthy live fishes were collected from the local fish market of Chittagong city. The

fishes were maintained in a glass aquarium (60×30×30 cm) containing tap water and acclimatized for 3-4 hours in laboratory condition. The average total length of *H. fossilis* was 10.82 ± 0.96 cm and weight was 9.12 ± 0.57 g respectively.

Blood films of both control and treated fishes (middle dose of each experiment) were collected by cardiac puncture with the aid of needle, taken on clean greaseless slides, smeared, air dried and stained in Leishmans stain, to observe the changes of blood cells. Blood was drawn in RBC and WBC pipettes with a haemocytometer to count the RBC and WBC. Blood was also collected in a haemometer to measure the haemoglobin content. Total number of RBC and WBC were estimated using the method of Raphael (1983). Haemoglobin was measured with Sahli's haemometer. The absolute blood parameters i.e. packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were evaluated following Bhatt and Farswan (1992).

The "t" test was applied to determine level of significance of statistical analysis made between the values of normal versus treated blood parameters of each of the plant seed extracts. Microphotographs were taken with a compound microscope (Microcos- Austria MC- 100 microscope) having photographic attachment (Canon Power Shot A-640 camera, Japan) under x1000 magnification.

Results and Discussion

***Haemolytic effects of S. saman, T. grandis* and *D. turbinatus* seed extracts on the blood parameters of *H. fossilis*:** The comparative analysis of the haematological data for the experimental and control fishes is given in Tables 1 and 2. The present investigation was done only with the fishes of the mid-dose. Exposure to different extracts of *S. saman, T. grandis* and *D. turbinatus* seeds produced decrease in RBC, haemoglobin content (Hb g and %), PCV, MCV, MCH, except WBC the number of which increased (Table 1). Comparative data of the haematological parameters of control and treated fishes showed that the toxic property of the plant seed extracts induced significant changes in the blood parameters of *H. fossilis*. The RBC counts, haemoglobin contents, PCV, MCV and MCH values of blood of treated fishes decreased and the WBC counts increased with all the experimental seed extracts. Generally all the blood parameters of the experimental fishes were reduced serially in the three extracts which were proved by "t" test (Table 2). The test applied to determine the level of significance of statistical analysis made between the blood parameters of normal versus treated fishes indicated that these three plant seed toxicants had definitely haemolytic properties.

The order of calculation of the affectivity of the solvents was control < distilled water < 50% ethyl alcohol < 100% ethyl alcohol. The "t" test showed significant ($P<0.05$) decrease in values of all the haematological parameters with all the three plant seed extracts of *S. saman*, *T. grandis* and *D. turbinatus*. However, the WBC values in all the three seed extract, Hb (g) value, PCV, MCV and MCH values of distilled water and

MCH values of 50% ethyl alcohol extracts of *S. saman* seeds, PCV and MCV values of distilled water and MCH values of 50% ethyl alcohol extracts of *T. grandis* seeds, RBC, Hb (% and g), PCV, MCV and MCH values of distilled water and MCH values of 50% ethyl alcohol extracts did not show significant variation from the control group. With all the three seed extracts variation were in the order of control < distilled water extract < 50% ethyl alcohol extract < 100% ethyl alcohol extract. Trend of toxicity on the basis of the blood parameters of the three seeds, was in the order: *T. grandis* > *S. saman* > *D. turbinatus* seeds (with respect to t' test).

Table 1. RBC, WBC, Hb, PCV, MCV and MCH of control and treated individuals of *H. fossilis* treated with *S. saman*, *T. grandis* and *D. turbinatus* seed extracts (Values are expressed as mean ± SE).

Seed	Treatment	RBC ($x10^6/mm^3$)	WBC ($x10^3/mm^3$)	Hb		PCV (g/dl)	MCV ($\mu m/m^3$)	MCH (μ/mg)
				%	(g/dl)			
S. saman	Control	6.05 ±0.01	3.57 ±0.05	80.00 ±1.52	12.28 ±0.19	36.84 ±0.58	74.43 ±1.46	132.31 ±2.44
	Distilled water	5.87 ±0.03	4.49 ±0.07	69.20 ±1.11	10.60 ±0.34	31.80 ±1.00	65.50 ±1.20	117.82 ±2.17
	50% ethyl alcohol	4.42 ±0.02	5.27 ±0.07	51.20 ±1.59	7.20 ±0.29	21.60 ±0.80	61.72 ±0.19	115.78 ±3.64
	100% ethyl alcohol	3.87 ±0.02	6.16 ±0.04	42.00 ±0.85	5.40 ±0.16	16.20 ±0.51	49.62 ±2.41	108.31 ±2.32
T. grandis	Control	6.29 ±0.02	3.88 ±0.01	70.40 ±1.46	10.92 ±0.31	31.52 ±1.18	52.10 ±1.64	146.25 ±4.98
	Distilled water	5.77 ±0.08	4.21 ±0.06	58.60 ±1.08	8.72 ±0.26	26.16 ±0.65	45.36 ±1.20	110.74 ±5.85
	50% ethyl alcohol	4.19 ±0.02	4.98 ±0.02	42.40 ±3.10	7.28 ±0.49	21.84 ±1.47	42.35 ±0.85	101.90 ±8.83
	100% ethyl alcohol	3.49 ±0.02	5.42 ±0.04	35.60 ±1.08	4.72 ±0.22	13.16 ±0.65	37.55 ±1.88	100.12 ±7.43
D. turbinatus	Control	5.94 ±0.02	3.40 ±0.02	68.00 ±1.72	10.52 ±0.38	31.56 ±1.13	64.69 ±2.04	146.83 ±6.43
	Distilled water	4.92 ±0.38	4.39 ±0.03	64.00 ±1.87	08.52 ±0.24	26.16 ±0.65	58.76 ±3.65	110.74 ±5.85
	50% ethyl alcohol	3.80 ±0.22	5.57 ±0.04	52.08 ±1.19	07.18 ±0.07	21.88 ±0.69	47.02 ±3.09	106.26 ±9.44
	100% ethyl alcohol	2.82 ±0.02	5.88 ±0.04	42.04 ±1.21	04.85 ±0.21	16.08 ±1.12	43.31 ±1.75	100.12 ±7.43

A decrease in the number of erythrocytes and increasing number of leucocytes was observed in *H. fossilis* toxicated with lethal and sub lethal concentrations of distilled water, 50% ethyl alcohol and 100% ethyl alcohol extracts of *S. saman*, *T. grandis* and *D. turbinatus* seeds. These findings support the view of Dhannekar *et al*. (1985), Bhatt and Farswan (1992), Hymavathi and Rao (2000) and Nasiruddin *et al*. (2011) who reported

similar decrease in all blood parameters except WBC. The reduction in these blood parameters is an indication of anemia caused by exposure to the plant seed extracts.

Table 2. Result of "t" test between control and *S. saman, T. grandis*, *D. turbinatus* seed extract treated blood parameters of *H. fossilis* (P-values indicate significance at 0.01 level).

Seed	Treatment	RBC	WBC	Hb		PCV	MCV	MCH
				%	g			
S. saman	Control vs. distilled water	5.38 P<0.01	-10.60 P>0.01	5.73 P<0.01	4.35 P>0.01	4.36 P>0.01	4.57 P>0.01	4.42 P>0.01
	Control vs. 50% ethyl alcohol	19.57 P<0.01	-20.00 P>0.01	13.10 P<0.01	14.50 P<0.01	14.26 P<0.01	8.64 P<0.01	3.77 P>0.01
	Control vs. 100% ethyl alcohol	103.61 P<0.01	-35.97 P>0.01	21.79 P<0.01	27.41 P<0.01	26.81 P<0.01	8.81 P<0.01	7.12 P<0.01
T. grandis	Control vs. distilled water	06.67 P<0.01	-5.89 P>0.01	6.40 P<0.01	5.43 P<0.01	3.98 P>0.01	3.23 P>0.01	4.62 P<0.01
	Control vs. 50% ethyl alcohol	75.00 P<0.01	-50.00 P>0.01	7.97 P<0.01	6.27 P<0.01	5.14 P<0.01	5.20 P<0.01	4.38 P>0.01
	Control vs. 100% ethyl alcohol	84.85 P<0.01	-42.79 P>0.01	19.16 P<0.01	16.16 P<0.01	13.62 P<0.01	5.85 P<0.01	5.16 P<0.01
D. turbinatus	Control vs. distilled water	2.67 P>0.01	-27.21 P>0.01	1.57 P>0.01	4.48 P>0.01	4.14 P>0.01	1.42 P>0.01	14.15 P>0.01
	Control vs. 50% ethyl alcohol	9.22 P<0.01	-69.00 P>0.01	7.61 P<0.01	8.68 P<0.01	7.30 P<0.01	4.78 P<0.01	3.55 P>0.01
	Control vs. 100% ethyl alcohol	141.73 P<0.01	-52.89 P>0.01	12.34 P<0.01	13.13 P<0.01	9.72 P<0.01	7.96 P<0.01	4.75 P<0.01

The anemic response could be as a result of destruction of erythrocyte or inhibition of erythrocyte production (Wintrobe 1978) or haemodilution as reported by Sampath *et al*. (1993). A decrease in the erythrocyte count or in the percent of haematocrit indicates its

developing anemia (Vosyliene 1999). Analysis of erythrocyte level has shown the presence of anemia or stress polycythemia which affect the transport of oxygen to critical tissue, whereas total leucocyte counts may indicate leukemia and leucocytosis, suggesting possible immune function alteration, which is caused by the increasing number of WBC .

***Blood cells of control H. fossilis* fish:** The blood smears of control *H. fossilis* indicated that the RBC were large and oval with distinct cell wall and centrally placed small circular nucleus surrounded by a thick broad rim of non granulated cytoplasm. WBC were ovoid or spheroid shaped cells with centrally placed large sized round nucleus and distinct cell wall with clear cytoplasm. The nuclei of WBC were comparatively larger than the nuclei of RBC. In both RBC and WBC the nucleus took dark blue stain and the cytoplasm took light pink to purple color stain (Plate 1A).

***Effects of S. saman seed extracts on the blood cells of H. fossilis* fish:** Both RBC and WBC were affected with the distilled water extract of *S. saman* seeds (Plate 1B). Clumping of blood cells was evident. RBC and WBC became deshaped with damaged cell wall. RBC were deshaped with granular and bare nuclei. Syncitium was evident to some extent. Pyknotic and bare nuclei of WBC were also found.

When the fishes were treated with 50% ethyl alcohol extract, damage of blood cells was evident (Plate 1C), showing deshaped RBC with swollen nuclei. Syncitium occurred. Some RBC were seen with granular and bare nuclei. Cytoplasm of RBC was vacuolated. WBC were swollen with bare and pyknotic nuclei.

With 100% ethyl alcohol extract extensive clumping in the blood cells were evident. RBC were with extensively damaged cell membrane, deshaped nuclei and syncitium occurred (Plate 1D). Bare and granulated nuclei of RBC were also evident. WBC were with damaged cytoplasm, with vacuoles in the matrix. Bare and pyknotic nuclei of the WBC were also observed.

***Effects of T. grandis seed extracts on the blood cells of H. fossilis*:** With distilled water extract of *T. grandis* seeds, RBC were found with damaged cell wall, swollen and deshaped nuclei. Granulated nuclei of RBC and slight cell syncitium were also evident. Deshaped WBC were seen with bare and pyknotic nuclei (Plate 1E).

As a result of the effect of 50% ethyl alcohol extract RBC were deshaped with damaged cell wall and swollen and granulated nuclei (Plate 1F). Slight syncitium in RBC matrix was evident. WBC were with dissolved cytoplasm and with irregular and deshaped nuclei. Some WBC were found to be with damaged cell wall and with swollen and pyknotic nuclei.

Exposed to 100% ethyl alcohol extract, extensive clumpings of both RBC and WBC were evident (Plate 1G). Cell membrane of RBC disintegrated and syncitium occurred. Vacuoles in the matrix and granulated nuclei of RBC were evident. WBC were shrunken with pyknotic, bare and deshaped nuclei.

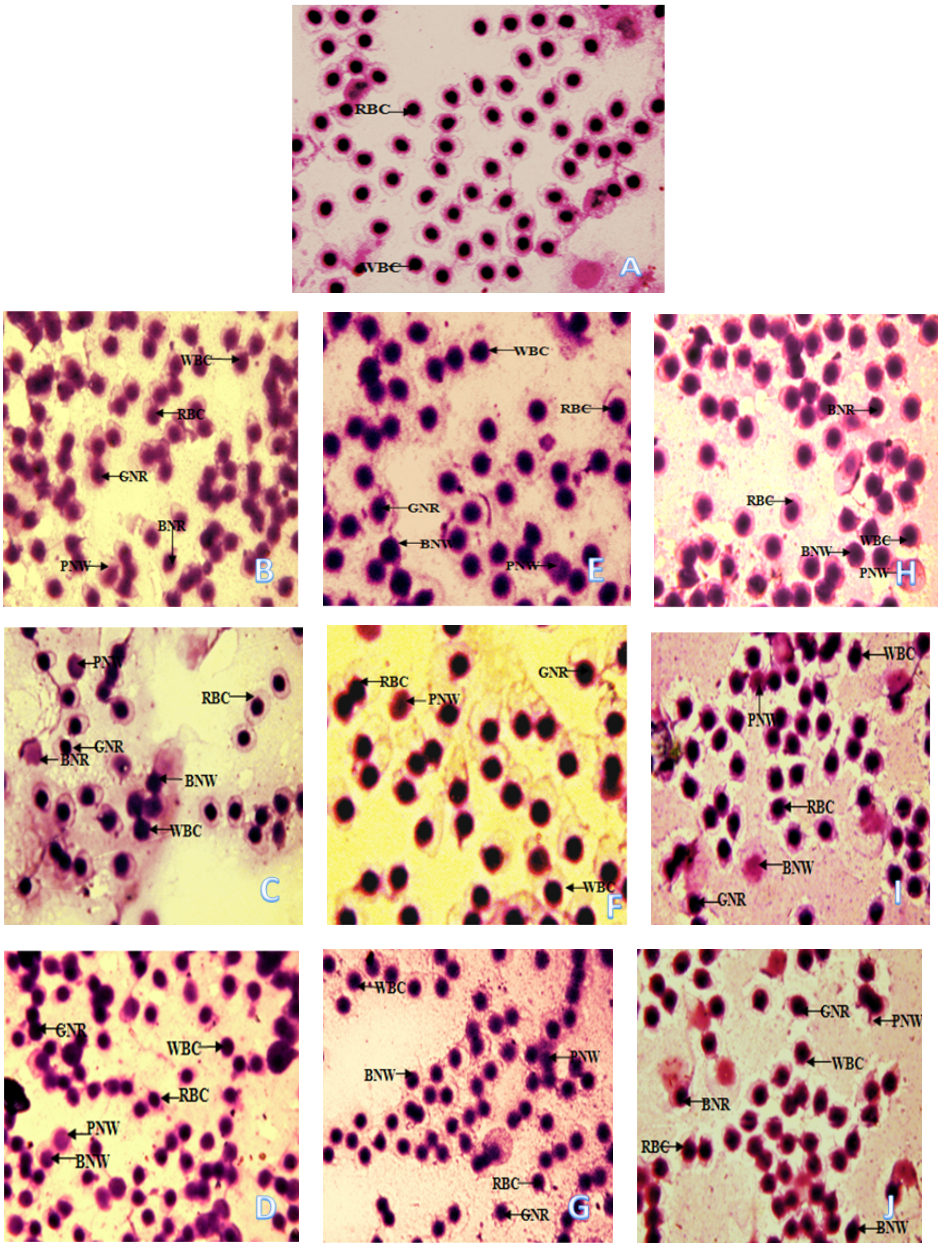

Plate 1. Microphotographs of blood cells of *Heteropneustes fossilis* (×1000). (A). Control; and treated with (B). Distilled water, (C). 50% ethyl alcohol, (D). 100% ethyl alcohol extracts of *S. saman* seeds; (E). Distilled water, (F). 50% ethyl alcohol, (G). 100% ethyl alcohol extracts of *T. grandis* seeds and (H). Distilled water, (I). 50% ethyl alcohol, (J). 100% ethyl alcohol extracts of *D. turbinatus* seeds. (BNR= bare nuclei of RBC, BNW= bare nuclei of WBC, PNW= pyknotic nuclei of WBC, GNR= granular bare nuclei of RBC).

***Effects of D. turbinatus seed extracts on the blood cells of H. fossilis*:** Slightly swollen, deshaped and irregular RBC with damaged cell wall were the principal changes in the blood of *H. fossilis* exposed to distilled water extract (Plate 1H J). Some RBC were damaged. Granules and vacuoles were seen in the cytoplasm of RBC matrix. Some were with bare nuclei. WBC were also found with bare and pyknotic nuclei.

RBC were shrunken, deshaped and disintregrated. Cell clumping and syncitium occurred, vacuoles in some RBC cytoplasm were evident when exposed to 50% ethyl alcohol extract (Plate 1I). Granulated nuclei of RBC were also observed. WBC were with deshaped, bare and pyknotic nuclei.

The effects of 100% ethyl alcohol extract were greatly noticeable (Plate 1J). Cell membrane of RBC disintegrated hence syncitium occurred. Bare and granular nuclei of RBC were evident. Bare and pyknotic nuclei of WBC were also evident. Cytoplasm of both RBC and WBC were reduced to some extent.

Morphological changes in blood cells observed in blood smear are one of the most specific indices of toxic effect on fish. From the study of blood cell slides, the blood smear in normal fishes were without haemolysis and that of treated fishes showed haemolysis of RBC and WBC in many ways, such as changes in shape, damage of cell membrane, swollen nuclei, clumping and formation of syncitium in RBC matrix, whereas nuclei of WBC were pyknotic and bare. Similar haemolytic effects of plant toxicants were found by Bhatt and Farswan (1992), and Nasiruddin *et al*. (2001, 2002, 2011). In the present study, from the study of blood film damages the order of toxicity followed the pattern: *D. turbinatus* > *S. saman* > *T. grandis* seed extracts.

The toxic substances in the medium changed the haemopoitic activity. The active ingredients of the three indigenous plant seeds influenced the dynamicity of haemopoesis in *H. fossilis*. The effects on blood parameters suggested that the physiology of the fishes was disturbed to an extent resulting in the death of the fishes whilst being toxified with these plant seed extracts. On the basis of haematological studies, it would be possible to forecast the physiological state of fish.

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GASTROINTESTINAL HELMINTHS IN PIGEON *COLUMBA LIVIA* (GMELIN, 1789)

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Abstract

A total of 60 pigeon, *Columba livia* (25 males and 35 females) were examined to observe helminth parasite infection. All the birds were found to be infected by eleven species of helminth parasites: four species of trematoda: *Echinostoma revolutum* (15%) *E. trivolvus* (5%), *Patagifer bilobus* (5%), *Ehinoparyphium recurvatum* (8.33%); six species of cestoda: *Hymenolepis columbae* (63.33%), *Raillietina echinobothrida* (100%), *R. bonini* (43.33%), *R. cesticillus* (100%), *Cotugnia celebesensis* (68.33%), *C. cuneata* (100%); and one species of nematoda: *Ascaridia columbae* (28.33%). Females showed slightly higher intensity of infestation than the males**.** Trematode parasites were found in intestine and rectum, cestode parasites were found in duodenum and intestine, nematode parasite *Ascaridia columbae* was found in caeca. Oesophagus, crop, proventriculus, gizzard, gallbladder, liver, kidney and muscles were free of parasites. Considering among seasons highest intensity of infection was found in autumn.

Key words: Helminth parasites, pigeon, prevalence, intensity, season.

Introduction

Pigeons (*Columba livia*) are among poultry species kept in the Bangladesh where they are a part of subsistence farming done by most poor families. However, little is known about their socio-economic importance, management and health aspects. Due to perceived little importance of pigeon little attention in term of research has been directed towards the species in Bangladesh. However, in many parts of the country pigeons are seen daily scavenging for food together with other poultry species. It's interaction with man and other domestic and wild birds, portends it as a potential carrier of zoonotic parasites.

The prevalence and intensity of parasitic infestations may be influenced by a number of epidemiological factors including host factors such as age, sex and breed and environmental factors such as climatic conditions (Nadeem *et al*. 2007). Investigations on chickens and ducks managed under similar conditions like pigeons have shown higher prevalence of gastrointestinal helminths (Muhairwa *et al.* 2007) which impair productivity and health of these birds.

As helminth parasites of poultry cause a great economic loss to poultry in Bangladesh, therefore, the present study has been designed to investigate the occurrence, prevalence, intensity, organal distribution and seasonal infestation of helminth parasites of pigeon.

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Materials and Methods

A total of 60 pigeon (25 males and 35 females) were randomly collected from February 2010 to January 2011. The pigeons were examined at the parasitology laboratory of Zoology Department, Dhaka University. The separated parts of the alimentary canal of the pigeons were taken in 0.85% normal saline solution to collect helminth parasites. Binocular dissecting microscope was used to detect and collect parasites. The trematode, cestode and nematode parasites were fixed with AFA solution and preserved in 70% alcohol. Before preparing permanent slides the parasites were removed from alcohol and cleaned in lactophenol. A suggestive method was used for the fixing, clearing and staining of the worms (Cable 1963). The collected helminths were identified according to Yamaguti (1958, 1959 and1961).

Prevalence of individual parasite species was calculated as a percentage of the host population that was infested with a specific parasite at a point in time (Thrusfield 1995). Intensity was calculated as number of parasites per infected birds.

Results and Discussion

Of the 60 pigeons examined, all the birds were found to be infested by helminth parasites (Table 1). Eleven species of helminth parasites were identified, of which four species of trematoda were *Echinostoma revolutum* 21 (15%), *E. trivolvus* 8 (5%), *Patagifer bilobus* 11 (5%), *Ehinoparyphium recurvatum* 17 (8.33%); six species of cestoda were *Hymenolepis columbae* 185 (63.33%), *Raillietina echinobothrida* 244 (100%), *R. bonini* 121 (43.33%), *R. cesticillus* 139 (100%), *Cotugnia celebesensis* 177 (68.33%), *C. cuneata* 213 (100%) and one species of nematode was *Ascaridia columbae* 49 (28.00%). According to Radfar *et al.* (2011), the prevalence of helminth *Ascaridia colombae*, *Cotugnia digonopora*, *R. magninumida* and *R. achinobothridia* were 16.66, 13.79, 18.62,

Table 1. Prevalence and intensity of helminth infections in *C. livia.* (n=60).

Name of the parasites	No. of host infected	Prevalence (%)	total no. of parasites collected	Intensity ± SD
Trematoda				
Echinostoma revolutum	09	15.00	21	2.33 ± 0.032
E. trivolvus	03	5.00	08	2.67 ± 0.298
Patagifer bilobus	03	5.00	11	3.67 ± 0.049
Ehinoparyphium recurvatum	05	8.33	17	3.40 ± 0.035
Cestoda				
Hymenolepis columbae	38	63.33	185	4.87 ± 0.061
Raillietina echinobothrida	60	100.00	244	4.07 ± 0.056
R. bonini	26	43.33	121	4.65 ± 0.027
R. cesticillus	60	100.00	139	2.32 ± 0.029
Cotugnia celebesensis	41	68.33	177	4.32 ± 0.029
C. cuneata	60	100.00	213	3.85 ± 0.045
Nematoda				
Ascaridia columbae	17	28.00	49	2.88 ± 0.065

32.35%, respectively. Natala *et al.* (2009) also reported that *Raillietina tetragona* (4.9%), *R. cesticillus* (3.0%), *R. echinobothrida* (7.6%), *Ascaridia columbae* (1.2%), *A. galli* (1.2%) and *Cappillaria anatis* (0.8%). The values found in the present study of helminth infestation were significantly higher than those of helminth infestation of pigeon observed by Radfar *et al.* (2011) and Natala *et al.* (2009). So far, information on the helminth parasites of *Columba livia* is scanty in Bangladesh, except that of Begum *et al.* (2008) who observed epidemiology and pathology of protozoan parasites in *Trichomonas gallinae* in the common pigeon (*Columba livia*). The present study thus provides baseline or preliminary information on the subject. Almost all the birds were found to be infested with different types of endoparasites throughout the year and each bird was found to harbour more than one type of endoparasites. The overall prevalence of endoparasites on pigeons in the present study appears to be high. Msoffe *et al.* (2010) in Tanzania also found high prevalence (79.5%) of helminth infection. The overall prevalence of various parasites differs greatly among the previous reports as well as when compared with present observation. Diversity of bird endoparasite assemblages may be related many factors, which may include home range, behaviour, size and roosting habit of the host. This may also be attributed to difference in the geographical areas and period of study.

Table 2. Organwise distribution of helminth parasites in *C. livia.* (n=60).

Name of the parasites	Duodenum	Intestine	Caecum	Rectum	Total no. of parasite
Echinostoma revolutum	-	3 (14.29%)	-	18 (85.71%)	21
E. trivolvus	-	1(12.5%)	-	7 (87.5%)	8
Patagifer bilobus	-	4 (36.36%)	-	7 (63.64%)	11
Ehinoparyphium recurvatum	-	2 (11.765)	-	15 (88.24%)	17
Hymenolepis columbae	91 (49.19%)	94 (50.81%)	-	-	185
Raillietina echinobothrida	184 (75.41%)	60 (24.59%)	-	-	244
R. bonini	69 (57.02%)	52 (42.98%)	-	-	121
R. cesticillus	52 (37.41%)	87 (62.59%)	-	-	139
Cotugnia celebesensis	89 (50.28%)	88 (49.72%)	-	-	177
C. cuneata	104 (48.83%)	109 (51.18%)	-	-	213
Ascaridia columbae	-	-	49 (100%)	-	49
Total no. of parasites	589	500	49	47	1185
(% of parasites in different organ)	(49.70)	(42.19)	(4.14)	(3.97)	

The helminth parasites were removed from different parts of the alimentary canal. Maximum infestation was found in duodenum. The rate of helminth infection in duodenum was 49.70%, in intestine it was 42.19%. Rectum was found to be less infected than the other organs (Table 2). Parasites of vertebrate hosts feed either on the digested contents of the host in the alimentary canal or the hosts own tissues. The duodenum and intestine seems to be a preferable site for helminth parasites (Marcov 1946). The

abundance of trematode and cestode parasites in the intestine may be related to their feeding behaviour as they are partially or completely devoid of digestive system. Hence, they absorb the simplest form of nutrients through the cuticle.

The results presented in Table 3 show that the overall prevalence of infestation was 100% in both sexes and the intensity of females was higher (20.09) than the males (19.28). The male and female birds had eleven endoparasite species each. *Raillietina echinobothrida*, *R. cesticillus* and *Cotugnia cuneata* showed 100% prevalence both in male and female birds. Highest intensity of infection (5.77) was found in *C. celebesensis* male and 4.46 was in *Hymenolepis columbae* female pigeon. Differences between the overall intensity of male and female hosts were very poor, so with this study, it has been proved that gender is not important in helminth infections in pigeon. It was found that the infection rates of male and female were very close to each other, which was statistically insignificant (P>0.05). Senlik *et al*. (2005) reported that the gender was not important factor for helminth infections in pigeons.

Table 3. Prevalence of endoparasites according to sex in *C. livia*. (Female (F) =35; Male (M) =25)

	No. of birds infested		Prevalence (%)		Total no.of endoparasites recovered		Mean intensity (± SD)	
	M	F	M	F	M	F	M	F
Echinostoma revolutum	3	6	12	17.14	8	13	2.66 ± 0.097	2.16 ± 0.052
E. trivolvus	1	2	4	5.71	3	5	3.00 ± 0.025	2.50 ± 0.082
Patagifer bilobus	3	0	12	0	11	0	3.66 ± 0.048	0
Ehinoparyphium recurvatum	0	5	0	14.29	0	17	0.00 ± 0.00	3.40 ± 0.036
Hymenolepis columbae	18	24	72	68.57	78	107	4.33 ± 0.097	4.46 ± 0.099
Raillietina echinobothrida	25	35	100	100	93	151	3.72 ± 0.052	4.31 ± 0.071
R. bonini	11	17	44	48.57	52	69	4.73 ± 0.094	4.06 ± 0.062
R. cesticillus	25	35	100	100	56	83	2.24 ± 0.067	2.37 ± 0.076
Cotugnia celebesensis	13	28	52	80	75	102	5.77 ± 0.008	3.64 ± 0.046
C. cuneata	25	35	100	100	90	123	3.96 ± 0.052	3.51 ± 0.045
Ascaridia columbae	7	11	28	31.43	16	33	2.29 ± 0.068	3.00 ± 0.027

Table 4. Seasonal infestation of helminth parasites in *C. livia.* (n=60)

Name of the Parasites	Autumn (n=15)				Winter (n=16)				Spring (n=17)				Summer (n=12)			
	No. of host infected	prevalence (%)	Total no. of parasites collected	Mean intensity	No. of host infected	prevalence (%)	Total no. of parasites collected	Mean intensity	No. of host infected	prevalence (%)	Total no. of parasites collected	Mean intensity	No. of host infected	prevalence (%)	Total no. of parasites collected	Mean intensity
Echinostoma revolutum	2	13.3	3	1.5 ± 0.230	2	12.5	5	2.5 ±0.039	3	17.65	8	2.67 ± 0.043	2	16.67	5	2.5 ± 0.039
E. trivolvus	0	0	0	0 ±0.00	2	12.5	5	2.5 ±0.039	1	5.88	3	3 ± 0.024	0	0	0	0 ± 0.00
Patagifer bilobus	0	0	0	0 ±0.00	0	0	0	0 ±0.00	2	11.76	7	3.5 ± 0.039	1	8.33	4	4 ± 0.113
Ehinoparyphium ecurvatum	1	6.66	4	4 ± 0.113	2	12.5	8	4 ±0.113	0	0	0	0 ± 0.00	2	16.67	5	2.5 ± 0.039
Hymenolepis columbae	10	66.6	35	3.5 ±0.03	15	93.75	81	5.4 ±0..07	13	76.47	69	5.31 ± 0.108	0	0	0	0 ± 0.00
Raillietina echinobothrida	15	100	98	6.53 ±0.073	15	93.75	74	4.93 ±0.08	15	88.23	28	1.87 ± 0.233	12	100	44	3.67 ± 0.087
R. bonini	0	0	0	0 ±0.00	0	0	0	0 ±0.00	15	88.23	76	5.07 ± 0.089	11	91.6	45	4.09 ± 0.071
R. cesticillus	15	100	52	3.47 ± 0.037	15	93.75	39	2.6 ±0.047	15	88.23	27	1.8 ± 0.199	12	100	21	1.75 ± 0.043
Cotugnia celebesensis	15	100	79	5.27 ±0.079	15	93.75	61	4.06 ±0.12	0	0	0	0 ± 0.00	11	91.6	37	3.36 ± 0.029
C. cuneata	15	100	82	5.46 ±0.075	15	93.75	57	3.8 ±0.052	15	88.23	43	2.87 ± 0.030	12	100	31	2.58 ± 0.046
Ascaridia columbae	10	66.6	32	3.2 ±0.02	7	43.75	17	2.43 ±0.10	0	0	0	0 ± 0.00	0	0	0	0 ± 0.00

R. echinobothrida, *R. cesticillus* and *C. cuneata* have 100% prevalence in autumn. *P. bilobus* and *R. bonini* showed the lowest prevalence, it was absent in autumn and winter but the prevalence of *R. bonini* in summer was 91.66% and in spring it was 88.23% (Table 4). *A. columbae* was absent in spring and summer. In previous studies (Senlik *et al*. 2005) helminth infections were more commonly observed during the autumn and winter and abundant rain along with a mild winter create a suitable environment for infestation and the development of helminth eggs. Maximum infection rate in pigeons was seen in October (Sari *et al.* 2008). In the present study, infections in autumn were highest supporting the finding of Sari *et al.* (2008) from domestic and mild pigeons of Turkey.

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ECOLOGY AND BREEDING BIOLOGY OF THE POND HERON, *ARDEOLA GRAYII* (SYKES, 1832) AND ITS CONSERVATION ASPECTS

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Abstract

Studies on ecology and breeding biology of the pond heron, *Ardeola grayii* was conducted from January to June, 2010 in Kalairpur Assim Union, Fulbaria under Mymensingh district. The breeding season of this bird ranged from February to June. The peak period for nesting was March. The pond heron choose the nesting site surrounding the human settlements near the pond and other water bodies. This species took 7 to 14 days to complete nest with an average of 10.8 days. The height of nest varied from 5.2 m to 10.9 m with an average of 7.82 m. The main nesting trees were bamboo (*Bambusa longispiculata*) and mango (*Mangifera indica*). Nesting materials were chiefly small sticks of mango, tamarind (*Tamarindus indica*), bel (*Aegle marmelos*) and leaves of bamboos. The clutch size varied from 3 to 4 eggs with an average of 3.7 eggs. The incubation period varied from 19 to 23 days with an average of 20.73 days. The hatching success was 90% in relation to the number of eggs laid. The fledging period of young varied from 25 to 29 days with an average of 26.73 days. Young individuals left the nest after 3 to 4 weeks. Results of this study indicate that the pond heron can breed successfully for their survival if indigenous plants trees are available to build their nest. So Government should make immediately an integrated conservation plan to protect this bird with the co-operation of national and international organizations.

Key words: Ecology, breeding biology, pond heron, conservation aspect

Introduction

The pond heron, *Ardeola grayii* is locally known as Kani bok/ kana bok / korchey bok and is common resident bird of Bangladesh. It is usually found to forage alone or in small, loose parties and occurs in all water bodies across the country (Ali and Ripley 1983). It is colonial nesting species, frequently found on the fresh water marshes, ditches, canals, riverbanks, paddy fields, jheels, tidal flats and other such natural wetlands. The pond heron chiefly feeds on animal matter, mainly on fishes, crustaceans, insects, amphibians and some vegetables on the ground (Ali and Ripley 1983). This heron species is an important biological component to maintain the food chain, ecosystem and can help to control the insect in the agricultural fields.

Some information of breeding biology of the pond heron, *Ardeola grayii* and other species of birds are available in the literatures and research papers (Baker 1922, Fletcher

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and Inglis 1936, Smythies 1953, Whistler 1963, Brown 1964, Ali and Ripley 1968, 1972, 1983, Henry 1971, Ali 1977, Husain 1979, Zacharias and Gaston 1982, Sarker 1987, Khan 1988, Ray 1992, Jaman *et al*. 1997, Jaman and Sahreen 2004). However, to the best of our knowledge, no detailed research work on the breeding ecology of this bird has been reported. Therefore, we conducted a detail research on the ecology and breeding biology of the pond heron. This study may help in the conservation of this bird species.

Materials and Methods

The study was carried out from January 2010 to June 2010. Information in detail on the breeding biology of 10 nests was collected. The study area was visited from two to four times in each month in order to collect sufficient data. The methods adopted were (i) selection of nesting trees and (ii) counting of nests and birds. Roosting and nesting trees of the pond heron were identified and listed. Vegetation and fauna of the study area were also recorded. Interview was conducted to local people to gather more information about the studied species. Density of nest was calculated per 12 km^2 area. Feeding ground was also surveyed to observe the feeding behaviour. For closer observations in mating, nest building, incubation, brooding, nestling and fledging of birds a portable hide and a pair of binoculars were used. The activities of the pond heron were also observed with the help of a pair of binocular. Digital camera was used to take photographs of various activities of the birds. For weighing and measuring the nests, eggs, nestling and young ones, an electric balance, spring balance, a wooden scale, a steel tape, a plastic tape and slide caliper were used. The eggs were marked as 1, 2, 3, 4, 5, etc. according to laying of eggs to determine the incubation period and hatching intervals. Measuring and weighing of eggs were done once in a week and records were kept in tabular form with dates. Any loss of nests, eggs, and nestling and factors affecting the damage of eggs and nestlings were also noted. Fledging period, breeding success and activities of young before and after left the nest were also recorded. Breeding season and inter action with other birds at that time were also observed and noted. Plastic bottle and formalin were used to keep and preserve the dead nestlings, eggs and food materials.

Study Area : The study area is situated under the Assim Union, Fulbaria Upazila in the district of Mymensingh and about 110 km north of Dhaka. It is predominantly agricultural landscape and the colony is on the backyard trees in the middle of rural settlement. The study area was divided into two 'paras' known as Uttarpara and Dokhinpara under the village Kalairpur. It is about 20 km far from Fulbaria Upazila. The total study area is approximately two acres. There are four ponds in Shakhidarpara and two ponds in Dakshinapara which are fulfilled by water in the rainy season. Water of the ponds dries up in winter. The approximate area of individual pond of Shakhidarpara is 9 decimal, 15 decimal, 8 decimal and 5 decimal and the approximate area of individual pond of Dakshinpara is 7 decimal and 3 decimal.

The study area supported a homestead forest including bushes, bamboos, grasses and several other tall and medium sized trees making good covers and shelters for the wildlife. During the study period the average temperature was 29°C, average humidity 79% and average rainfall was 298 cm. The dominant vegetations were *Aphanamixis polystachya, Cedrella toona, Bambusa longispiculata, B. vulgaris, Trichosanthes bracteata, Derris indica, Samanea saman, Albizia procera, A. richardiana Cassia fistula, Bombyx ceiba, Ficus hispida, F. lacor, Artocarpus heterophylus, Pinus longifolia, Eucalyptus citriodora, Syzygium cumini, Mollolus philippense, Croton tiglium, Miliusa velutina, Solanum torvum, Mangifera indica, Anona squamosa, Polyalthia longifolia, Phoenix sylvestris, Delonix regia*, *Aegle marmelos* and *Musa paradisiaca*.

Results and Discussion

***Breeding season*:** Breeding season of the pond heron, means the time of a year during which the animals form pair, mate, build nest, lay eggs and rear the nestling and young. The breeding activities of the pond heron started from February and continued up to June. No breeding activities were found during the rest of the year. The peak nesting record was found in March when the average temperature was about 29°C, humidity 79% and rainfall 298 cm.

Baker (1922) mentioned that the breeding season of the pond herons started from December to March in Southern India and June to August in Northern India and Myanmar. Ali (1977) stated that the breeding season in North India was between April and September, December to February in the South.

***Pair formation*:** Pair formation is the first step of breeding biology (Welty 1975). Pairing of the pond herons occurred between February and March. Most of the pairing takes place in February. In this time they like to stay close together all the time. But in the non breeding season they prefer to stay separately. Information was scanty about the pair formation of the pond herons. Sexes were not recognized by earlier authors although some authors mentioned that both sexes were alike.

***Sex recognition*:** Both males and females were looked alike except in the breeding season. The head and neck became yellowish brown in the breeding season. The back became deep maroon with long feathers over the tail. The plumages became longer and white. The tarsal region became reddish in colour. The rest of the body became white. The male pond heron was found to be bigger than the female.

***Nest site selection*:** In the present study the male and the female selected the nesting site. The pond heron choose the nesting site surrounding the human settlements near the pond and other water bodies for finding food easily. They preferred the top branches of the trees. Generally tree-forks were the favorite sites for nest. This bird builds their nest with other birds like night herons, egret or other storks, etc and form a nesting colony. Nesting trees

were – bamboo and mango. Whistler (1963) stated that the pond herons preferred to build nests in trees, usually at a good height from the ground. Henry (1971) mentioned that the pond herons preferred to build their nest at varying heights mostly under the tree canopy. Ali and Ripley (1968) mentioned that the nest site was placed in the same sort of sites as used for day time roosts. Ali (1977) found the nesting sites of the pond herons in colonies in canopy of large trees or screw pine brakes and near the water bodies. Ali (1977) stated that the pond herons build their nests in upper canopy of bamboo thickets, bushes and trees.

***Nest*:** The nest was generally a large platform of petioles of leaves and sticks without lining. The shape of the nest might be circular or round or of any other shape suiting the shape of the branch on which the nest was lodged. But its inner side always remained characteristically cup shaped by elevating sides and lowering middle of the nest where the eggs were placed. In the study we found all nests were similar in size and shape.
Birds make their nests mainly to protect themselves, their eggs and particularly their developing young from predatory animals and from adverse weather during the breeding season (Welty 1975).

***Nesting trees*:** In total 150 nests were counted in the colony. Among them, 100 nests were recorded at the village side and 50 nests in the other side. The nesting trees were mainly bamboo, mango, krishnachura and bahera, etc.

***Nesting materials*:** The nest was generally a large platform of twigs and sticks with a core depression in the centre. The pond heron mainly used small sticks of bamboo as nest materials. Besides this they commonly found to use leaves and branches of Tetul (*Tamarindus indica*), Koroi (*Albizia procera*), mango, pakur (*Ficus relgiosa*) and bel (*Aegle marmelos*).

***Collection of nestling materials*:** The pond heron collected nesting materials from the neighboring trees. The collection of nest material was rapid in the morning and gradually declined towards noon. In the rainy day the collection of nest material was subsided. They picked up nesting materials and quickly carried by beak to the nesting tree and untiringly to build their nest. Only male partner took part in the collection of nesting material and the female stayed in the nest and took part in nest building.

***Nest building*:** Like black drongo (Jaman *et al*. 1997) both the sexes of the pond heron shared and worked hard in building their nest but the female pond heron was more active than the male. In the earlier part of construction the nest materials were arranged in a criss - cross fashion, resulting a circular platform. When the platform was three to four sticks, the additional sticks were set on it tangentially and converted it into a shallow cup.

The outer cup was done by both the male and the female though the female did the final construction of the inner cup with its breast, wings, bill and legs. The cups of all the nests were without lining.

***Time required for nest building*:** The pond heron took 7 to 14 days to build the nest (average: 10.8 days, N = 10 nests) (Fig. 1). They took comparatively more time in nest building at the starting of the breeding season than late of the breeding season. The minimum height of ten nests from the ground was 5.2 m and the maximum was 10.9 m (average: 7.82 m).

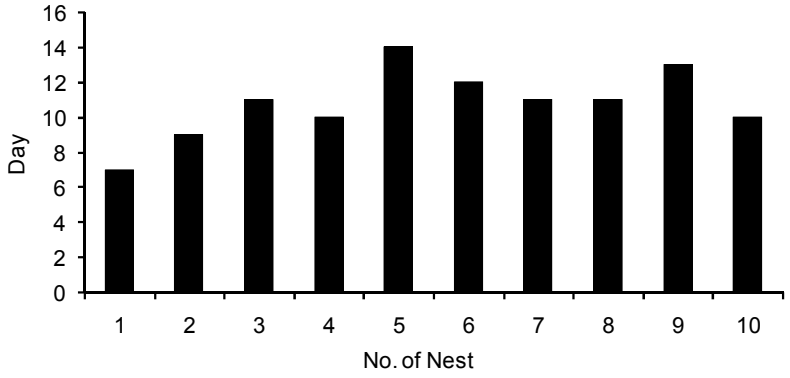

Fig. 1. Total time (days) required for nest building height of the nests.

***Measurement of nests*:** The length of the nests varied from 31.5 to 36 cm (average: 33.58 cm, N = 5). The breadth of the nests varied from 24 to 28 cm (average: 7.94 cm). The depth of the nests cup was 2.4 to 3.5 cm (average: 2.82cm). The weight varied from 175 to 320 g (average weight: 247 g).

***Mating*:** Mating is the vital fact for breeding biology. For mating they need a quiet environment. In the present study, the mating of the pond heron was observed in the morning and late afternoon. Mating took place on the branches of the trees, or on the ground and even in the nests, sometimes on the ground near the water body and even when the female was sitting on the nest. Before mating the male and the female sat closely together. At that moment the male scratched the female's forehead for several times. Suddenly the male rode over the female's back by constantly fluttering his wings and tried to mate with her. The male bird bent his tail along the left or right side of the female's tail and brought their cloaca close together. Mating took place in this fashion. Generally mating lasted for 20 to 30 seconds.

***Egg Laying*:** During the present study first egg laying was observed in late March and it was continued until April. The gap between the completions of laying of eggs varied from 1 to 2 days.

***Clutch size and time of egg laying*:** The clutch size is the complete set of eggs laid by one female which are brooded simultaneously (Thomson 1964). The pond heron has one clutch size in a year in nature. Its clutch size varied from 2 to 4 with an average of 3 eggs. Most of the eggs were laid at late night or early in the morning. Usually this species laid one egg a day until the clutch was completed. The gap between egg laying varied from 1 to 2 days. It was found that two eggs were not laid in the same day. The shapes of the eggs were broad, longish oval and somewhat pointed towards the smaller end. The colour of egg was usually pale greenish blue without any marking as was mentioned by Ali and Ripley (1972). The egg color changed into dark before hatching.

***Measurements and weight of eggs*:** In total 30 eggs were measured. The maximum length was 42 mm and the minimum length was 34 mm and the maximum breadth was 37 mm and minimum breadth was 29 mm (average length/breadth: 39.52/32.56 mm). The average weight of eggs was 20.05 g (Table 1). The maximum and the minimum weight were 23.5 g and 17 g, respectively. It was found that the weight of the eggs gradually decreased with the time of incubation. The decreasing rate was very high for infertile eggs.

Table 1. Estimated measurement of eggs.

Sl. No. of nest	Clutch size	Weight of eggs (g)				Mean weight (g)	Average weight (g)	Maximum weight (g)	Minimum weight (g)
		1st	2nd	3rd	4th				
1	3	21	20.3	22	-	21.5			
2	3	17	23.5	22	-	20.83			
3	2	20	22	-	-	21			
4	3	18	22	20	-	20			
5	4	21	22	19	17	19.75	20.05	23.5	17
6	3	19	18	20	-	19			
7	3	21.5	22	18.5	30	20.67			
8	2	19	17	-	-	18			
9	4	19	20.5	22	21.5	20.5			
10	3	22	18.5	18.5	-	19.67			

***Incubation*:** Incubation is the process by which the heat necessary for embryonic development is applied to an egg after it has been laid (Thomson 1964). In case of the pond heron, incubation started as soon as the first egg was laid and continued up to the hatching. It was found that the incubation was not continuous in the day time and the eggs remained uncovered for sometimes. The female pond heron played a vital role for incubation. She incubated the eggs continuously at night and the male shared during the

day time. When the female went out in search of food, the male entered the nest and sat on the eggs until the female came back. The female spent 97.25% time and the male spent 2.75% time in incubation. Ali and Ripley (1968) mentioned that both sexes took part in incubation. During incubation, the incubating bird changed their face at several directions and moved after a few minutes interval. Sometimes they transferred the position of eggs with their bills.

The percentages of incubating time in 5th, 10th, 15th, and 20th day were 87.77%, 85%, 82.22% and 79.16%, respectively (Table 2). In this study, the incubation period was considered from laying of first egg to its hatching (Welty 1975). The incubation period varied from 19 to 23 days with an average of 21.01 (SD = 0.86) days.

Table 2. Duration of incubating time in the day time.

Sl. No. of Nest	Day	Incubating time (Min.)												Incubation time (Min.)	Percentage of time (%)
		6-7 am	7-8 am	8-9 am	9-10 am	10-11am	12-13 pm	13-14 pm	14-15 pm	15-16 pm	16-17 pm	17-18 pm	18-19 pm		
1	5th	53	57	49	55	51	58	50	48	54	56	52	49	632	87.77%
2	10th	52	56	53	47	55	50	51	58	50	45	46	49	612	85.00%
3	15th	44	55	51	45	54	52	48	51	42	47	50	53	592	82.22%
4	20th	40	47	52	50	49	52	53	50	42	43	44	48	570	79.16%

***Hatching*:** Out of 30 eggs, 27 hatched and the remaining 3 (10%) were lost due to various causes. The percentage of hatching success was 90% in relation to eggs laying. Out of 30 eggs, 2 (2.41%) were hatched in April and 25 (92.59%) in May. Generally the eggs hatched in order in which they were laid. The minimum gap between hatching of eggs was 1 day and the maximum gap was 3 days.

***Post hatching*:** The newly hatched young was naked. The eyes were closed. They were too weak and completely dependent on their parents. The featherless body was blackish in colour and the abdomen was almost transparent. The mouth cavity of hatchling was reddish in colour.

Out of 10 nests studied, six nests contained three broods and four nests contained two broods (Fig. 2A). The average brood size was 2.7. The parents covered the young for protection from the sun, rain, and predator as well as to incubate the rest of the eggs. The role of the male and the female in brooding was same as in incubation. The frequency decreased with the increase of the nestling's age. The brooding period varied from 19.8 to 21.3 days (Fig. 2B).

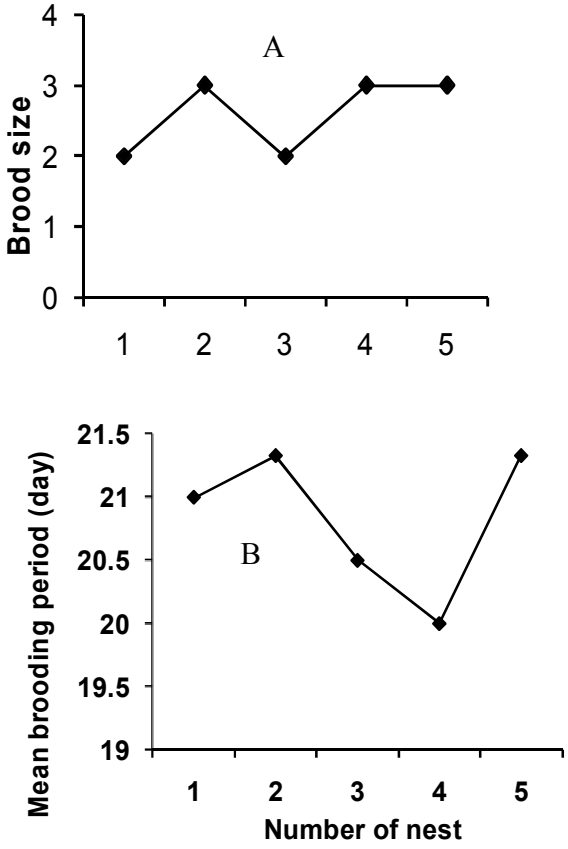

Fig. 2. **A.** Brood size and B. brooding periods of the pond heron.

***Feeding of nestlings*:** The nestling did not take food for the first 24 to 36 hours. Afterwards, parents collected food and fed the nestlings regularly. When the parents entered into the nest, the nestling made characteristic food beginning noise and opened their mouth cavity widely, pointing towards the parents by stretching the neck as much as possible, and then the parents fed the nestlings by the process of regurgitation. After collection of food the pond herons used to sit on the branch of tree nearby the nest and then entered into the nest for feeding. Sometimes the male transferred some food directly to the mouth of the female for feeding the nestlings. The frequency of feeding was not continuous in whole day. It was the highest in morning and afternoon and at noon. The frequency of feeding visits to the nestlings in a day was found to vary from 8 to 11 times (Fig. 3).

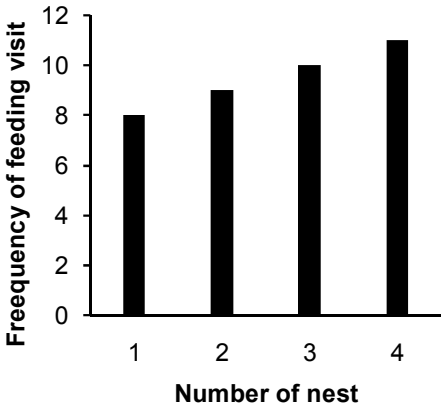

Fig. 3. Different feeding time in the day.

***Growth and development of nestlings*:** After hatching the nestling began to grow and increased in weight gradually. But the rate of increase was not equal in all days of nestling life. The daily weight of nestlings increased continuously up to the age of two weeks (Fig. 4). Afterwards the weight decreased to some extent before leaving the nest. Decrease of weight was due to the factors like human interferences, starvation, natural calamities, etc. The mean weight of newly hatched nestlings was 16 g (N=12). The maximum growth rate of nestling in weight was 228 g at the 15th day after hatching (Fig. 4). The time required for opening of the eyes was 3 to 5 days and the average time was 3.9 days. The opening was first marked by a very small aperture on the bare skin over both the eyes.

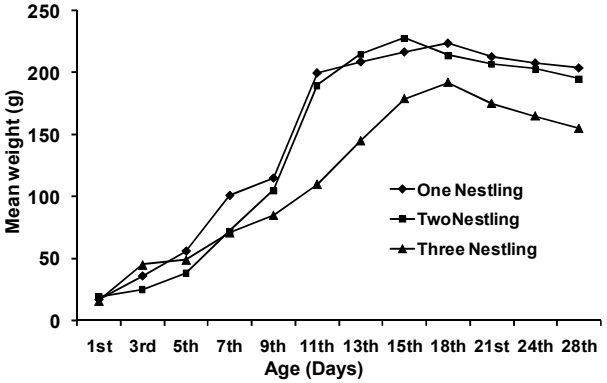

Fig. 4. Average growth curve of broods having one nestling, two nestlings and three nestlings.

***Loss of nestlings and nest sanitation*:** The pond heron was very careful about the sanitation of their nest. The broken shells were removed by the parents after hatching. Both the male and the female shared in nest sanitation. None nestling was destroyed.

***Fledging period*:** In the study period, the fledging period of the young varied from 25 to 29 days on an average 26.73 days. Out of 27 nestlings, 27 (100%) fledged successfully. The fledging success was 100% in relation to hatching and 90% in relation to egg laying.

***Breeding success*:** The breeding success in relation to the number of eggs laid was calculated to be 90%.

***Dispersal*:** After the successful fledging the young remained in their nest from 2 to 3 weeks. They visited the feeding ground with their parents or went alone to forage and returned to the nest. They finally left the nest and nesting tree after 4 weeks.

***Threats and conservation aspects*:** Although the pond heron is not listed under the threatened species in Bangladesh but their population has been decreasing day by day due to human interferences. The pond herons are colonial breeder. They mainly build their breeding colony near human settlement in rural areas where human disturbances are low. But unfortunately the human activities are the main threat, such as cutting of roosting and nesting trees, destroying the nest and egg, catching young animals, illegal hunting as game bird and industrialization. We should take awareness program for the local people, so that they can stop illegal catching of birds and their nestlings for meat. We should not destroy the breeding environment of the pond heron where they choose for nesting colony. Wildlife preservation Act 1973 should be implemented by the Government authority to protect this important piscivore bird.

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SPECIES COMPOSITION OF LIMNETIC ZOOPLANKTON FROM THE SOUTHERN COASTAL AREAS (MATHBARIA AND BAKERGANJ) IN BANGLADESH

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Abstract

We studied the coastal zooplankton community structure of six waterbodies of Mathbaria and Bakerganj from January 2008 to June 2009. In total 35 zooplankton species were identified under 26 genera under 20 families under 8 orders from Mathbaria. Among them 6 were protozoans, 24 were rotifers, 3 were copepods, 1 was cladocerans and 1 was ostracods. From Bakergonj a total of 42 zooplankton species were identified under 23 genera under 17 Families under 7 orders. Among them 3 were protozoans, 25 were rotifers, 7 were copepods, 6 were cladocerans and 1 was ostracods. Results showed that abundance of rotifera group from both area was higher in Mathbaria (64.86%) and Bakerganj (60.98%) than other groups while abundance of ostracoda of Bakerganj area was lowest.

Keywords: Diversity, Mathbaria, Bakerganj, zooplankton, abundance.

Introduction

Plankton are the most sensitive component of aquatic ecosystem and thereby act as an essential biological indicator for water quality. Thus the study of their taxonomic diversity, number, biomass, and physiological parameters could provide background information on the ecological status of a given body of water and its particular region. Again, the zooplankton itself being a primary consumer in the second trophic level play an important role in the aquatic food chain as well as contributor to the next trophic level. The main foods of rui, catla and catla-rui hybrid were plankton in origin (Mozumder and Naser 2009). Several investigations have been made on the community structure of zooplankton in inland aquatic ecosystems of Bangladesh (Habib *et al.* 1984, Patra and Azadi 1987, Chowdhury *et al.* 1989, Kabir *et al.* 1996, 1997, Bhuiyan and Nessa 1998, Hasan *et al.* 2001, Chowdhury and Mamun 2006, Islam 2007, Kabir and Naser 2008, Ahmed *et al.* 2011). It has recently been shown that the aquatic and free-living pseudopod, *Acanthamoeba castellanii* is not a predator to *V. cholerae* O139 rather the bacterium has shown an intracellular compatibility with this host. Thus, a possible role of *A. castellanii* as an environmental reservoir of *V. cholerae* has been proposed

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(Abd *et al.* 2007). An association has also been established between viable and 'viable but non-culturable' *V. cholerae* (Islam *et al.* 1996) and the chitinous layer of zooplankton (Nahar *et al.* 2012), particularly the copepods, which is believed to serve as a reservoir of the bacterium (Islam *et al.* 1996). *V. cholerae* is naturally present in the environment and is autochthonous in many coastal and estuarine ecosystems (Colwell and Spira 1992). This bacterium is strongly associated with both plankton forming commensal or symbiotic relationships (Colwell and Huq 1994).

Alam *et al*. (2006) has meanwhile established that the coastal ecosystem of southern Bangladesh usually form a point source were the outbreak of cholera epidemics might associate with the zooplankton population. Thus the role of zooplankton in the survival and proliferation of *V. cholerae* in the coastal aquatic ecosystem of the Bay of Bengal is now an important query. But, no study has so far been conducted on the *V. cholerae* associated zooplankton that exist in the stated coastal ecosystem of Bangladesh. The present study about the species composition of limnetic zooplankton from southern coastal area like Mathbaria and Bakerganj in Bangladesh will help the future scientists to find any association of zooplankton species with *V. cholerae* and thereby to dissolve the related public health hazard.

Materials and Methods

Mathbaria of Pirozpur district is located adjacent to the Bay of Bengal, approximately 400 Km southwest of Dhaka. The river, Baleshwar, flows along the western boundary of Mathbaria, on its other side a tropical mangrove forest of the Sundarbans is located. On the other hand, Bakerganj is adjacent to Barisal district.

Ponds or waterbodies which were socially used for sources of drinking or domestic water were selected. Sampling of water from 3 ponds of Mathbaria and 2 ponds of Tulatali river of Bakerganj were sampled monthly from January 2008 to June 2009. For zooplankton study 100 liters of water were filtered successively through 64µm mesh nylon nets (Millipore Corp., Bedford, MA) and 50 ml of the concentrates were collected initially as a crude measure of zooplankton. The collected zooplankton samples were fixed in buffered formaldehyde (4%) and were brought to the laboratory of Department of Zoology, University of Dhaka for further analysis.

For qualitative and quantitative study, zooplankton samples were observed under a compound microscope (Axioskop 40, Japan) in a Sedgeweak-Rafter cell. The specimens were identified as far as to genera or species level with the help of different literatures (Edmonson 1959, Needham and Needham 1961, Mellanby 1971, Tonapi 1980, Ali and Chakraborti 1992, Battish 1992, Bhouyain and Asmat 1992,). Plankton communities were characterized quantifying the abundance of all species on a logarithmic 'DACFOR' scale (Paramu and Ravichandran 2007). Where D = dominant, up to 500 individuals; A = abundant, up to 100 individuals; C = common, up to 50 individuals; F = frequent, up to

10 individuals; O = occasional, up to 3 individuals; R = rare, 1 individual; and NF = absent from sample.

Results and Discussion

From the present study, a total of 35 zooplankton species were identified under 26 genera, under 20 Families and under 8 orders from Mathbaria. Among them 6 were protozoans, 24 were rotifers, 3 were copepods, 1 was from cladocera and 1 from ostracoda (Table 1).

Table 1. Species composition of limnetic zooplankton from Mathbaria and Bakerganj, Bangladesh.

Group	Order	Family	Species Name	Mathbaria			Bakerganj		
				DACFOR scale					
				Site-1	Site-2	Site-3	Site-4	Site-5	Site-6
Protozoa									
	Arcellinida	Arcellidae	*Arcella sp.*	1	NF	NF	NF	NF	NF
	Arcellinida	Difflugiidae	*Difflugia sp.*	5	4	5	4	2	
	Hymenostomatida	Glaucomidae	*Glaucoma sp.*	1	1	1	NF	NF	4
	Arcellinida	Centropyxidae	*Centropyxis sp.*	1	1	2	NF	NF	1
	Euglenida	Euglenidae	*Phacus sp.*	NF	4	4	NF	NF	NF
	Euglyphida	Euglyphidae	*Pareuglypha sp.*	NF	4	NF	NF	NF	NF
			Unidentified (Nebela sp.)	NF	NF	1	NF	NF	NF
Rotifera									
	Ploima	Asplanchnidae	*Asplanchna priodonta*	NF	NF	4	4	1	5
		Brachionidae	*Brachionus angularis*	2	1	1	1	2	4
			B. caudatus	NF	NF	3	6	3	3
			B. calyciflorus	NF	NF	NF	2	NF	3
			B. diversicornis	NF	NF	1	6	3	3
			B. donneri	NF	NF	NF	1	NF	NF
			B. forficula	1	NF	3	6	4	2
			B. falcatus	NF	NF	4	4	3	4
			B. nilsoni	NF	NF	NF	NF	NF	1
			B. quadridentatus	NF	NF	1	NF	NF	2
			B. urceolaris	NF	NF	NF	1	NF	3
			Colurella sp.	1	NF	NF	NF	NF	NF
		Testudinellidae	*Filinia longiseta*	3	3	2	4	1	1
			F. opolinesis	NF	1	1	6	2	1
			F. terminalis	NF	1	1	3	2	
			Harrirgia sp.	NF	NF	NF	NF	NF	1
			Pompholyx sulcata	NF	4	1	NF	NF	NF
			Horaella sp.	1	2	2	3	1	3
			Keratella cochlearis	NF	2	NF	1	3	
			K. tropica	1	1	2	6	3	2
			Testudinella patina	NF	1	1	3	NF	3
		Hexarthridae	*Hexarthra sp.*	NF	1	3	4	NF	3
		Lecanidae	*Lecane luna*	1	NF	NF	1	NF	NF
			Lepadella sp.	NF	NF	1	NF	NF	NF
			Manfredium sp.	NF	1	1	NF	NF	NF
			Monostyla sp.	NF	1	1	1	1	1
			Monogononta sp.	NF	NF	NF	NF	NF	2
		Synchaetidae	*Polyarthra vulgaris*	4	4	4	6	3	5
		Philodinidae	*Rotaria neptunia*	NF	1	1	NF	NF	NF

Group	Order	Family	Species Name	Mathbaria			Bakerganj		
				DACFOR scale					
				Site-1	Site-2	Site-3	Site-4	Site-5	Site-6
		Trichocercidae	*Trichocerca sp.*	NF	1	1	2	1	4
			Unidentified rotifer	1	1	1	3	3	5
Nauplii			*Nauplius*	5	5	5	5	4	4
			Metanauplius	4	4	4	5	4	4
Copepoda									
	Cyclopoida	Cyclopidae	*Cyclops sp.*	3	4	4	4	3	2
			C. nanus	NF	NF	NF	3	1	1
			C. varicans	NF	NF	NF	1	NF	NF
			C. vernalis	NF	NF	NF	NF	1	1
			Mesocyclops sp.	NF	NF	NF	3	1	NF
	Calanoida		*Diaptomus gracilis*	3	4	2	NF	1	NF
			Diaptomus sp.	4	3	2	4	3	2
Cladocera									
	Diplostraca	Bosminidae	*Bosmina sp.*	NF	NF	NF	NF	1	NF
		Sididae	*Diaphanosoma sp.*	3	3	3	2	2	3
		Daphniidae	*Daphnia lumholtzi*	NF	NF	NF	3	NF	NF
Ostracoda									
	Podocopida	Cyprididae	*Heterocypris sp.*	NF	1	NF	NF	NF	1

A total of 42 zooplankton species were identified under 23 genera and under 17 Families and under 7 orders from Bakerganj. Among them 3 were protozoans, 25 were rotifers, 7 were copepoda, 6 were cladocera and 1 was ostracoda (Table 1).

Results show that zooplankton species abundance of rotifera from both area (64.86% in Mathbaria and 60.98% in Bakerganj) was highest compared to other groups and diversity of ostracoda of Bakerganj area was lowest (Fig.1). Rahman *et al.* (2006) made similar observations from Hamil beel, Bangladesh where rotifer was dominant and protozoans were 3rd or 4th in abundance.

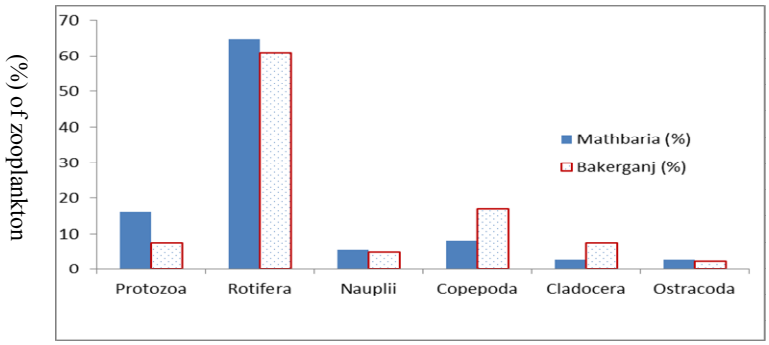

Fig. 1. Composition (%) of zooplankton species in Mathbaria and Bakerganj during January 2008 to June 2009.

Composition of zooplankton at 6 sites (3 sites of Mathbaria and 3 sites of Bakerganj) presented in Table 1 shows that the most abundant and common zooplankton species in

all sites were *Brachionus angularis,* followed by *Filinia longiseta, Horaella sp., Keratella tropica, Polyarthra vulgaris, Cyclops* sp.*, Diaptomus* sp. and *Diaphanosoma* sp.

On the basis of abundance range (DACFOR Scale), rare species (R = 1 indiv.) were *Heterocypris* sp. *Bosmina* sp., *Cyclops varicans*, *C. vernalis*, *Rotaria neptunia, Monostyla bula*, *Manfredium* sp., *Lecane luna*, *Brachionus nilsoni* and *Arcella* sp. Ehsan *et al*. (1997) stated that Cladocerans were less in abundance in winter months in Chanda beel, Bangladesh. Distribution of zooplankton was not uniform in all sites (Rahman *et al*. 2006). The number of zooplankton species varied from 19 to 33 in Mathbaria and 27 to 32 at Bakerganj. Highest number of zooplankton was recorded at site 3 (33 species) of Mathbaria and the lowest at site 1 (19 species) of Mathbaria (Fig. 2). The variation of species number and differences of total zooplankton in two geographical locations were `acceptable as they may be influence by environmental factors of the waterbodies.

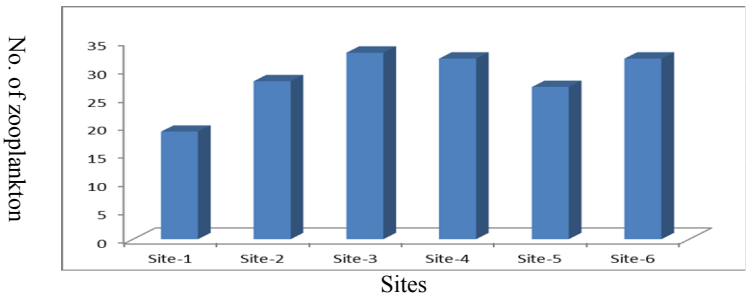

Fig 2. Total number of zooplankton species and their distribution at Mathbaria during January 2008 to June 2009

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— Short Communication

AIRBORNE FUNGAL AND PTERIDOPHYTIC SPORES IN CHITTAGONG UNIVERSITY CAMPUS, CHITTAGONG

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Airborne spores, including fungi and pteridophyte, are the cause for environmental pollution and are sometimes injurious to plants and animals including human beings. These spores which are major component of the aeroallergens are found to be suspended in the air. They cause widespread upper respiratory tract and naso-bronchial allergy including asthma, hay fever, eye, skin and respiratory disorders. For proper diagnosis and treatment of respiratory allergy disorders due to airborne spores, the aerobotanical data (i.e. concentration, frequency and variations of airborne plant particles) of an area is a pre-requisite. In Bangladesh Badya (1989) and Badya and Pasha (1991) first initiated aeropalynological investigation in Chittagong. Spore concentration in a location varies from month to month and year to year and therefore, monitoring of seasonal variation of airborne spores is essential towards proper diagnosis and treatment of allergic disorders.

The Chittagong University Campus was selected as sampling area.The airborne spore survey of Chittagong University Campus area was carried out by Gregory's Sampler method (Gregory 1961). This sampler is grouped under the impaction using wind movement by vertical and inclined microscopic slide. From October 2006 to September 2007, two slides smeared with glycerine jelly, were placed in Gregory's Sampler at 10 m height from the ground level. With an interval of every twenty four hours, the slides were collected from the trap and covered with 18 x18 mm cover glass. The covered areas were examined under microscope on collection or a few days afterwards. The trapped spores were studied on daily, monthly, seasonal and then yearly basis.

Airborne fungal spores survey: In all 13 types of airborne fungal spore, including some unidentified, had been counted and identified. A total of 4,449 spores was counted and was listed month-wise along with the percentage contribution of each to the total fungal spore. The result is presented in Table 1. Some of the identified spores are presented in Plate I.

Only the species of *Alternaria* and *Drechslera* occurred throughout the 12 months of survey. Spores of *Curvularia, Cladosporium, Mucor, Torula* and *Tetraploa* species also occurred throughout the year except a few months. *Penicillium* spp. spores occurred from April to July with a peak in April. *Mucor* spores were recorded in all the months except in July. Spore types of *Diplodia, Diplodina, Menispora* and *Mastigosporium,* were also

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recorded intermittently during the survey period. The newly reported fungal spores in the atmosphere of the Campus were *Diplodia, Diplodina, Mucor, Menispora, Mastigosporium, Torula and Tetraploa*.

Regarding the length of time present in the air the main but common fungal spore types were *Alternaria, , Mucor, Tetraplora, Curvularia, Torula* and *Cladosporium*. In Table 1 the monthly variation of spore concentration is depicted where the highest spore catch was observed in June. The other peak incidence occurred during the month of February. In between these two months (June and February) of incidence, the other months also showed very high in comparison to rest of the years. The commonest fungal spores encountered were of the species of *Mucor* amounting to 16.57% of the total fungal spore count, followed by *Alternaria* (8.86%), *Penicillium* (7.15%), *Torula* (5.44%), *Menispora* (5.12%) and *Drechslera* (3.55%). The minimum incidence of fungal spore was *Diplodina* with an average of 1.75%. Many of the fungal spores were not possible to identify, which are classified into "unidentified type", with an average of 41.67% (Table 1).

Table 1. Monthly incidence of airborne fungal spores in Chittagong University Campus.

Sl No.	Name of fungi	Months with total spore count												Total count	Total In %
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep		
1	*Mucor*	2	34	3	64	329	187	42	35	17	-	15	9	**737**	**16.57**
2	*Alternaria*	3	19	17	9	54	107	45	51	67	6	4	12	**394**	**8.86**
3	*Cldosporium*	13	-	7	-	5	9	5	7	17	8	19	7	**97**	**2.18**
4	*Curvularia*	-	9	2	6	8	4	4	2	32	2	8	3	**80**	**1.80**
5	*Diplodia*	-	-	-	-	-	-	-	7	40	-	21	24	**92**	**2.07**
6	*Diplodina*	-	-	-	-	4	7	-	8	24	-	15	20	**78**	**1.75**
7	*Drechslera*	5	25	16	20	21	11	5	12	15	3	18	7	**158**	**3.55**
8	*Mastigosporium*	-	-	-	2	38	-	-	8	26	-	5	13	**92**	**2.07**
9	*Menispora*	-	-	-	26	59	16	-	37	-	47	29	14	**228**	**5.12**
10	*Penicillium*	-	-	-	-	-	-	180	70	25	15	16	12	**318**	**7.15**
11	*Tetraploa*	-	5	2	4	12	17	7	6	16	2	5	3	**79**	**1.78**
12	*Torula*	4	13	-	-	13	36	24	21	59	17	38	17	**242**	**5.44**
13	Unidentified	61	28	45	86	225	160	128	232	676	114	53	46	**1854**	**41.67**
	Monthly total	**88**	**133**	**92**	**217**	**768**	**554**	**440**	**496**	**1014**	**214**	**246**	**187**	**4449**	
	Total in %	**1.98**	**2.99**	**2.07**	**4.88**	**17.26**	**12.45**	**9.89**	**11.15**	**22.79**	**4.81**	**5.53**	**4.20**		

Badya (1989) reported only 5 genera of fungal spores from the same location, of which, *Alternaria* was the most abundant while *Mucor* was most abundant in the present study. In this study, least abundant fungal spore was *Diplodina*, while *Curvularia* was in previous study. A total of 5 fungal spores was common in both the surveys, while the spores of *Menispora*, *Diplodia*, *Diplodina*, *Mucor*, *Torula*, *Tetraploa* and *Mastigosporium* were found to be additional in this study.

Here, the frequency of spores of fungi was found maximum in the month of June and minimum in October, while in the previous survey by Badya (1989), the frequency of spores of fungi was found maximum in the month of July and minimum in February. This

difference may be due to variation of spore counting method along with the changes in fungal vegetation over time. Moreover, the presence of fungal spores in the air is greatly influenced by changing weather or climatic factors (Hjelmroos 1993).

A total of 1,854 fungal spores was unidentified in the present study, while in the previous study the unidentified fungal spore number was not taken into consideration.

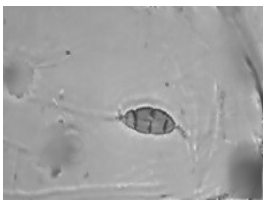
Some fungal spores are reported to be allergenic. In this investigation the trapped allergenic fungal spores were *Alterneria, Mucor, Penicillium, Curvularia, Drechslera* and *Cladosporium.*

Airborne fungal spores are known to be responsible for the diverse human allergenic disorders, particularly type-I and type-III allergenic reactions. Fungal spores have clearly implicated in allergenic asthma and adverse effect on symptomatic health, particularly among asthmatic patients. Here, in this study many other fungal spores were identified to be distinctly allergenic as reported by many workers (De Lara *et al.*, 1990, Rosas *et al.*, 1990). Further studies with these spores of fungi both identified and unidentified, will shed some light in this line of research in future.

Airborne Pteridophytic spores survey : During the present study, 5 types of airborne pteridophytic spores were identified and counted (Plate I**)**. Altogether four types of pteridophytic spores were identified upto genus/species level and the remaining was grouped into unidentified type (Table.2). The monthly incidence of airborne pteridophytic spores were recorded (Table 2). The highest incidence was observed in the month of February, followed by January, December and May. The lowest incidence was observed in October.

Table 2. Monthly incidence of airborne pteridophytic spores in Chittagong University Campus.

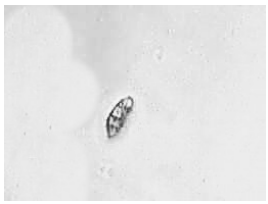
SL No.	Name of Pteridophyte	Months with total spore count												Total count	Total in %
		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep		
1	*Christella dentata*	-	-	-	-	-	3	5	51	28	15	6	8	**116**	**17.63**
2	*Ophioglossum petiolatum*	-	-	-	-	-	-	2	1	7	17	25	22	**74**	**11.25**
3	*Pteris vittata*	2	9	78	75	73	24	-	-	2	-	3	7	**273**	**41.49**
4	*Selaginella* spp	-	-	4	5	-	-	-	-	-	-	-	-	**9**	**1.37**
5	Unidenfied types	2	5	3	14	51	25	11	28	12	5	16	14	**186**	**28.27**
	Monthly total	**4**	**14**	**85**	**94**	**124**	**52**	**18**	**80**	**49**	**37**	**50**	**51**	**658**	
	Total in %	**0.61**	**2.13**	**12.92**	**14.29**	**18.84**	**7.90**	**2.74**	**12.16**	**7.45**	**5.62**	**7.60**	**7.75**		



Alternaria

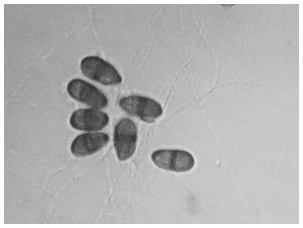
Diplodina

Cladosporium



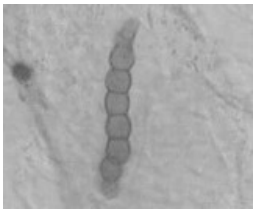
Mastigosporium

Curvularia

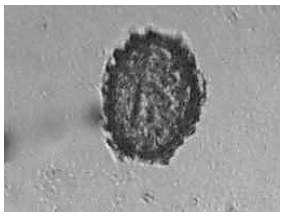


Diplodia

Drechslera

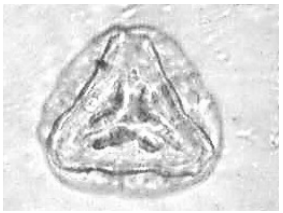


Torula

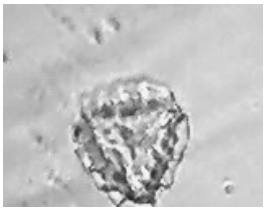


Christella dentata

Ophioglossum petiolatum



Pteris vittata



Selaginella

PLATE I. Trapped airborne fungal and pteridophytic spores.

A total of 658 pteridophytic spores were trapped during the survey. The maximum contribution was made by *Pteris vittata*, followed by *Christella dentata* and *Ophioglossum petiolatum*. The minimum contribution was made by *Selaginella* spp. A total of 186 spores remained unidentified.

All the identified pteridophytic spores occurred intermittently in the air of the location throughout the survey (Table 2). *Pteris vittata* was found as dominant spore type and occurred highest in the month of December, followed by January and February. In the previous study made at the same location, Badya (1989) reported 1319 pteridophytic spores. But, all the family/generic level identification was not made by him.

Here, maximum concentration of pteridophytic spores was observed in the month of February and minimum in October. In the previous survey (Badya 1989) maximum concentration was observed in the month of July and minimum in February. The difference may be due to changes in the spectrum of fern vegetation and variation of local climatic factors. This is the first report of generic/species level identification of airborne pteridophytic spores in Bangladesh so far.

In this study, 28.27% pteridophytic spores remained unidentified. If these spores could be identified, the spore types might further be increased to 8 to 10 types. So, it is apparent that the atmosphere of the location is also containing a remarkable amount of pteridophytic spores which, like fungi, also may be the causative of respiratory allergy of the local people.

It may be mentioned that a good number of reports are available mentioning seasonal variation of airborne fungal (Konger and Baruah 1958, Chakraverty 1974, Kulshrestha and Chauhan 2003, Arora and Jain 2003) and pteridophytic (Devi and Jamil 1979, Devi and Khare 1981)) spores and their allergenicity (Hausen and Schulz 1978, Geller-Bernstein *et al*. 987, Bunnag *et al.* 1989, Kasprzyk *et al*. 2004). So, more extensive and intensive investigations and monitoring should be made on the aerobiology and allergenicity of the prevailing pteridophytic spores along with the fungal spores of Bangladesh for longer period of time.

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