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CONTENTS

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INSECT PESTS OF SOYBEAN (GLYCINE MAX L.), THEIR NATURE OF DAMAGE AND SUCCESSION WITH THE CROP STAGES

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Abstract

Thirty nine species of insect pests were found to infest soybean crop at their different growth stages in Noakhali region of Bangladesh during January to May, 2010 and 2011. Among the recorded pest species, six species namely, hairy caterpillar, *Spilarctia obliqua* (Walker); leaf roller, *Lamprosema indicata* F; common cutworm, *Spodoptera litura* F; pod borer, *Helicoverpa armigera* (Hubner); stem fly, *Ophiomyia phaseoli* (Tryon) and white fly; *Bemisia tabaci* Genn. were considered as the major pests while the rests were of minor importance on the basis of population densities per plant, nature and extent of damages, and yield reductions. Most of the major and minor pests appeared in the crop during vegetative to flowering stages (30-50 Days after sowing) and the maximum insect population and their infestation occurred during flowering and pod formation stages of the crop throughout the study period.

Key words: Insect pests, Soybean, *Glycine max,* Damage, Succession, Crop stages

Introduction

Soybean (*Glycine max* L.) is one of the most important crops which is grown for oil and protein in both the rabi and kharif seasons. Seeds of soybean contain about 42% protein and 20% oil and provide 60% of the world supply of vegetable protein and 30% of the edible oil (Fehr 1989). In Bangladesh this crop is comparatively new but soybean oil is very much popular as edible oil. However, recently the crop gained popularity in the poultry industry and its cultivation expands day by day. In the Greater Noakhali region (Noakhali and Laxmipur districts) soybean is the major oilseed crop and is extensively cultivated. Cultivation of soybean covered about 55,000 hectares of land and produced about 90,000 metric tones of seeds during the period 2009-2010 in Bangladesh (Anonymous 2011). One of the major constraints to the successful soybean production in Bangladesh is the damage caused due to insect pests. Research experiences reveal that 15 - 20 percent of the total soybean production is lost directly or indirectly by the attack of insect pests every year (Biswas 2008).

In order to evolve economically feasible, ecologically sound and socially acceptable pest management strategies, detailed information on the pest complex, their status and sequence of appearance during the crop period, losses and type of damage are of great importance (Jayanthi *et al*. 1993). In Bangladesh, check lists of insect pests of soybean and their succession in relation to crop stages are scanty. Only the list of some insect

pests of soybean in Bangladesh was recorded (Sardar and Debnath 1984, Kaul and Das 1986, Ali 1988, Begum 1995, Biswas *et al.* 2001 and Biswas 2008). Therefore, the present research work was undertaken to record the insect pests of soybean, their nature of damage, incidence, infestation and time of appearance with the crop stages.

Materials and Methods

The research work was conducted in the field of Subarna Char, at Noakhali and in the laboratory of Oilseed Research Centre, BARI, Gazipur during rabi 2009-2010 and 2010-2011 crop seasons. The survey was conducted from one hectare land soybean field cultivated by the farmers with the supervision of scientists of Oilseed Research Centre, BARI, Joydebpur, Gazipur. The experimental plot measured 10 m X 10 m. The seeds of soybean were sown in the plots on 4th week of December of 2009 and 2010. There were four replications and plots were selected following a Randomize Complete Block Design. The rows and plants were spaced 30 cm and 10 cm apart, respectively. The recommended agronomic practices for raising the crop were maintained following the work of Mondal and Wahhab (2001).

Observation on species of insect pests with their population per plant was recorded from seedling to matured stage of the crop from 10 randomly selected samples of the plants in each plot. The time of appearance of the pests were observed and recorded. The nature of damage and feeding behaviour of the insects were carefully observed and their photographs were taken in the crop fields and in the laboratory. The recordings of data were included visual observations, hand tens, and hand picking of insects from the standing crops during 7:00-10:00 a.m and 4:00-6:00 p.m at weekly intervals. Some insects were also collected by aspirators for laboratory studies. The collected insects were preserved in the insect box and vial having 75% alcohol for identification. Relative population of insect was counted as suggested by Biswas *et al.* (2001). The collected insects were also reared in the laboratory at an ambient temperature (24-34^0 C) in cages and preserved in the insect boxes. The insects (specimens) were preliminarily identified following Maxwell- Lefroy (1909), Borror *et al.* (1975), Fletcher (1985), Nair (1986), Singh (1990) and Biswas (2008). The insects were graded as major and minor on the basis of their population density per plant, nature and extent of damage of the crop and the yield reduction. The insect pests were also grouped as stem feeders, leaf feeders, leaf roller, sap sucker and borer on the basis of their feeding behaviour.

Results and Discussion

Pest complex of soybean: Thirty nine species of insect pests belonging to seven orders and 22 families were found to infest at the different growth stages of soybean crop in Noakhali region, Bangladesh during rabi seasons of two consecutive years 2009-10 and 2010-11(Table 1). Of these, only six species namely, hairy caterpillar, *Spilarctia obliqua* (Walker); leaf roller, *Lamprosema indicata* F.; common cutworm, *Spodoptera litura* F.;

Table 1. Insect pests recorded from soybean crop ecosystems at Noakhali region, Bangladesh during 2009-10 and 2010-11 crop seasons.

SL no.	Common Name	Scientific Name	Order	Family	Feeding behaviour
01.	Hairy caterpillar	*Spilarctia obliqua* (Walker)	Lepidoptera	Arctiidae	Leaf eater
02.	Hairy caterpillar	*Anarsia ephippias* (Mullar)	Lepidoptera	Arctiidae	Leaf eater
03.	Common cutworm	*Spodoptera litura* Fab.	Lepidoptera	Noctuidae	Leaf eater & cutter
04.	Common cutworm	*Spodoptera exigua* Fab.	Lepidoptera	Noctuidae	Leaf eater & cutter
05.	Leaf roller	*Lamprosema indicata* F.	Lepidoptera	Pyralidae	Leaf roller & eater
06.	Stem fly	*Ophiomyia phaseoli* (Tryon.)	Diptera	Agromyzidae	Stem borer
07.	Shoot fly	*Melanagromyza obtuse* Mach	Diptera	Agromyzidae	Shoot borer
08.	White fly	*Bemisia tabaci* Genn.	Diptera	Aleyrodidae	Sap sucker
09.	Pod borer	*Helicoverpa armigera* (Hub)	Lepidoptera	Noctuidae	Pod eater
10.	Black cutworm	*Agrotis ipsilon* (Hufn.)	Lepidoptera	Noctuidae	Stemcutter
11.	Leaf miner	*Stomopteryx* spp.	Lepidoptera	Gelechiidae	Miner &eater
12.	Semilooper	*Plusia orichalcea*(Fab.)	Lepidoptera	Noctuidae	Leaf eater
13.	Green grasshopper	*Attractomorpha crenulata* F.	Orthoptera	Acrididae	Leaf eater
14.	Longhorn grass hopper	*Phaneroptera gracilli* Bur.	Orthoptera	Tettigonidae	Leaf eater
15.	Green stink bug	*Nezara viridula* L.	Heteroptera	Pentatomidae	Sap sucker
16.	Stink bug	*Chrysocoris stolli* F.	Heteroptera	Pentatomidae	Sap sucker
17.	Green stink bug	*Dolycoris indicus* Stal.	Heteroptera	Pentatomidae	Sap sucker
18.	Pod bug	*Eusarcocoris* sp.	Heteroptera	Pentatomidae	Sap sucker
19.	Stink bug	*Coptosoma cribrarium* F.	Heteroptera	Plataspidae	Sap sucker
20.	Coreid bug	*Leptocorisa* spp.	Heteroptera	Coreidae	Sap sucker
21.	Coreid bug	*Riptortus pedestris* F.	Heteroptera	Coreidae	Sap sucker
22.	Red cotton bug	*Dysdercus cingulatus* F.	Heteroptera	Pyrrhocoridae	Sap sucker
23.	Aphid	*Aphis craccivora* (Koch)	Homoptera	Aphididae	Sap sucker
24.	Leaf hopper	*Aphannus sordidus* F.	Homoptera	Jassidae	Sap sucker
25.	Jassid	*Empoasca* sp.	Homoptera	Jassidae	Sap sucker
26.	Jassid	*Amrasca biguttula* (Ishida)	Homoptera	Jassidae	Sap sucker
27.	Mealy bug	*Pseudococcus corymbatlus*	Homoptera	Coccidae	Sap sucker
28.	Mealy bug	*Pseudococcus filamentosus*	Homoptera	Coccidae	Sap sucker
29.	Brown hopper	*Nilaparvata lugens*	Homoptera	Jassidae	Sap sucker
30.	Thrips	*Frankliniella schultzei*	Thysanoptera	Thripidae	Sap sucker
31.	Black weevil	*Cyrtozemia cognate* Marsal	Coleoptera	Culculionidae	Leaf eater
32.	Grey weevil	*Myllocerus discolor* Boh.	Coleoptera	Culculionidae	Leaf eater
33.	Weevil	*Tenymecus indicus* Fst.	Coleoptera	Culculionidae	Leaf eater
34.	Weevil	*Chaetocnema* sp.	Coleoptera	Halticidae	Leaf eater
35.	Pumkin beetle	*Aulacophora* sp.	Coleoptera	Chysomelidae	Leaf eater
36.	Leaf beetle	*Oulema* sp.	Coleoptera	Chysomelidae	Leaf eater
37.	Leaf beetle	*Monolepta signata* Olv.	Coleoptera	Chysomelidae	Leaf eater
38.	Epilachna beetle	*Epilachna 12-punctata*	Coleoptera	Chysomelidae	Leaf eater
39.	Girdle beetle	*Oberia brevis* S.	Coleoptera	Chysomelidae	Leaf eater

pod borer, *Helicoverpa armigera* (Hubner); stem fly, *Ophiomyia phaseoli* (Tryon) and white fly, *Bemisia tabaci* Genn. were considered as the major pests while the rests were of minor importance on the basis of population densities per plant, nature and extent of damages and yield reductions. The population density per plant of major and minor insects and their rate of infestation on soybean plant is presented in Table 2. The population density per plant of major insects namely, *S.obliqua, L. indicata*, *.S. litura*, *H. armigera*, *O. phaseoli* and *B.tabaci* ranged from 0.50-0.60, 2.00-2.50, 1.50-1.80, 0.35-0.40, 0.40-0.50 and 4.00-5.00, respectively in 2010 while it ranged from 0.45-0.55, 1.50-2.00,1.20-1.50 ,0.30-3.50, 0.30-0.40 and 3.00-3.50, respectively in 2011. Similarly, percent plant infestation by the major insects namely, *S.obliqua, L. indicata*, *.S. litura*, *H. armigera*, *O. phaseoli* and *B. tabaci* ranged from 70-80, 95-100, 90-100, 35-40, 40-45, 96-100, respectively in 2010 while it ranged from 60-70, 95-100, 80-90, 30-35, 35-40 and 95-100, respectively in 2011. Most of the number of major and minor pests per plant of soybean with their infestation rate was found higher in 2010 than in 2011 (Table 2). About 100 percent plant was infested by leaf roller, white fly, leaf hopper and leaf beetle throughout the study period. The higher insect population and their infestation in 2010 may be due to higher temperature, lower relative humidity and rainfall in 2010 than 2011 which provided suitable conditions for the population build-up of the insect pests.

Among the minor pests, green stink bug (*Nezara viridula* L.), semilooper (*Plusia orichalcea* Fab.), Black cutworm (*Agrotis ipsilon* (Hufn.), leaf miner (*Stomopteryx* spp.), green grasshopper (*Attractomorpha crenulata* F.), pod bug (*Eusarcocoris* sp.) and aphid (*Aphis cracraccivora*) became occassionally important and caused serious damage to the soybean crop. Aphid, jassid and whitefly are also important as vectors for transmission of viral (YMV) diseases (Biswas 2008).

On the basis of feeding behaviour, 17 species were grouped as sap sucker, 15 as leaf eater, 2 as leaf roller and eater, and the remaining five such as, *Helicoverpa armigear*, as pod borer and eater, *Stomopteryx* sp. as leaf miner, *Agrotis* sp. as stem cutter, *Ophiomyia phaseoli and Melanagromyza obtusa* as stem or shoot borers.

Nature of damage of the important pests: The 1st and 2nd instar larvae of *S. obliqua* damaged the soybean leaves and shoots and gregariously occurred in the same plants and leaves. Later on, 3rd and onward instars dispersed and moved from one plant to another and fed on the older leaves, stems, shoots, flowers and pods causing serious damage to the plants. The yellowish green larvae of leaf roller rolled the leaves of soybean plants and several may be webbed together and feed inside. Damaged leaves became silvery-brown papery. About 2-3 larvae remained in a single folded leaf. The pale green larvae of *S. litura* damaged the leaves and shoots by feeding voraciously. Infested leaves beared irregular holes, growth of the plant was arrested, flower and pod formation was hindered. Very small larvae of the pest bore into the stem through the petiole. They feed on cortex and pith of the stem causing tunneling of the stems and died. The green larvae of *H.*

armigera feed on leaves and tender shoots firstly; later on they bore pods and feed inside. The green stink bugs suck sap from the tender shoots, leaves and pods resulting distorted leaves and pods.

Succession of the pests: The succession of the major insect pests of soybean crop during 2010 and 2011 at Noakhali region is presented in Fig.1. Soybean crop was first attacked by leaf beetle, *Monolepta signata*, black beetle, *Cyrtozemia cognata*, epilachna beetle, *Epilachna* 12 *Punctata*, *E.* 28 *punctata*, pumkin beetle, *Aulacophorai* sp., black cutworm, *Agrotis ipsilon*, leaf hopper, *Aphannus sordidus*, jassids, *Empoasca* spp. at the seedling stage and their infestation continued up to pod formation stage of the crop during January 2010 and 2011. After 2-3 weeks, leaf roller, *Lamprosema indicata* F. common cutworm, *Spodoptera litura* F. hairy caterpillar, S. *obliqua* , pod borer, *Helicoverpa armigera* Hubner, green stink bug, *Nezara viridula* L., rice bug and other pentatomid bugs were frequently observed from flowering to maturity of the crop and recorded on February to April 2010 and 2011.The bugs were also important which suck sap from the tender part of the stems, leaves and pods. The white fly and aphids were noticed from vegetative to pre-maturity of the crop and act as vectors of yellow mosaic virus (YMV) and leaf curled viruses that were seriously affected plant growth and yield of soybean crop in both the years. Stem fly was observed from seedling to pod formation stage of the crop while pod borer damage was recorded at the flowering and pod pre-maturity stage in both the years (Fig. 1).

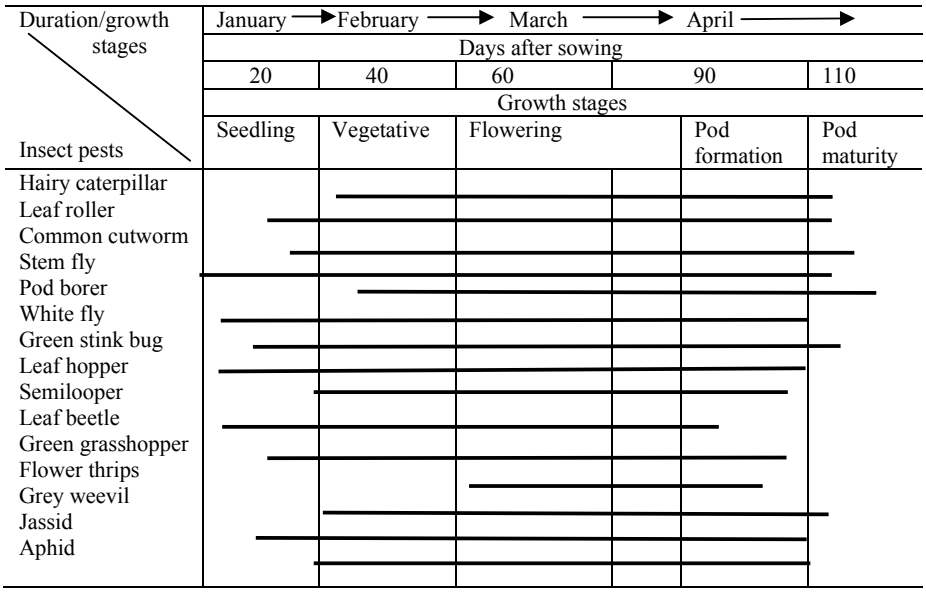

Fig. 1. Succession of important insect pests of soybean in relation to crop stages during 2010 and 2011 at Noakhali.

- The duration of occurrence of each species on the crop is shown by horizontal lines.

The most damaging insects were hairy caterpillar, leaf roller, common cutworm, pod borer and stem flies those were found to damage during vegetative, flowering and pod formation stages (30-65 DAS) of the crop. The yield loss caused by these pests has been estimated more than 25% in Bangladesh. In the soybean field infestation of insect pests like stem flies, bugs, aphids and white fly were frequently observed. Stem flies (*Ophiomyia phaseoli* and *Melanagromyza sojae*) were the major pests of soybean and about 30% loss occurred due to attack of these pests in this country. About 25-30% pod was damaged by pod borer at the pod formation to prematurely of the crop. About 100 % soybean plant and 70% leaf were infested by leaf roller and common cutworm and hairy caterpillar (Table 2).

Table 2. Population density per plant and percent infestation of some important insect pests of soybean crop during 2010 and 2011 at Noakhali region.

Name of insects	No. of insect /plant		Plant infestation%		Stage of severe infestation
	2010	2011	2010	2011	
Hairy caterpillar	0.50-0.60	0.45-0.55	70-80	60-70	F-P
Leaf roller	2.00-2.50	1.50-2.00	95-100	95-100	V-F
Common cutworm	1.50-1.80	1.20-1.50	90-100	80-90	V-F
Stem fly	0.40-0.50	0.30-0.40	40-45	35-40	S-V
Pod borer	0.35-0.40	0.30-0.35	35-40	30-35	F-M
White fly	4.00-5.00	3.00-3.50	95-100	95-100	V-P
Green stink bug	0.60-0.80	0.50-0.80	70-75	65-70	V-P
Leaf hopper	3.50-4.50	3.00-4.00	95-100	95-100	V-P
Semilooper	0.30-0.40	0.25-0.30	40-50	35-40	V-P
Leaf beetle	2.50-3.50	2.00-3.00	96-100	95-100	S-F
Green grasshopper	0.30-0.40	0.25-0.30	40-45	35-40	V-M
Flower thrips	5.00-6.00	4.00-5.50	96-100	95-100	F
Grey weevil	0.25-0.30	2.00-0.25	25-30	20-25	V-P
Jassid	2.50-3.50	2.00-3.00	96-100	95-100	V-P
Aphid	4.50-5.50	4.00-4.50	60-70	50-60	V-P

Data were recorded on average of 30 soybean plants.
V- Vegetative, S-Seedling, F-Flowering, P-Pod formation, M-Maturity.

The insect pests of soybean were recorded in Bangladesh by several scientists. Alam (1976) listed only four species of insect pests attacking the soybean. Of these, leaf roller (*L. indicata* F.) and mealy bug (*Pseudococcus corymbatlus*) were important. Sardar and Debnath (1984) recorded 15 species of insects of soybean crop in Bangladesh. Of these, bean bug, leaf roller, hooded hopper caused serious damage. Kaul and Das (1986)

recorded 14 species of insect pests attacking soybean in Bangladesh. Of these, hairy caterpillar, cutworm, leaf roller (*L. indicata* F.) and bug (*N.viridula* L.) were recorded as major pests. From the survey report of Ali (1988) in the northern Bangladesh, it is revealed that 47 species of insect pests had been recorded in different stages of soybean crop in that area. Of these, 12 species were considered as serious pests. Begum (1995) listed 9 species of insects in soybean, Das (1998) recorded two major pests namely, hairy caterpillar and stem fly. Biswas (2008) recorded 35 species of insect pests attacking soybean at Gazipur all of which were also recorded in this study. Biswas *et al.* (2001) reported that leaf roller (*L. indicata*) and hairy caterpillar (*S. obliqua*) were the major pests of soybean and about 80% plant and about 60% leaf were infested by the attack of these pests.

The succession of appearances of the insect pests on soybean showed that the population of different pest species occurred in an overlapping manner and the crop was under the continuous attack of one or more pests. Most of the major and minor pests appeared in the crop during vegetative to flowering stages (30-50 Days after sowing) and the maximum infestation occurred during flowering and pod formation stages of the crop in both the years. Although most of the insects recorded from soybean crop during the study period have been considered as minor, it is not unlikely that any one of the minor pests may attain the status of a major pest depending upon the environmental conditions and changing cropping pattern.

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BIOINFORMATICS ANALYSIS OF NOVEL NON-CODING MOTIFS IN PATHOGENIC BACTERIAL GENOME

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Abstract

Antibiotic resistance of MRSA (Methicillin Resistant *Staphylococcus aureus*) has been evolved through the rapid and diversified changes in the genetic structure of the bacterial strains. The onset of new resistant strains makes the diagnosis, prognosis, treatment and control process more difficult. The study was started with MRSA252, the most diverse strain of *S. aureus* and then elaborated to 14 other strains. By screening out the complete genome sequences of MRSA, 274 regions of 362,792bp non-coding unique sequences were found. Among those, sequences of less than 500bp length are mostly important to use in diagnostic purposes. Functional analysis and comparison with few other pathogens were done to find correlations.

Key words: *Staphylococcus aureus*, Non-coding DNA, Antibiotic resistance, Sequence analysis, Functional analysis

Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the major 'superbugs' in nosocomial and community acquired infections. The extent of infection may range from simple to life threatening and this has become of great concern in hospitals (Am J Infect Control 1999). Although they are named after the antibiotic methicillin, MRSA strains show resistance against a number of antibiotics, including the Penicillin family (methicillin, dicloxacillin, nafcillin, oxacillin, etc.), tetracycline, minocycline, vancomycin, streptomycin and to some toxic metals (Neu1992).The most common mechanism of resistance against penicillin involves the production of penicillinase or β-lactamase, which breaks the β-lactam ring of the penicillin molecule (Lyon and Skurray 1987). Another mechanism introduces the product from *MecA* gene, which synthesizes penicillin binding proteins (PBP2a and PBP2') and upon binding inactivates penicillin and methicillin (Ponting *et al.* 2009). Other mechanisms include production of protonated amide or hydroxyl groups by interacting with 30S ribosomal RNA and efflux the drugs out from the cell (Hiramatsu 2004). Bacterial strains having plasmid, and/or genomic islands may carry resistance genes to different antibiotics. Yet most of the mechanisms are not properly understood (Neu1992 and Strommenger *et al.* 2003). These bacteria can confer resistance through mutation, gene alteration or gene transfer (i.e. horizontal gene

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transfer). Naturally evolving bacteria may also gain or lose functions that could turn them into resistant strains (Neu1 992 and Hiramatsu 2004). One of the most fascinating characteristics of these bacteria is their ability to change their non-coding region of the genome as per necessity e.g. under stressful condition (Ponting *et al.* 2009).

MRSA transmission occurs through skin contact and as it can be highly infectious, prevention, control and treatment is necessary as soon as it is detected. Early detection of the pathogens and their resistance can prevent the infection from becoming worse. It is preferable to have a diagnostic system that needs a small amount of sample and can give an accurate and specific result in the shortest possible time (Holden *et al.* 2004). Detection of MRSA is commonly done by a micro-dilution assay of a bacterial broth culture which gives phenotypic antibiotic resistance data and takes a minimum of 2-3 days incubation time. ELISA and scanning electrochemical microscopy (SECM) coupled with ELISA can be good methods for detecting MRSA (Thornsbery *et al.* 1983, J Hosp Infection 1998 and Dequaire *et al.* 1999). The latter needs a sample of 5.25pg/mL and takes a day for incubation. Molecular methods are now sophisticated and popular because of the reliability. Polymerase chain reaction (PCR) and multiplex PCR are the fastest ways of identifying MRSA infections. Multiplex PCR can identify genes in different groups of antibiotics. These procedures can take 2.5 to 6 hours for detection with the smallest amount of DNA. The main problems with these systems are the presence of inhibitors and the amplification of non-targets, which can give false negative results (Kasai *et al.* 2000 and Strommenger *et al.* 2003). Detection of *MecA* gene is also used in many hospitals which can be done by either by radio labeling, PCR amplification or simply by enrichment culture broths (Kitagawa *et al.* 1996).But this method is not very well established as it can only detect nasal and wound infections(Brown *et al.* 2005 and Oberdorfer *et al.*2006). Another DNA screening can be done to find the Panton-Valentine leukocidin (*PVL*) gene, which is also a toxin producer in MRSA infection (Shrestha *et al.* 2002).

The non-coding DNA which were previously referred to as “junk sequences” are now found to carry great value as they have regulatory roles, can play an important role in protein folding and can be promoter or operator binding sites as well. They also give information regarding evolution (Oberdorfer *et al.* 2006).In recent times thousands of different types of RNAs which are transcribed from non-coding regions of the genome have been found to play regulatory roles (Locey and White 2010). These non-coding RNAs are also found to be associated with disease, from microbes to humans. Compared to eukaryotes, prokaryotic genomes contain a very small amount of non-coding DNA, which mainly serves as the inter-genic region and typically has regulatory functions. Studies have found that approximately 6-14% of most of the bacteria and archaeal genome has non-coding part and has a positive selective pressure on the evolutionary process (Mercer *et al.* 2009). MRSA has a highly gene dense genome (84% coding region) containing approximately 2.9Mb DNA (Rogozin *et al.* 2002). Analysis of the

genome sequence using bioinformatics tools may provide valuable information before heading the laboratory for experimentations. This study was done with an aim of finding novel motifs of MRSA non-coding sequences with significance, which can be used for diagnostic purposes.

Materials and Methods

The complete genome of bacteria and other related sequences were downloaded from NCBI nucleotide database (*www.ncbi.nlm.nih.gov › NCBI › DNA and RNA*) and the Welcome Trust Sanger Institute's own genome database (www.sanger.ac.uk/ *resources/databases/*). For sequences comparison and functional study, different online tools and software were used (Johnson *et al.* 2008). Artemis, ACT (Artemis Comparison Tool, www.sanger.ac.uk/resources/software/artemis), Inter ProScan (http:// www. ebi.ac.uk/Tools/pfa/iprscan/), AmiGO (http:// amigo.geneontology.org/cgi-bin/amigo/ go.cgi), BLAST, BLASTn, BLASTx, (*blast.ncbi.nlm.nih.gov,* version 2.2.23) were the major tools used for this study purpose (Altschul *et al.* 1990, Rutherford *et al.* 2000, Korf *et al.*2003 and Carver *et al.* 2005). PERL and BioPerl (www.bioperl.org) were used for the bioinformatics analysis (Tisdall 2001).

Fourteen *Staphylococcus aureus* strains were taken under consideration as per their availability on reliable databases and the relatedness with the diseases symptoms (Data retrieve date: June to August, 2011). Table 1 represents one strain susceptible to the antibiotic Methicillin and other commonly used antibiotics (MSSA476) and other 13 strains which are resistant to several antibiotics (including Methicillin).

The complete process of the method of research was organised as follows: 1. Getting sequences of MRSA252 where no MSSA476 common sequence is present; 2. Screening out (Using non-redundant databases) the unique non-coding MRSA252 sequences; 3. Finding conserved non-coding sequences in 14 strains of MRSA (Table 1) which have no match with MSSA476; 4. Comparing MRSA non-coding sequences with other pathogenic bacteria and 5. Sequence to function analysis.

Windows and Linux operating system were used for convenience. Bioperl and Perl were used for the analysis. In Bioperl, there are already prepared commands, available to use in biological study. The major commands of Perl used here was the 'subroutines', 'hash', 'formatdb', 'makeblastdb' etc.

The obtained sequences were in different sizes. Those were then taken to compare with some other pathogenic bacteria randomly. From 100% BLASTn match, few organisms were taken and their sequences were downloaded and crosschecked with NCBI prokaryotic genome database (Data retrieve date: June to August, 2011 by Nishat Shayala). Overview of the sequence searching and matching process is presented in Fig. 1. Detailed information of the pathogens was used for functional analysis. Again Perl was used for comparison and finding the non-coding genes around the coding region.

Table 1. List of *Staphylococcus aureus* strains taken for this study.

Name of strain	Accession no	Compete genome Size	Source
Staphylococcus aureus 04-02981	CP001844.2	2,821,452bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* COL chromosome	NC_002951.2	2,809,422bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* JH9	CP000703.1	2,906,700bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* MRSA252 chromosome	NC_002952.2	2,902,619bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* MSSA476	NC_002953.3	2,799,800bp circular DNA	Sanger Institute
Staphylococcus aureus subsp. *aureus* Mu50	NC_002758.2	2,878,529bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* MW2	NC_003923.1	2,820,462bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* N315	NC_002745.2	2,814,816bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* NCTC 8325 chromosome	NC_007795.1	2,821,361bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* str. Newman chromosome	NC_009641.1	2,878,897bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* T0131	CP002643.1	2,913,900bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* TCH60	CP002110.1	2,802,675bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* USA300_FPR3757	CP000255.1	2,872,769bp circular DNA	NCBI (nucleotide database)
Staphylococcus aureus subsp. *aureus* USA300_TCH1516	CP000730.1	2,872,915bp circular DNA	NCBI (nucleotide database)

Searching nearby genes and protein products of non-coding sequences and analysing their functions were done to find if there was any impact of that region on the coding sequences. ARTEMIS and ACT not only give the sequence data but also statistical and graphical presentations and compare sequences from any format. MRSA252 was taken and compared with 3 other *Staphylococcus* species.

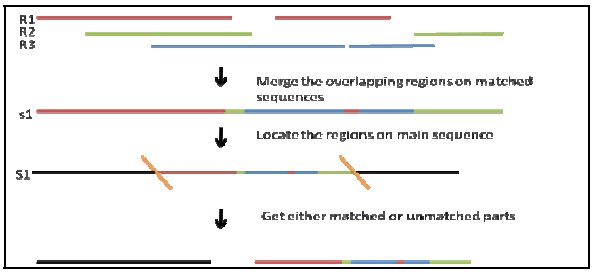

Fig.1. Overview of the working process. R1, R2 and R3 are the regions of the matched sequences after the BLAST search. Their overlapping regions are merged in the next step. Finally that portion is either removed or taken out of the main sequence to get the expected output. In every step after the BLAST search this basic procedure is used with the help of the Perl programming language.

InterProScan is as simple as BLAST and with only a sequence input can search for every major domain and protein family database, looking for single peptides, finding transmembrane domains and showing low complexity regions. It also assigns sequences to InterPro family and tells about the gene ontology terms that apply to it. The names of proteins found in three species that matched sequences with MRSA252 were taken for function prediction. From Artemis, the amino acid sequences in FASTA for the genes/locus were taken to search in InterProScan. From the InterProScan result for each protein, their GO (Gene Ontology) was searched through the GO link and through "AmiGO" for the cellular component, biological process and molecular functions (Quevillon *et al.*2005).

Results and Discussion

Uncommon in other prokaryotic pathogens *Staphylococcus aureus* has a complex genome structure with combination of genes and mobile genetic elements which can also be found on their plasmids (Weigel *et al.*2003). This research work started by looking into the non-coding sequence of *S. aureus* which was found to be 16% of their genome in the previous study (Rogozin *et al.* 2002).

Results from the search for unique sequence of MRSA252 strain which excluded common MSSA476 sequences came out with 492,331bp unique MRSA252 sequences of the total 2.9Mb genome (Table 2). From this large unique region 363,767bp sequences were found to be non-coding in MRSA252 (Table 2). Conserved sequences among the 14MRSA strains were found to have a total sequence length of 362,792 bp. A total of 274 sequence pool was formed with variant sequence length ranges. Among those, 139 regions of sequences had lengths less than 500 bp, 36 had lengths between 500 to 1000 bp, 29 had lengths between 1000 to 1500 bp and remaining had lengths above 1500 bp (Fig. 2). The mean length of conserved sequences was 1324.058 bp (Table 2).

Table 2. Unique non-coding sequence among the MRSA252, MSSA476 and 14 other MRSA strains.

Outputs	MRSA252-MSSA476	Non coding MRSA252	Conserved non coding 14 MRSA strains
Unique sequence < 500 bp	92	225	139
Unique sequence length between 500-1000 bp	25	63	36
Unique sequence length between 1000-1500 bp	10	29	29
Unique sequence length > 1500 bp	53	75	70
Total :	180	392	274
Mean length:	2735.17bp	1010.46bp	1324.06bp

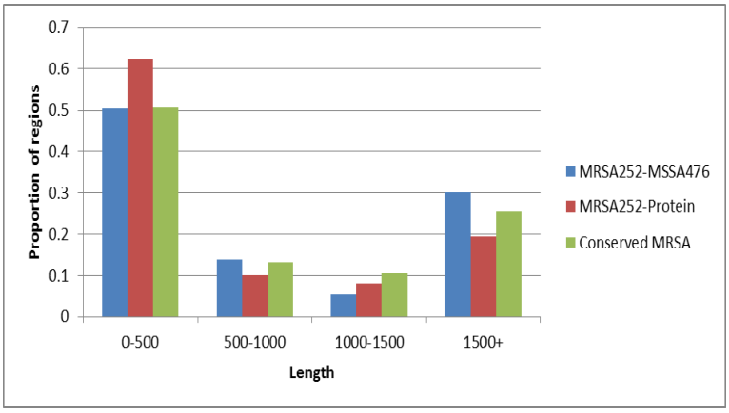

Fig. 2. Vertical data on bar diagram represent the number of regions in percentage and the horizontal data represent the corresponding length. This is a comparative analysis of how the sequence length and region number varied during the screen out process. Blue bar shows the result of first analysis where only MRSA252 sequences were present without MSSA476 common sequences. Red bar shows the non-coding sequences of MRSA252 and the green bar shows the conserved non-coding sequences of MRSA strains.

Elaborate search for conserved MRSA non-coding sequences, 362,792bp sequences with 100% identity showed that these sequences were almost the same as the MRSA252 non-coding sequences with very small differences in lengths and also were consistent with the previously published data. An interesting aspect of this study result was the length of the non-coding unique sequences, which were more than the average length found in other bacteria. Compared to eukaryotes, prokaryotes contain very less number of non-coding sequences to avoid complexity in the inter-genic regions and use the maximum spaces in the cellular compartment. The non-coding sequence can be deleted or modified depending on the necessity of the organism which includes the actions in case of selective pressure

for antibiotic resistance (Comeron 2001). More than average non-coding sequences found in this study suggest that these sequences might act as regulatory elements and/or can perform roles on protein folding (Taft *et al.* 2010).

In this study most of the strains matching with MRSA conserved sequences were from *Staphylococcus* family. Three species were selected from the matching sequences for functional analysis. Of the three selected species two belonged to different species (*Salmonella enterica* subsp. *entericaserovarSchwarzengrund* str. CVM19633, and *Streptococcus gallolyticus* UCN34) and one was *Staphylococcusaureus* subsp. *aureus* MSSA476 plasmid pSAS.

Results from ACT (Artemis Comparison Tool) show that the BLAST search matched (Fig. 3) MRSA252 in region between 39,658 bp to 97,899 bp and in *Salmonella enteric* subsp. *enteric serovarSchwarzengrund* str. CVM19633 in region between 46,011 bp to 46,588 bp of their complete genome with 100% identity. This matching comes about just after the *MecA* gene of MRSA252 and after SeSa_B0001 locus in *Salmonella enteric* CVM19633.

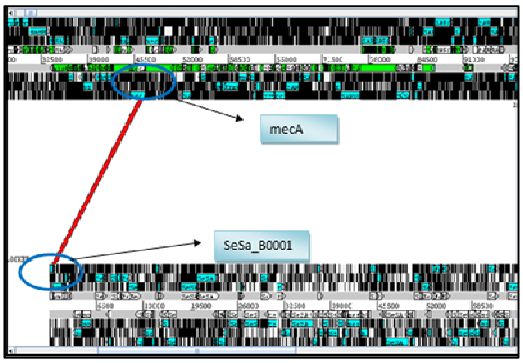

Fig. 3. *Salmonella enteric* CVM19633 genome and MRSA252 genome compared with BLAST result. Bluecircles show the nearby genes of the target non-coding sequence.

Blast result for MRSA252 matched with MSSA476pSAS plasmid with 100% identity (Fig. 4) in regions extending from 743,760 bp to 787,721 bp. The regions are from 3,729 bp to 3,770 bp and 7,735 bp to 7,766 bp with 100% identity and score 42 and 32 correspondingly. MSSA476 plasmid with a nearby gene locus pSAS04 was found to match with the first sequence whereas no known gene locus was found close to match with MRSA252 strain. The second match between 7,735 bp to 7,766 bp of the non-coding sequence of MSSA476 plasmid had the gene locus pSAS13 nearby and that matched with MRSA252 non-coding regions near to SAR0007 locus (Fig. 4).

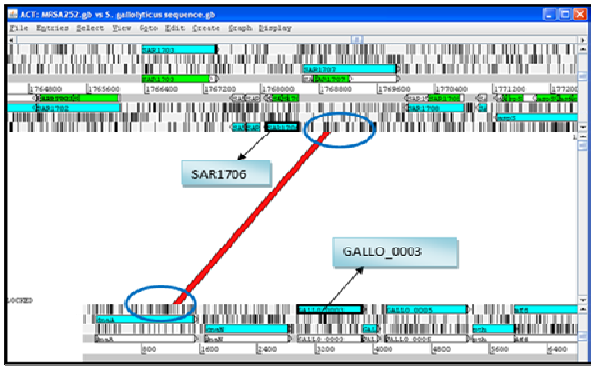

Fig. 4. Comparison between the genomes of MSSA476 pSAS and MRSA 252. Blue circles indicate the nearby locus of the non-coding matching sequences.

Match between MRSA252 and *Streptococcus gallolyticus* found in region 37,077 bp to 93,774 bp and in regions between 1,220 bp and 1,320 bp.Gallo_0003 gene loci was found in the close proximity of *S. gallolyticus* and in MRSA252 the nearby locus found was SAR1706 (Fig. 5).

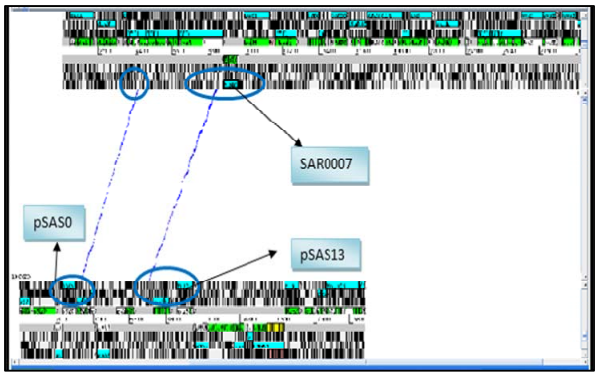

Fig. 5. Comparison between *S. gallolyticus* genome and MRSA252 genome. Blue circles indicate the nearby locus of the non-coding matching sequences.

A simple tabular comparative presentation (Table 3) of the three selected species with MRSA252 shows their gene/locus name, accession number with protein products.

InterProScan analysis results show the biological functions of the genes found at selected regions with the GO (Gene Ontology) terms. Table 4 represents a list of gene/loci involved in biological processes, molecular functions, cellular processes and name of other species where these genes can be found.

Table 3. Comparative matching list of three pathogens with MRSA252.

Query Species: *S. aureus* subsp. MRSA252				Matched Species: *Salmonella enteric* subsp. enteric *serovarschwarzengrund* str. CMV19633 plasmid pCMV19633_110			
Accession	Region	Locus/ gene	Protein product	Accession	Region	Locus/gene	Protein product
NC_002952	39658-97899	*MecA*	Penicillin binding protein	NC_011092	46011-6588	SeSa_B0001	protein SamA
				Matched Species: S. *aureus* subsp. *aureus* MSSA476 plasmid pSAS,			
	743760-787721	-	-	NC_005951	3729 - 3770	Nearby locus: pSAS04	Similar to *Neisseria gonorrhoeae* replication
	743760-787721	SAR0007	Similar to Lactococcuslactis hypothetical protein ycfG		7735-7766	Nearby locus: pSAS13	hypothetical protein
				Matched species: *Streptococcus gallolyticus* UCN34, complete genome			
	37077-93774	SAR1706	Similar to Bacillus halodurans hypothetical protein BH1259	NC_013798	1220-1320	Nearby locus: GALLO_0003	diacylglycerol kinase

Table 4. List of functions of the genes found near to the non-coding regions.

Gene/locus	Protein product	Biological process	Molecular function	Cellular component	Species
MecA	Penicillin binding protein	GO: 0009273: peptidoglycan-based cell wall biosynthesis, GO:00046677: response to antibiotics	GO:0008658: Penicillin binding	-	*E.coli*, *S.aureus*
SeSa_B0001	SamA	-	GO:0003677: DNA binding,	-	*S. enteric* CMV19633
pSAS04	Replication protein	GO:0006270: DNA-dependent DNA replication	GO:0003887: DNA-directed polymerase activity	GO: 0005727: Extra chromosomal circular DNA	MSSA476 plasmid pSAS
pSAS13	Hypothetical protein				MSSA476 plasmid pSAS
SAR0007	ycfG	Carbohydrate related kinase			*Lactococcuslactis*,
SAR1706	Hypothetical protein BH1259				*Bacillus halodurans*,
GALLO_0003	Diacylglycerol kinase	Activation of protein kinase C activity by G-coupled receptor protein signalling pathway	Diacylglycerol kinase activity		*Streptococcus gallolytcus*

This result indicates that most of the unique sequences found in MRSA252 belong to the non-coding region and the small percentage that is found in the coding region code for the *MecA* gene, the major gene responsible for the resistance to antibiotics. Other than the *MecA* gene in the coding region, this study did not find any known function of the non-coding regions which might be associated with other genes or gene products for defined functional purposes.

Functional information provided by the Inter ProScan and GO depicts *MecA* gene as a penicillin binding protein which changes the conformation of protein-drug complex upon binding with antibiotics from the penicillin family. Other function of *MecA* includes peptidoglycan-based cell wall synthesis in organisms like *E. coli*. Protein product locus SeSa_B0001 found to be matched in *Salmonella enteric* plasmid codes for SamA which is a DNA binding protein that can bind with single or double stranded DNA.

Two matched areas with 100% identity between MRSA252 and *Staphylococcus aureus* subsp. *aureus* MSSA476 plasmid pSAS show that even though there were matched sequences they did not share any functional similarities between species. The first match pSAS04, which was found nearby the gene locus of a non-coding region in MSSA plasmid, was defined as a replication protein having polymerase activity in DNA directed manner and resided in an extra-chromosomal circular DNA. For this matched region no noticeable gene locus was found in MRSA252 strain. pSAS13 locus was the second matched region that was found in MSSA476 pSAS plasmid and no defined function was reported from that region. In case of MRSA252 strain the matched region was SAR0007 which coded the protein ycfG. The function of this protein was found to be a carbohydrate kinase in different *Lactococcuslactis* strains. Through BLAST analysis a single match was found between MRSA252 and *Streptococcus gallolyticus* with 100% identity where Gallo_0003 gene locus of *S. gallolyticus* and *SAR1706* locus of MRSA252 strain, respectively. Gallo_0003 has molecular function as a diacylglycerol kinase which is a secondary messenger in signaling pathway. It activates protein kinase C by coupling with G protein. SAR1706 locus is defined as transcription regulator Rrf-2 with no further information.

Though the non-coding sequence matched with different regions among the selected species, in every case they were found near genes which are involved in different molecular and cellular functions. The result from these comparisons among the different species suggests that there is no functional relationship among them and also with MRSA252 strain. Although non-coding sequences compared within the MRSA strains found similar functions, no relation was found with other pathogens. It would have been more useful if common non-coding sequences were found in other species and as well as in MRSA strains; implying a specific and universal biological or regulatory function of that specific region.

Even though no regular functional correlation was found among the pathogens, these unique non-coding sequences can be used for detection and diagnostic purposes with higher specificity and accuracy. Further investigation of these novel non-coding sequences might reveal unknown information about the MRSA species. Studies searching open reading frames and then comparing them for functional analysis might provide new insight about the functions and pathogenicity as well. Understanding and information from the in-silico laboratory can ease the process of investigation of these pathogens in wet laboratory where DNA microarray based studies could be another approach for studying Methicillin Resistant *Staphylococcus aureus* (MRSA).

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EFFECT OF HERBICIDES ON THE GROWTH, YIELD COMPONENTS AND YIELD OF BR11 PADDY

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Abstract

Eleven treatments with three herbicides were applied on BR_{11} paddy field to control weeds and also to study the growth, yield components and yield. The effect of herbicides was found to be positive in controlling the weed species and in increasing the yield components and yield. The maximum number and length of tillers, length of panicle, area of flag leaves, number and percentage of filled grains, grain and straw yield per hectare were found at T_3 when normal dose of Rifit 500 EC was applied. Different doses of Machete 5G were also found effective in controlling weeds and increasing in yield.

Key words: Weed, Herbicides, Paddy, Aman Season

Introduction

Weeds cause problems in rice cultivation by reducing yield of rice (Smith 1970).Losses due to weeds in Aus rice, range from 58% to complete failure of the crop (Mian and Ahasan 1969 and BRRI 1981).Weed competition was most severe ranging from 10 to 20 days after emergence of paddy. Competition of weeds is a major constraint to the productivity of wet-seeded rice because rice has no growth advantage over weeds. Grass weeds are also more difficult to hand weeding of wet seeded rice because of their similar morphology to that of rice.

The traditional methods of weed control in rice field in Bangladesh are land tillage and hand weeding which are time consuming and expensive as well. These involve a large number of man power which during the peak period is very difficult to hire (Chowdhury *et al*.1995). Therefore, the uses of herbicides are easier to control weeds in paddy field and comparatively involve less cost. In the present study three herbicides namely, Rifit 500 EC (Pretilachlor), Ronstar 25 EC (Oxadiazon) and Machete 5G (Butachlor) were applied in different doses in BR_{11} paddy grown in Aman season and their effect was studied on the control of weeds and ultimate yield components and yield of paddy.

Materials and Methods

The seeds of BR_{11} paddy were sown in seed bed of 3 m x1m in the Botanical garden, University of Chittagong. 200 g of seeds were evenly sown in seed bed on 17 July 2004. Watering and weeding were done in the seed bed when required. The field for transplantation was prepared in the same area by ploughing and cross ploughing and leveled properly. The field was divided into 33 plots, each measured 4 m x 4 m.

There were eleven treatments each with three replications (block) where Complete Randomized Design (CRD) was maintained. 24 days old seedlings were transplanted from seed bed to the field on 10 August 2004. Two healthy seedlings were transplanted per hill. Row to row and hill to hill distance were maintained as 20 cm x 20 cm and there were 400 hills per plot. The fertilizers were used in the experimental fields as per following schedule: a. During last ploughing as basal dose (100 g Urea, 160 g TSP and 168 g MP); b. 35 day after transplantation (DAT) (110 g Urea); c. At booting stage (110 g Urea). Rifit 500 EC, Ronstar 25 EC and Machete 5G used in the present investigation as herbicide are presented in Table 1. A brief description of these herbicides is given below:

a) Rifit 500 EC: It is applied on saturated soil and pre transplanting at any time up to weed emergence. It works as cell division inhibitor. It is a selective herbicide. It is taken up readily by the hypocotyls, mesocotyls and coleoptiles. It controls annual grasses, sedges and broad leaved weeds.

b) Ronstar 25 EC: In transplanting rice, the depth of water should be at least 3 to 5 cm deep and be maintained at this level for 2 to 5 days after application. It inhibits Protoporphyrinogen oxidase. It works on annual grasses and broad leaves.

c) Machete 5G: It is a protein synthesis inhibitor. It works as selective and systematic herbicide, absorbed primarily through germinating shoots and secondarily by roots; translocated throughout the plant, with higher concentration in the vegetative parts than the reproductive parts. It controls annual grasses and some broad leaves.

Table 1. Schedule of application of Rifit 500 EC, Ronstar 25 EC and Machete 5G.

Treatment	Herbicides	Dose	Time of Application
T_1 (control)	No herbicide was used	Not applicable	Not applicable
T_2	Only hand weeding was done	Not Applicable	Not applicable
T_3	Rifit 500 EC	1.6ml/800ml water @1L/ha	3 days after transplantation
T_4	Rifit 500 EC	Double the dose of T3	3 days after transplantation
T_5	Rifit 500 EC	Half of the dose of T3	3 days after transplantation
T_6	Ronstar 25 EC	3.20ml/800ml water @2L/ha	3 days after transplantation
T_7	Ronstar 25 EC	Double the dose of T6	3 days after transplantation
T_8	Ronstar 25 EC	Half of the does of T6	3 days after transplantation
T_9	Machete 5 G	40gm/plot@25kg/ha	3 days after transplantation
T_{10}	Machete 5 G	Double the dose of T9	3 days after transplantation
T_{11}	Machete 5 G	Half of the dose of T9	3 days after transplantation

All the herbicides were applied in the BR11 paddy field three days after transplantation as per the product pamphlet. The hand weeding was done at 45 (W_1) and 75 (W_2) days after transplantation and during harvest (W_3) in all the treatments. In control, the weeds were collected during harvest. A total of 35 weed species of 28 genera under 13 families was

found to occur in the present experiments. Among the species, the prevalence of *Fimbristylis miliacea* was highest. *Ludwigia adscssendens, Marsilea quadrifolia, Fimbristylis miliacea, Schoenoplectus erectus, Cyperus difformis,Cynadon dectylon and Monochoria vaginalis* were obtained in all the treatments.

The paddy was harvested 117 days after transplantation, and the following data were recorded. For each treatment, five hills of the Paddy from each block were taken to evaluate the following parameters: a) Number of tillers per hill. b) Length of the tillers. c) Area of flag leaves (measured maximum length × breadth) .d) Length of panicle. e) Fresh weight of straw f) Fresh weight of grain g) Straw and grain yield h) Number of filled and unfilled grains per panicle (Three panicles in each block) and i) 1000- grains weight .

For chemical analysis 100 g of straw and grains were taken from each plot separately and then dried and grinded to powder. Powdered plant samples (straw and grains) were digested as modified Microkjeldahl method, and then NPK were determined as described by Jackson (1973). ANOVA was done to show the significant differences among the treatments following Little and Hills (1977).

Results and Discussion

Different doses of Rifit 500 EC, Ronstar 25 EC and machete 5G were used in BR_{11} paddy field and their effects were studied on the growth, ultimate yield components and yield in Aman season. The results presented in Table 2 showed that in comparison to control all the treatments showed significant number of tiller. The number of tillers per hill increased significantly in all the treatments from T_1 due to application of herbicides and hand weeding. The highest number of tillers per hill was found at T_3 (Table 2). The length of tiller decreased significantly in all the treatments from T_1 and T_2 except T_3. The highest length per tiller was found at T_3. The increase of length of tiller of the present investigation due to the application of Rifit 500 EC was found to be consistent with the findings of Awan *et. al.* (2001). The area of flag leaves increased significantly in all the treatments from T_1. The highest value was at T_3 followed by T_8, T_7, T_{10}, T_6, T_5, T_9, T_4, T_{11}, and T_2. Except T_2 the length per panicle increased significantly in all the treatments from T_1.

The highest length of panicle was found at T_3 followed by T_8, T_5, T_4, T_7, T_8, T_6, T_9 and T_{11}. The number of filled grains of a panicle was found to increase significantly in all the treatments from T_1. The highest number of filled grains was obtained at T_3 followed by T_8, T_7, T_{10}, T_5, T_9, T_{11}, T_6, T_2 and T_4. The number of unfilled grains per panicle was found to be significantly lower in all the treatments from T_1. The lowest number of unfilled grains was found at T_3 followed by T_8, T_7, T_{10}, T_5, T_6, T_9, T_4, T_{11} and T_2. The percentage of filled grains was highest at T_3 followed by T_8, T_7, T_{10}, T_5, T_6, T_9, T_{11}, T_4 and T_2.The number of tillers, length of panicle area of flag leaves, number of filled

grains, and percentage of filled grains increased in almost all the treatments from T_1. The significantly increased values of the above parameters were found at T_3.

Table 2. Effect of Rifit 500 EC, Ronstar 25 EC and Machete 5G on different parameters of BR_{11} paddy.

Treatments	Number of tillers/hill	Length/tiller cm	Length/ panicle cm	Area of flag leaves cm^2	Number of grains/panicle		%of filled grains*
					Filled (F)	Unfilled (UF)	
T_1	7.73	95.47	18.83	25.10	84.69	16.40	83.78
T_2	10.80	84.31	19.08	31.61	106.33	14.17	88.24
T_3	12.25	97.15	23.08	36.79	131.93	9.09	93.55
T_4	9.17	90.12	21.27	32.41	103.92	13.40	88.58
T_5	11.00	95.82	22.10	32.68	110.10	12.73	89.64
T_6	10.60	88.59	20.70	33.86	107.47	12.73	89.41
T_7	10.82	89.93	21.12	34.79	113.27	12.00	90.42
T_8	11.10	92.40	22.17	35.82	115.18	10.92	91.34
T_9	10.33	86.01	20.32	32.63	108.94	13.07	89.29
T_{10}	11.41	89.43	20.75	34.10	111.13	12.47	89.91
T_{11}	10.25	84.39	19.52	32.29	108.76	13.66	88.84
$LSD_{0.05}$	0.90	1.22	0.4	0.11	1.7	0.4	0.02
$LSD_{0.01}$	1.27	1.73	0.57	0.16	2.42	0.57	0.03

* calculated value.

The fresh weight of straw per block increased significantly in all the treatments from T_1. The highest weight of straw per block was at T_3 (Table 3). The fresh weight of grains per plot was found to increase significantly in all the treatments from T_1 that shown in Table 3. The highest value was found at T_3 followed by T_8, T_7, T_{10}, T_6, T_5, T_9, T_4, T_{11} and T_{12} (Table 3). The total fresh weight of straw and grain per plot increased significantly in all treatments from T_1. The highest value was found at T_3 followed by T_8, T_7, T_{10}, T_6, T_5, T_9, T_{11}, T_2, and T_4 (Table 3) . The present findings corroborate with the findings of Budhar and Tamilseivan (2002) who had observed significantly higher grain and straw yield over hand weeding by the use of herbicides. The straw and grain ratio decreased significantly in all the treatments from T_1 and the lowest value was at T_4. Except T_4 the straw yield per hectare increased significantly in all the treatments from T_1. The highest yield was obtained at T_3 followed by T_8, T_7, T_{10}, T_6, T_5, T_9, T_4, T_{11} and T_2.

The grain yield per hectare increased significantly in all the treatments from T_1. The highest yield was found at T_3 followed by T_8, T_7, T_{10}, T_6, T_5, T_9, T_4, T_{11} and T_2. Matsunaka (1970) had also reported the significant increase in rice yield due to use of herbicides over hand weeding. The 1000 grain weight was found to be significant in all the treatments from T_1 except T_6 and the highest 1000-grain weight was found at T_3 followed by T_{10}, T_9, T_{11}, T_5, T_8, T_7, and T_4 (Table 3).

Table 3. Effect of Rifit 500 EC, Ronstar 25 EC and Machete 5G on different parameters of BR_{11} paddy.

Treatments	Fresh weight of straw & grains/plot			Straw and grain ratio (S/G)	Straw yield* t/ha	Grain yield* t/ha	1000- grain weight g
	Straw(S) kg	Grain(G) kg	Total (S+G)				
T_1	9.58	4.43	14.01	2.16	5.99	2.77	22.35
T_2	11.52	7.32	18.84	1.57	7.20	4.58	23.16
T_3	14.13	9.1	23.23	1.55	8.83	5.69	24.49
T_4	10.05	7.63	17.68	1.32	6.28	4.77	23.36
T_5	13.28	7.98	21.26	1.66	8.30	4.99	24.07
T_6	13.38	8.18	21.56	1.64	8.36	5.11	22.40
T_7	13.82	8.45	22.27	1.64	8.64	5.28	23.40
T_8	14.00	8.93	22.93	1.57	8.75	5.58	23.79
T_9	13.10	7.75	20.85	1.69	8.19	4.84	24.26
T_{10}	13.72	8.38	22.10	1.64	8.58	5.24	24.29
T_{11}	11.85	7.35	19.20	1.61	7.41	4.60	24.10
$LSD_{0.05}$	0.63	0.13	0.02	0.02	0.42	0.08	0.41
$LSD_{0.01}$	0.89	0.18	0.03	0.03	0.59	0.11	0.58

* calculated value.

The N concentration of straw increased significantly in all the treatments from T_1. The highest value was obtained at T_8 followed by others (Table 4). The P concentration of straw showed significantly in all the treatments from T_1. The highest value was at T_4 followed by others. The highest value of K in straw was at T_8 followed by others .The total NPK concentrations of straw increased significantly in all the treatments from T_1. The highest value was at T_8 followed by others. In N:P:K of straw, N concentration was found to be maximum at T_3. The P concentration of N:P:K was maximum at T_{10}. The K concentration of N:P:K was found maximum at T_2. The N concentration of grains increased significantly in all the treatments from T_1. The highest value was at T_{11} followed by T_9, T_8, T_4, T_5, T_3, T_6, T_7, T_{10} and T_2. The P concentration of grains was found significantly in all the treatments from T_1. The highest value was at T_6 followed by T_{11}, T_{10}, T_4, T_5, T_9, T_8, T_2, T_3, and T_7. Except T_7 the K concentration of grains was found to be increased significantly in all the treatments from T_1. The total NPK concentration of grains significantly increased in all the treatments from T_1. The highest value was at T_{11}. In N:P:K of straw, N concentration was found to be maximum at T_9. The P concentration of N:P:K was maximum at T_1. The K concentration of N:P:K was found to be maximum at T_2 (Table 4).

The individual increase of N, P and K was found in all the treatments from the unwedded control (T_1) but no definite trend of increase was observed. However, the total NPK concentration was observed maximum at T_8 when half of the dose of Ronstar was applied. The total NPK concentration of grain was found maximum at T_{11} when Machete half dose was applied.

Table 4. Effect of Rifit 500 EC, Ronstar 25 EC and Machete 5 G on N, P and K concentrations** on straw and grains of BR_{11}.

Treatment	Straw					Grain				
	N	P	K	Total NPK	N:P:K*	N	P	K	Total NPK	N:P:K*
T_1	0.47	0.20	0.66	1.33	35.34:15.04:49.62	0.42	0.54	0.43	1.39	30.21:38.85:30.93
T_2	0.63	0.30	0.95	1.88	33.51:15.96:50.53	0.91	0.68	0.73	2.32	39.22:37.07:31.46
T_3	0.84	0.32	0.87	2.03	41.37:15.76:42.86	1.12	0.65	0.57	2.34	47.86:27.78:24.36
T_4	0.70	0.58	0.72	2.00	35.00:29.00:36.00	1.19	0.76	0.63	2.58	46.12:29.46:24.42
T_5	0.70	0.54	0.94	2.18	32.11:24.77:43.12	1.19	0.76	0.65	2.60	45.77:29.23:25.00
T_6	0.70	0.43	0.81	1.94	36.08:22.16:41.75	1.12	0.82	0.72	2.66	42.10:30.83:27.07
T_7	0.84	0.55	0.95	2.34	35.89:23.50:40.59	1.12	0.64	0.49	2.25	49.78:28.44:21.78
T_8	1.00	0.52	1.03	2.55	39.21:20.39:40.39	1.26	0.69	0.65	2.60	48.46:26.54:25.00
T_9	0.85	0.54	0.95	2.34	36.32:23.08:40.59	1.38	0.73	0.54	2.65	52.07:27.55:20.38
T_{10}	0.64	0.57	0.72	1.93	33.16:29.53:37.30	1.1	0.79	0.54	2.43	45.27:32.51:22.22
T_{11}	0.7	0.57	0.91	2.18	32.11:26.15:42.74	1.4	0.80	0.50	2.70	51.85:29.63:18.52
$LSD_{0.05}$	0.01	0.03	0.05	0.06		0.05	0.04	0.04	0.07	
$LSD_{0.01}$	0.02	0.04	0.07	0.08		0.07	0.06	0.06	0.10	

* Calculated value, ** g% of dry weight basis

In the present experiment, T_3 was found to be most effective in increasing the yield components and yield of BR11 paddy. At T_4, double the dose of Rifit 500 EC was used where the yield did not increase accordingly. This may have toxic effect of the double the dose of Rifit. So, normal dose of Rifit 500 EC (1.6ml/800ml water @ 1L/ha) may be recommended.

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IN VITRO CONTROL OF FIVE PATHOGENIC FUNGI ISOLATED FROM GROUNDNUT (ARACHIS HYPOGAEA L.)

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Abstract

Efficacy of five plant extracts namely, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Tagetes patula* and *Zingiber officinale* was evaluated against five pathogenic species of fungi isolated from groundnut *in vitro*. These were *Colletotrichum acutatum*, *Colletotrichum dematium*, *Colletotrichum orbiculare*, *Colletotrichum* sp. and *Fusarium semitectum.* Colony growth of *C. dematium* was completely checked with *Allium sativum* at all the concentrations used (5, 10 and 20 %). Similarly *A. cepa*, *A. sativum* and *A. indica* completely inhibited the colony growth of *C. orbiculare* at the same concentrations used. *T. patula* and *Z. officinale* also showed appreciable inhibition in colony growth of five species of fungi at 10 and 20% concentrations.

Key words: *In vitro*, Control, Pathogenic fungi, Groundnut

Introduction

Groundnut, one of the principal economic crop of world occupies 13th position among fruit crops (Varnell and Mccloud 1975), 4th place among the oilseed crops in respect to both area and production next to soybean, sunflower and cotton (Weiss 1983). Groundnut is the second major oil crops in Bangladesh covering an area of 76 thousand ha. producing 1.2 million MT of nuts. Bangladesh produces 46000 MT of groundnut (BBS 2007). Increase in the production of this crop can help to minimize the shortage of edible oil in our country. It is the richest plant source of thiamin (B_1). Groundnut contains at least 13 different types of vitamins and also rich in 26 essential minerals. Incidence of disease is the most important obstacle for groundnut production. Fungi can be rendered as the most harmful microorganism and so far, 46 fungal diseases were recorded on groundnut and 67 (aprox.) fungi were associated with various symptoms type (Wikipedia 2012). In Bangladesh, groundnut suffers from many diseases out of which 14 are fungal, two are viral, nine are nemic and one is mycoplasma disease (Ahmed 1985, Baker *et al.* 1980, Fakir 1980 and Talukder 1974). Shamsi and Sharmin (2012) recorded ten types of symptoms on eighteen varieties of groundnut during the period of December 2010 to May 2012. This investigation also revealed that a total of 48 species of fungi representing 24 genera was associated with 18 vareites of *Arachis hypogaea*. To protect groundnut from diseases, one must have knowledge on etiology of disease, isolation and identification of causal organism, prevention and control measure. Much research works have been carried out on management of diseases of groundnut in different parts of the

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world (Ambang *et. al*. 2011, Sing *et al.* 1997 and Sunker *et al.* 2005). In Bangladesh very little work has been done to protect groundnut from the incidence of diseases (Mia *et al.* 2007, Bakr *et al*. 2009 and Sharmin 2012). Control of plant diseases by using plant extract having antifungal properties has recently gaining appreciable importance to plant pathologists. Intensive research has been done in this field to avoid the hazardous impact of pesticides and agro-chemicals on ecosystem. On account of their non phytotoxicity, biodegradability and renewable nature such substances appear to be the ideal antifungal agents (Baker *et al.* 1980). Present study was undertaken to (i) find out the association of the fungi with groundnut (ii) determine the pathogenic potentiality of the fungi and (iii) evaluate antifungal potentiality of some botanicals *in-vitro* against most frequently isolated fungi from groundnut.

Materials and Methods

Collection of samples: During the period of December 2010 to May 2012, 18 varieties of groundnut plant were grown in field plot of Botanical garden, Curzon Hall, University of Dhaka. Samples were collected from Botanic Garden (Curzon Hall campus), University of Dhaka and BARI, Gazipur. Collected samples were examined and symptoms were recorded. After microscopic observation fungi were isolated from healthy and diseased samples following the Blotter" and"Tissue planting" method on PDA medium (CAB 1968). Specimens were preserved in the Herbarium of Mycology and Plant Pathology laboratory, Department of Botany, University of Dhaka. The varieties used in the experiment were: GN, BB- 8, DG- 2, B- 5, B- 6, B- 7, BN- 1, BN- 2, BN- 3, BN- 4, DHAKA- 1, BARI- 5, BARI- 6, BARI- 7, BARI- 8, BARI- 9, GN_1 and GN_2. Isolated fungi were tested for their pathogenic potentiality. In the present investigation 48 species of fungi were isolated from groundnut and identified following Standard Literature (Booth 1971, Ellis 1971, 1976, Sutton 1980, Ellis and Ellis 1997, Barnett and Hunter 2000). Isolated fungi were tested for their pathogenic potentiality following modified "detached leaf technique" (Azad and Shamsi 2011). *Cercospora arachidicola* S. Hora, *Pheoisariopsis personata* Berk and M.A., *Puccinia arachidis* Speg. and *Sclerotium rolfsii* are well documented pathogens of groundnut. In this experiment five fungi namely *C. acutatum* Simmonds*, C. dematium* (Pers. Ex. Fr.) Grove., *C. dematium orbiculare* (Berk. & Mont.) Arx., *Colletotrichum* $sp._1$ and *Fusarium semitectum* Berk. & Rav. were found to be pathogenic to groundnut. Efficacy of five plant extracts namely *Allium cepa* L.*, A. sativum* L.*, Azadiracta indica* L.*, Tagetes patula* L. and *Zingiber officinale* L. was evaluated against these five pathogenic fungi following poison food techniques (Grove and Moore 1962).

Preparation of plant extracts: The desired parts of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation (Table 1). In case of leaves and bulbs, extracts were prepared by crushing known weight of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized mass of a plant part was

squeezed through four folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 minutes to remove particulate matter. The supernatants were filtered through Whitman filter paper and the filtrate was collected in 250 ml Erlenmeyer flasks. In this method, the requisite amount of the filtrate of each plant extract was mixed with PDA medium and sterilized in an autoclave at 121°C for 15 minutes.

Table 1. The particulars of plant extracts used in this experiments.

Plant species	Native name	Family	Plant part used
Allium cepa	Onion	Liliaceae	Bulb
Allium sativum	Garlic	Liliaceae	Bulb
Azadiracta indica	Neem	Meliaceae	Leaf
Tagetes patula	Marigold	Asteraceae	Leaf
Zingiber officinale	Ginger	Zingiberaceae	Rhizome

The medium thus prepared was poured into sterilized Petri plates and was allowed to solidify. Each Petri plates was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing culture of the test pathogen. In control set, a Petri plate containing PDA medium with the requisite amount of distilled water instead of a plant extract was also inoculated with agar disc of the test pathogen in the same way as described above. Three replications were maintained for both treatment and control sets. The inoculated Petri plates were incubated at 25 ± 1°C. The radial growth of the colonies was measured after 5 days of incubation.

The percentage growth inhibition of each test pathogen was calculated by using the following formula

$$I = \frac{C - T}{C} \times 100$$

Where, I = percent growth inhibition, C = growth in control and T = growth in treatment

Results and Discussion

Use of plant extracts against plant pathogenic fungi and plant diseases is relatively a recent approach. Five fungi, isolated from leaflets of groundnut showing anthracnose, Colletotrichum leaf spot and rotting symptom were found to be pathogenic to the plant. This is the first report of anthracnose, Colletotrichum leaf spots caused by *Colletotrichum* spp. from Bangladesh. The isolated fungi were *Colletotrichum acutatum, C. dematium, C. orbiculare, Colletotrichum* sp. and *Fusarium semitectum.* Efficacy of five plant extracts namely *Allium cepa, A. sativum, Azadiracta indica, Tagetes patula* and *Zingiber officinale* was evaluated against those five fungi. The extent of inhibition, however, varied among the fungi.

The vegetative growth of *C. acutatum* showed highest (69 %) inhibition with *T. patula* at 20 % followed by 53 and 46 % inhibition at 10 and 5 % concentration respectively. *Z. officinale* showed 36 % inhibition of the fungus at highest concentration (20 %). Lowest

inhibition of the fungus such as 20, 14 and 11 % was encountered at 20, 10 and 5 % concentrations respectively (Fig. 1). This observations are in agreement with the observations made by Sing *et al.* (1997) and Mala *et al*. (1998).

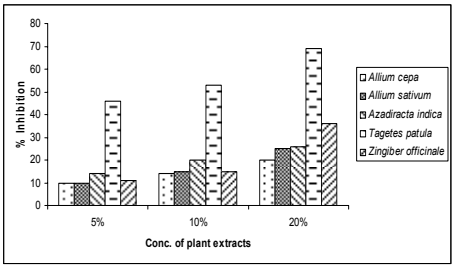

Fig.1. Effect of plant extracts on growth of *Colletotrichum acutatum.*

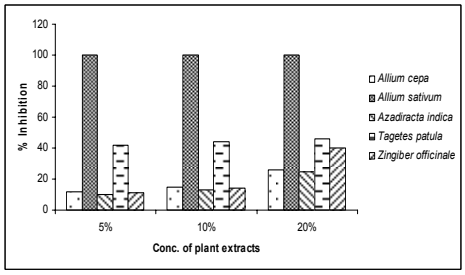

Fig. 2. Effect of plant extracts on growth of *Colletotrichum dematium*.

Colletotrichum dematium was completely checked with *A. sativum* at all the concentrations used (5, 10 and 20 %). Less inhibition of the fungus was recorded when treated with 10, 13 and 25 % of *A. indica* at 5, 10 and 20 % concentrations (Fig. 2). Higher fungitoxicity of *A. sativum* was also reported by Misra and Dixit (1976). Seed borne pathogens of jute were effectively controlled by *Allium sativum* (Ahmed and Sultana 1984). Shovan *et.al.* (2008) recorded 89.44 % inhibition of *C. dematium* isolated from anthracnose of Soybean.

The growth of *C. orbiculare* was completely inhiteded by *A. cepa*, *A. sativum* and *A. indica* at all the concentrations used (5, 10 and 20 %). *Z. officinale* and *T. patula* checked 45 and 34 % colony growth of the fungus respectively at 20 % concentration (Fig. 3).

Colonies of *Colletotrichum* sp. was 57 % inhibited *by Z. officinale* at 20 % concentration followed by *T. patula*, 51 % inhibition of the colony at the same concentration. *A. cepa* and *A. indica* showed 39 % inhibition of the fungus at 20 % concentration. *A. sativum* inhibited 30, 36 and 45 % colony growth of the fungus at 5, 10 and 20 % concentrations respectively (Fig. 4).

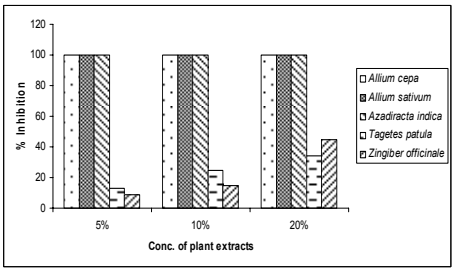

Fig. 3. Effect of plant extracts on growth of *Colletotrichum orbiculare.*

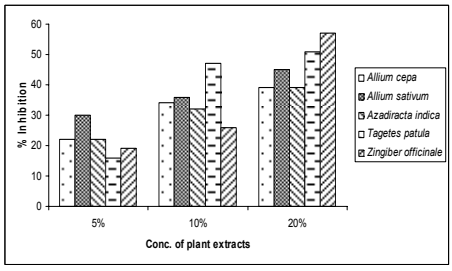

Fig. 4. Effect of plant extracts on growth of *Colletotrichum* sp.

Plant extract of *T. patula* inhibited 81 % vegetative growth of *Fusarium semitectum* at 20 % concentration followed by 62 and 33 % at 10 and 5 % concentration respectively. *Z. officinale* checked 71, 61 and 42 % colony growth of the fungus at 20, 10 and 5 % concentrations respectively. *A. sativum* showed 62, 44 and 38 % inhibition of the fungal colony at the above mentioned concentrations. *Allium cepa* inhibited 53, 41 and 34 % colony growth of the fungus at 20, 10 and 5 % concentrations respectively. *A. indica* showed lowest inhibition of the fungus 49. 43 and 20 % at 20, 10 and 5 % concentrations. Methanol extract of *T. patula* inhibited growth of three pathogenic fungi *Botrytis cineria*, *Fusarium moniliformae* and *Phythium ultimum* (Mares *et al.* 2004) (Fig. 5).

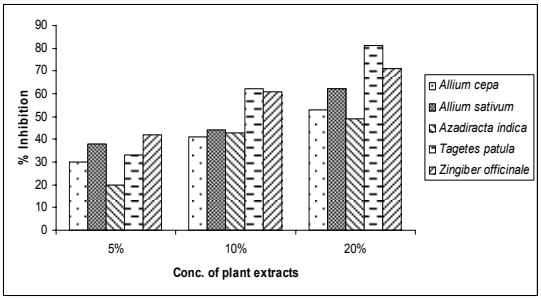

Fig. 5. Effect of plant extracts on growth of *Fusarium semitectum.*

In home and abroad, cultural practice and chemical control have been practiced against leaf spot, rust and stem rot diseases of groundnut, but this is the first approach of controlling the causal agents of anthracnose, Colletotrichum leaf spot and leaf rot of groundnut with botanicals *in vitro* .

Allium sativum completely inhibited the growth of *C. dematium* at all the concentration used (5, 10 and 20 %). *Allium cepa*, *A. sativum* and *A. indica* completely inhibited the growth of *C. orbiculare* at the same concentration used (5, 10 and 20%). *T. patula* and *Z. officinale* also showed appreciable inhibition in colony growth of five species of fungi at 10 and 20 % concentrations. Among the five plant extracts used *A. cepa*, *A. sativum* and *A. indica* showed excellent results in controlling the redial diameter of the colonies of *C. orbiculare* at 5 % concentration. In addition to *C. orbiculare, A. sativum* also exhibited complete inhibition of *C. dematium* at the same concentration. Hadian (2012) reported that *Allium sativum* and *A. indica* at 100% concentrations inhibited growth of *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* two pathogenic fungi causing wilting disease of tomato.

This is the first report of evaluation of plant extracts against *Colletotrichum* spp. and *F. semitectum* isolated from groundnut. The present findings on the antifungal activities of these plant extracts may, in future, open a new horizon in plant disease control.

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SURVEY ON THE INCIDENCE AND SEVERITY OF COMMON SCAB OF POTATO IN BANGLADESH

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Abstract

A survey on the incidence and severity of common scab (*Streptomyces* sp.) of potato was made in the major potato growing districts of Bangladesh during the cropping season of 2007-2008. A total of 150 fields was surveyed for collection of potato common scab infected samples. It was observed that there was a lot of variation in disease severity and its incidence in different districts of Bangladesh. Highest per cent scab incidence (71.41 %) was recorded in Kahalo Upazila (Bogra) and lowest (13.79 %) in Pirgacha Upazila (Rangpur). Disease incidence also varied among the tested varieties namely, Cardinal, Diamant, Granola, Binella and Raja. Highest incidence was observed in Cardinal (54.08 %) followed by Binella (50.71 %) and it was lowest in Raja (3.07 %). Cardinal and Binella were found to be highly susceptible and Raja was resistant to common scab disease. Diamant, the commercial variety showed medium susceptible reaction to the disease.

Key words: Survey, Incidence, Common scab, Potato, Bangladesh

Introduction

Potato, the world's fourth most important food crop after wheat, maize and rice, provides balanced source of starch, vitamins and minerals to many communities in the global villages (Rowe 1993). In Bangladesh potato is the third largest crop after rice and wheat. It is used primarily as a vegetable and has potential as a staple food. Potato cultivation in the Bengal was promoted by a British Governor in 1770s and then it was a well established garden vegetable. Annual consumption of potato has been growing rapidly, from around 7 kg per capita in 1990 to more than 25 kg in 2005 (FAO 2007).

In Bangladesh, so far as many as 57 diseases in potato have been recorded (Hossain *et al*. 2008*)*. Among them late blight, stem rot /sclerotium rot, wilt, common scab, potato leaf roll and mosaic are the most important diseases (Ahmed *et al*. 2000). Common scab is widely distributed in Bangladesh which gives ugly appearance to wear potatoes. Though the disease does not cause appreciable reduction in yield, it can cause great loss due to reduction of market value of tuber (Dutt 1997). Moreover, infected seed tubers serve as the primary sources of inoculum for the next season (Anonymous 2009).

The incidence, severity, etiology, epidemiology and control of common scab have been investigated extensively in many countries of the world and the disease has been the topic of various reports in Bangladesh during the end of the 18th century (Rahman 1990). In Bangladesh potato common scab was initially a minor disease but now has become a major potato disease and incidence of the disease is increasing day by day. The information of potato common scab is not available on the incidence of disease and its severity in different agro- ecological regions and the susceptibility of commercially cultivated potato in Bangladesh. Keeping all these in view, the present investigation was undertaken to study the regional variations on the disease incidence, severity and susceptibility of common scab disease of commercially cultivated potato in Bangladesh.

Materials and Methods

Fifteen Upazilas, three from each district of Bogra, Rangpur, Rajshahi, Munsiganj and Comilla were selected for the survey. The Upazilas and districts located into eight different Agro-ecological zones of Bangladesh (Anonymous 2005). The disease incidence (DI), disease severity (DS) and percentage of disease index (PDI) were recorded during the potato harvesting season of December 2007 to February 2008. The sampling areas are shown in Fig 1.

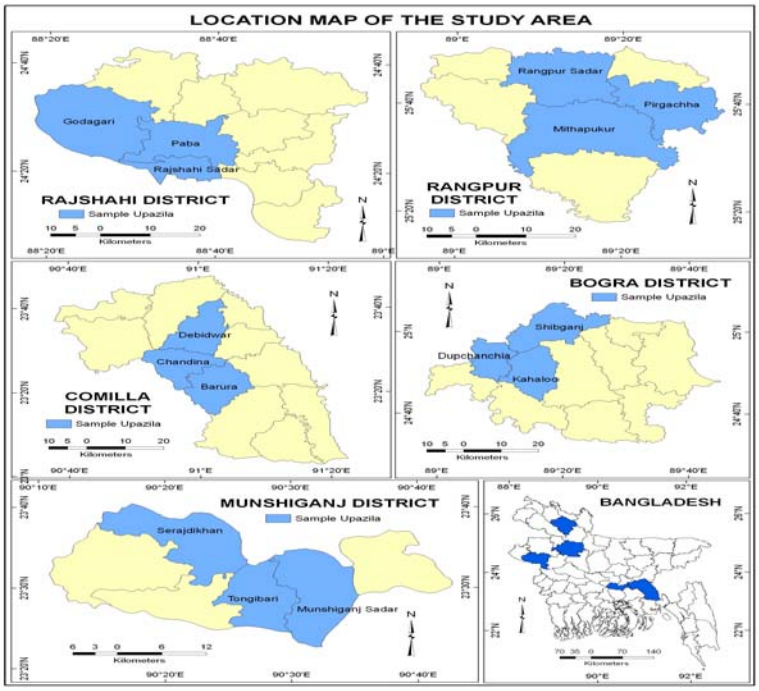

Fig. 1. Location map of the study area for common scab disease incidence in Bangladesh.

Three Upazilas from each district and ten potato fields from each Upazila were randomly selected. Therefore, a total of 150 fields was selected for sampling during potato harvesting season. During the survey, ten sampling points from a field were selected. In each point ten tubers were collected at random and composite it. Therefore, a total of 100 tubers from each field was collected for observation. Cultivars grown in selected fields were also recorded.

Disease incidence was calculated on the basis of number and weight of infected tubers and it was expressed in percentage. Disease severity was recorded based on the symptom as shown on the surface of the tuber. One hundred tubers were selected randomly, categorized into 0-5 scale (Liu *et al*. 1995) and finally percentage of disease incidence was calculated following the formula of Goswami *et al.* (2002). Disease severity was recorded according to disease severity rating scales where 0 = No symptom, 1 = Very small lesions, 2 = Small superficial lesions, 3 = Periderm broken, 4 = Light pitted and 5 = Deep pitted.

Randomly selected potato cultivars were grouped as least susceptible, medium susceptible, highly susceptible and comparatively resistant. Grouping of potato cultivars was done based on the potato common scab index. Average PDI value of each potato variety was calculated and then the class was determined. Susceptibility of potato cultivars to common scab was grouped according to Marais and Vorster (1988) which is given below:

Scab Index	Class
< 08	Comparatively resistant
08 - 15	Least susceptible
15 - 20	Medium susceptible
20 – 24	Highly susceptible
>24	Very highly susceptible

Results and Discussion

Under field condition common scab of potato was identified by observing the symptoms of the disease. The disease was found to produce different types of symptoms on the surface of the tubers. The lesions appear as small reddish brown, water- soaked lesions on the tuber periderm. As the pathogen continues to colonize the tuber, the host develops wound periderm resulting in slightly raised lesions composed of rough and corky tissues. These spots coalesce to form irregularly shaped patches which are usually tan to brown in color and rough in texture. The patches became cracked as the infection progresses and developed a star-like appearance. In deeper lesions, the tuber periderm was ruptured. Typical lesions on susceptible potato variety are presented in Plate 1.

Plate 1. Lesion types of common scab on potato tubers.

The percentage of disease incidence of potato tubers is represented in Table 1 based on number and weight. Disease incidence in number and weight basis was highest at Kahaloo (73.3 and 71.41 %) and lowest (13.6 and 13.79 %) at Pirgacha. Disease incidence at Kahaloo was followed by Paba (67.9 and 69.40 %), Shibganj (61.3 and 61.72 %) and Dhupchaciya (56.0 and 55.32 %). The disease incidence at Chandina and Mithapukur were 22.23 and 28.70% respectively (Table 1).

Table 1. Prevalence and severity of common scab of potato in fifteen Upazilas of five districts during potato harvesting season in 2007- 2008.

District	Upazila	Disease Incidence (%) of infected tuber by No.	Disease Incidence (%) of infected tuber by Wt.	Range (%) of infected tuber by wt.	PDI
Rajshahi	Paba	67.9	69.40	26 – 95	22.52
	Godagari	31.4	37.15	7 – 55	15.04
	Rajshahi sadar	41.90	46.88	18 – 64	22.78
Bogra	Dhupchaciya	56.0	55.32	43 – 66	15.06
	Shibganj	61.3	61.72	29 – 100	24.44
	Kahaloo	73.3	71.41	37 – 100	28.76
Rangpur	Pirgacha	13.6	13.79	04 – 31	3.64
	Mithapukur	26.8	28.70	13 – 47	9.62
	Rangpur sadar	38.3	44.11	20 – 78	13.02
Munsiganj	Munsiganj sadar	24.1	31.68	9 – 44	8.26
	Tongibari	29.6	32.88	10 – 68	11.4
	Serajdekhan	27.9	32.91	18 – 40	10.82
Comilla	Barura	41.6	47.29	17 – 90	23.9
	Chandina	21.4	22.23	9 – 75	12.9
	Dabedhar	40.9	45.38	16 – 56	24.28

Highest scab incidence was observed in Cardinal (54.08 %) followed by Binella (50.71 %) and both the varieties were highly susceptible to common scab disease. Diamant was medium susceptible potato variety where the incidence to common scab was 40.96 %. The incidence of common scab in Granola was 38.96 %. Lowest scab incidence was recorded in Raja (3.07 %) which showed resistant reaction (Table 2).

Table 2. Scab index and incidence of potato cultivars screened in 2008 at farmer's field.

Cultivar`s name	Scab index	Scab incidence (%)	Class
Diamant	16.955	40.96	Medium susceptible
Cardinal	22.955	54.08	Highly susceptible
Granola	13.072	38.96	Least susceptible
Binella	13.044	50.71	Highly susceptible
Raja	0.800	03.07	Comparatively resistant

From the Fig. 2 it is apparent that among the surveyed districts the highest scab incidence was recorded in Bogra (62.81 %) and lowest in Rangpur (28.86 %). The scab incidence was 51.14, 38.3, and 32.49 % in Rajshahi, Comilla and Munsiganj respectively. The highest percentage of disease index (PDI) was also recorded in Bogra (22.75 %) followed by Comilla (20.36 %), Rajshahi (20.11 %) and Munsiganj (10.16 %). Lowest PDI was recorded in Rangpur (8.76 %).

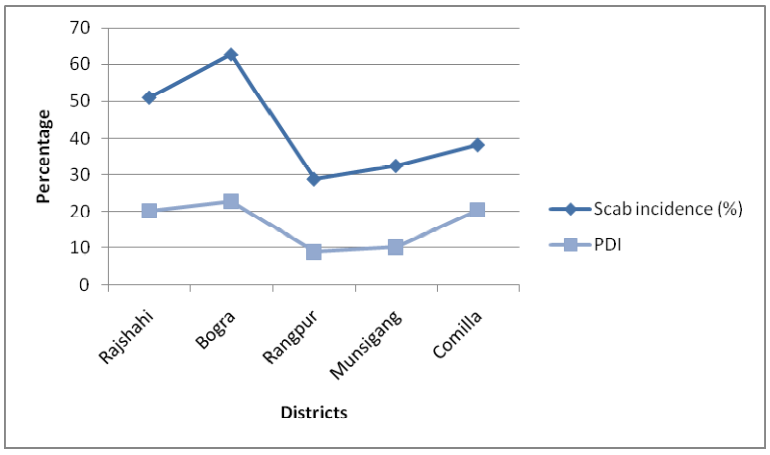

Fig. 2. Percentage of scab incidence and disease index of five districts.

Scab incidence and PDI were higher in Rajshahi and Bogra districts. Higher scab incidence in Rajshahi and Bogra might be due to cultivation of susceptible variety *viz*. Cardinal, Diamant and Granola. Mono- crop (potato) cultivation in these districts might

be another reason for increasing inoculam pressure of this disease. The pathogen survives as spores or mycelium within crop debris. It can remain viable in soil from a decade to up to 20 years (Kritzman *et al*. 1996).

High soil pH is favorable for scab disease incidence. High soil pH (above 7) is present in Rajshahi and Bogra districts. The soil pH level of Rangpur, Munsigang and Comilla districts are 4.6, 4.7 and 4.1 respectively (Anonymous 2005). Both the disease incidence and PDI were higher in Rajshahi and Bogra districts compared to Rangpur, Munsigang and Comilla districts. Disease development increases with soil pH from 5.0 to 8.0 (Goto 1985). The variation of the occurrence of scab and its severity in different districts of Bangladesh might be due to cropping system, soil moisture, soil texture and soil pH (Loria 1991).

From this survey it appears that the occurrence of common scab and its severity varies from fields to fields and locations to locations. It was also observed that the most popular variety Diamant and Cardinal showed medium susceptible and highly susceptible reaction to common scab disease respectively which indicates the necessity of finding out suitable control measures of the disease. An integrated disease management approach that includes cultural, biological and chemical control methods, in the field and in storage, may be necessary for long-term control of common scab disease. Research should also be undertaken in order to develop resistant variety against scab disease.

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OPTIMIZATION OF CULTURE CONDITIONS FOR THE PRODUCTION OF XYLANASE BY TWO THERMOPHILIC FUNGI UNDER SOLID STATE FERMENTATION

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Abstract

Two thermophilic fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 were studied under solid state fermentation (SSF) using wheat bran for the production of thermostable xylanase. The optimum time required for the production of xylanase was found to be 4 days and 7 days for *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 respectively. The optimum temperatures for the production of xylanase by *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 were 45°C and 50°C respectively. The maximum activity of xylanase (1.685 IU/ml and 0.075 IU/ml) was exhibited by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 at pH 7.0 and pH 4.0 respectively. The optimum moisture content for maximum xylanase production was 90% for both fungi.

Key words: Xylanase, Thermophilic fungi, *Thermomyces lanuginosus* and *Rhizomucor pusillus*

Introduction

Microbial technology is applied to produce xylanolytic enzymes for improving the quality of inferior lignocellulosics using thermophilic fungi under solid state fermentation. Although most of the sources of xylanase are reported to be produced by mesophilic fungi (Gattinger *et al*. 1990 and Yang *et al*. 2006), one of the major problems encountered in the utilization of these enzymes is their poor temperature stability and low hydrolysis rate of the lignocellulosics (Akhtar *et al*. 2006).

Thermophilic fungi carry out enzymatic hydrolysis at elevated temperature over prolonged period of time due to their inherent superior thermostability (Akhtar *et al*. 2006 and Yang *et al*. 2006). Moreover, thermostable enzymes can be recovered and purified at ambient temperature. Diffusion and other chemical processes of thermostable enzymes are accelerated at high temperature resulting increased reaction rate. On the otherhand, large scale fermentation with thermophiles is technically and economically more feasible than mesophilic counterparts (Khan *et al*. 1996).

Thermostable lignocellulolytic enzymes such as xylanase has significant potential applications in biodegradation of various industries including chemicals, fuel, food, brewery and wine, animal feed, fiber, textile and laundry, pulp and paper and agriculture (Ghatora *et al*. 2006, Howard *et al*. 2003, Senthilkumar *et al*. 2005 and Virupakshi *et al*. 2005). In the present study, an attempt has been made to investigate two thermophilic

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fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 for the production of thermostable xylanase to optimize some of the cultural parameters such as temperature, pH, incubation time and moisture content under solid state fermentation for maximization of the production of enzymes.

Materials and Methods

Two thermophilic fungi, *Thermomyces lanuginosus* BPJ-10 and *Rhizomucor pusillus* BPJ-2 were isolated and identified at the laboratory of Plant Physiology and Biochemistry, Department of Botany, Jahangirnagar University, Savar, Dhaka. The fungi were cultured on PDA media and maintained at 50°C and 45°C temperature for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively. The cultures were incubated for five days and the spores of the cultures were washed with sterile water and the resulting suspention (2.5×10^5 spores per ml) was used as inocula. These fungi were employed for the production of xylanase under solid state fermentation described by Halim *et al*. (2001) and Mohiuddin (1992). To optimise the enzyme production following cultural conditions were investigated by using wheat bran as a solid substrate. Wheat bran (10g) was taken in each of the 250 ml conical flask. The substrates were mixed thoroughly with distilled water for maintaining the suitable amount of moisture content. The flasks were then properly cotton plugged and autoclaved at 121°C for 15 minutes. Each flask was inoculated with 2 ml of spore suspension. To find the effect of temperature for maximization of enzyme production, inoculated flasks were incubated at temperature ranging from 35° to 60°C. To investigate the effect of incubation period for maximization of enzyme production, the inoculated flasks were incubated for 10 days at 45°C and 50°C for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively. Enzyme was extracted and assayed at every 24 hours starting from the 1st day upto 10th day of inoculation. For the determination of the effect of moisture content on enzyme production 50, 60, 70, 80, 90 and 100% distilled water were added with solid substrate. The pH of the initial culture was adjusted to 4, 5, 6, 7, 8 and 9 for both fungi to find its optimum value.

After optimum incubation time (7 days for *T. lanuginosus* BPJ-10 and 4 days for *R. pusillus* BPJ-2) 100 ml of 1% NaCl solution was added to the culture and mixed properly to each fermented biomass. In order to disperse the mycelia bound biomass, the flasks with moldy substrate was shaken in a shaker for 45 minutes at 150 rpm. The fermented slurry was first filtered with nylon cloth and then extracts were filtered with Millipore membrane filter paper (0.25μ). Clear filtrate was obtained and centrifuged at 4500 rpm for 15 minutes. The clear supernatants were used for the determination of enzyme activity. The amount of reducing sugar was estimated by Dinitro salicylic acid (DNS) method (Millar 1959). Xylanase activitiy was assayed by standard procedure of Mohiuddin (1992). The enzyme activity was calculated and expressed in International Unit (IU). One IU is the amount of enzyme which liberates 1 μmol of reducing sugar per minute under assay conditions. The enzyme activities were measured from average of

three replicates for each parameter. The relative percentage of xylanase activity was calculated by taking the maximum activity as 100%.

Results and Discussion

The optimum time required for xylanase production was 4 days and 7 days for *R. pusillus* BPJ-2 and *T. lanuginosus* BPJ-10 respectively. The maximum activities of xylanase for *T. lanuginosus* BPJ-10 was 1.49 IU/ml, whereas the maximum activities of xylanase for *R. pusillus* BPJ-2 was 0.085 IU/ml (Table 1). The activity of xylanase was reached at the peak on 7th day of incubation by *T. lanuginosus* BPJ-10 and on 4th day of incubation by *R. pusillus* BPJ-2. Then it declined sharply for both fungi (Fig.1). Akhtar *et al*. (2006) and Rashid *et al*. (2005) also reported that the optimum time required for the maximum production of xylanase was 7 days for *T. lanuginosus*.

Table 1. Effect of incubation period on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Incubation period (days)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
1th	1.6	0.085	0.2	0.004
2th	9.2	0.49	1.76	0.031
3th	18.6	0.992	3.2	0.057
4th	21.8	1.163	4.76	0.085
5th	24.2	1.29	2.56	0.046
6th	26.2	1.397	1.28	0.023
7th	28	1.49	0.76	0.014
8th	13.4	0.715	0.56	0.01
9th	11	0.587	0.28	0.005
10th	6.8	0.363	0.2	0.004

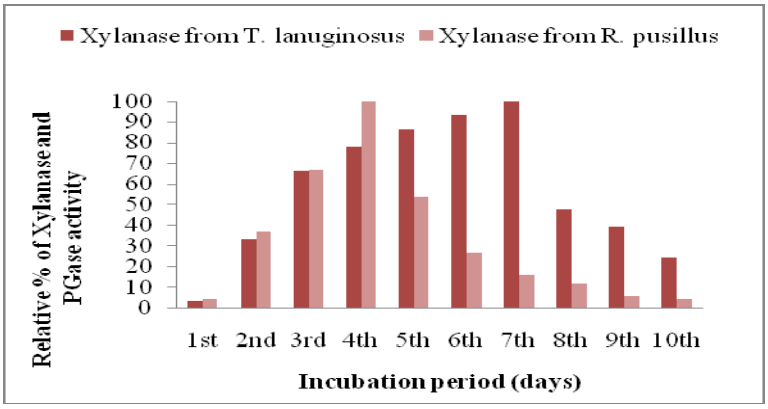

Fig.1. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different incubation periods.

Moisture content in solid substrate is an important controlling factor for enzyme production in solid state fermentation (Nagai and Nishio 1980). An increase in moisture content adversely affects the enzyme production. This is probably due to poor diffusion of oxygen and release of toxic metabolites. The higher percentage of moisture content might be required at high temperature. After 4 to 5 days of incubation moisture content decreased in the substrate due to utilization of moisture by fungi. The problem of drying out of the medium can be overcome by maintaining a constant relative humidity at 50-60% in the incubator. In case of *T. lanuginosus* BPJ-10, xylanase exhibited the maximum activity (1.013 IU/ml) at 90% moisture content (Table 2). On the other hand, Akhtar *et al*. (2006) observed that 80% moisture content was congenial to the growth of the fungus and xylanse production by *T. lanuginosus*. Another fungus *R. pusillus* BPJ-2 also exhibited xylanase activity (0.078 IU/ml) at 90% moisture content (Table 2). The relative percentage of xylanase activity was calculated by taking the maximum activity as 100%. It increased gradually from 50% to 90% moisture content and then started to drop the trend of increase for both fungi (Fig. 2).

Table 2. Effect of moisture content on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Different Moisture Content (%)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
50	1.4	0.074	1.28	0.023
60	12	0.64	2.24	0.040
70	15	0.8	3.12	0.055
80	16.8	0.896	3.72	0.066
90	19	1.013	5.52	0.078
100	15.8	0.843	2.56	0.045

Average of three replicates.

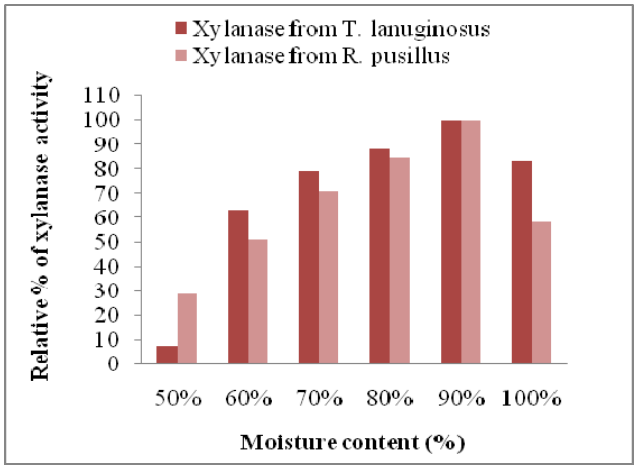

Fig. 2. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different moisture levels.

The fungi were grown at different temperature ranging from 40 to 60°C to find out the optimum temperature for growth and maximum enzyme production. The optimum temperature for the maximum production of xylanase (1.568 IU/ml) was 50°C for *T. lanuginosus* BPJ-10 and the optimum temperature for the maximum production of xylanase (0.089 IU/ml) was 45°C for *R. pusillus* BPJ-2 (Table 3). The relative percentage of xylanase activity was calculated by taking the maximum activity as 100% and it was exhibited at 50ºC and 45ºC temperature for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively (Fig. 3).

Table 3. Effect of different temperature on production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Temperature (ºC)	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
35	8	0.426	2.4	0.043
40	25	1.333	4.24	0.075
45	27	1.44	5.04	0.089
50	29.4	1.568	3.84	0.068
55	24	1.28	1.4	0.025
60	6	0.32	0.28	0.005

Average of three replicates.

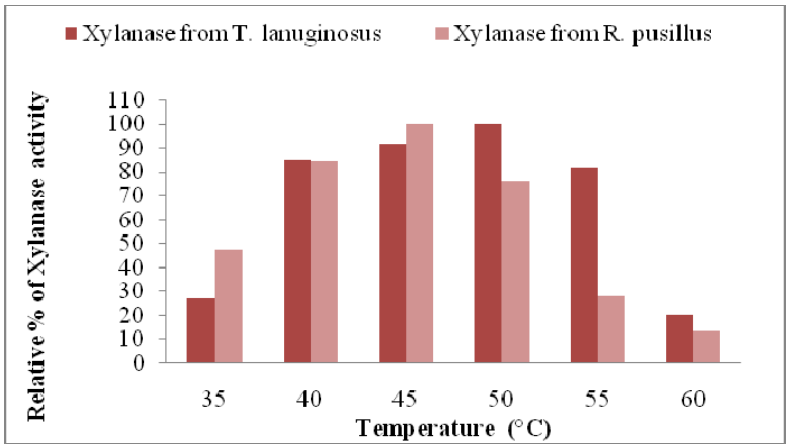

Fig. 3. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 in different temperatures.

Reports from many workers revealed that maximum thermophilic fungi and other microorganisms showed their higher performance between 50 to 55°C temperatures (Debsarma 1989, Bodine and Stutzenberger 1992 and Nowab 1992) which corroborates with the result of the present study. According to Akhtar *et al.* (2006) the optimum temperature found for the maximum production of xylanase by *T. lanuginosus* was 55°C.

Initial pH of culture medium played an important role on the production of enzyme. Thermophilic fungi grew satisfactorily in a minimal medium if the pH of the medium was controlled between 5.5 and 7.0 (Maheshwari *et al.*2000). *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 exhibited good performance in pH range of 4 to 8. However, the maximum activity of xylanase (1.685 IU/ml) was exhibited at pH 7.0 in case of *T. lanuginosus* BPJ-10 (Table 4). On the other hand, the maximum activity of xylanase (0.075 IU/ml) was exhibited at pH 4.0 in case of *R. pusillus* BPJ-2 (Table 4). The percentage of xylanase activity was calculated by taking the maximum activity as 100% and it was found at pH 7 and pH 4 for *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 respectively (Fig. 4). Akhtar *et al*. (2006) reported that the highest activity of xylanase from *T. lanuginosus* was at pH 6.5. According to Gomes *et al*. (1993) the required pH for the maximum production of xylanase by *T. lanuginosus* lies between 7 and 7.5 and Nawab (1992) found it between 6.0 and 7.25. The results of the present study agree well with those findings.

Table 4. Effect of different level of pH on the production of xylanase by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2.

Different level of pH	Name of the fungi			
	T. lanuginosus BPJ-10		*R. pusillus* BPJ-2	
	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)	Xylose conc. (mg/ml)	Xylanase activity (IU/ml)
pH 4	20.6	1.099	4.2	0.075
pH 5	24.2	1.290	3.64	0.069
pH 6	30	1.6	3.36	0.059
pH 7	31.6	1.685	1.88	0.033
pH 8	24.2	1.290	1.44	0.026
pH 10	18.4	0.981	0.56	0.001

Average of three replicates.

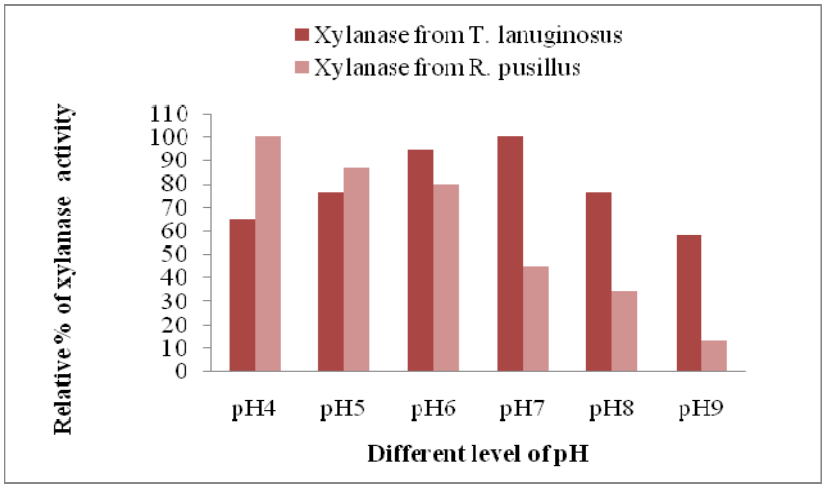

Fig.4. Relative % of xylanase activity by *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 at different pH.

It may be concluded from the present findings that the thermophilic fungi, *T. lanuginosus* BPJ-10 and *R. pusillus* BPJ-2 have potential capability of producing xylanase under the physiological conditions and the cultural environment described above. Though both fungi exhibited maximum activity of xylanase in the same level of moisture content (90%), their level of temperature and pH were different. Moreover, *R. pusillus* BPJ-2 exhibited maximum activity of xylanase on 4th day of incubation whereas it was exhibited by *T. lanuginosus* BPJ-10 on 7th day of incubation, which reveals that *R. pusillus* BPJ-2 is more economical than that of *T. lanuginosus* BPJ-10 in the respect of time duration. Besides these, *T. lanuginosus* BPJ-10 is more potential thermophilic fungi

than *R. pusillus* BPJ-2 with regard to xylanase producing capacity in solid state fermentation.

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PHYSICO-CHEMICAL CHARACTERIZATION OF SILT PREPARED FROM BIJOYPUR SOIL

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Abstract

Silt obtained from fractionated Bijoypur soil based on particle size (53~140μm) was characterized by SEM (Scanning electron microscopy), LIBS (Laser induced breakdown spectroscopy), XRD (X-Ray diffraction) and FT-IR (Fourier transform infra-red spectroscopy). pH_{zpc} (Zero point charge pH) of silt was also determined by titrimetric method. The scanning electron micrograph of silt was taken in two different magnifications. Micrographs show that the surface of silt is slightly homogenic in nature and the particle size varied between 50 and 100 μm. Elemental analysis of silt was performed by LIBS. According to this Fe, Si, Ti, Cu, Zn and Na are present in silt. XRD analysis indicates that silt fraction of Bijoypur soil is closely similar to kaolinite but it contains significant proportion of quartz. FT-IR analysis shows the presence of Zn=O, O-H, Al-O-Si, Fe-O, Al-OH and Si-O bonds. The pH_{zpc} value of silt was obtained as 6.39 ± 0.02 indicating neutrality of the surface.

Key words: Silt, Characterization, SEM, LIBS, FT-IR, XRD, pH_{zpc}

Introduction

Clay minerals are generally defined as very fine grained, natural, earthy material aggregates consisting essentially of the hydrous silicate of alumina. This hydrous silicate becomes hard when dried or fired. Sometime the aluminum silicate contains variable amounts of iron, magnesium, alkali metals, alkaline earths and other cations having structural similarity to the micas and form flat hexagonal sheets (Sjöberg *et al.*1990). Clay minerals can be fractionated to different categories (Day 1965, Gee and Bauder 1986 and Schmidt *et al.* 1999). Based on particle size, three different fractions as sand, silt and clay can be obtained from soil. Silt is soil or rock of a grain size between sand and clay. Silt may occur as a soil or as suspended sediment (also known as suspended load) in surface water. It may also exist as soil deposited at the bottom of a water body. Silt is created by a variety of physical processes capable of splitting the generally sand-sized quartz crystals of primary rocks by exploiting deficiencies in their lattice (Moss and Green 1975). These involve chemical weathering of rock and regolith, and a number of physical weathering processes such as frost shattering and haloclasty (Goudie and Viles 1995). The main process is abrasion through transport, including fluvial comminution, aeolian attrition and glacial grinding (Wright *et al.* 1998). Mineralogically, silt is

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composed mainly of quartz and feldspar. Sedimentary rock composed mainly of silt is known as siltstone. Silt is easily transported in water or other liquids and is fine enough to be carried long distances by air as 'dust'. Silt and clay contribute to turbidity in water. Silt is transported by streams or by water currents in the ocean. Silt deposited by annual floods along the Nile River, created the rich and fertile soil that sustained the Ancient Egyptian civilization. Silt obtained through the fractionation of soil can be characterized by several analytical techniques e.g. elemental analysis, surface charge / surface pH, phase, molecular analysis.

The objective of the present investigation was to characterize the surface of silt by SEM and pH_{zpc}, to analyze different elements present by LIBS, molecular bonding analysis by FT-IR and phase analysis by XRD.

Materials and Methods

Bijoypur soil was characterized by Miran *et al.* (2008); investigation was carried out for soil sample dried at 120ºC and calcined at 500ºC and characterized by FT-IR, TGA/DTA and XRD. Bijoypur clay fraction was characterized by Zaker *et al.* (2013); by SEM, LIBS, FT-IR, XRD and pH_{zpc}.

Silt was prepared from the fractionation of Bijoypur (Netrokona) soil. Different methods are available for the fractionation of soil samples (Day 1965; Gee and Bauder 1986; Schmidt *et al.* 1999). But in the present investigation, Hydrometer method (Day 1965) was used.

A small portion of fractionated silt was separately taken in a Scanning Electron Microscope (SEM) sample holder and made it platinum coated using a Pt-coated auto system (JFC-1600, JEOL, Japan). Platinum coated clay was placed in the SEM sample chamber and SEM picture was taken at 20 kV with 2000 and 30,000 magnification and presented in Fig.1.

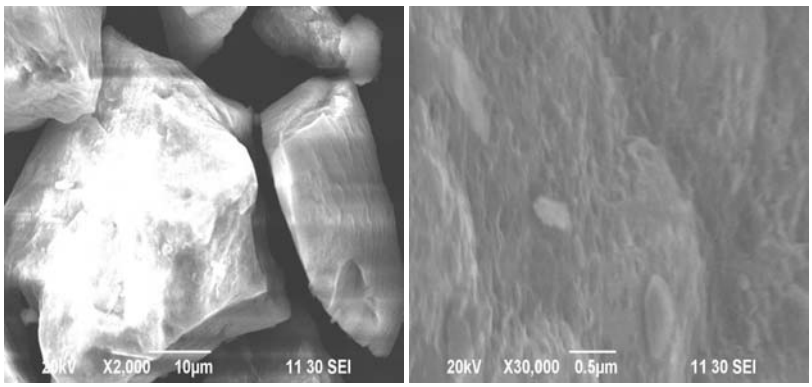

Fig. 1. SEM micrograph of silt with 2000 and 30,000 magnifications.

The prepared silt was air dried for several days. Then these were crushed and ground for making powder. The powder samples were then reserved in plastic jar. For LIBS technique, about 0.5-1.0 g silt was mixed with 1-2 drops of glue and small pellet was made. This was air dried before use. LIBS spectroscopy can be produced from high intensity laser pulse interacting with the sample producing a plasma plume that evolves with time from the point of impact of the incident laser pulse. The laser pulse usually lasts for 5 to 20 ns. LIBS spectra were taken in the spectral range of 200-900 nm for silt fraction using two gratings, One of them was 2400 ruling/mm grating blazed at 240 nm (for 200-350 nm) and the other was a grating with 600 ruling/ mm blazed at 500 nm (for 350-900 nm). The characteristic emission lines with respect to NIST atomic spectral reference data-2010 for corresponding elements with oxidation states are presented in Table 1.

Table 1. Characteristic emission lines of elements present in silt.

No	Element	Charge state*	Characteristic emission lines (wavelength, nm) for identification of the elements
1	Si	I	212.357, 221.062, 221.643, 251.437, 288.170, 298.732
2	Ti	I	263.108, 295.580, 390.387, 429.946, 453.324
		II	308.780, 316.242, 316.842, 323.409, 323.614, 326.121, 334.817, 337.165, 338.261
3	Fe	I	251.612, 252.417, 294.747, 296.659, 297.286, 302.029, 438.249
		II	227.629, 234.318, 238.174, 273.949, 274.924, 275.571
4	Cu	II	766.340
5	Zn	II	467.586, 471.778, 480.597
6	Na	I	588.884, 589.487, 261.173

* I and II imply neutral and singly ionized states of the atoms respectively.

FT-IR spectra of silt were taken from the Centre of Excellence, University of Dhaka. This was carried out by Shimadzu FT-IR spectrometer (IR prestige 21) equipment using potassium bromide pellet. The pellet was prepared by mixing 1.0 mg of finely ground dry sample and 200.0 mg of spectroscopic grade dry KBr. The mixture was ground thoroughly in an agate mortar and pressed between a pair of special dice under a pressure of 8-9 tons using hydraulic press for 5 minutes connected with vacuum pump for removal of CO_2. The spectra were recorded between 500- 4000 cm^{-1} with 2 cm^{-1} resolution. Usually 10 scans were recorded. FT-IR spectrum is presented in Fig. 2 and spectral data of silt were compared with standard data for different bonds with different vibrational modes as shown in Table 2.

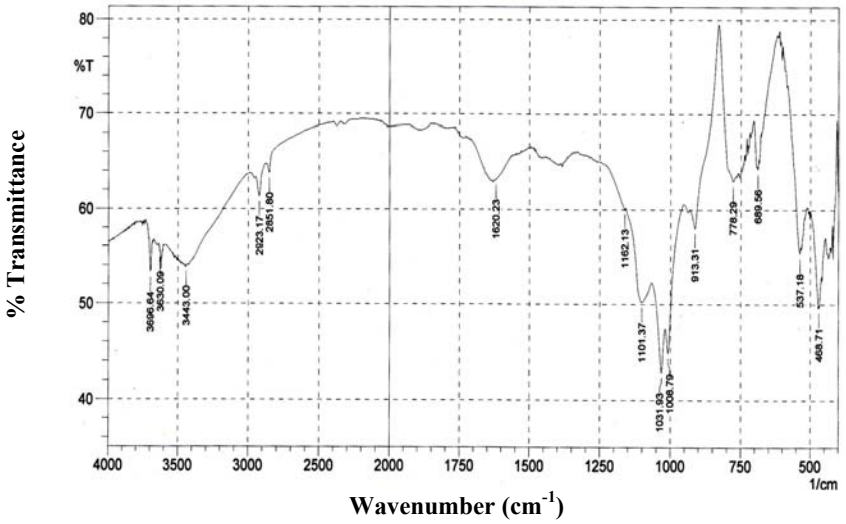

Fig. 2. FT-IR spectrum of Bijoypur silt fraction.

Table 2. FT-IR analysis for Bijoypur silt fraction.

Functional group	standard	Silt	Comment
O-H (stretching vibration)	3710-3200	3696, 3630, 3443	O-H present
Zn=O (plane bending)	1650-1350	1620	Zn=O may present
Si-O (plane bending)	1150-900	1101, 1031, 1008, 913	Si-O present
Al-OH (bending)	900-600	778, 689	Al-OH present
Fe-O (bending)	460-490	468	Fe-O may present
Al-O-Si (skeletal vibration)	550-450	537, 468	Al-O-Si may present

X-ray powder pattern of silt sample was recorded with a Philips PW-1380 X-ray generator operating at 40 kV and 30 mA and an XDC-700 Guinier- Hägg focusing camera using Cuk$_{\alpha 1}$ radiation. An exposed time of 1 hour was used and the film was processed using commercially available developer and fixer. The d-values of the diffraction lines in the films were calculated manually and presented in Table 3.

Table 3. X-ray data of silt along with the data from JCPDS for kaolinite, quartz, chlorite, Illite and clay fraction of Bijoypur soil.

d-values of silt fractionated from Bijoypur soil, A^o	d-values of clay fractionated from Bijoypur soil (Zaker *et al.* 2013) A^o	d-values of Kaolinite A^o	d-values of Quartz A^o	d-values of Chlorite A^o	d-values of Illite A^o
4.25	7.08	7.1	4.32	7.70	10.0
3.35	4.25	4.41	3.38	4.78	5.02
2.47	3.35	3.56	2.50	4.44	4.48
2.28	2.58	2.55	2.30	3.50	4.44
2.23	2.43	2.49	2.16	2.56	3.46
2.13	2.28	2.43	2.01	2.50	3.34
1.98	2.11	2.38	1.84	2.34	3.20
1.82	1.82	2.33	1.70	1.98	2.99
1.68		2.20	1.57	1.66	2.56
1.55		1.98			2.00
1.45		1.79			1.49
1.39		1.67			
1.38		1.66			
1.29		1.54			
1.26		1.49			

The pH of silt surface at zero point charge (pH_{zpc}) was determined by the method suggested by titrimetric process (Huang and Ostovic 1989). 0.1 g of silt was added to four identical portions of 40 mL 0.1 M NaCl solution of pH 7. The mixtures were agitated for 24 hours in thermo mechanical shaker (SWB-20, HAAKE, Fisons Ltd, Germany). Two bottles of suspension were titrated; One with 0.05 M HCl and another with 0.05 M NaOH using micro burette (± 0.01 mL). During the titration, the constant pH reading was taken carefully. From other two bottles, the supernatants after filtration were titrated similarly; one with 0.05 M HCl and another with 0.05 M NaOH as described above. From above titration four curves were obtained for the plot of pH versus volume of acid or alkali. Using these plots in a same scaled single graph differences in volume (ΔV) at constant pH for suspension and supernatants were estimated. Using these data, ΔV versus pH plot was obtained for 0.1 M NaCl. Similarly another plot was obtained for 0.01 M NaCl solution. These two plots intersect at a point defined as surface zero point charge pH (pH_{zpc}) of silt (Fig. 3).

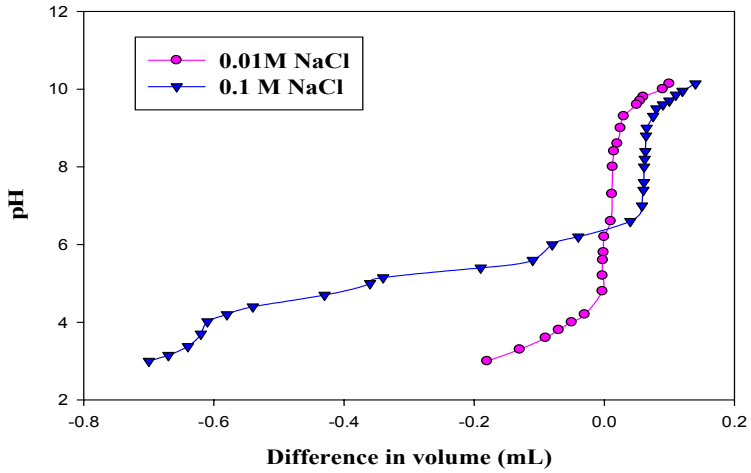

Fig. 3. Net titration curves for silt in presence of different concentrations of NaCl. The curves intersect at the pH_{zpc}.

Results and Discussion

Silt particles fractionated from Bijoypur soil presented in Fig.1 show the SEM micrograph of silt by two different magnifications such as 2,000 and 30,000. It indicates that the fractionated silt's particle size and shapes are not uniform. There were many pores and cavities in surface which make the surface to act as a moderate adsorbent (Gurses *et al.* 2006). SEM micrographs of clay fraction of Bijoypur soil (Zaker *et al.* 2013) also indicated strong adsorbent properties in surface. In comparison to clay, silt has fewer pores and cavities on surfaces. Particle size of silt varied between 50 to 100 µm which is higher than the particle size of clay.

LIBS were used to analyze the presence of element in the silt. Table 1 shows the list of elements present in the silt sample (NIST Atomic Spectra Ref. Data 2010). Comparing with LIBS spectra of clay fraction of Bijoypur soil, it was observed that there were lower number of emission lines for identification of elements detected and lines for Sn and Co are absent here (Zaker *et al*. 2013).

Different vibrational modes of bonding of silt were investigated using FT-IR spectroscopy. Fig. 2 shows the FT-IR spectrum of fractionated silt. Respective vibrational mode for different functional groups analyzed with standard FT-IR data (Xu and Axe 2005 and Ozcan and Ozcan 2004) is presented in Table 2. FT-IR analysis of silt and clay of Bijoypur soil when compared with that of Zaker *et al.* (2013) no differences were found.

X-Ray powder diffraction (XRD) method was also used to compare the silt with several clay minerals such as kaolinite, illite, quartz and chlorite. The d- values of XRD pattern of silt were estimated and compared with standard values of clay minerals supplied by JCPDS (Joint Committee on Powder Diffraction Standards). The values are shown in Table 3. Comparing the d-values, it can be concluded that the silt is mainly quartz, (Silicon oxide, SiO_2) containing small amount of Kaolinite type of mineral (Aluminum silicate hydroxide, $Al_2Si_2O_5(OH)_4$, trace amount of Chlorite, (Sodium Aluminum Silicate Hydroxide Hydrate, $Na_{0.5}Al_6(Si, Al)_8O_{20}(OH)_{10}.H_2O$) and Illite, (Potassium Aluminum Silicate Hydroxide, $(K, H_3O)Al_2Si_3AlO_{10}(OH)_2$. d-values of fractionated silt when compared with fractionated clay, it was observed that a large number of d-values are obtained in case of silt. Quantitatively the values are not same as d-values of clay (Zaker *et al.* 2013).

The pH of the zero point charge is the pH of the solution at which the charges on the surface (-ve or +ve) are balanced by the addition of acid or alkali. In Fig. 3 the point of intersection of two curves is the pH_{zpc} of silt and it was estimated as 6.39 ± 0.02. The value is almost equal to the pH_{zpc} of clay (6.40±0.02). The pH_{zpc} of silt indicated that the surface of silt acts as a positive charge at 6.39±0.02 and below, whereas negative charge at pH is higher than 6.39±0.02. These results indicate that surface of silt fraction is almost neutral suggesting that the cationic adsorbate would favor basic medium and anionic adsorbate would favor acidic medium.

It can be concluded that the particle size and shapes of silt are not uniform and the sizes are in between 50 to 100 µm. Many metals bonded with oxygen are present in silt. XRD lines and d-values indicate that silt fraction resembles more to quartz than kaolinite.

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BIOLOGY OF THE ANGOUMOIS GRAIN MOTH, SITOTROGA CEREALELLA (Oliver) ON STORED RICE GRAIN IN LABORATORY CONDITION

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Abstract

The experiment was conducted in the laboratory of the Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka during the period from May 2009 to April 2010 to study the biology of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) in Bangladesh. The ovipositional period, incubation period, larval period, pre-pupal period and pupal period of Angoumois grain moth were 3.67 days, 5.5 days, 25.2 days, 3.0 days and 5.0 days, respectively; male and female longevity of moth were 8.0 and10 days, respectively. The lengths of all five larval instars were 1.0 ± 0.00, 2.0 ± 0.02, 4.0 ± 0.06, 5.0 ± 0.03 and 4.0 ± 0.06 mm, and the widths were 0.10 ± 0.0, 0.4 ± 0.0, 0.6 ± 0.01, 0.8 ± 0.02 and 1.0 ± 0.09 mm, respectively. The length and width of the pre-pupa and the pupa were 4.0 ± 0.02, 3.5 ± 0.01 mm and 1.20 ± 0.05, 1.50 ± 0.03 mm respectively. The length of male and female was 11.2 ± 0.09 and 12.07 ± 0.06 mm respectively.

Key words: Biology, Angoumois grain moth, *Sitotroga cerealella*, Stored rice grain

Introduction

Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae) is a primary colonizer of stored grain in subtropical and warm temperate regions of the world. Rice is the most important cereal crop and staple food in Bangladesh. The demand for rice is constantly rising in Bangladesh with nearly 2.3 million people being added each year to her population of about 120 million (Anon. 2001). About 90% of the population of Bangladesh depends on rice for their major food intake (Anon. 1981). The farmers store more than 65% of the total produced rice till the next season for their food, feed and seed purposes. Insect pests damage different types of stored grains including rice causing serious loss to national economy. Among them Angoumois grain moth is one of the most serious pests of stored rice (unhusked) at post harvest level. At the time of harvest the panicle shows no sign of infestation usually, and the first adults emerge some weeks later in storage. A large quantity of unhusked rice is stored at farmer's level which is badly damaged by *S. cerealella*, which is an extremely efficient seed penetrator (Cogburn 1975). Angoumois grain moth, *S. cerealella* also known as the rice moth or paddy moth is one of the most dominant species in the stored paddy (Prakash *et al.* 1984). In Bangladesh it is locally known as "surui poka". In the bag stored with the paddy, it appeared to be the major and number one pest. It not only infests the grains in storage,

but also in field conditions, which enhances its ability to damage (Douglus 1941). The newly hatched caterpillar bores directly into the grain and typically remains inside the grain for both larval and pupal development. The larvae of this pest tunnel inside the kernels are causing substantial damage and are rendering the grain more susceptible to secondary insect pests (Weston and Rattlingourd 2000). Before pupation the larva constructs a chamber just under the grain seed coat, forming a small circular translucent window. Pupation takes places within the chamber inside a delicate cocoon. Adults fly well and cross-infestation occurs readily, but they are short-lived and generally survive only for 5-12 days, and in suitable stores breeding may be continuous throughout the year (Hill 1990).

Materials and Methods

The study was conducted in the laboratory of the Department of Entomology, Sher-e-Bangla Agricultural University, Dhaka during the period from May 2009 to April 2010. Parboiled rice variety BR-11, collected from farm store house of Sher-e-Bangla Agricultural University, was used for this investigation. Male and female adult moths were also collected from farm store house of the same University. Male and female adult moths were sorted out under a simple microscope by observing their abdominal tergites. In males, the abdomen is thinner, pointed and blackish when viewed from the ventral side whereas in females, the abdomen is bulky and long without any blackish coloration and size of the body (male is smaller than female). The comparative biology of *Sitotroga cerealella* was studied on rice grains in laboratory conditions by maintaining them at room temperature. The collected moths were enclosed in plastic jars (measuring 25×30 cm) for mating and oviposition.

Collection of the eggs of *S. cerealella*: For the collection of fresh egg mass, the rearing of *S. cerealella* was done by using wheat grain (only for rearing purposes) as a diet in a special mass rearing chamber. From *S. cerealella* mass rearing chamber, thousands of adults were collected and kept them in a glass cylinder. The top of the cylinder was covered by 32 mesh net .Adults were kept in a cylinder for one day for mating and subsequent egg laying on the glass cylinder. In the consecutive days the eggs laid on the wall of the cylinder were brushed and sieved to collect fresh eggs along with body parts of moth. Then the body parts of moth were cleaned and fresh eggs were obtained. The collected eggs of *S. cerealella* were kept into a glass tube with labeling and stored in a refrigerator at 4^0C temperature to ensure continuous supply for future study.

Biology of *S. cerealella*: The eggs of *S. cerealella* were laid on pieces of white paper placed on Petridish for hatching. The newly hatched larvae were transferred in Petri dishes with grains. The morphological characteristics of the larvae and pupae were studied and recorded during the period of larval and pupal development respectively.

Different growth and development stages of *S. cerealella*, such as incubation period, larval period, pupal period and adult longevity were studied during the experimental period. The incubation period was assessed based on the time the egg was laid and the time the larva was emerged from a particular egg. Larval and pupal periods were recorded by cutting infested grains with the help of a blade and observed under the microscope. The emerging adults of *S. cerealella* were kept in the glass tube until death and the adult longevity was recorded.

Length and width: The length and width of different stages of the insect were observed under a sterio- microscope and measured with the help of slide calipers.

Results and Discussion

Mating and Oviposition: The Angoumois grain moth , *S. cerealella* adults used to start their mating after passing 24 hrs of emergence. On rice grains, a single female moth laid eggs from 42 to 213 with an average of 109 eggs throughout its life (Table1). The eggs were laid singly or in groups of 4-7 depending upon the season and ovipositional site. Fletcher and Ghosh (1919) observed that a female laid 120-350 eggs on paddy grains and other cereals and also on depressions, cracks, crevices and holes of storage structures and godowns. Dhotmal and Dumbre (1982) reported that from 41 to 58 eggs were laid by a female on different rice varieties in a laboratory test and found that fine grain varieties were preferred for egg laying. On an average, oviposition period was found to be 3.67 ± 0.01 days (Table 2). Newly hatched egg was white in color, but gradually changed to

Table 1. Number of eggs of *Sitotroga cerealella* laid at different days on rice grains in the laboratory condition (temperature 27^0 C and 70 - 72 % RH).

Insect	Number of eggs laid				Total number of eggs laid
	1st day	2nd day	3rd day	4th day	
1st pair	150	50	10	0	210
2nd pair	28	56	22	07	113
3rd pair	12	23	07	0	42
4th pair	34	37	22	02	96
5th pair	37	57	16	10	120
6th pair	30	28	15	06	79
7th pair	26	17	0	0	43
8th pair	37	23	08	0	68
9th pair	43	35	17	11	106
10th pair	143	57	10	03	213
				Mean = 109 ± 57.23	

reddish brown with age. It measured about 0.5 mm in diameter. The egg was oval shaped and hatched within a week. An average incubation period was 5.5 ± 0.03 days (Table 2), but in summer season incubation period was from 2 to 3 days and in winter season it ranged from 5 to more (overall, incubation period depends on temperature and relative humidity). Hatching was reported to take 11 days at 17.3^{0}C temperature and 68.3% R.H. (Germanov 1982). Unmated females have also been reported to lay eggs within a day of emergence (Ayertey 1975). Prakash *et al*. (1981) reported that for egg lying female prefers a rough surface than a smooth one in stored rice.

Table 2. Developmental period of different life stages of *Sitotroga cerealella* on rice grain in the laboratory.

Development stage	Duration (days)	Statistics
Oviposition	3.67 ± 0.01	P<0.002
Incubation	5.5 ± 0.03	P<0.004
Larval Period		
1st Instar	3.2 ± 0.09	P<0.000
2nd Instar	4.0 ± 0.11	P<0.012
3rd Instar	10.0 ± 0.23	P<0.004
4th Instar	6.0 ± 0.07	P<0.031
5th Instar	3 .0 ± 0.03	P<0.001
Pre-pupal	3 .0 ± 0.05	P<0.098
Pupal	5 .0 ± 0.08	P<0.019
Adult Longevity		
Male	8.0 ± 0.13	P<0.000
Female	10.0 ± 0.32	P<0.005

Larval period: The larvae developed through five instars. The newly hatched larvae of all instars were yellowish white in colour with light brown head. The stadia of the 1st, 2nd, 3rd, 4th and 5th instar larvae were 3.2 ± 0.09, 4.0 ± 0.11, 10.0 ± 0.23, 6.0 ± 0.07 and 3.0 ± 0.03 days, respectively (Table 2). The lengths of all five larval instars were 1.0 ± 0.00, 2.0 ± 0.02, 4.0 ± 0.06, 5.0 ± 0.03 and 4.0 ± 0.06 mm, respectively and the widths were 0.1 ± 0.0, 0.4 ± 0.0, 0.6 ± 0.01, 0.8 ± 0.02 and 1.0 ± 0.09 mm, respectively (Table 3).

The tiny larva lives inside a grain. It crawls around for sometimes and soon finds a comparatively weaker spot or a crack or split in the husk through which it enters the grain. Larval migration is reported as being up to 10 cm horizontally and 5 cm vertically (Germanov 1982). After entering the grain, the larva often turns and practically closes the entry hole with a silken web. The larval life then begins in an environment of plenty of food and safety and continues in that state till it is fully grown to about 5 mm within two

or three weeks. At this time the grain is practically hollow filled with faeces and other refuge. The larva then cuts out a circular exit hole leaving over it just a sort of cap (Anon. 2001). Germanov (1982) described four larval stages during his studies under conditions of mass rearing and reported that larval stages I, II, III and IV were observed on the 9th, 12th, 18th and 20th days respectively after grain infestation at 22.3^0 C temperature and 65.8% RH. Duration of larva was found to be from 13.66 to 19.33 days in different rice varieties and development was faster in fine-grained varieties (Dhotmal and Dumbre 1982). Full-grown larvae spin silken cocoons around them in hollows in the grain and become inactive for two days before pupation (Crombie 1943).

Table 3. Morphometirics of different life stages of *Sitotroga cerealella* on rice grain in the laboratory.

Life Stage	Size (mm)	
	Length	Width
Egg	0.8 ± 0.00	0.5 mm in diameter
Larval Instars		
1st Instar	1.0 ± 0.00	0.10 ± 0.00
2nd Instar	2.0 ± 0.02	0.40 ± 0.00
3rd Instar	4.0 ± 0.07	0.60 ± 0.01
4th Instar	5.0 ± 0.03	0.80 ± 0.02
5th Instar	4.0 ± 0.06	1.00 ± 0.09
Pre-pupa	4.0 ± 0.02	1.20 ± 0.05
Pupa	3.5 ± 0.01	1.50 ± 0.03
Adult wing span		
Male	11.2± 0.09	
Female	12.7± 0.06	

Pupal period: Pupa is brown coloured, develops inside silken cocoon. Total 3.0 ± 0.05 days were required for pre-pupal stage and 5 ± 0.08 days for pupal stage under the laboratory condition (Table 2). The pre-pupa measured 4.0 ± 0.02 mm in length and 1.20 ± 0.05 mm width and the pupa measured 3.5 ± 0.01 mm in length and 1.50 ± 0.03 mm width in rice grain (Table 3). Generally, pupal period is 4-7days (Crombie 1943). The moth, on emergence, pushes off the cap on the circular exit hole. Germanov (1982) reported pupation on the 15th day after infestation at 22.3^{0}C temperature and 68.8% relative humidity.

Adult Period: The adult was a good filter, gray or buff coloured moth, usually nocturnal in habit. The longevity of females was more than that of males. The adult longevity was 8 ± 0.13 days for male and 10 ± 0.32 days for female (Table 2). The length of male was 11.2 ± 0.09 mm and 12.07 ± 0.06 mm for female in rice grain (Table 3). From Table 4, we observed that moth development stage duration also varied in different temperatures. The highest developmental period (25.05 days) of *S. cerealella* was found from the lowest temperature 20^{0}C which was statistically different from other different temperatures and closely followed by the temperature 24^{0}C (22.60 days). The lowest

developmental period (17.42 days) of *S. cerealella* was found from 32^{0}C temperature. With the increase of temperature moth development stage duration also decreased significantly (Table 4). According to Hill (1990), total larval development of *S. cerealella*

Table 4. Development of *Sitotroga cerealella* at different constant and cycling temperatures at 50-65% relative humidity.

Temperature (^{0}C)	Number observed	Mean developmental period (Days±SE)
20	58	25.05 ± 0.21^a
24	68	22.60 ± 0.21^b
28	67	20. 93 ± 0.17^c
32	55	17.42 ± 0.23^d
*20/32	56	19.29 ± 0.26^e

Means of developmental period followed by the same letter are not significantly different (Duncan's Multiple Range test at 5% level); * Estimated from cycling temperature data.

can be completed by 19 days at 30^{o}C and 80 % relative humidity. Temperature limits for the development are 16^{o}C and 35^{o}C and humidity between 50 -90 % which seem to have little effect on the rate of development. Grewal and Atwal (1967) concluded that 25 – 30^{o}C and 80% RH are most favourable for development, survival and reproduction of the Indian strain of *S. cerealella*. The highest population increase of *S. cerealella* occurred at 30^{o}C. High relative humidity and temperatures higher than 30^{o}C are not suitable for development of this pest (Hansen *et al.* 2004). The wing span was 10-15 mm, body length 5-10 mm with grayish/yellowish, darker spots on forewings. The apex of hind wings was fringed with hairs, which was sharply pointed towards the tips and widely separated so that abdomen is partially visible. Adults mate 24 hrs after emergence. The shape of their abdomen distinguished male and female. In males, the abdomen was thinner, pointed and blackish when viewed from the ventral side whereas in females the abdomen was bulky and long without any blackish colouration.

However, The Angoumois grain moth, *Sitotroga cerealella* is one of the most serious pests of stored rice (unhusked) at post harvest level. The moth develops through egg, five larval instars, pupa, pre-pupa and adult stages.

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STATUS AND PROSPECTS OF AQUACULTURE PRACTICES IN BARURA UPAZILA, COMILLA, BANGLADESH

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Abstract

The present study was carried out in Barura upazila under Comilla district to assess the aquacultures status in 2008 and its future prospects. The upazila has no river but one Jolmohal (Carzon khal) occupying 700 ha, 7494 deghi and pukur (1836.77 ha), 6 khal (490 ha), 625 commercial and khas pukur (428.07 ha), 56 fish farms (92.57 ha) and paddy-cum fish culture (777.62 ha) which altogether formed vast fishery resources. In addition, 16 hatcheries with production capacity of 5350 kg and 56 nurseries with production capacity of 271.15 metric ton support necessary stocking fries and fingerlings for the above resources. In total 35% people are involved with aquaculture in the upazila. Thai Pangus (*Pangasiadon hypopthalmus*) and carp (rui, katla, mrigal etc.) are dominated cultured species in the study area. Semi-intensive culture systems are mostly used in this area**.** The upazila fulfills its own demand and the surplus fishes are sold to the neighboring districts. Only the Thai Koi (*Anabas testudineus)* was found to be trading overseas.

Keywords: Barura Upazila, Aquaculture resources, Production, Future prospects

Introduction

Inland waters of Bangladesh is blessed with vast water area in the form of ponds, canals, ditches, flood plain, haors (natural depression), baors (ox-bow lake), rivers, estuaries etc. covering an area of 5.31 million ha in which only ponds and ditches occupy an area of 2.42 lac ha. Fish production from this water body during the year 2009-2010 was 23.8 lac MT whereas, the total country fish production in the same year was 29 lac MT. This is 82.16% of the total fish production (DoF 2011). Only 7.71% ponds all over the country are used for commercial venture and the rest are used for non-commercial practices. Whereas, the production rate from this sector can be raised many folds through proper pond management techniques using the existing carrying capacity of different types of ponds in relation to species stocked and selection of species. In this respect, Barura upazila of Comilla district with an area of 241.69 sq. km. may be a model for such study. The present study was aimed to assess the present status and probable scope to enhance the production of captive fishing and aquaculture at Barura upazila, Comilla.

Materials and Methods

Barura upazila located under the Comilla district in Bangladesh lies between $90^0.57^{'}$E and $91^0.08^{'}$ E longitude and $23^0.13^{'}$ N and $23^0.27^{'}$ N latitude. Fifteen unions of the upazila

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are potentially rich for aquaculture due to presence of 16 hatchery, 56 nursery and 7494 ponds. Among these aquaculture resources, union like Uttar Shilmuri, Dakshin Shilmuri, Bobhanipur, Choddhaia and Addra usually practiced traditional culture technique and thus production is comparatively poor. On the other hand, rest of the unions practice semi-intensive and improved traditional system and therefore getting higher production, stimulating more and more people and as such most of the hatchery, nursery and production ponds are located in these areas.

The survey was conducted over a period of one year from January to December 2010, based on both primary and secondary data collected from various sources (field observation, questionnaires, key informants, journal, and document and report book). Data were collected using questionnaires and Participatory Rapid Appraisal (PRA) tools including Focus Group Discussion (FGD) with the fishermen and the womenfolk in each union. Questionnaire survey and key informants (KI) interviews were conducted at different levels of fisherman, administrative and sectoral officials namely Upazila Nirbahi Officer (UNO), Upazila Fisheries Officer (UFO) and Upazila Agricultural Officer (UAO). Secondary data such as topographic map, land use data, climate, etc. were collected from annual reports, documentary of relevant agencies, official books, journals and local people.

Results and Discussion

Production pond: In Barura upazila there are about 7494 ponds occupying a total area of 1836.77 ha and about 484 paddy-cum fish culture farms occupying area of 777.62 ha. The production capacity is about 20 MT per ha. In spite of that only 7.71% ponds are used for commercial purpose while, large number of commercial fishermen have to depend on lease for fish culture (Table 1).

Hatcheries: Hatchery plays an important role in aquaculture production. In the study area there are two Thai koi *(A. testudineus)* hatcheries, one shing (*H. fossilis)*, one magur (*C. batrachus)*, one monosex tilapia (*O. niloticus*) and eleven carp hatcheries. All of these hatcheries are private owned and the owners usually prefer locally available PG hormone for induced breeding. The production capacity of those hatcheries is 5350 kg/year and that can fulfill the local demands of fry and the excess sells to neighboring upazila. However, six unions are well known for hatchery activities though maximum fry production comes from the Jalam, Phoroshaba and Uttar Khosbas unions and altogether fulfill the demand of the upazila (Table 2).

Table 1. Different phases of aquaculture activities at union level.

Union	No. of commercial ponds	Area of commercial ponds (ha)	No. of non-commercial ponds	Area of non-commercial ponds (ha)	No. of professional fisherman	Farmers involved in paddy cum fish culture	Area of paddy cum fish culture (ha)	No. of total ponds	Area of total ponds (ha)
Adda	50	55.3	445	65.16	87	40	51.78	495	120.46
Addra	48	49.48	456	70.73	66	41	53.7	504	120.21
Aganagor	21	73.87	486	46.2	46	41	48.68	507	120.07
Babhanipur	27	25.5	444	86.7	36	24	34.86	471	112.2
Choddhaia	28	46.9	326	39.17	38	11	24.52	354	86.07
Dakshin Khoshbas	38	39.72	447	75.88	51	32	40.37	485	115.6
Dakshin Silmuri	11	13.95	449	86.75	15	10	19.58	460	100.7
Dewra	41	48.25	421	61.76	50	26	35.12	462	110.01
Galimpur	78	136.21	631	33.4	91	38	54.33	709	169.61
Jalam	48	117.06	443	27.14	59	28	46.4	491	144.2
Lakhipur	39	63.5	433	47.94	44	28	55.5	472	111.44
Phoroshaba	37	73.87	556	66.24	75	41	48.68	593	140.11
Uttar Khoshbas	72	134.55	491	35.65	93	76	192.3	563	170.2
Uttar Pholilgacha	20	40.9	455	68.54	55	40	56.3	475	109.44
Uttar Shailmuri	20	37.3	423	68.15	22	8	15.5	443	105.45
Total	578	956.36	6906	879.41	828	484	777.62	7484	1835.77

Table 2. Hatchery distribution at union level.

Union	Species	Production capacity/year (kg)	Number of hatchery
Uttar khosbas	Kai (*A. testudineus)*	350	04
	Shing (*H. fossilis)*	350	
	Magur (*C. batrachus)*	300	
	Carp hatchery	750	
Phoroshaba	Kai (*A. testudineus)*	650	03
	Monosex tilapia	700	
	Carp hatchery	200	
Galimpur	Carp hatchery	400	02
Choddhaia	Carp hatchery	700	01
Jalam	Carp hatchery	450	05
Lakhipur	Carp hatchery	500	01
Total		5350	16

Nursery pond: In total, 56 nursery farms and 273 nursery ponds within 92.57 ha of water areas contribute about 271.65 metric ton. An average size of these nursery ponds was found to be 0.339 ha and with the average production capacity of 0.995 lac 21 day's nursing fry/year for sell. Market price of these fry varies as Rui (*Labeo rohita)* Tk.200-250/kg; cattla (C*atla catla)* Tk.150-200/kg; Tilapia (*Oreochromis niloticus*) Tk.100-150/kg and Thai koi *(A. testudineus)*, shing (*H. fossilis)*, magur (*C. batrachus)* fries vary from Tk.350-500/kg. Union level distribution of nursery pond and their production capacity are presented in Table 3.

Table 3. Distribution of nursery pond at union level.

Union	Number of ponds	Ponds area (ha)	Production/year (lac Mt)
Adda	2	0.70	2.50
Addra	27	8.42	21.50
Aganagar	3	0.81	5.00
Bhabanipur	23	10.70	23.00
Choddhaia	21	6.42	13.50
Dakshin Khosbas	7	2.00	8.50
Dakshin Shilmuri	8	3.70	8.65
Deora	37	13.45	41.50
Galimpur	23	8.12	22.00
Jalam	55	16.50	48.50
Lakhipur	13	4.50	15.00
Payalgachha	10	3.50	12.00
Phoroshaba	19	6.05	21.00
Uttar Khosbas	6	2.20	9.50
Uttar Shilmuri	19	5.50	19.00
Total	273	92.57	271.15

Culture species: However, various species of fish were available in the study area but, only few preferred for culture. Only carps were cultured in previous years but at present Thai koi (*A. testudineus)* and Thai pangus (*P. hypopthalmus*) are being cultured in sufficient quantities either as mono-or poly culture with other carps throughout the upazila (Table 4).

Culture system: Fish culture in Barura upazila are characterized by extensive (traditional), improved traditional (semi-intensive) and intensive system. In traditional method of fish culture, fishermen did not stock any fry rather depended on natural fry to entry through the opening section of the embankment along with the flood waters. Thus, the stocked fishes were not specifically selected, predators were not eliminated, ponds were not fertilized and even no supplementary feeds were supplied there in. As a result, the average harvest from these types of ponds was very low and about 10-15% compared to intensive system. Mean while, the fishermen improved the already used traditional culture system in the name of improving traditional (semi-intensive) system. In this

Table 4. Distribution of cultured fish in different union.

Scientific name	Local name	Pond/Paddy field	Culture Area
Anabas testudineus	Koi	Pond	Partial (Uttar & Dakshin Khosbas, Aganagar)
Anabas testudineus	Thai Koi	Pond	All upazila
Aristichthys nobilis	Bighead carp	Pond	Whole upazila
Barbodes gonionotus	Silver barb	Pond, Paddy Field	Partial (galimpur, lakhipur)
Cirrhinus mrigala	Mrigal	Pond	Partial (Jalam,Chidda)
Clarias batrachus	Magur	Pond	Partial (Uttar Khosbas, Aganagar)
Ctenopharyngodon idellus	Grass carp	Pond	Whole upazila
Cyprinus carpio	Common carp	Pond	Whole upazila
Heteropneustes fossilis	Singh	Pond	Partial (Payalgachha, Phoroshaba,Uttar Khosbas)
Hypophthalmichthys molitrix	Silver carp	Pond	Whole upazila
Labeo rohita	Rui	Pond, Paddy Field	Whole upazila
Oreochromis mossambicus	Tilapia	Pond, Paddy Field	Partial (Uttar & Dakshin Khosbas, Aganagar)
Oreochromis niloticus	Tilapia	Pond, Paddy Field	Whole upazila

system, all the ponds were repaired, stock fry along with natural predator and non predator stock, given some supplementary feeds (not regular), as well as in necessity water was changed from nearby natural sources. This approach has enhanced about 45-50% production than traditional culture system. In recent years further improvement in pond aquaculture has gained depending on previous self made technique by introducing scientific strategies of pond preparation (liming, repair dam), species selection, stocking density, feed application, fertilizers, water exchange and proper management. In this system, water supply is fully dependent on deep tube-well, healthy fry from hatchery and feed from market with the target of maximum production of fish than other two systems. Yearly production of fish, its demand, deficit and consequent supplies are presented in Fig. 1.

People involved in aquaculture: The population of Barura upazila is about 3,47,222 but only 30-35% people are involved in aquaculture. Uttar Khosbas, Adda, Jalam, Phoroshoba and Galimpur unions are especially dominated area where 35-45% people are involved in aquaculture because most of the hatchery, nursery and production ponds are located in those areas (Table 5). The production capacity is almost 40%.

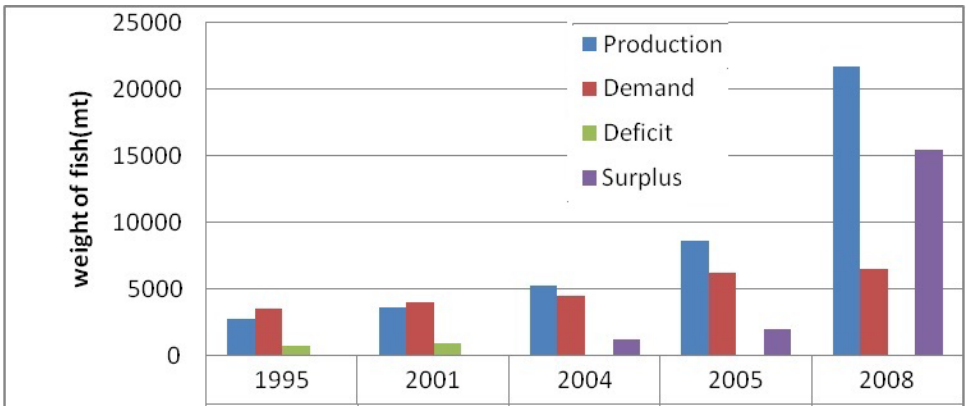

Fig. 1. Yearly production from 1995-2008.

Table 5. No. of people involved in different phases of aquaculture production at union level.

Union	Fish farmer	Hatchery owner	Nursery owner	Production pond owner	Paddy cum fish farmer	Fisherman (jellay)	Transporter	Stockiest	Trader	Seller	Labors	Total	Percentage (%)
Adda	780	-	1	87	40	1800	1225	437	630	675	2275	7950	7.5
Addra	650	-	4	66	41	1685	1150	528	476	650	2000	7250	6.9
Aganagar	480	-	1	46	41	1630	790	492	685	535	1650	6350	6.0
Bhabanipur	450	-	5	36	24	1625	735	315	775	540	1795	6300	6.0
Choddhaia	400	1	3	38	11	1605	720	400	737	550	1835	6300	6.0
Dakshin Khosbas	450	-	1	51	32	1580	900	453	553	480	2050	6550	6.0
Dakshin Shilmuri	310	-	2	22	8	1180	700	723	985	395	1275	5600	5.3
Deora	600	-	7	50	26	1650	1005	697	375	625	2015	7050	6.7
Galimpur	800	2	7	91	38	1750	1320	592	650	750	2600	8600	8.1
Jalam	680	5	8	59	28	1695	1145	480	650	635	1865	7250	6.9
Lakhipur	495	1	3	44	28	1550	885	537	487	595	1875	6500	6.2
Payalgachha	360	-	3	55	40	1670	1100	475	622	610	2115	7050	6.7
Phoroshaba	700	4	5	75	41	1850	1275	520	490	690	2350	8000	7.6
Uttar Khosbas	830	3	2	93	76	2100	1360	526	285	785	2850	8900	8.4
Uttar Shilmuri	375	-	4	15	10	1630	690	641	750	485	1450	6050	5.7
Total	8360	16	56	828	484	25000	15000	7816	9150	9000	30000	105700	100

Different project activities and credit: Various extension projects and fisheries training program organized by NGO's like Proshika, Jobo Unnyaon, Ansar VDP, BRDB, Babro, Obolombon always help the farmers to improve their culture technique. Besides, the Fisheries department offered financial support incurred from the UNDP, FAO and ADB. Similarly, various NGO's provide small loan along with training. These all made a better

link between the farmers and experienced personnel to enable the application of appropriate technology (Table 6).

Table 6. Development project activities in Barura upazila.

Donor	Project Title	Implementing Agency (IA)	Project Objectives
ADB	Integrated Fisheries activities project (1st & 2nd stage).	MOFL	To remove poverty
FAO	Fourth fisheries project activities:	MOFL	Establishment of an ideal fisheries village.
UNDP	Enterprises development project by National Packaging Activities:	DOF	Give loan and mixed carp culture training. Give loan for pangus culture training.
UNDP		DOF	Fish culture training by establishing village team.
UNDP	Various activities of fisheries training and extension project	DOF	Traditional fish culture training by local NGO in a village level.
UNDP		DOF	To teach the high school level student from class vi-x.
ADB		MOFL(UFO)	Fish culture activities in pond.
ADB		MOFL(UFO)	Fish culture activities in paddy land.
ADB	Fish culture extension project (stage-2) activities at upazila level.	MOFI(UFO)	Fry production and trained business man.
ADB		MOFL(UFO)	Fish culture extension activity in community based water body.
ADB		MOFL(UFO)	Develop Ideal Fishermen and Village Fish Culture extension association
SEAFDEC	Pilot project for semi-intensive culture system.	MOFL	Project operated by RIMP staff to make a model for semi-intensive culture of fish to increase production and thus profitability, and at the same time teach the fish farmers how to conserve the resources.

Credit for investment in aquaculture has traditionally come from non-institutional sources, most commonly from family members, village money lenders, fish brokers, fry/fingerlings suppliers and fish merchants. Pond owners frequently receive fingerling on credit from nursery operators where the cost of credit is high. In the usual banking system, there is also a provision for credit in aquaculture from different Bangladesh Bank nominated Government Banks like Sonali, Janata, Agrani, Rupali, Bangladesh Krishi Bank, etc. About 90% of the pond fish culture credit was granted by Bangladesh Krishi Bank with a recovery rate of 20% (Ahsanullah 1989) and this option usually goes to large-scale farmers. The main constrain of this loan to marginal farmers were the security

or guarantee ensuring and relatively higher administrative costs for smaller loans. Thus, supervised credit is at present the only available alternative to replace the traditional ways of providing security, namely collateral or mortgage (Bhuiyan and Chowdhury 1995). Multi-ownership is another problem for getting institutional credit in fish production. Bank officials are not familiar with all kinds of fisheries activities and not equally trained in identifying credit request. The weekly or fortnightly return of NGO's credit is another problem for collection of these loans from the fish production farms. Whereas, the ultimate goal of this production is to meet the protein demand, solve the employment problems and improve socio-economic condition of fish farmer's community.

In Barura both small scale and large scale aquaculture are being practiced but small scale culture has been getting more popularity day by day because of its low investment and high production rate. The success of such aquaculture largely depends on the extension activities provided by different NGO's and Government agencies. Major parts of these cultured fish production were found in market for domestic consumption while the rest (especially *Anabas testudineus*) for export. Identical to fish production, large number of people were also involved in the fish marketing channel beginning from the farmers then processors, traders, intermediaries, day laborers and transporters. Four categories of market trend were involved in the distribution of fish such as primary market, secondary market, higher secondary market and central fish market.

The techniques and strategies so far followed in the production of fish in all the union of the studied upazila need further upgradation in both above stated sides. In this case, the experiences from 'The National Fresh Water Aquaculture Plan' Bhubaneswar, India would be a guide line (Gopakumar *et al*. 1999). At the same time, several recommendations as suggested in several seminars and symposiums from the end of last century may propose afresh for the uplifting of fish production and other related activities of stated upazila.

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PESTICIDE RESIDUES IN SOME SELECTED POND WATER SAMPLES OF MEHERPUR REGION OF BANGLADESH

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Abstract

Water samples collected from some selected ponds of Meherpur region were studied for the presence of organophosphorus and carbamate pesticide residues. High performance liquid chromatography (HPLC) technique was used to determine the concentration levels of those residues. The results obtained indicate slight contamination of some of the water samples with residues of diazinon, chlorpyriphos (organophosphorus insecticide) and carbofuran (carbamate insecticide). The residues level of diazinon and chlorpyriphos ranged from 0.033 to 0.079 ppm and 0.010 to 0.471 ppm respectively. Among carbamate pesticides, carbofuran identified in two samples ranged from 0.0143 to 0.0387 ppm, and carbaryl was not detected in any of the samples. Limit of detection (LOD) was 0.01 ppm. However, the residue level was also within the acceptable ranges according to the WHO guideline value of water quality. But, the presence of such residues is indicative of weak regulatory control which is required to be in place for safety of the environment and to stop further aggravation of the situation.

Key words: Pesticide**,** Residue, Organophosphorus, Carbamate, Pond water

Introduction

The economy of Bangladesh is mainly based on agriculture. So, agriculture is her economic backbone with the production accounting for about one third of the gross domestic product. In Bangladesh, 40% of the crop loss can be attributed to attack by pests and insects which is a significant loss (Bagchi *et. al.* 2008). The widely cultivated high yielding variety is highly vulnerable to pests and diseases. So the use of pesticide is now an inherent part of agriculture for pest control. Thus, agrochemicals, including pesticides are considered a critical aid in improving agricultural production and the prevention of crop losses daring pre and post harvest (Rahman *et al.* 1995).

Due to use of pesticides for agriculture the pond water around the paddy fields may get contaminated. Although pesticide is beneficial for pest control but it also poses a harmful effect to our environment such as the pollution of pond water. After application of pesticide in the crop field the pesticide is degraded in the soil by the soil microorganisms in some extent but many of the toxic pesticide is transported into surface and ground water by agricultural run off rain water from the crop field. Ultimately, the surface and ground water might be highly contaminated due to this agricultural run off

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pesticide (Bagchi *et al*. 2008). Use of pesticides has to be controlled to avoid contamination of food supplies and ecological imbalance, but present measures taken in Bangladesh are inadequate and farmers rarely implement standards, allowing sale of unregistered pesticides and misuse (Matin *et al.* 1998).

Apart from being occupationally hazardous, indiscriminate use of pesticides in developing world is today posing a serious threat to human health. So, in this connection, monitoring pesticide residues is one of the most important aspects in minimizing potential hazards to human health (Bagchi *et al.* 2008). So, the levels of pesticide residues in various environmental samples should be monitored routinely and effective measures must be adopted to control the use of pesticides for saving the surface water as well as for minimizing human health hazards. Hence, the present study was undertaken to investigate the extent of contamination from pesticide residues (i.e. organophosphorus and carbamate) and their concentration levels in water samples of different locations and propose a sound recommendation for minimizing the pesticide contamination of pond water in those locations of Bangladesh.

Materials and Methods

Reagents: During this investigation analytical grade hexane (Merck, Germany); acetone (extra pure, BDH, England); anhydrous Sodium Sulphate (Merck,Germany); Florisil (Magnesium Silicate, Sigma,USA, mesh 60-100) and diethyl ether(analytical grade) were used.

Equipment: Rotary vacuum evaporator (Type -350, USA); High Performance Liquid Chromatograph (HPLC, Waters), Detector-Waters 486 and Pump- Waters 515 were used.

Operating condition of HPLC (UV mode): Detector:Ultra Violet Detector (Fixed wave length), Column: C_{18} , Flow rate: 0.5 mL /min, Mobile phase: Acetonitrile: Water (65: 35), Wave length : 254 nm, Injection volume: 20 µL.

Collection and Preservation of water samples: Water samples were collected from some selected ponds of Meherpur area of Bangladesh. Samples were then taken to the laboratory as quickly as possible in glass containers and kept in freezing condition until extraction to avoid degradation.

Extraction of Water: Extraction of water sample (500 mL each) was performed with 100 mL double distilled hexane in a separatory funnel with shaking for 5 minutes. Hexane extract was separated and collected in evaporating flasks. Two further extractions with 25-mL hexane were done. The combined hexane extract was treated with 5-g anhydrous sodium sulphate to remove traces of water. The water free extract was evaporated to a small volume (Approx. 1 mL) and transferred to a glass-Stoppard test tube followed by complete evaporation of solvent (About to dried sample) under a mild stream of nitrogen.

Clean up: The extract was subjected to clean-up using florisil column chromatography (DFG Manual of Pesticide Residue Analysis, 1987) . The top 1.5 cm of the florisil

column was packed with anhydrous sodium sulphate. Elution was done with 2% diethyl ether in hexane (5 mL/min). The eluate was concentrated in a rotary vacuum evaporator and transferred to glass-stoppard test tubes. Solvents were completely removed under mild nitrogen flow. The evaporated sample was dissolved in acetonitrile and then made to volume 1 mL in a volumetric flask for high performance liquid chromatography (HPLC).

Sample Analysis: Injections of the aliquots (usually 20 µL) were done by micro syringe into HPLC. Identification of the pesticide was carried out in relation to the retention time of the pure analytical standard supplied by International Atomic Energy Agency (IAEA). Quantification was made with a standard curve of the relevant (standard) pesticide and r^2 =.0.978345.

Analysis was done by HPLC (Detector-Waters 486, Pump- waters 515), which was used for the detection of organophosphorus, chlorpyriphos and carbamate pesticide residues.

Chromatographic Determination: For pesticides residues analysis, aliquot was injected by microlitre syringe into the High Performance Liquid chromatography (HPLC) fitted with UV Detector. The wave length was fixed at 254 nm for organophosphorus and carbamate pesticides.

Extraction efficiency / recovery: Analytical procedures employed were found to be satisfactory and average recoveries between 71 and 93% were obtained for diazinon, chlorpyriphos and carbofuran pesticides from the water samples (the fortifications were made in the concentration range 0.02-0.2 ppm level) indicating the suitability of the methodology.

Results and Discussion

Twenty water samples collected from the most vulnerable sites of Meherpur region of Bangladesh were analyzed for the presence of pesticide residues and the data were compared with the results of FAO/WHO recommended level. According to the results of this study, some water samples were found to be contaminated with organophosphorus (diazinon, chlorpyriphos) and carbamate (carbofuran, carbaryl) insecticide residues to some extent. Among the organophosphate pesticides, malathion, chlorpyriphos and diazinon were analyzed. Malathion was not detected in any of the analyzed samples during the present investigation. But, diazinon was found to be present in three water samples; one from Amzhupi union (WS-01; 0.0328 ppm), another from Kutubpur union (WS-11; 0.0790 ppm) and the last one from Amdaho union (WS-19; 0.0775 ppm). The residue of chlorpyriphos was detected in only two samples, one from Kutubpur union (WS-11; 0.0107 ppm), and another from Amdaho union (WS-19; 0.0143 ppm). The results are presented in Table 1. This result matches the survey data which suggest the widespread use of diazinon and chlorpyriphos by the farmers of areas under

investigation. Because, as a background study, the farmers of the study area informed that they did not apply any organochlorine pesticide but a lot of organophosphate (diazinon and chlorpyriphos) and carbamate pesticides have been applied by them in the cultivating areas. Similar findings were reported by Fatta *et al*. (2007) in their study.

Table 1. Amount of organophosphorus (OP) and carbamate pesticide residues in pond water samples.

Sample No	OP pesticide residues in water sample (ppm)		Carbamate pesticide residues in water sample (ppm)	
	Diazinon	Chlorpyriphos	Carbofuran	Carbaryl
WS1	0.0328	ND	ND	ND
WS11	0.0790	0.0107	0.0387	ND
WS19	0.0775	0.0143	0.0143	ND

W = Water, S = Sample, ND = Not Detected.

Matin *et al.* (1998) stated that DDT, DDE and dieldrin were present in some surface water samples obtained from the irrigated crop fields at Gaibandha. Groundwater samples from Nayarhat (hand tube well) were apparently free from residues. In most cases, residual levels were found to be within WHO guideline values for drinking water quality (WHO 1993). However, water samples from a irrigated crop field of Begumganj were found to contain DDT residues at 19 μg/L, which was above the WHO guideline value (Matin *et al.*1998). It was reported that since, Bangladesh is an agro-based country, huge number of pesticides especially carbamate and organophosphorus are used regularly in large quantities in the crop fields. Twenty insecticides, 18 fungicides and 2 rodenticides are being used in our agricultural and public health sectors (Sattar 1985). In the coastal region, large amount of various pesticides are used in the paddy fields, dry fish preservation and horticulture and in betel leaf cultivation. Those pesticides are washed down to the nearby canals, tributaries, rivers and ultimately find their way into the coastal water (Islam *et. al.* 2001). It is reported that 25% of the total amount of pesticides used in Bangladesh may reach the coastal water and pollute the sea (Khan and Talukder 1993). Also, Shoeb *et al*. (2009) reported in a study that organochlorine pesticides as DDTs (0.432 ppm) were found in dry fish used as feed ingredient from shrimp cultivation areas of Bagerhat.

Among the carbamate group, carbofuran was identified in two water samples; one from Kutubpur union (WS-11) and another from Amdaho union (WS-19) and the concentration level of carbofuran was 0.0387 ppm and 0.0143 ppm respectively. The results are presented in Table 1. However, residual levels of carbofuran in all the samples were fairly below the WHO guide line values. But, in a study, Bagchi *et al.* (2008) observed the highest concentration of carbofuran residue (2.208 ppm) in pond water samples of Bangladesh. Carbaryl was not present in any environmental water samples in

the present study. This suggests that both carbofuran and carbaryl might not frequently be used by the local farmers of the selected areas and if used, those might be used in a well-controlled manner.

Carbamate pesticides are rapidly taken up by plants through the roots from soil and water and are translocated mainly into the leaves. The main metabolite in plants has been identified as 3-hydroxycarbofuran (Thapar *et al*.1995). Field studies have indicated a half-life of 26 to 110 days in soil. Carbamate pesticides are degraded in water by hydrolysis, microbial decomposition and photolysis (WHO 2003 and Thapar *et al*. 1995).

The present findings are relatively less than the findings of others in terms of organophosphorus in water (Sanghi and Sarma 2005). But, the presence of such compounds indicates lapses in regulatory control, which endanger the environment. Although the Pesticide Rule, 1985 of Bangladesh strictly prohibited any kind of unauthorized use of pesticides but because of lack of strong monitoring and proper evaluation facilities this regulation did not work effectively to control indiscriminate use of hazardous pesticides.

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DIVERSITY OF FRUIT AND TIMBER TREE SPECIES IN THE COASTAL HOMESTEADS OF SOUTHERN BANGLADESH

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Abstract

In a study conducted in three southern districts (Bhola, Borguna and Patuakhali) of Bangladesh, a total of 69 tree species was recorded from the homegardens, of which 32 were fruit tree and 37 were timber tree species. Among the fruit tree species, coconut, betel nut, mango, jackfruit, guava, velvety apple were found in more than 80% households. The stocking of fruit trees per homestead was found highest for betel nut (265) followed by velvety apple (212), mango (38) coconut (25), jackfruit (20) and guava (9). Among the timber tree species, rain tree, mehogoni, raj koroi were most prevalent and found in more than 65% homesteads. The stocking of timber trees/ homestead was found highest for mehogoni (79) and then for rain tree (57), raj koroi (29) and katbadam (6).

Key wards: Fruit tree, Timber tree, Diversity, Coastal, Homesteads

Introduction

Bangladesh is one of the most densely populated countries in the world with a population of 152.5 million and with an annual growth rate of 1.37 (BBS 2011). There are 32.07 million homesteads in Bangladesh and over 74% of the population lives in the rural areas. Approximately 7% area (0.53 million ha) of the total 8.4 million ha of cultivable land in Bangladesh is occupied by homesteads which is extremely productive (BBS 2005). Homesteads play a vital role in providing timber, fuelwood, fodder, and fruits. Record of 70% of timber, 90% of fuelwood, 48% sawn and veneer logs and almost 90% of bamboo requirement is available from homegardens of Bangladesh (Uddin *et al.* 2002). But state forest of Bangladesh covers 2.52 million ha of lands, representing 17% of the countries land area and supplying only 12% wood (Poffenberger 2000). It is difficult to meet the country's huge demand for timber, fuel, fruit and fodder from the state forests. Villages of Bangladesh have a long heritage of growing timber and fruit trees along with other perennial shrubs and herbs (Rahman *et al.* 2009). The homegardens of Bangladesh is a source of livelihood for many farmers and serve as safety net during the time of hardship and natural disaster. Most of the homesteads of landlord houses contained improved cultivars of different fruits and other aesthetic plants, which are very much important from horticultural and breeding point of view. Homesteads represent a land use system involving deliberate management of multipurpose trees and shrubs in limited association with seasonal vegetables (Fernandes and Nair 1986).

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The coastal region of Bangladesh covers an area of about 47,201 km^2 extending along the Bay of Bengal. This region now covers 19 coastal districts facing, or in proximity to, the Bay of Bengal (Islam *et al*. 2006). The coastal and offshore areas include tidal, estuaries and river floodplains in the south along the Bay of Bengal. There are numerous old and new islands of varying size. The coastal zone constitutes 20% of the area and 28% of the population of Bangladesh (Islam 2004a). Agricultural labourers, small farmers, fishermen folk and the urban poor make up 71% of the 6.85 million households (Ahmad 2004).

The cultivable areas in coastal districts are affected by varying degrees of soil salinity. It has been recognized that 8,142 km^2 (5.5% of the country) land is salt affected and it is increasing at the rate of 146 km^2 per year (SRDI 2003). Although homesteads are the main source of fruit and timber production in the coastal areas but increased salinity hinders growth and survivability of trees in this region. Salinity causes unfavorable environment and hydrological situation that restrict the normal growth and crop production throughout the year (Haque 2006). The effect of salinity causes significant reduction in vegetation in the salt affected areas (Dutta and Iftekhar 2004). Besides, majority of the farmers cultivates their homesteads by different fruit and timber species in unplanned way. It is necessary to develop sound plans and procedure for planting more prevalent fruit and timber tree species in scientific way. So, exploration of existing timber and fruit tree species adaptive with changing climatic condition is needed first to have a clear understanding of the homgardens. Adaptability of a species and its suitability to a site is indicated by its frequency and growth. Therefore, this study was carried out to identify fruit and timber tree species and their distribution pattern in the coastal homesteads of Bangladesh.

Materials and Methods

The coastal zone of Bangladesh forms the lowest landmass and is a part of the delta of the extended Himalayan drainage ecosystem and covers 19 coastal districts. Among them three districts namely Bhola, Borguna and Patuakhali were selected for the present study. These coastal districts lie between the latitude $22^0$10'-$22^0$39'N and longitude $90^0$39'-$91^0$05'E. The climate is humid. Temperature ranges between 18^0 and 32^0 Celsius. The amount of rainfall varies between 2000-2500 mm at Borguna, 2500-3000 mm at Patuakhali and 3000 mm at Bhola district (Siddiqi and Khan 2004). Soils in the delta consist primarily of sands, silts, silty sands, sandy silts and clayey silts (Anon 1987). The delta soils occur in the coastal region of the Ganges tidal flood plain, the young Meghna estuarine flood plain and the old Meghna estuarine flood plain. The estuarine floodplain landscape occupies Bhola and Borguna districts. The landscape has been formed by the combined actions of rivers Meghna, Brahmaputra and Ganges. Usually, silty and clay deposits are finely stratified, and sandy deposits, as well as mixed sandy and silty deposits are coarsely stratified. The greater part of Borguna district consists of Gangetic meander floodplain soil having non-calcareous grey floodplain soil. It includes recent

accretions as well as the young and old Meander floodplain deposits. The soils of these areas are slightly saline (0.5-9.9 ds/m) and the pH values range from 5.8-7.8 and soil organic matter varies between 1.2 and 3.6%. Salinity of the soil and water at this region decreases toward north and increases toward east and west taking Bhola in the centre (Hassan 1999). Almost all the soils are silty to clayey in texture.

Two upazillas from each of three districts and five villages from each upazilla were selected. Then five homesteads from each village and 25 homesteads from each upazillas, totally 50 homesteads from each district were randomly selected (Table 1). Thus a total of 150 homesteads were selected from 3 districts. A multistage random sampling method was applied to select representative villages for the study. Sampling was done at four levels: district, upazilla, village and homestead.

Table 1. Name and location of the sampled area.

Sl.no.	District	Upzilla	Village
1	Bhola	Char-Fashon	Shahabajpur, Zinnagor, Aslampur, Nurabad and Aminabad.
		Lalmohon	Goneshpur, Satdarun, Romaganj, Collegepara and Lalmohon.
2	Barguna	Barguna Sadar	Gazi Mahmud, Borobaliatali, Amlokitala, Keorabunia and Amtoli-Nimtoli.
		Amtoli	Rahamatpur, Boythakata, Gotkhali, Sotonasnapara and Mohishvanga
3	Patuakhali	Kolapara	Panjupara, Misripara, Porgoja, Shirajpur and Bipinpur.
		Galachipa	Char Nandail, Majgram, Gramardan, Kamarhaola and Badarpur

Information was collected through a semi structured questionnaire and field survey which included interviews, group discussion and field observations. The respondents from selected homestead were interviewed with this pre-formulated questionnaire. Information was recorded through interview of family members like head of the family, housewife and others. The name and number of different timber and fruit tree species from sapling to big trees were recorded from the selected homesteads. The survey was conducted during January-March 2010. Microsoft Excel was used to process and analyze the collected data.

Results and Discussion

The size of the homesteads varied from home to home. It ranged from 0.08-2.53 ha in Bhola, 0.04-2.14 ha in Borguna and 0.08-2.51 ha in Patuakhali district. A total of 69 different tree species (both fruit and timber) were identified in all three districts of which, 61 species were found in Bhola, 55 species in Borguna and 61 species in Patuakhali districts (Table 2). Abedin and Quddus (1990) reported that the number of plant species (excluding vegetable species) in the coastal areas was higher (70 spp) than those found in the homesteads of Tangail (52 spp), Ishurdi (34 spp) Jessore (28 spp), Patuakhali (20 spp)

Rajshahi (28 spp) and Rangpur (21 spp) district respectively. Anam (1999) reported only 28 tree species in the plain area of Barind tract.

Table 2. Species richness of fruit and timber tree species at different coastal districts.

Sl. no.	District	Total sampled area (ha)	No. of fruit tree species	No. of timber tree species	Total
1	Bhola	36.49	31	30	61
2	Borguna	20.83	30	25	55
3	Patuakhali	21.17	30	31	61
	All	-	32	37	69

Fruit tree species diversity: The fruit tree species diversity was almost same in three coastal districts. A total of 31 different fruit tree species in Bhola, 30 species in Borguna and 30 species in Patuakhali districts was recorded. In all study areas 32 different fruit tree species were identified (Table 2). Vernacular, English and scientific names of recorded fruit tree species and their occurrence in the households are presented in Table 3.

The study showed that the total number of fruit trees/ homestead was 794, 666 and 356 in Bhola, Borguna and Patuakhali districts respectively. Bhola possessed a larger number of fruit tree stocking /homestead. This site falls under the estuaries of Meghna which are less saline due to fresh water flow during rainy season.

The occurrence of different fruit tree species in terms of percentage of households was calculated and is presented in Table 3. In Bhola district, coconut was found in 100% households, followed by betel nut, mango (98%), jackfruit (96%), guava (94%), velvety apple (92%), pummelo (80%), hog plum (72%) and palmyra palm (62%). In Borguna district, coconut was found in 96% households, followed by mango (94%), betel nut, guava (88%), jujube/ ber, jackfruit (84%), pummelo, velvety apple (78%), date palm (70%) and palmyra palm (66%). In Patuakhali district, coconut and mango were found in 100% households, followed by guava (94%), jackfruit (92%), betel nut (90%), palmyra palm, pummelo (74%), and velvety apple (72%). The result also revealed that the average percentage of households for all three districts coconut was found in 98.67% homesteads followed by mango (97.33%), betel nut and guava (92%), jackfruit (90.67%), velvety apple (80.67%), pummelo (77.33%) and palmyra palm (67.33%).

In Bhola district, the highest number of fruit trees/ homestead was found for betel nut (516) and then for velvety apple (115), mango (59), jackfruit (33) and coconut (31). In Borguna, the highest number of trees/ homesteads was found for velvety apple (362) followed by betel nut (199), mango (24) and coconut (20). In Patuakhali, the highest number of trees/ homestead was recorded for velvety apple (160) followed by betel nut (78), mango (31), coconut (24) and jackfruit (16). From the average data for all three districts the highest number of trees/ homestead were recorded for betel nut (265) followed by velvety apple (212), mango (38), coconut (25), jackfruit (20) and guava (9) (Table 4). The result revealed that some species were abundant and some others were scare in the homesteads. This is probably due to farmer's choice to some economically important fruit bearing species like coconut, betel nut, mango, guava etc. These species

grow well in the southern districts and fruit production is also high. Therefore, farmers planted more seedlings with some selected fruit tree species for earning more money.

Table 3. Percentage (%) of homesteads containing fruit tree species in the coastal areas.

Sl. no.	Name of fruit tree species			% homestead containing fruit tree species			
	Local	English	Scientific	Bhola	Borguna	Patuakhali	Mean of all districts
1	Narikel	Coconut	*Cocos nucifera*	100	96	100	98.67
2	Supari	Betel nut	*Areca catechu*	98	88	90	92.00
3	Bilati gab	Velvety apple	*Diospyros discolor*	92	78	72	80.67
4	Aam	Mango	*Mangifera indica*	98	94	100	97.33
5	Kanthal	Jackfruit	*Artocarpus heterophyllus*	96	84	92	90.67
6	Peyara	Guava	*Psidium guajava*	94	88	94	92.00
7	Jambura	Pummelo	*Citrus grandis*	80	78	74	77.33
8	Tal	Palmyra palm	*Borassus flabellifer*	62	66	74	67.33
9	Amra	Golden apple	*Spondias pinnata*	72	28	46	48.67
10	Tentul	Tamarind	*Tamarindus indica*	54	54	54	54.00
11	Boroi	Jujube	*Zizypus mauritania*	56	84	60	66.67
12	Jamrul	Wax apple	*Syzygium samarangense*	54	60	62	58.67
13	Kamranga	Carambola	*Averrhoa carambola*	54	40	44	46.00
14	Lebu	Lemon	*Citrus limon*	56	54	60	56.67
15	Khejur	Date palm	*Phoenix sylvestris*	40	70	56	55.33
16	Kalojam	Blackberry	*Syzygium cumini*	32	42	68	47.33
17	Dewa	Monkey jack	*Artocarpus lakoocha*	22	40	38	33.33
18	Chalta	Elephant apple	*Dillenia indica*	36	24	32	30.67
19	Letchu	Litchi	*Litchi chinensis*	26	44	42	37.33
20	Jalpai	Indian olive	*Elaeocarpus floribundus*	24	28	28	26.67
21	Amloki	Aonla	*Emblica officinalis*	16	24	30	23.33
22	Ata	Bullock's heart	*Annona reticulata*	40	12	14	22.00
23	Kaophal	Cowa	*Garcinia cowa*	24	8	10	14.00
24	Bel	Wood apple	*Aegle marmelos*	10	14	34	19.33
25	Sofeda	Sapota	*Achras sapota*	8	26	32	22.00
26	Dalim	Pomegranate	*Punica granatum*	12	18	24	18.00
27	Sarifa	Custard apple	*Annona squamosa*	16	16	18	16.67
28	Gab	Riverebony	*Diospyros peregrina*	6	-	12	6.00
29	Kamala	Orange	*Citrus chinensis*	8	10	10	9.33
30	Kadbel	Elephant's foot apple	*Feronia limonia*	2	2	12	5.33
31	Gulapjam	Rose apple	*Syzygium jambos*	2	-	-	0.67
32	Malta	Sweet orange	*Citrus sinensis*	-	2	-	0.67

Bangladesh has a number of varieties of tropical and sub-tropical fruits. About 70 different kinds of fruit are grown in Bangladesh of which 90% fruits come from the homesteads (Islam 2004b). Rahman *et al.* (2009) observed that mango and jujube were in 100% homesteads in Hatiya island followed by coconut (98.7%), guava (97.5%), betel

Table 4. Distribution of fruit tree species in three coastal districts of Bangladesh.

Sl. no.	Name of fruit tree species		Bhola		Borguna		Patuakhali		Mean of all districts
	English	Scientific	Total no. of trees	Trees/ home stead	Total no. of trees	Trees/ home stead	Total no. of trees	Trees/ home stead	Trees/ home stead
1	Coconut	*Cocos nucifera*	1575	31.5	992	19.84	1220	24.4	25.25
2	Betel nut	*Areca catechu*	25823	516.46	9938	198.76	3926	78.52	264.58
3	Velvety apple	*Diospyros discolor*	5732	114.64	18109	362.18	7999	159.98	212.27
4	Mango	*Mangifera indica*	2929	58.58	1190	23.80	1556	31.12	37.83
5	Jackfruit	*Artocarpus heterophyllus*	1638	32.76	597	11.94	805	16.10	20.27
6	Guava	*Psidium guajava*	285	5.70	684	13.68	379	7.58	8.99
7	Pummelo	*Citrus grandis*	172	3.44	161	3.22	224	4.48	3.71
8	Palmyra palm	*Borassus flabellifer*	276	5.52	401	8.02	359	7.18	6.91
9	Golden apple	*Spondias pinnata*	80	1.60	27	0.54	44	0.88	1.01
10	Tamarind	*Tamarindus indica*	80	1.60	133	2.66	172	3.44	2.57
11	Jujube	*Zizypus mauritania*	81	1.62	139	2.78	76	1.52	1.97
12	Wax apple	*Syzygium samarangense*	54	1.08	78	1.56	72	1.47	1.37
13	Carambola	*Averrhoa carambola*	53	1.06	52	1.04	52	1.04	1.05
14	Lemon	*Citrus limon*	218	4.36	139	2.78	130	2.60	3.25
15	Date palm	*Phoenix sylvestris*	336	6.72	322	6.44	306	6.12	6.43
16	Blackberry	*Syzygium cumini*	50	1.00	86	1.72	107	2.14	1.62
17	Monkey jack	*Artocarpus lakoocha*	27	0.54	33	0.66	42	0.84	0.68
18	Elephant apple	*Dillenia indica*	52	1.04	24	0.48	68	1.36	0.96
19	Litchi	*Litchi chinensis*	20	0.40	60	1.20	46	0.92	0.84
20	Indian olive	*Elaeocarpus floribundus*	22	0.44	30	0.60	22	0.44	0.49
21	Aonla	*Emblica officinalis*	20	0.40	22	0.44	30	0.61	0.48
22	Bullock's heart	*Annona reticulata*	66	1.32	9	0.18	15	0.3	0.60
23	Cowa	*Garcinia cowa*	30	0.60	8	0.16	8	0.16	0.31
24	Wood apple	*Aegle marmelos*	9	0.18	17	0.34	37	0.74	0.42
25	Sapota	*Achras sapota*	6	0.08	22	0.44	20	0.40	0.31
26	Pomegranate	*Punica granatum*	7	0.14	15	0.30	20	0.40	0.28
27	Custard apple	*Annona squamosa*	19	0.38	11	0.22	13	0.26	0.29
28	Riverebony	*Diospyros peregrina*	35	0.70	-	-	38	0.76	0.49
29	Orange	*Citrus chinensis*	8	0.16	7	0.14	14	0.28	0.19
30	Elephant's foot apple	*Feronia limonia*	1	0.02	2	0.04	6	0.12	0.06
31	Rose apple	*Syzygium jambos*	4	0.08	-	-	-	-	0.03
32	Sweet orange	*Citrus sinensis*	-	-	1	0.02	-	-	0.01
	Total :		39708	794.12	33305	666.18	17806	356.16	605.49

nut (96.2%) and jackfruit (95%). Abedin and Quddus (1990) found mango at 95% homesteads of Tangail and above 67% homesteads of Ishurdi, Jessore and Rangpur district. Momen *et al*. (2006) recorded a total of 33 plant species from the homegarden on an off-shore Sandwip island, of which 19 were fruit and 14 were timber tree species. They stated that betel nut was the highest in number (4.72 stems/household) and guava (2.02 per household). It was also observed that 98.5% of households possessed betel nut followed by coconut (96.3%) and lemon (93.3%). Rahman *et al*. (2009) recorded 28 fruit species in the homestead of Hatiya island of Noakhali district. Among them banana, mango and jujube were found in 100% homesteads followed by coconut (98.7%), guava (97.5%), betel nut (96.2%) and jackfruit (95%). They reported that black berry and jujube were found highly diverse fruit species followed by mango and jackfruit.

Uddin *et al*. (2002) studied plant biodiversity in the homesteads of saline areas of greater Noakhali district. They found 17 fruit species in the study areas. Coconut was found in 98.63% household followed by mango (96.72%), betel nut (93.44%), banana (90.16%), guava (85.24%) and date palm (80.32%). Alam *et al*. (1990) observed that mango, jackfruit, coconut and banana were available at more than 65% homesteads in Jessore. Alam and Masum (2005) found 34 fruit species, 24 timber species and 21 fuel wood species in the Sandwip offshore island. They mentioned that coconut, betel nut, guava, date palm and mango were cultivated in more than 75% of the homesteads.

Timber tree species diversity: A total of 30 timber species in Bhola, 25 species in Borguna and 31 in Patuakhali districts was recorded. In all three districts, 37 different timber tree species were identified (Table 5). Almost 13 timber tree species were found common in all districts. The mean number of timber trees/ homestead was 214, 169 and 205 in Bhola, Borguna and Patuakhali respectively (Table 6).

The percentage of households containing different timber tree species was calculated and presented in Table 5. In Bhola district, rain tree was found in 96% households, followed by mehogoni (74%), raj koroi (54%), bamboo grove (50%), katbadam (46%) and sada koroi (40%). In Borguna district, rain tree was found in 78% households, followed by raj koroi (64%), mehogoni (60%) and katbadam (54%). In Patuakhali district, mehogoni was found in 92% households followed by rain tree and raj koroi (86%) and sada koroi (56%). The result also showed that the average percentage of households for all three districts rain tree was found in 86.67% homesteads followed by mehogoni (75.33%), raj koroi (68%), katbadam (44%) bamboo grove (44%) and sada koroi (42%).

In Bhola district, the highest number of timber trees/ homestead was found for mehogoni (73) and then for rain tree (66) and raj koroi (31). In Borguna, the highest number of trees/ homestead was found for rain tree (67) and then for mehogoni (50), raj koroi (29) and katbadam (13). In Patuakhali, the highest number of trees/ homestead was recorded for mehogoni (115) followed by rain tree (36) and raj koroi (28). From the average data for all three districts the highest number of trees/ homestead was found for mehogoni (79) and then for rain tree (57), raj koroi (29) and katbadam (6) (Table 6). Momen *et al*.

(2006) recorded 14 timber tree species in the homegarden on an off-shore Sandwip island. They observed that the mean number of trees for rain tree/ household was the highest (3.57) followed by kala koroi (2.07) and sada koroi (1.62). They stated that 92.1% household contained rain tree followed by kala koroi (91.3%) and sada koroi (90.1%).

Table 5. Percentage (%) of homesteads containing timber tree species in the coastal areas.

Sl. no.	Name of timber tree species		% homestead containing timber tree species			
	Local	Scientific	Bhola	Borguna	Patuakhali	Mean of all districts
1	Mehogoni	*Swietenia macrophylla*	74	60	92	75.33
2	Rain tree	*Samanea saman*	96	78	86	86.67
3	Raj koroi	*Albizia richardiana*	54	64	86	68.00
4	Kala koroi	*Albizia lebbeck*	14	6	28	16.00
5	Sada koroi	*Albizia procera*	40	30	56	42.00
6	Neem	*Azadirachta indica*	8	30	34	24.00
7	Simul	*Bombax ceiba*	18	16	18	17.33
8	Sonalu	*Cassia fistula*	2	8	4	4.67
9	Karanja	*Pongamia pinnata*	12	16	14	14.00
10	Payra	*Pithecellobium dulce*	30	4	6	13.33
11	Akashmoni	*Acacia auriculiformis*	16	10	28	18.00
12	Katbadam	*Terminalia catappa*	46	54	32	44.00
13	Bamboo grove	*Bambusa sp.*	50	38	44	44.00
14	Babla	*Acacia nilotica*	2	-	2	1.33
15	Sisso	*Dalbergia sissoo*	10	-	8	6.00
16	Segun/Teak	*Tectona grandis*	2	-	4	2.00
17	Pitraj	*Aphanamixis polystachya*	12	-	4	5.33
18	Sonboloi	*Thespesia populnea*	6	-	-	2.00
19	Sundari	*Heritiera fomes*	8	6	-	4.67
20	Gewa	*Excoecaria agallocha*	4	4	2	3.33
21	Ipil-Ipil	*Leucaena leucocephala*	8	28	24	20.00
22	Mander	*Erythrina sp.*	18	2	4	8.00
23	Aurjune	*Terminalia arjuna*	4	2	4	3.33
24	Debdaru	*Polyalthia longifolia*	2	6	-	2.67
25	Khoir	*Acacia catechu*	2	8	14	8.00
26	Eucalyptus	*Eucalyptus camaldulensis*	2	4	2	2.67
27	Jial badhi	*Lannea coromandelica*	4	4	14	7.33
28	Bot	*Ficus bengalensis*	2	2	-	1.33
29	Bohera	*Terminalia belerica*	2	-	-	0.67
30	Ponial	*Calophyllum inophyllum*	4	-	-	1.33
31	Jarul	*Lagerstroemia speciosa*	-	4	2	2.00
32	Bokain	*Melia sempervirens*	-	2	2	1.33
33	Sheora	*Streblus asper*	-	-	4	1.33
34	Gamar	*Gmelina arborea*	-	-	2	0.67
35	Hijol	*Barringtonia acutangula*	-	-	6	2.00
36	Kadam	*Anthocephalus chinensis*	-	-	4	1.33
37	Jhao	*Casuarina equsetifolia*	-	-	2	0.67

Table 6. Distribution of timber tree species in three coastal districts of Bangladesh.

Sl. no.	Name of timber tree species		Bhola		Borguna		Patuakhali		Mean of all districts
	Local	Scientific	Total no. of trees	Trees/ homestead	Total no. of trees	Trees/ homestead	Total no. of trees	Trees/ home stead	Trees/ home stead
1	Mehogoni	*Swietenia macrophylla*	3637	72.74	2523	50.46	5739	114.78	79.33
2	Rain tree	*Samanea saman*	3318	66.36	3376	67.52	1805	36.1	56.66
3	Raj koroi	*Albizia richardiana*	1531	30.62	1429	28.58	1412	28.24	29.15
4	Kala koroi	*Albizia lebbeck*	189	3.78	11	0.22	81	1.62	1.87
5	Sada koroi	*Albizia procera*	144	2.88	103	2.06	244	4.88	3.27
6	Neem	*Azadirachta indica*	12	0.24	31	0.62	50	1	0.62
7	Simul	*Bombax ceiba*	21	0.42	14	0.28	16	0.32	0.34
8	Sonalu	*Cassia fistula*	50	1	17	0.34	4	0.08	0.47
9	Karanja	*Pongamia pinnata*	263	5.26	51	1.02	51	1.02	2.43
10	Payra	*Pithecellobium dulce*	174	3.48	8	0.16	10	0.2	1.28
11	Akashmoni	*Acacia auriculiformis*	131	2.62	38	0.76	350	7	3.46
12	Katbadam	*Terminalia catappa*	215	4.3	630	12.60	89	1.78	6.23
13	Bamboo grove	*Bambusa sp.*	161	3.22	42	0.84	104	2.08	2.05
14	Babla	*Acacia nilotica*	2	0.04	-	-	12	0.24	0.09
15	Sisso	*Dalbergia sissoo*	75	1.5	-	-	49	0.98	0.83
16	Segun	*Tectona grandis*	2	0.04	-	-	16	0.32	0.12
17	Pitraj	*Aphanamixis polystachya*	45	0.9	-	-	55	1.10	0.67
18	Sonboloi	*Thespesia populnea*	107	2.14	-	-	-	-	0.71
19	Sundari	*Heritiera fomes*	255	5.1	17	0.34	-	-	1.81
20	Gewa	*Excoecaria agallocha*	18	0.36	4	0.08	1	0.02	0.15
21	Ipil-Ipil	*Leucaena leucocephala*	19	0.38	95	1.9	62	1.24	1.17
22	Mander	*Erythrina sp.*	238	4.76	7	0.14	4	0.08	1.66
23	Aurjune	*Terminalia arjuna*	13	0.26	3	0.06	3	0.06	0.13
24	Debdaru	*Polyalthia longifolia*	7	0.14	19	0.38			0.17
25	Khoir	*Acacia catechu*	1	0.02	10	0.20	21	0.42	0.21
26	Eucalyptus	*Eucalyptus camaldulensis*	6	0.12	12	0.24	2	0.04	0.13
27	Jial badhi	*Lannea coromandelica*	31	0.62	25	0.5	40	0.80	0.64
28	Bot	*Ficus bengalensis*	1	0.02	1	0.02	-	-	0.01
29	Bohera	*Terminalia belerica*	2	0.04	-	-	-	-	0.01
30	Ponial	*Calophyllum inophyllum*	10	0.20	-	-	-	-	0.07
31	Jarul	*Lagerstroemia speciosa*	-	-	6	0.12	1	0.02	0.05
32	Bokain	*Melia sempervirens*	-	-	2	0.04	1	0.02	0.02
33	Sheora	*Streblus asper*	-	-	-	-	17	0.34	0.11
34	Gamar	*Gmelina arborea*	-	-	-	-	13	0.26	0.09
35	Hijol	*Barringtonia acutangula*	-	-	-	-	4	0.08	0.03
36	Kadam	*Anthocephalus chinensis*			-	-	2	0.04	0.01
37	Jhao	*Casuarina equsetifolia*	-	-	-	-	2	0.04	0.01
		Total :	10678	213.56	8474	169.48	10260	205.00	196.02

Nath *et al*. (2004) found that rain tree was the most dominant timber tree species grown in the coastal homesteads of Sitakunda Upazilla. Uddin *et al*. (2002) found 16 timber species in greater Noakhali coastal district. mehogani, jial badhi and neem were found at more than 50% household. Alam and Masum (2005) observed that mehogani, raintree, sada koroi and segun/teak were common in most of the homesteads in Sandwip.

Effect of salinity on tree crops: The diversity and distribution pattern of the plant species are influenced by macro and micro environmental factors of the homesteads. Most fruit trees are relatively sensitive to salinity with little exception and few other species believed to be moderately salt tolerant. It is generally believed that growth and yield of woody fruit crops suffer from both osmotic effect and toxicities caused by chloride or sodium accumulation (Bernstein 1980). The vegetation coverage is reducing due to increasing soil salinity in different countries. But there are some terrestrial plants that can grow well in saline soil. In this study, some fruit tree species were found growing well in more or less saline condition. The common dominant species in all districts of the study areas are coconut, betel nut, velvety apple, mango, jackfruit, guava, date palm and palmyra palm. From the available information, coconut and date palm are high salt or strong salinity (12.1-16.0 dS/m) tolerant species in the coastal areas of Bangladesh (Dutta and Iftekhar 2004). Nandy *et al.* (2002) reported that coconut is highly adaptive in moderately saline zone (8.1-12.0 dS/m) for embankment plantation. According to the farmer's opinion, coconut, velvety apple, tamarind, date palm are high salt tolerant species. On the other hand, guava, mango, bullocks heart, lemon, palmyra palm, carambola, pummelo are moderately salt tolerant species.

This information might help to understand the tree diversity, and selection of salt tolerant species of any environmental stress condition. Present study reveals only occurrence and diversity of different fruit and timber tree species in the coastal regions, but intensive research should be undertaken to improve homegardens for more productivity.

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MINERALOGICAL COMPOSITION OF SOILS FROM URIR CHAR − A TINY OFFSHORE ISLAND OF BANGLADESH

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Abstract

Mineralogical composition of three surface soils from Urir Char − an environmentally vulnerable small offshore island of Bangladesh − was determined by X-ray diffraction analysis. In the bulk soils layer silicates and quartz were the dominant minerals while the quantity of feldspars was around ten percent. Quantity of weatherable minerals was very high in the soils. Illite was the dominant mineral in the clay fraction of the soils. The second dominant clay mineral was smectite which comprised around one-fourth of the clay fraction closely followed by chlorite with a small quantity of kaolinite mineral. Soil vermiculite and mixed layer minerals were absent in these soils. These soils had a mixed mineralogical composition with a high cation exchange capacity and percent base saturation. Texturally the soils were silty loam. Exchangeable Ca^{++}/ Mg^{++} ratio was less than unity. Magnesium solonization was considered as the dominant pedogenic process along with gleization. There was problem of salinity as the elevation of land was around one meter above the mean sea level. Natural calamities like tropical cyclones and tidal surges were highly devastating as the island was completely washed with sea water.

Key words: Urir char, Offshore island, Clay mineralogy, Soil properties

Introduction

Urir Char is a small offshore island of Bangladesh located to the eastern side of the mouth of the Meghna estuary near the northern tip of the Bay of Bengal. This island was formed by the sediments derived from the Ganges-Brahmaputra-Meghna (GBM) river system. Urir char is divided into two parts − old charland which is protected by polders and is periodically flooded in the monsoon months by rain water. The other part is a freshly deposited new mud flat which is tidally affected. Bank erosion is little although the island is surrounded by water on all sides but the natural environment is quite fragile. The major natural calamites are devastating tropical cyclonic storms that come occasionally along with tidal surges.

Urir Char island is roughly an oval shaped land mass and lies between Sandwip upazila of Chittagong district and Companyganj upazila of Noakhali district (Fig. 1). Administratively, it is a union under Sandwip upazila of Chittagong district. This island is roughly10 Km long in the north-south direction and about 7 Km wide in the east-west direction comprising an area of about 10000 ha of land and has a population of more than 9000 (Khan 2007). Because of the environmental vulnerability it is sparsely populated

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and rice cultivation is the main occupation of the islanders with fishing and cattle/buffalo farming as secondary activities. More than 30 % of the island has been brought under plantation mangrove forest under the coastal afforestation programme of Bangladesh Forest Department.

Urir Char is located between 22°34′00″ - 22°43′15″ N latitudes and 91°18′38″ - 91°24′43″ E longitudes. Physiographically, it is an isolated coastal plain of Holocene period with an elevation of only 0.5-1.0 m above mean sea level (SRDI 1997). Agroecologically, this island has been designated as the Meghna Estuarine Charland (FAO-UNDP 1988). Soils of this island have developed on silts deposited by the Lower Meghna river, which is a mixture of the sediments derived from the GBM river system. The climate of the area is humid tropical monsoon with a mean annual rainfall of more than 3000 mm and over 90 percent of which falls during the months of March to October (SRDI 1997). Water supply from rainfall is enough to wash out salts from the soils. Strong tropical cyclones along with devastating tidal surges are the common natural hazards which lash the island occasionally. Rice is the principal crop grown in the island and Rabi/ fallow –Aus/fallow – transplanted Aman is the usual cropping pattern. Accretion and erosion occur along the sides of the island and new deposition of raw muds is vegetated with plantation mangrove forest at suitable time.

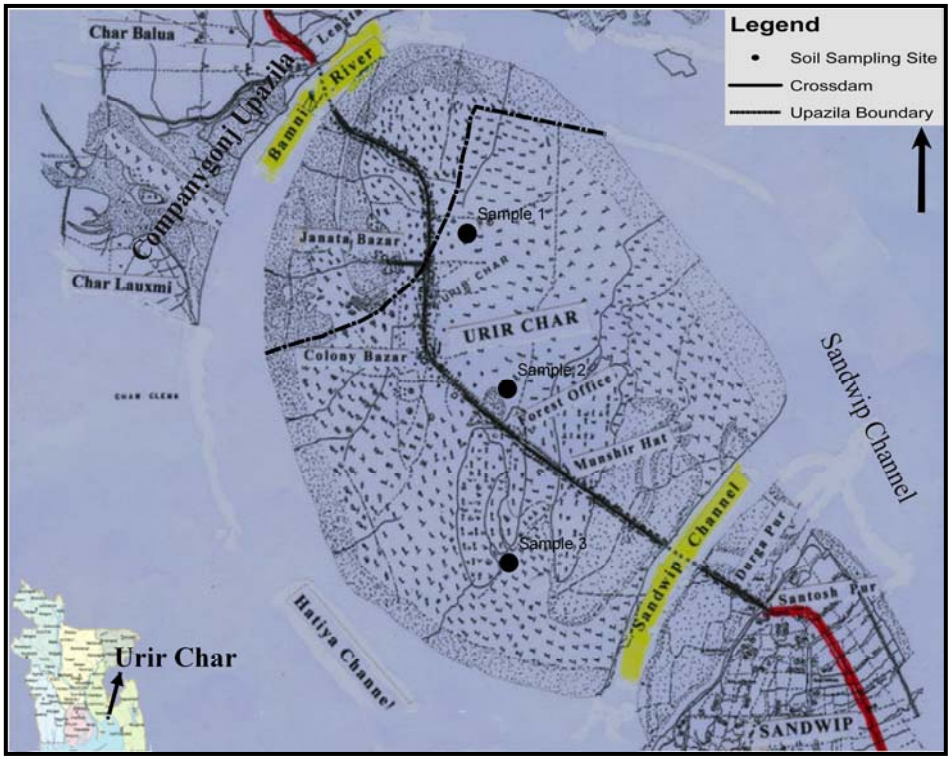

Fig. 1. Sketch map of Urir Char showing soil sampling site (source: modified from Khan 2007).

The soils of Urir Char were surveyed by Soil Resources Development Institute and identified the soils as belonging to Ramgati series (SRDI 1997). Mineralogical investigation of soils is essential for characterizing the soils, as well as to ascertain the mineralogical impact on soil management and productivity and also to understand the nature of pedochemical weathering. The present paper attempts to report information on some basic soil properties and the bulk and clay mineralogical composition of some selected soils from Urir Char.

Materials and Methods

Three surface soil samples (0-15 cm depth) from three selected locations of the oldest parts of the island, where rice cultivation is being practised, were collected for mineralogical investigations. The locations of the soil sampling sites have been recorded using a GPS and marked in a sketch map of Urir Char (Fig. 1). Soils in the older part of this island where cambic horizon have developed have been characterized as Inceptisols, mostly Endoaquepts (Soil Survey Staff 1999). The collected soil samples were physically and chemically analysed following standard methods and its results are presented in Table 1. The mineralogical analysis of the soils was carried out in the laboratory of the Institute of Applied Geology, University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria.

The mineralogical composition of bulk soil sample (< 2mm) as well as separated clay (< 2μm) fractions were determined. For bulk mineral analysis soil samples were prepared according to the "backload procedure" and analysed in a Philips X-ray-diffractometer (PW 1710, a long fine focus tube) with Ni filtered Cu-Kα radiation (45kV, 40mA) from 2° to 70° 2θ. The measuring time was 1 sec. in step-scan mode with a step size of 0.02°. Semi -quantitative mineral composition of the soil samples was estimated using the method described by Schultz (1964). The clay mineralogical studies were performed by removing organic materials by oxidation with H_2O_2 (Kunze and Dixon 1986) and iron oxides by citrate –dithionite- bicarbonate method (Mehra and Jackson 1960). Clay fraction was separated by sedimentation method and the separated clay was saturated with Mg^{++} and K^+ ions using 1 *M* $MgCl_2$ and KCl solutions. The oriented clay samples on ceramic plates were scanned with a Philips 1710 X-ray diffractometer (XRD) with Ni filtered Cu- Kα radiation (at 45 kV, 40 mA) at a scanning speed of 2° 2θ per minute from 2° to 40° 2θ. The identification of clay minerals was generally based on the methods outlined by Thorez (1975), Brindley and Brown (1980), Moore and Reynolds (1997) and Wilson (1989). Semi-quantitative estimations of minerals were carried out using the corrected intensities of characteristic X-ray peaks (Riedmüller 1978).

Results and Discussion

The soils of Urir Char are young, loamy textured with high percentage of silt and are alkaline in reaction with high cation exchange capacity (CEC). Percent base saturation of

the soils is very high (Table 1). Exchangeable Ca^{++}/ Mg^{++} ratio is less than unity. Karim and Bhuyian (1963) reported this kind of results in some soils from the coastal area of Chittagong and called the pedogenic process as magnesium solonization. The exchangeable Na^{+}/ K^{+} ratio is near 3 which may be due to the fact that these soils are sometimes flooded by sea water. Exchangeable sodium percentage (ESP) in the soils is high but did not exceed 15 in any of the soils. The dominant soil forming processes are magnesium solonization along with gleization.

The semi-quantitative estimation of the minerals in the bulk soils indicates that the layer silicate minerals comprise the major portion (around 59%) of the soils (Figs. 2 and 4). Quartz comprises about one third the bulk of which was present in the sand and silt

Table 1. Some physical and chemical properties of the soils from Urir Char.

Properties	Results		
	Soil 1	Soil 2	Soil 3
GPS coordinates (Sampling location)	22°41′21.408"N 91°20′39.326"E	22°39′07.777"N 91°20′38.683"E	22°37′05.709"N 91°20′07.845"E
pH (H_2O)	7.6	7.7	7.5
ECe (dS m^{-1})	6.0	5.8	7.5
Organic Carbon (%)	1.88	1.95	1.79
Sand (%)	22	19	20
Silt (%)	54	58	55
Clay (%)	24	23	25
Textural Class	Silt loam	Silt loam	Silt loam
Exch. Na^{+} ($cmol^{+}kg^{-1}$)	1.87	1.67	1.93
Exch. K^{+} ($cmol^{+}kg^{-1}$)	0.63	0.58	0.53
Exch. Ca^{++} ($cmol^{+}kg^{-1}$)	7.41	7.98	8.08
Exch. Mg^{++} ($cmol^{+}kg^{-1}$)	8.05	8.13	8.29
Cation exchange capacity ($cmol^{+}kg^{-1}$)	19.88	19.97	20.45
Base saturation percent (BSP)	90	92	92
Exch. Ca^{++}/ Mg^{++} ratio	0.92	0.98	0.97
Exch. Na^{+}/ K^{+} ratio	2.96	2.87	3.54
Exchangeable sodium percentage (ESP)	9.4	8.3	9.4

fractions. The 3rd dominant mineral in the soil is feldspars (k-feldspar and plagioclase) which comprise on an average 12 percent. Plagioclase feldspars are much higher than K-feldspars in all the studied soils (Figs. 2 and 4). Calcite mineral was not found in soils of Urir Char island although the sediments were derived from the Ganges, the Brahmaputra and the Meghna rivers together. The sediments of the Ganges river are known to be calcareous (Brammer 1996). The absence of calcite mineral in the sediments of Urir Char suggested that this mineral was probably depleted during transportation, sedimentation

and diagenesis stages. Begum *et al.* (2004) studied the mineralogical composition of some soils from the Monpura island located in the lower Meghna estuary and reported that the layer silicate minerals comprise the major portion of these soils followed by quartz and feldspar. Huizing (1971) examined minerals in the sand fraction across the soils of the Bengal basin and reported that the floodplain soils were rich in feldspars, micas and amphiboles. These were mainly derived from crystalline rocks of the Himalayan mountain range.

One startling fact is that almost 70 percent of the minerals present in the soils are moderately to easily weatherable (Fig. 2). Occurrence of such a high quantity of weatherable minerals in these soils may indicate that they are chemically reactive and possess high nutrient reserve.

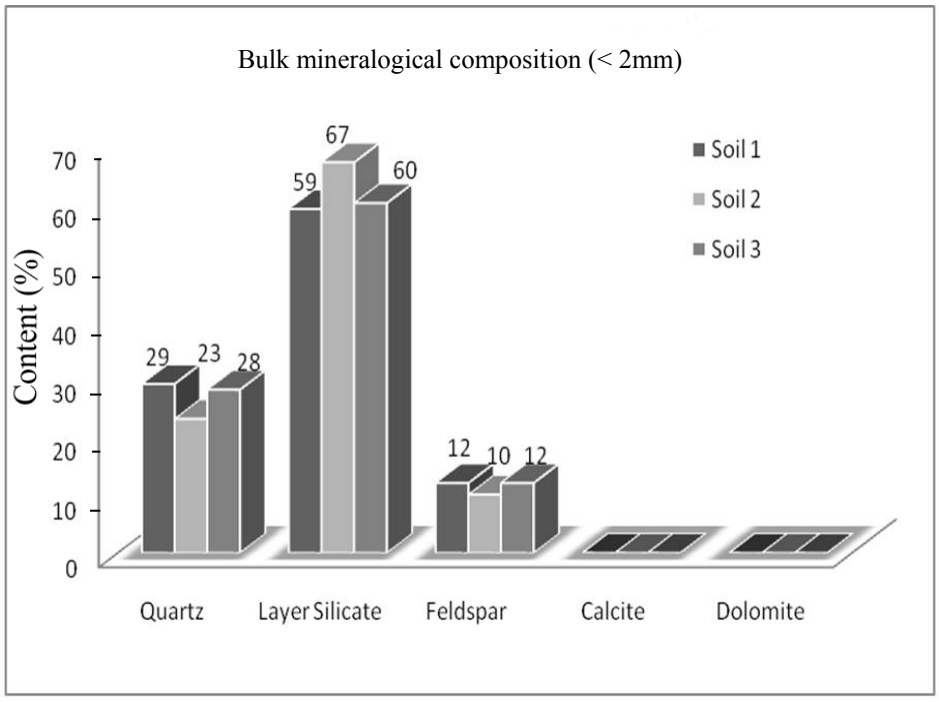

Fig. 2. Mineral composition of bulk soil samples from Urir Char.

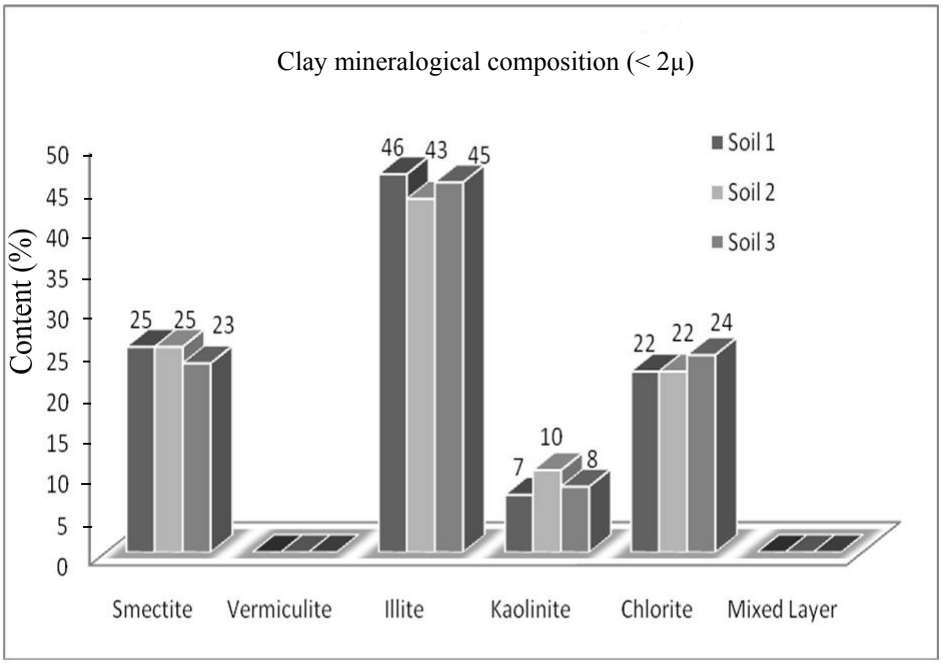

Fig.3. Mineral composition of clay fraction of surface soils from Urir Char.

The semi-quantitative estimation of clay minerals showed the dominance of illite in the clay fraction of the soils followed by smectite which comprised around one-fourth of the clay fraction (Figs. 3 and 5). Chlorite was the third dominant mineral in the clay fraction of the soils (23%) with a small quantity (around 8 %) of kaolinite mineral. The absence of vermiculite in the clay fraction of the soil indicates that illite is not transforming to form expanding lattice minerals such as vermiculite. Almost one fifth of the clay fraction is occupied by chlorite which is thought to be derived from the parent material as an allogenic mineral and may remain unaffected by the present cycle of pedochemical weathering processes. Absence of interstratified minerals in the clay fraction is also interesting to note that mica is not being weathered to form expanding lattice minerals and that the minerals in the clay fraction were mostly inherited from the parent materials. It is possible that the soils of Urir Char were developed on sediments in which weathering processes have not advanced enough to significantly alter the silicate clay minerals. The presence of smectite mineral in the soils indicates that the soils were formed on alluvium derived from the combined sediments of the Ganges Brahmaputra and Meghna rivers. These results appears to be in conformity with the findings of White (1985) and Moslehuddin *et al.* (1998) in some GBM river floodplains soils of Bangladesh where they reported the occurrence of more than 20% smectite mineral in the clay fraction of the soils.

The mineralogical composition indicates that the soil materials of the Urir Char island were mainly allogenic and their transformation process appears to be in the initial stage only. Occurrence of high quantity of weatherable minerals also indicates the occurrence of weak weathering and a considerably high nutrient reserve.

The clay mineral composition further indicates that the soils of Urir Char have a mixed mineralogy with significant quantity of smectite. With a mineralogical composition as this, the soils of Urir Char are expected to demonstrate a favourable physical as well as physicochemical condition for their agronomic use. Smectite mineral may have a high potassium fixing capacity. More K application may be recommended for enhancing the productivity of soils. The soils are medium textured and have an illite -smectite-chlorite clay mineralogical composition and consequently may be graded as having a 'good'

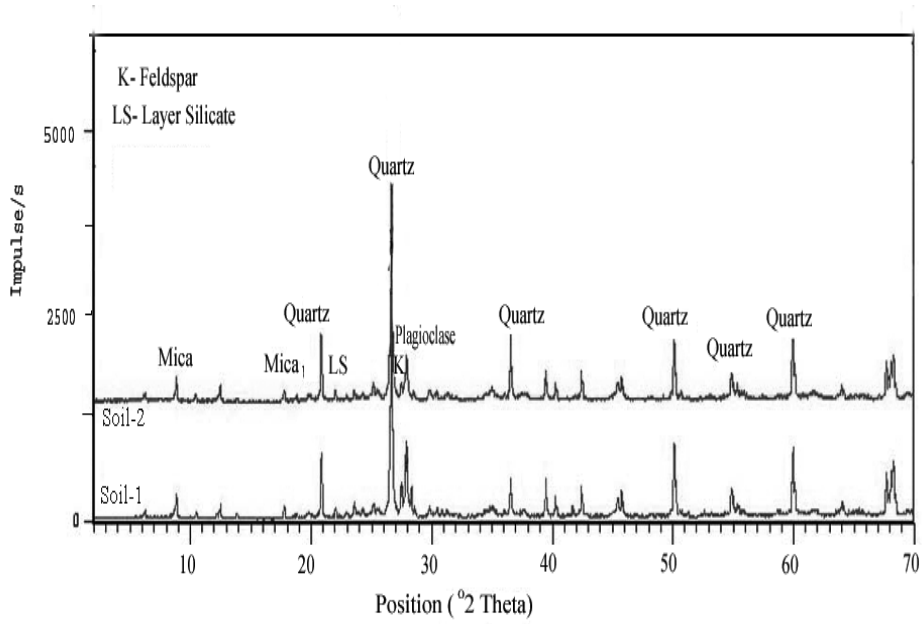

Fig. 4. X-ray diffractogram of bulk soil samples from Urir Char.

inherent potentiality which means good buffer capacity and good level of nutritional reserve. Therefore, these soils are easy to manage and are expected to support good crop growth and high production. Egashira and Yasmin (1990) also evaluated the inherent potentiality of some floodplain soils on the basis of their type and amount of clay minerals and noted that the soils having silt loam texture and high quantity of illite and smectite minerals could be rated as having a 'good' inherent potentiality. Hussain (1998) reported that clay mineralogical compositions in Bangladesh soils have little adverse effect on their management properties. According to him, mixed mineralogy is a boon

and this mineralogy class is the most ubiquitous and, by far, cover the largest area of agricultural land in this country. Quantity of minerals having extreme physical properties such as high COLE (coefficient of linear extensibility) is quite small in this country. It is clear from the present study that the soils have few limitations from their mineralogical aspect.

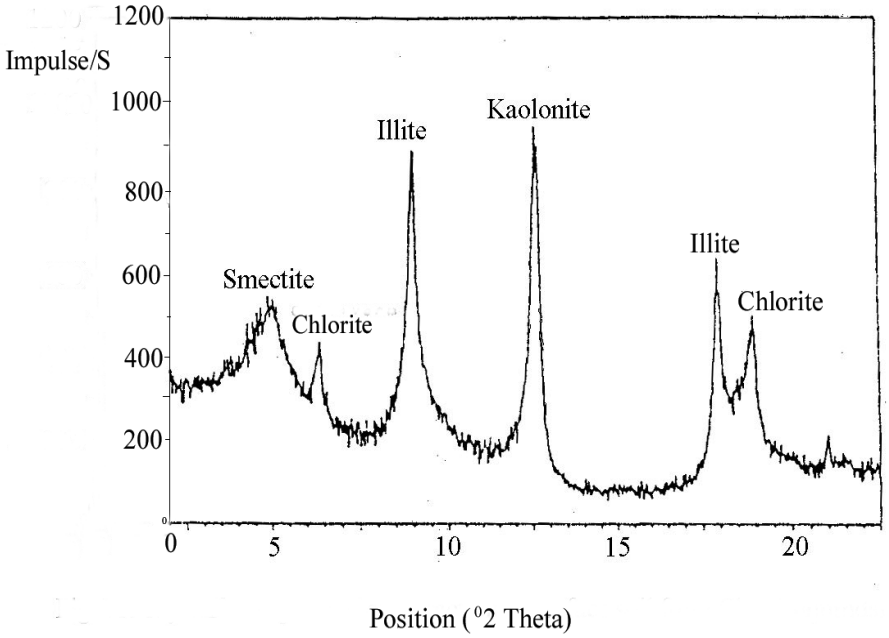

Fig. 5. X-ray diffractogram of clay fraction of a surface soil from Urir Char.

Each person in Urir Char has more agricultural land in his share in comparison to that in the mainland Bangladesh, but there are some serious natural limitations such as low elevation from the sea level and the seasonal occurrences of tropical cyclones. In reality Urir Char has fragile swampy land with some hazards and human habitation there should be discouraged and restricted.

Perhaps, it will not be quite irrelevant to put a note of warning that plans are in place to increase the area of Urir Char by building cross dams (Khan 2007). The adverse impacts of this cross damming system on the nearby lands and islands have not been examined closely and sufficiently. The presently severe and unstoppable erosion along the eastern bank of Bhola may possibly be distantly related to these cross dams, as river morphology is not seriously and efficiently studied in this country of rivers by experts.

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HISTOPATHOLOGICAL EFFECTS OF EXTRACTS OF TWO INDIGENOUS PLANTS, PONGAMIA PINNATA (L.) PIERRE AND CLERODENDRUM VISCOSUM (VENT.) ON THE CAT FISH, HETEROPNEUSTES FOSSILIS (BLOCH)

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Abstract

Piscicidal properties of part extracts (seed, leaf and bark) of two indigenous plants, *Pongamia pinnata* (L.) Pierre and *Clerodendrum viscosum* (Vent.) were studied on *Heteropneustes fossilis* (Bloch). A number of histopathological lesions was observed in the tissues of intestine, gill and liver of the fish which were treated with 50% ethyl alcohol extract of the two plant parts toxicants. The principal degenerative changes in the intestine were: disintegrated serosa, swollen and partially ruptured muscularis layer, vacuolated submucosa, damaged mucosa and distended and coalesced villi. The principal changes in the gills included vacuolated and disrupted epithelial cells and disorganized gill filaments, shrunken and oedamatous distensions in the primary lamellae, swollen, shortened and coalesced secondary lamellae and distorted and disintegrated gill arches. The changes in the liver included compactly or loosely arranged hepatic cells, reduced or swollen hepatic artery, disintegrated hepatic vein, blood coagulated portal vein, dilated and swollen central vein and dispersed sinusoids. The most toxic extract for *P. pinnata* was the leaf extract and for *C. viscosum* was the seed extract in three organs. Among the extracts of three plant parts (seed, leaf and bark) *P. pinnata* was found to be more toxic than *C. viscosum.*

Key words: Histopathology, Plant toxin, *Heteropneustes fossilis*, *Pongamia pinnata*, *Clerodendrum viscosum*

Introduction

To investigate the impact of toxicants on the ecosystem, it is necessary to study the histopathological effects of toxicants on fish organs. Histological examinations which show pathological alterations upon exposure to toxicants have been useful to assess disease problems (Chen and Kumlin 1989), nutritional stress (Kapoor *et al.* 1975 and Williams and Nickol 1989), environmental stress (Srivastava *et al.* 1982) as well as physiological adaptations to salinity changes (Cataldi *et al.* 1987). The fresh water fishes show dissimilar pattern of responses when exposed to toxicants (Mount 1968 and Gardner and Laroche 1973). The extent of damage varies with body parts, nature of the toxicants, medium and duration of exposure (Vijayamadhawan and Iwai 1975). To

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understand the impact of toxicants in aquatic ecosystems, it is necessary to study the histopathological effects of poisons on different fish organs. By histopathological studies we can observe the abnormalities in the tissue structure.

Some information is available on the histopathological effects of plant piscicidal compounds on fish organs. Changes in the tissues as a result of induced toxins i.e., plant piscicides involve only a few reports (Bhatt 1991, 1992, Fafioye *et al.* 2004, Obomanu *et al.* 2007, Verma *et al.* 2007 and Olaifa *et al*. 2008). In Bangladesh, research work on histopathological effect of plant toxicants in various organs of fishes is limited excepting those of Latifa *et al.* (2002), Latifa and Begum (2009) and Nasiruddin *et al.* (2005, 2007, 2008, 2009, 2011).

The present study evaluated the efficacy of the extracts of the two experimental plant parts on the histopathological alterations of the organs- intestine, gill and liver of the test fish, *H.fossilis*, the effects being induced by 50% ethyl alcohol extracts of seed, leaf and bark of *P. pinnata* (Karenja) and *C. viscosum* (Vat) plants. The objective of the study was to see the toxic impact of the dry seed, leaf and bark extracts of the two experimental plants on the histopathological changes of intestine, gill and liver of the fish.

Materials and Methods

During the investigation, the predatory fish, *H. fossilis* was used to ascertain the histopathological changes so as to determine the toxic effects of *P. pinnata* and *C. viscosum* plant part extracts (seed, leaf and bark). In order to extract the toxicants, seed, leaf and bark of *P. pinnata* and *C. viscosum* were well dried in diffused sun heat and preserved in airtight jars. The plant parts were pounded using metallic mortar and pestle, then crushed into fine powder with an electric grinder and sieved through 0.0025 cm^2 mesh size sieve. Required amount of plant part powder was weighed in an electronic pan balance to mix with 50% ethyl alcohol solvents. The desired concentrations of different test solutions were obtained by appropriate dilution of the stock solution (APHA 1976).

The histopathological effects of dry plant part extracts were observed for 24 hours exposure, the tissues were taken from the fishes that were treated with the second highest concentrations of 50% ethyl alcohol extract. The histopathological effects of *P. pinnata* on the tissues were observed from the fishes that were treated with 500 ppm, 1250 ppm and 600 ppm of dry seed, leaf and bark extracts respectively. In case of *C. viscosum* the doses were 300 ppm, 750 ppm and 1000 ppm of dry seed, leaf and bark extracts respectively. A controlled set was similarly maintained in tap water which was free of any kind of extract. Pieces of tissues of intestine, gill and liver were collected from both control and experimental fishes and kept in saline water and fixed in Bouin's fluid overnight. After 18-20 hours, tissues were preserved in 70% ethyl alcohol. Dehydration was done in progressively graded alcoholic series and embedded in melted paraffin wax. The embedded tissues were trimmed and placed into rotary microtome to get transverse

and longitudinal sections of the tissues at 3-4 μ thickness. Dewaxing, hydration and staining with haematoxylin and eosin (aqueous) of the sections were carried out and finally mounted with DPX and cover slip.

The prepared tissues were studied with a compound microscope (x10). The photomicrographs of desired stained histopathological sections were taken with an Axiovert 25 CFL (Germany) microscope fitted with a SLR Canon camera (Japan). Fuji colour films were used for taking the photographs. Microphotographs of desired areas of the sections were taken at magnification factors using eyepiece 10 x and objective 10 x.

Results and Discussion

Histology of intestine of control H. fossilis: The transverse section of intestine of control *H. fossilis* showed the four basic layers: serosa, muscularis, submucosa and mucosa. Serosa was the outer layer of the intestine. It was very thin and made up of single layer of peritoneal cells. Cognominal muscle fibre and circular muscle fibre made the muscularis mucosa. Longitudinal muscle fibre was the thin layer. It was the outer layer of muscularis mucosa. Circular muscle fibre was the thick layer. It was the inner layer of muscularis mucosa. The submucosa consisted of loose connective tissue fibre and simple fold or villi and was made up of single layer of columnar epithelium, which was formed of absorptive and goblet cells. Villi consisted of absorptive and muscle secreting cells (Plate 1A).

Histopathological changes in the intestine of H. fossilis treated with 50% ethyl alcohol extracts of dry seed, leaf and bark of P. pinnata: Treatment with dry seed extract, in the treated intestine serosa was more or less intact but partially damaged; muscularis was swollen to an extent; submucosa was organized but vacuolated and swollen; lamina propria was normal; mucosa was fused, disorganized, disintegrated and coalesced; and villi were slightly swollen and fused with each other, irregular and extensively damaged (Plate 1B). Treatment with dry leaf extract, the serosa was not intact but partially damaged; muscularis was organized and swollen to an extent; submucosa was proliferated and disintegrated very much; lamina propria was reduced; mucosa was disorganized and disintegrated very much; and villi were disorganized, disintegrated, proliferated, elongated and ruptured (Plate 1C). With dry bark extract treatment, serosa was more or less intact but disintegrating in some portion; muscularis was not so organized and slightly swollen and proliferated in some places; submucosa was not organized but proliferated; lamina propria was disintegrated; mucosa was compact, swollen, proliferated and coalesced; and villi were elongated, swollen and also fused with each other (Plate 1D).

Histopathological changes in the intestine of H. fossilis treated with dry seed, leaf and bark extracts of C. viscosum: Treatment with dry seed extract, serosa was more or less intact; muscularis was organized but swollen; submucosa was slightly proliferated but swollen; lamina propria was not organized but swollen; mucosa was shrunken, swollen,

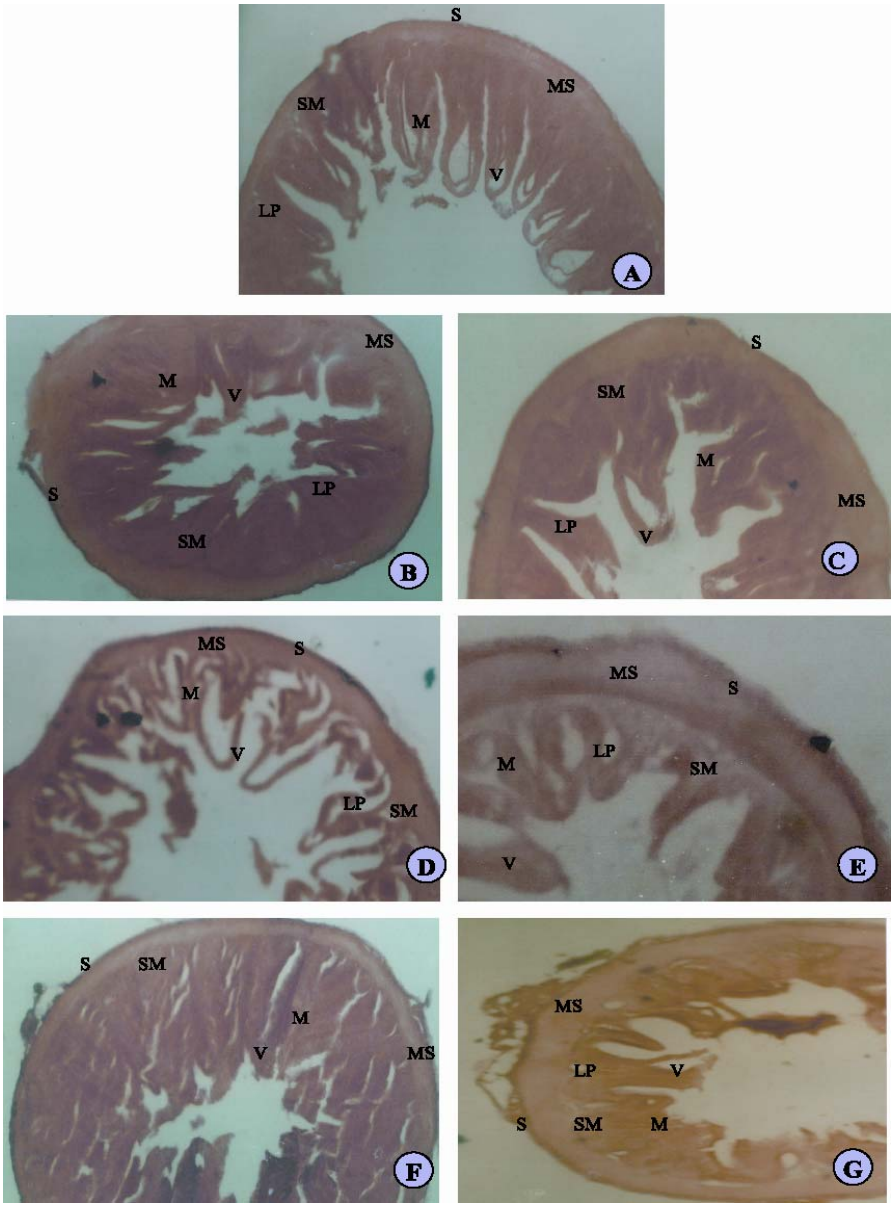

Plate 1. (a) Photomicrographs of the T. S. of the intestine of the *H.fossilis* in control, (b) Treated with dry seed, (c) Dry leaf and (d) Dry bark of *P. Pinnata* and (e) Treated with dry seed, (f) Dry leaf and (g) Dry bark of *C. viscosum* (S- serosa, Ms- Muscularis, Sm- submucosa, M- mucosa, Lp- lamina Propria and V- villi) (x100).

deshaped, irregular and coalesced; and villi were extensively swollen, fused in some places and damaged (Plate 1E). Being treated with dry leaf extract, serosa was more or less intact but proliferated in some portion; muscularis was swollen to an extent; submucosa was slightly extended; lamina propria was normal; mucosa was disorganized and fused in some portion; and villi were compact, slightly swollen and elongated (Plate 1F). After treatment with dry bark extract, serosa was not intact but partially damaged; muscularis was swollen to an extent; submucosa was normal to slightly proliferated; lamina propria was swollen; mucosa was disorganized, swollen and coalesced, some were shrunken and damaged; and villi were disintegrating, shortened, swollen, also fused with each other and damaged (Plate 1G).

Histology of gill of control H. fossilis: The control gill had normal morphology of the hyaline cartilaginous rods in each filament. Four pairs of reddish gill filaments and leaf like primary gill lamellae were found in *H. fossilis.* Primary gill lamellae consisted of secondary gill lamellae. Primary lamellae were borne by gill arches. Single large squamous epithelial cells and numerous mucus cells lay scattered on both sides of the epithelial cells of the normal gill lamellae. The gill lamellae were semicircular, attached basally and partially, overlapped one another on either side of the filament. They were supported by gill rays, which were partly bony and were connected with gill arch and with each other by fibrous ligaments. For gaseous exchange the surface of each gill filament was thrown into numerous small folds, which increased the sum total surface area of the gills (Plate 2A).

Histopathological changes in the gill of H. fossilis treated with dry seed, leaf and bark extracts of P. pinnata: After treatment with dry seed extract, gill filaments were more or less organized; primary gill lamellae were more or less swollen; secondary gill lamellae were swollen, coalesced and destroyed in some regions; gill rays were highly swollen and damaged in some regions; and gill arch was proliferated and distorted with vacuolations (Plate 2B). After treating with dry leaf extract, the gill filaments were more or less organized; primary gill lamellae were more or less swollen; secondary gill lamellae were slightly swollen and coalesced in some regions; gill rays were swollen and damaged; and gill arch was quite normal and organized (Plate 2C). With dry bark extract treatment, gill filaments were not well organized; primary gill lamellae were swollen and moderately shrunken; secondary gill lamellae were coalesced and swollen at the base, atrophied and shrunken at the apex; gill rays were swollen and damaged extensively; and gill arch was damaged and disorganized to some extent (Plate 2D).

Histopathological changes in the gill of H. fossilis treated with dry seed, leaf and bark extracts of C. viscosum: When the fishes were exposed to dry seed extract, gill filaments were swollen, distended and disorganized; primary gill lamellae were swollen and disorganized; secondary gill lamellae were swollen and extended at the base and atrophied and shrunken at the apex; gill rays were damaged at the base and swollen at the

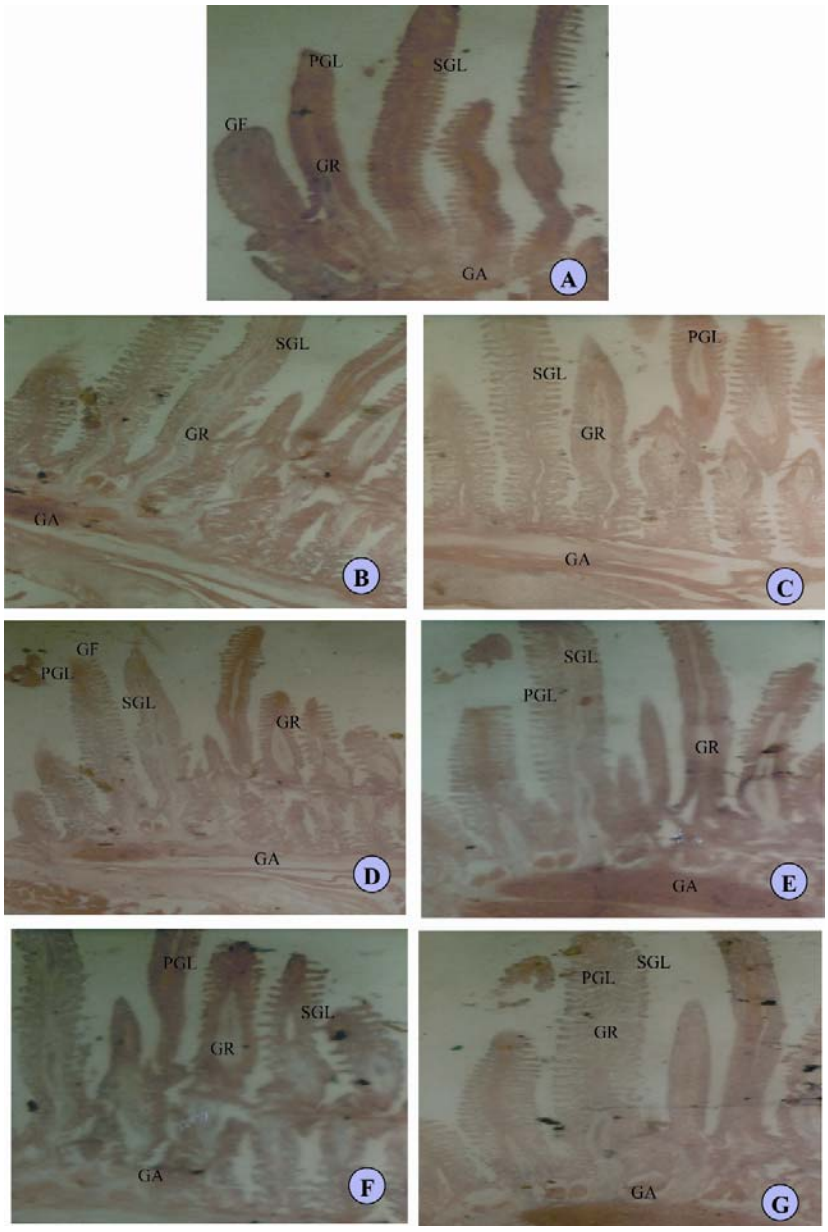

Plate 2. (a) Photomicrographs of the T. S. of the gill of the *H.fossilis* in control, (b) treated with dry seed, (c) dry leaf and (d) dry bark of *P.pinnata* and (e) treated with dry seed, (f) dry leaf and (g) dry bark of *C. viscosum* (Gf- gill filaments, pgl- primary gill lamellae, sgl- secondary gill lamellae, gr- gill ray and ga- gill arch) (x100).

tip; and gill arch was more or less organized (Plate 2E). After treatment with dry leaf extract, gill filaments were disorganized and damaged; primary gill lamellae were extensively swollen and distended; secondary gill lamellae were coalesced and swollen and also destroyed in some areas; gill rays were highly swollen; and gill arch was proliferated, distorted and vacuolated (Plate 2F).With dry bark extract, gill filaments were more or less organized; primary gill lamellae were swollen in some regions; secondary gill lamellae were swollen and coalesced; gill rays were highly swollen in some places; and gill arch was more or less organized (Plate 2G).

Histology of liver of control H. fossilis: Control liver had normal arrangement with components of numerous polyhedral hepatic cells with a prominent nuclei and granular cytoplasm. This structure indicated their secretory nature. But these cells had no definite arrangement. Hepatic cells radiated outwards from the central vein and constituted the parenchyma. Blood sinusoids separated the parenchymatous cells. There were no definite cell cords and no hepatic lobules. Portal vein, hepatic artery, hepatic vein, central vein and bile duct spread throughout the liver. Hepatic ducts and ductules and blood capillaries were seen between the spaces of hepatic cells (Plate 3A).

Histopathological changes in the liver of H. fossilis treated with dry seed, leaf and bark extracts of P. pinnata: Upon exposure to dry seed extract, hepatic cells were compact and condensed; hepatic artery was elongated and disintegrated; hepatic vein was dilated with clotted blood; portal vein was much distended; central vein was with clotted blood; blood vessels were reduced and shrunken; and blood sinusoids were not clear (Plate 3B). Treatment with dry leaf extract, the hepatic cells were swollen, vacuolated and disintegrated; hepatic artery was deshaped, disintegrated and narrowed in size; hepatic vein was deshaped, disintegrated and with coagulated blood; wall of portal vein was destroyed, disintegrated, deshaped and with coagulated blood; central vein was dilated with clotted blood; blood vessels were shrunken and disintegrated; and blood sinusoids were irregularly distributed (Plate 3C). Treatment with dry bark extract, the hepatic cells were swollen and pyknotic with vacuolation of the cytoplasm; hepatic artery was shrunken with coagulated blood; wall of hepatic vein was destroyed; portal vein was dilated with clotted blood; central vein was normal in size; blood vessels were normal; and spaces of blood sinusoids did not fill the cavities suggesting decreased blood supply (Plate 3D).

Histopathological changes in the liver of H. fossilis treated with dry seed, leaf and bark extracts of C. viscosum: When the fishes were exposed to dry seed extract, the hepatic cells were more or less organized, much swollen and pyknotic, some were also vacuolated; hepatic artery was elongated in shape and slightly distended; hepatic vein was with coagulated blood; portal vein was deshaped and disintegrated; blood vessels were normal but some with coagulated blood; and blood sinusoids were irregularly distributed (Plate 3E). Due to treatment with dry leaf extract, the hepatic cells were swollen

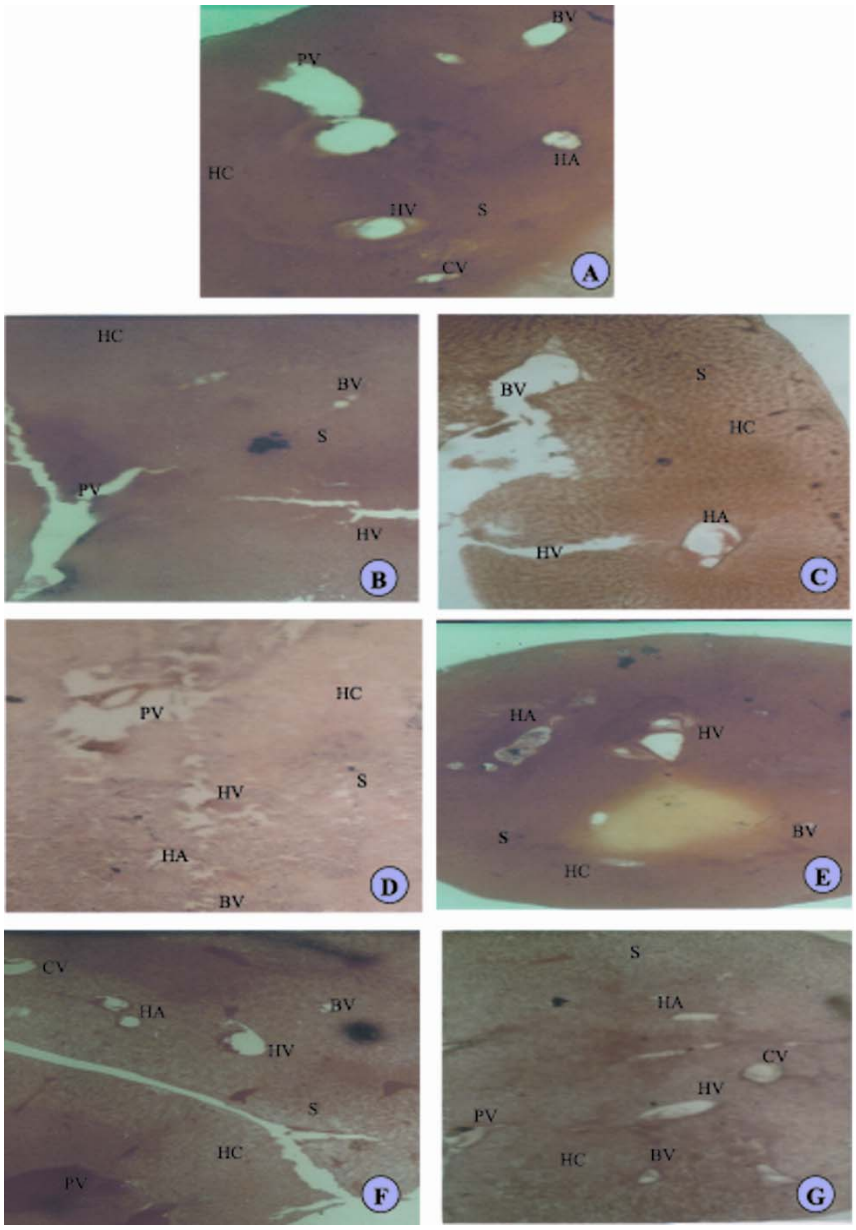

Plate 3. (a) Photomicrographs of the T. S. of the liver of the *H.fossilis* in control, (b) treated with dry seed, (c) dry leaf and (d) dry bark of *p.pinnata* and (e) treated with dry seed, (f) dry leaf and (g) dry bark of *C.viscosum* (hc- hepatic cells, ha- hepatic artery, hv- hepatic vein, pv- portal vein, cv- central veins, s- blood sinusoids and bv- blood vessels) (x100).

and compact; hepatic artery was reduced and distorted in shape and with coagulated blood; hepatic vein was deshaped and disorganized; portal vein was slightly distended with clotted blood; central vein was reduced in size but with clotted blood; blood vessels were shrunken; and blood sinusoids were not organized (Plate 3F). Treatment with dry bark extract, the hepatic cells were very much swollen and pyknotic, some were also vacuolated; hepatic artery was reduced in size and elongated; hepatic vein was reduced with coagulated blood; portal vein was elongated and with coagulated blood; central vein was shrunken with clotted blood; blood vessels were reduced; and blood sinusoids were regularly distributed (Plate 3G).

The histopathological changes in the intestine, gill and liver of *H. fossilis* of the present observation showed similarities with the findings of Nasiruddin *et al.* (2008, 2009 and 2011), when the test fishes were treated with *Acacia auriculaeformis, Mesua ferrea, Madhuca indica* and *Tabarnaemontana divaricata* plant part extracts (seed, leaf and bark). In the present experiments, it was observed that the most affected organs were the gill and intestine and then liver. Necrosis of the intestinal wall might have interfered with the normal function of digestion and absorption. Malfunctioning of intestine might result in total disorder in the digestive system leading to starvation. Gill is one of the most susceptible organs affecting the respiratory and osmoregulatory ability of the fish. It might be said that the increased acute toxicity of the toxicant lead to hyperventilation in fishes which was due to contact of gill surface to the increased amount of toxicant in the water. Furthermore, histopathological changes in the gill structure could impair respiratory function by reducing the surface area for gaseous exchange. Finally, gill alterations are related to gill function disorders, which might affect the physiology or cause death of fish (Smart 1976). It is also inferred that lesion in the gill morphology could lead to functional alteration and interference with fundamental processes such as osmoregulation and gaseous exchange in the toxicant exposed fishes. Liver is the major organ of detoxification. The histopathological changes also lead to a reduction of functional efficiency of the liver, leading to malfunctioning. These disorders in the affected organs cause malfunctioning of the organs as well as the systems resulting in physiological disturbance which in turn might cause death of the fish.

A comparative study of these two plant part extracts on the basis of histopathology was made on *H. fossilis*. While observing the histopathological effects it was seen that the effect of *P. pinnata* leaf extracts showed greatest affectivity followed by seed and bark extracts, whilst the effect of *C. viscosum* seed extracts showed greatest affectivity followed by bark and leaf extracts.

In the present investigation, on the basis of histopathology, the trends of toxicity of the three plant part extracts of *P. pinnata* on the three studied organs were: in the intestine – effects of leaf > seed > bark extracts; in the gill – effect of leaf > seed > bark extracts; and in the liver – effect of leaf > bark > seed extracts. Thus, on comparison, it is seen that the leaf extract was the most effective on the three organs, than seed extract followed by

bark extract. In case of *C. viscosum* the trends of toxicity on the basis of histopathology of the three plant part extracts on the three studied organs were: in the intestine – effect of seed > bark > leaf extracts; in the gill – effect of seed > bark > leaf extracts; and in the liver – effect of seed > leaf > bark extracts. Thus, on comparison, it is seen that the seed extract was the most effective on the three organs followed by bark and leaf extracts.

The observed altered histological changes were as a result of various pathological or toxicological factors. The active ingredients of the plant parts of *P. pinnata* and *C. viscosum* caused histopathological necrosis in the intestine, gill and liver of *H. fossilis* and influenced the physiological activities of the fish resulting in death.

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PREVALENCE OF DIFFERENT PROTOZOAN PARASITES IN PATIENTS VISITING AT ICDDR'B HOSPITAL, DHAKA

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Abstract

Gastrointestinal protozoan parasites are important cause of diarrhoeal illness in Bangladesh. The present study was conducted to observe the prevalence of different human gastrointestinal parasites in patients visiting a Hospital in Dhaka. Several classical techniques were employed to diagnose the causal agents which include *E. histolytica, G. intestinalis, Cryptosporidium sp.* and *A. lumbricoides* etc. A total of 540 samples from outdoor patients of ICDDR'B Hospital at Dhaka was examined where different protozoan parasites including *E. histolytica* (1.11%), *G. intestinalis* (0.37%), and *Cryptosporidium sp*. (4.44%) were detected. Several diagnostic tools were employed that include ELISA, acid fast staining and trichrome staining techniques. Age and sex-specific susceptibility and seasonal incidence pattern were also assessed.

Key words: ICDDR'B, Hospital, Gastro intestinal parasites, Outdoor patients

Introduction

Diarrhoeal diseases are one of the leading causes of mortality and morbidity worldwide including Bangladesh. Dhaka is the capital of the country that has highly densed population. There are many slums with poor sanitation and lack of access to drinking water making the slum-dwellers more vulnerable to different water-borne diarrhoeal illnesses. The elderly people and children are the most susceptible group as thought to have reduced immunity to different pathogens causing diarrhoea.

Since long time, diarrhoeal diseases were considered as a leading public health problem, particularly in children in Bangladesh (Lima and Guirrant 1992). Early studies in rural Bangladesh also indicated persistent diarrhoea in children as a concern for public health (Huttly *et al.* 1989). A separate recent study also indicated that *E. histolytica, C. hominis, C. parvum and G. lamblia* assemblage A infections are important causes of diarrhoeal illness in Bangladesh population. The prospective case-control study was performed which involved a total of 3,646 case patients and 2,575 control subjects with asymptomatic infection (Haque *et al.* 2009). Cryptosporidiosis has long been considered as an important pathogen causing diarrhoea in Bangladesh (Shahid *et al.* 1987). The very

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first report of cryptosporidiosis in Bangladesh indicates possible zoonotic transmission as reported from calves, animal handlers and associated family members at a dairy farm in Savar (near the capital city, Dhaka) (Rahman *et al.* 1985). A prospective study on the urban slum in capital city, Dhaka reported that malnutrition significantly increases the risk of cryptosporidiosis along with some enteropathogen (Mondal *et al.* 2009). In another study, association of enteric protozoan-associated diarrhoeal illness with that of the nutritional status and growth of preschool children in Bangladesh was investigated. (Mondal *et al.* 2006). Several other investigators have reported different aspects of prevalence of *Cryptosporidium* and their harmful effects in the country (Haque *et al.* 2007, Stroup *et al.* 2006 and Khan *et al.* 2004).

Stool examination for intestinal protozoan parasites and helminths is one of the most frequently performed examinations in parasitological laboratories. Most of protozoan parasites usually excrete through stool in both cyst and vegetative stages. The present study was undertaken to find out the prevalence of protozoan parasites of gastrointestinal tract in patients with diarrhoeal illness at a Dhaka hospital while optimising suitable methods for their identification to aid disease diagnosis.

Materials and Methods

The present study was carried out during May 2006 to April 2007 and several classical laboratory techniques were used to detect the protozoan cysts or trophozoites. The stool samples were collected from patients visiting the ICDDRB Dhaka Hospital with complains of diarrhoeal illness. Patient visiting the clinic were supplied with clean sterilized vials for stool collection and all relevant data were recorded. Upon submission, the stool samples were carried to the Parasitology Laboratory, Laboratory Sciences Division (LSD) at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB) Dhaka for microscopic examination. Stool samples were preserved in 10% formal saline and also stored specimens frozen (-20°C) which could not be performed within 72 hours of collection. The stool samples were examined for trophozoid, cysts of protozoan parasites using microscopy and additional diagnostic procedures were followed.

For direct smear analysis, a minute portion of the stool was diluted with normal saline (0.9%) on a slide and later covered with a cover slip. Later the smear was examined under a binocular microscope in different objectives (X10 and X40). As a complementary approach, several classical staining techniques were used during this study. These include trichrome stain and modified acid-fast stain as described elsewhere. In addition ELISA tests were also performed during this study.

Results and Discussion

One of the aims of the present study was determining the prevalence of protozoan cysts or trophozoites from gastrointestinal tract such as *Entamoeba histolytica, Giardia*

intestinalis, Cryptosporidium sp. among the hospital samples. A total of 540 stool samples was examined and analysed to understand the overall prevalence of different protozoa. The findings revealed that *Cryptosporidium sp.* was prevalent in most stool specimens (4.44%) compared to *E. histolytica* (1.11%) and *G. intestinalis* (0.37%) as shown in Fig. 1.

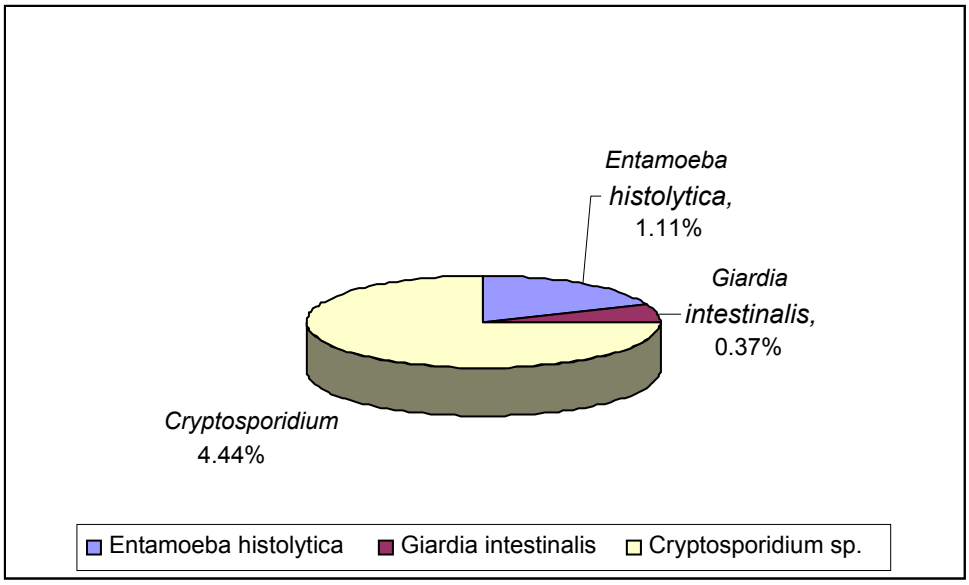

Fig. 1. Percent prevalence of gastrointestinal parasites among patients of ICDDRB hospital.

The sex-specific susceptibility was also assessed during this study which showed that male patients were preferred victim compared to their female counterparts (data not shown). However the age-specific data analysis showed that 21–25 years age group was more vulnerable to *E. histolytica* infection and >35 years age group was more prone to suffer from giardiasis (Figs. 2 and 3). A different scenario was found in cryptosporidiosis, which was found in maximum number among patients of age group 26-30 years and lowest in patients of 11-15 years age group (Fig. 4).

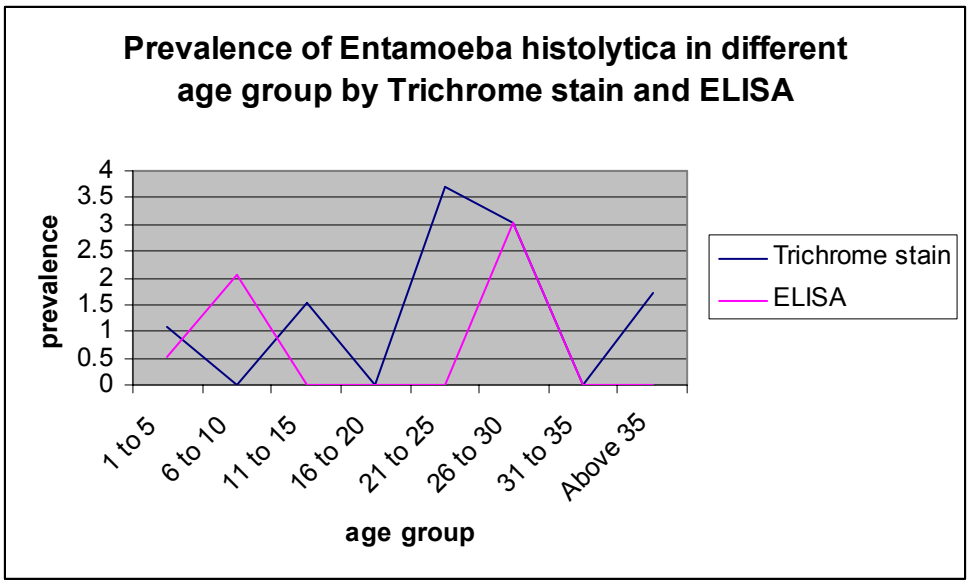

Fig. 2. Prevalence of *E. histolytica* according to different age groups.

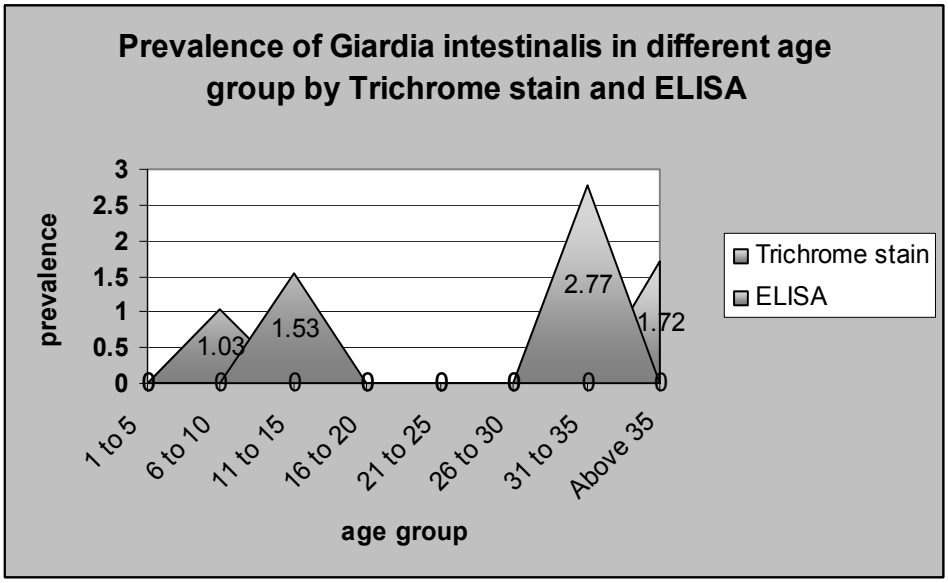

Fig. 3. Prevalence of *G. intestinalis* according to different age groups.

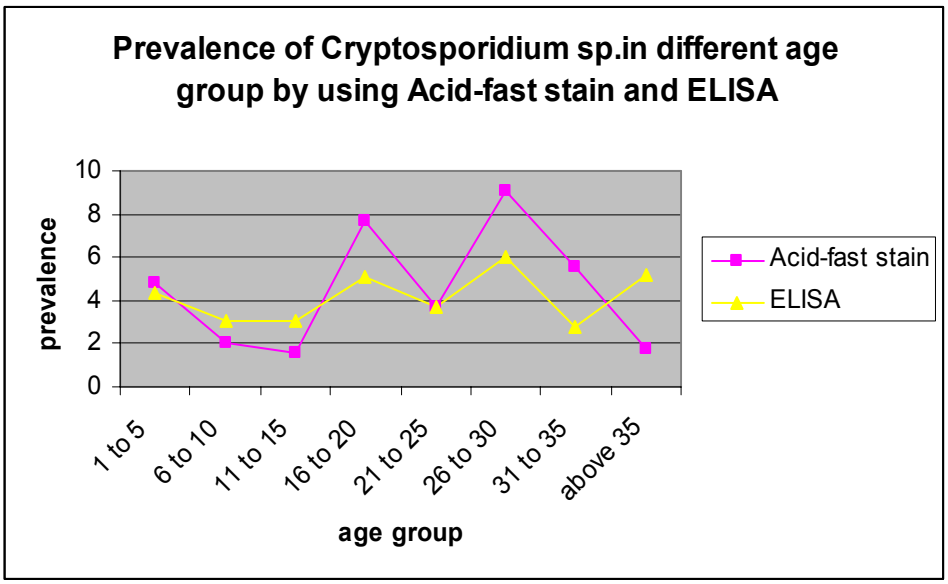

Fig. 4. Prevalence of *Cryptosporidium* sp. according to different age groups.

During the study, seasonal variations in prevalence of protozoan parasites among hospital patients were also investigated. The case records were used to organize the positive cases according to the month of the year. The analysis revealed that June was the most important month of the year when maximum number (11.1%) of cases were found or reported. On the contrary, lowest (2.04%) number of cases were recorded in January which is usually winter season in Bangladesh (Fig.5).

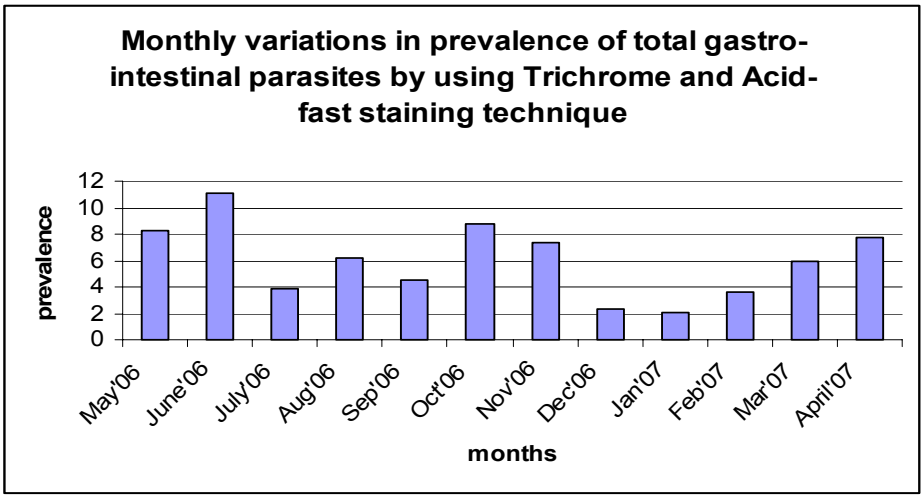

Fig. 5. Monthly variations in prevalence of total gastrointestinal parasites.

Protozoa are among the most important pathogens that can cause infections in immuno-compromised hosts. Over recent decades , parasitic protozoa have been recognized as having great potential to cause waterborne and food borne disease. *Entamoeba histolytica, G. intestinalis* and *Cryptosporidium* spp. are not only three of the most important and common diarrhoea-causing parasitic protozoa, but they often have similar clinical presentations.

Comparatively few studies have been directed to investigate the prevalence and incidence of gastrointestinal protozoan infection in human in Bangladesh. Some of the recent reports have indicated cryptosporidiosis most important among different diarrhoea causing organisms. Surveillance study (over three years of time) from the same hospital recorded 3.5% (n=1949) cases contributed by the same pathogen (Bhattacharya *et al.* 1997). Another year-long study from the same hospital identified 3% (n=1382) incidence of *Cryptosporidium* oocysts in the diarrhoeal stool samples (Rahman *et al.* 1990). During this present study we have recorded 4.44% (n=540) cases of cryptosporidiosis which is somewhat similar with previous reports. Another important observation is the high incidence of positive cases during summer which could be due to increased drinking water intake by the people giving rise to more water-borne outbreaks. Likewise during winter season, the incidence of diarrhoeal illness is reduced and we have recorded least number of cases in winter during this study.

Age and sex-specific vulnerability is an important issue for prevalence of diarrhoeal illness and various factors can contribute to this fact. They may include different biochemical factors like hormones, enzymes and specific proteins or other genetic or immunologic factors along with food habit, culture etc. Further study can highlight these different attributes which can increase our understanding of the transmission of these protozoan pathogens and their further control strategies.

In conclusion, diagnosis of protozoan parasitic cases heavily rely on suitable approach and use of modern molecular tools like PCR, Real time PCR can be more sensitive for accurate identification of pathogens causing diarrhoea in any clinical setting. During present study we have found ELISA approach more sensitive (data not shown) and further optimization of classical staining techniques can be an added advantage to diagnose protozoan parasites efficiently.

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— Short Communication

ECO-ENVIRONMENTAL CHANGES OF HAIL HAOR WETLAND RESOURCES UNDER SYLHET BASIN OF BANGLADESH DUE TO SEDIMENTATION: A GIS APPROACH

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Bangladesh is a country of vast wetland resources covering an area of about seven to eight million hectares, which is about 50% of the total land surface including the flood plains. The greater part of northeast region is taken up by the wetland basin, which comprises the floodplains of the Meghna river tributaries, and is characterized by the presence of numerous large, deeply flooded depressions, between the rivers known as *haors*. The geology, hydrology, soil characteristics, and socio-economic attributes of the *haor* basin also contribute to distinguish it from its adjacent hilly lands. It is believed that the basin is technically active and is undergoing subsidence (Morgan and McIntire 1959). During monsoon the basin receives huge discharges of large number of rivers flowing down from the hills of Assam and Meghalaya and takes the form of a vast inland sea. Sylhet basin covers a large number of *haors* and wetlands and among those *Hakaluki haor, Tanguar haor, Hail haor* etc cover an extensive area (WRI 1990). This basin is an extensive alluvial plain supporting a variety of wetland habitats. It contains about 47 major *haors* and more than 6,000 *beels*, or freshwater lakes, nearly half of which are seasonal (Haque 2008). The major rivers in the study area are the Surma and the Kushiyara and their tributaries Manu, Khowai, Jadukhata, Piyain, Mogra, Mahadao and Kangsha which have formed the dense drainage network of the *haors*. These hilly rivers coming down from the Khasia and Jaintia hills in Meghalaya carry high volumes of water as they come from some of the rainiest places in the world. The main objective of this paper is to investigate the eco-environmental changes of the *hail haor* wetland ecosystem using GIS technology.

Hail haor is located in the *Sylhet* basin between the *Balishira a*nd *Satgaon* hills under the Moulvibazar district of Bangladesh which lies between 24°18'-24°26'N latitude, 91°38'-91°45'E longitude**.** The *haor* originates from the surrounding hill streams. It is a large shallow lake in a saucer-shaped depression, bounded in the south, east and west by low hills and in the north by the plains of the *Manu and Kushiara* rivers. The *haor* is almost encircled by a chain of tea estates and natural forest blocks. The river *Gopla* flows through the wetland in a north-south direction. In order to compare the area of water body, two time series data sources have been taken. The first one is the topographic maps of Messers Capital Air Survey Ltd. Canada during 1974-75 and revised on the ground during 1982-83 and verified during 1987-88 and also 1989-90. The second one is the

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Land and Soil Resources Utilization Guides of SRDI (2003). In addition, aerial photo of 1984 was used for some verification. The scale used for these two data sources was 1:50,000. ARC/GIS version 7.1 was used in preparing the maps and the subsequent spatial analysis.

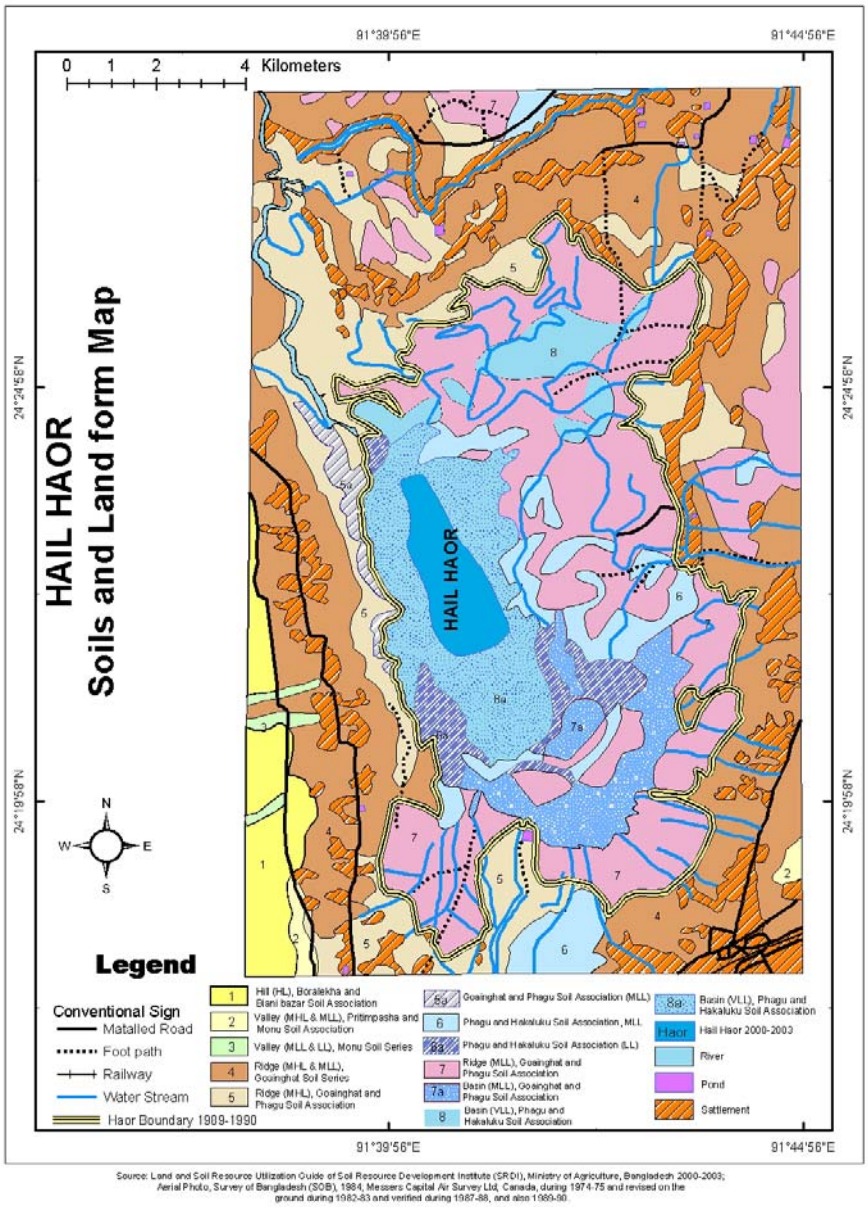

Fig. 1: Soils and landform map of *Hail haor* under Sylhet basin of Bangladesh.

It is quite evident that a huge amount of sedimentation has taken place in the study sites. This phenomenon of sedimentation over a time periods introduces new soil boundaries along with HYV rice crops which enhance the shrinkage of the water body. The sedimentation process is accelerated with the flow of upstream water from the surrounding hilly areas. As a result, Goainghat-Phagu soil association and Phagu-Hakaluki soil association emerge (Fig. 1) where HYV *boro* rice cultivation is gaining popularity with ultimately shrinking the bio-resources of this wetland. The study revealed that the area covered by wetlands in the *hail haor* has been significantly reduced over the period from 1989 to 2003 (Figs. 1 and 2)). According to GIS analysis using PAT (Polygon Attribute Tables) files, total *hail haor* area was about 10,000 ha in 1989, covering the mapping units of 6, 6a, 7, 7a, 8 and 8a (Fig. 1) . In 2003, the area of this water body was reduced and the area becomes 5,200 ha covering the mapping unit 6a, 7a and 8a (Fig. 1). In 2010, this water ecosystem also decreased significantly and the area of which becomes 2000 ha (Fig. 2). The rate of reduction of the water body is alarming but in the dry season, the area of the Hail haor shrinks further which become 900 ha. The study also shows that considerable changes have occurred due to sedimentation and as a result depth and duration of inundation has changed. This change shows a positive impact on agricultural aspects enhancing emergence of new soil boundaries and serious negative impact on eco-environmental aspects i. e. degradation /reduction of wetland ecosystem. Similar findings were also reported by Hoq and Shoaib (2003) and Sultana *et al*. (2009) in accretion of land for edaphic use in some flood prone areas of Bangladesh (Erickson *et al.* 1993). Sedimentation has taken place in low lying areas where grazing land emerges in course of time in some flood prone areas (Ullah *et al*. 2006). Land use mapping of the surrounding areas of the *Hail haor* revealed that 46.0 percent is under tea estates, 28.0 percent is forest land and 13.0 percent is privately managed pineapple or other citrus garden (SRDI 2003). These citrus and pineapple gardens disproportionately contributed to siltation because the local farmers habitually grew pineapple and citrus fruits in rows running up-down slope accelerates soil erosion.

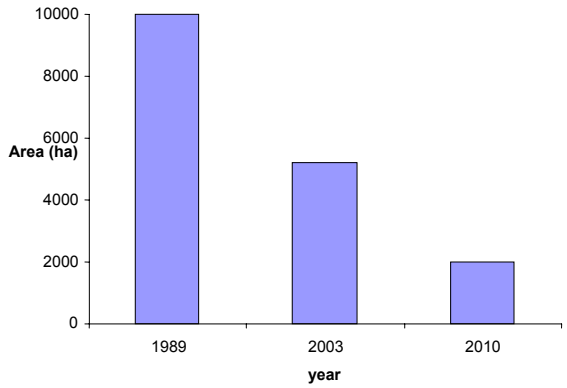

Fig. 2. Decreasing trends of *Hail haor* wetland ecosystem over the two decades.

Poor land management in the neighboring hills results in serious soil erosion where pineapple is grown in lines up-downslope. In a study MACH (2004) revealed that *Hail haor* carried over 2,00,000 m^3 of sediment just in July, 1999. In 2001, silt loads of 22 hill stream carried 50,000 tons, suggesting that the total of 59 active hill stream carried over 1,00,000 tons of silt into *Hail haor* each year. Deposition of 8-15 cm of silt in one year was recorded near the outfalls of the hill streams, suggesting that the *haor* bed is rising on an average by about 5 cm per year (MACH 2004). *Hail haor* is changing rapidly, the fringes of the *haor* are rapidly being filled in and it is apprehended that in near future it may disappear.

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