JOURNAL OF
THE ASIATIC SOCIETY OF BANGLADESH
SCIENCE

ISSN 1016-6947

Vol. 46	No. 2	December 2020

THE ASIATIC SOCIETY OF BANGLADESH

THE ASIATIC SOCIETY OF BANGLADESH

Journal of the Asiatic Society of Bangladesh (Science)

Journal of the Asiatic Society of Bangladesh, Science is published twice a year (June and December comprising one volume) in English. Original research papers dealing with all branches of science with special reference to Asiatic studies by the members of the Society are published. Any non-member of the Society may publish a research paper if it is communicated by a member of the Society.

SUBSCRIPTION

Annual per volume:			Single issue:		
Inland	:	Tk. 500.00	Inland	:	Tk. 250.00
Foreign	:	US$ 40.00	Foreign	:	US$ 20.00

CORRESPONDENCE

All correspondence should be addressed to The General Secretary, The Asiatic Society of Bangladesh, 5 Old Secretariat Road (Nimtali), Ramna, Dhaka-1000, Bangladesh.
Phone : 9576391, E-mail : asbpublication@gmail.com, www.asiaticsociety.org.bd

PRINTED BY

SRL Printing Press
8/12 Babupura, Nilkhet, Dhaka-1205

JOURNAL OF
THE ASIATIC SOCIETY OF BANGLADESH
SCIENCE
(J. Asiat. Soc. Bangladesh, Sci.)

Vol. 46 **No. 2** **December 2020**

CONTENTS

Guidelines to authors

The Journal of the Asiatic Society of Bangladesh publishes original research papers related to any field of sciences from anywhere in the world.

All manuscripts written in English and typed on one side of a good quality paper (A4) with one and a half space and 2.54 cm margins on all sides should be submitted in duplicate (one copy without identity of the authors) to the Editor. However, authors from overseas countries may submit soft copy of the paper. Regarding publication of the paper after review, the decision of the Editorial Board shall be final. All papers accepted by the Editorial Board are subjected to editorial modifications.

The manuscript of a full paper, preferably be limited within 12 typed pages (A4) including tables, figures, graphs, references, etc. For short communication it should not exceed five pages.

The manuscript should contain the following sub-titles in sequence: Title, Abstract, Key words, Introduction, Materials and Methods, Results and Discussion, Acknowledgements, if any, and References. In case of **'Short Communication'** no sub-titles are necessary.

Title: It should be brief and specific. The manuscript will have a separate title page giving name of the paper, authors name and address and a running head.

Abstract: It should not exceed 200 words containing the gist of results only.

Key words: Should not exceed 6 words and it should be after Abstract.

Text: The text of the article should be clear and precise. Introduction should be concise, precise and relevant to the study. Methods already published should be indicated by references. In case of modifications of a method give explicit descriptions. Results should be stated concisely and presented logically referring to tables and graphs. The same data should not be used both for the tables and for the graphs and/or figures. Simple data may be presented as a text instead of Tables and Figs. Each result should be followed by discussion or interpretation (in any case the Results and Discussion must not be separated) with appropriate reference(s). Discussion should deal with the obtained results and related results published nationally and internationally. Concluding remarks (without heading) must be given (that tally with the objective) in the last paragraph. The references in the text should be arranged chronologically.

Units: All measurements must be given in SI (International System) or SI-derived units.

Tables, Graphs and Figures: Number of tables, graphs and figures should be minimum. However, these should be typed separately. The graphs and figures should be drawn giving statistical analysis and properly labelled. The figures and graphs should be properly drawn with bold, solid lines so that these could be reduced up to half or less of the original. The photographs should be submitted on glossy papers or in JPEG format. Symbols in the graphs should be distinct and labels should be readable. Coloured photographs are also accepted.

References: In the text, references should be cited within brackets quoting author's surname and the year of publication in the appropriate place e.g. (Alva and Edwards 1990, Kabir 2005 and Khan *et al*. 2008). References should be arranged alphabetically according to author's surname at the end of the paper. Names of journals and books should be in italics giving edition, year of publication and name of the publishers.

Examples:

Alva, A.K. and D.G. Edwards. 1990. Response of lupin cultivars to concentration of Ca and Al in dilute nutrient solutions. *J. Pl. Nutr.* **13**: 57-76.

Amin, F.B., Z.N. Tahmida Begum and R. Mandal, 2011. Impact of potassium and zinc on growth of cyanobacteria in seasonally flooded rice field of Meghna floodplain. *J. Asiat. Soc. Bangladesh, Sci*. **37**(2): 251-255

Helyar, K.R. and W.M. Porter. 1989. Soil acidification, its measurement and processes involved. *In:* Robson, A.D. (ed.) ‘Soil Acidity and Plant Growth’, Academic press, pp. 61-101.

Kabir, S.M. 2005. Acid sulfate ecosystem and their sustainable management. Unpublished Ph.D. thesis. Department of Soil, Water and Environment. Univ. of Dhaka, Bangladesh.

Khan, H.R., S.M. Kabir, M.M.A. Bhuiyan, F. Ahmed, S.M.A. Syeed and H.-P. Blume, 2008. Response of Mustard to Basic Slag and Aggregate Size Treatments under Modified-Plain-Ridge-Ditch Techniques Used for the Reclamation and Improvement of Cheringa Acid Sulfate Soil. *Soil and Environ*. **27**(1): 1-10.

Khoiyangbam, R.S. and N. Gupta. 2012. Introduction to Environmental Science. Publ. The Energy and Resources Institute, New Delhi.

The author(s) must submit one computer composed hard copy of the revised paper along with the CD in MS Word version 8.

Final galley proofs, if possible, will be sent to authors. No alternations or additions in the text are desirable at this stage.

Off-prints

A contributor of each paper is entitled to get 10 off-prints free of cost.

Declaration

While submitting a paper the individual authors are to sign a declaration to the effect that (i) the work reported has been carried out by them and they prepared the manuscript, (ii) they take public responsibility for the contents of the paper, (iii) the paper has not been published before in any referred scientific journal or has not been submitted to such journal for publication.

Review of books

Books for review should be sent to the Editor in duplicate.

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 117-131, December 2020

GENETIC CHARACTERIZATION OF SWAMP EEL OF BANGLADESH THROUGH DNA BARCODING AND RAPD TECHNIQUES

MD MINHAZUL ABEDIN, MD MOSTAVI ENAN ESHIK, NUSRAT JAHAN PUNOM, MST. KHADIZA BEGUM AND MOHAMMAD SHAMSUR RAHMAN*

Department of Fisheries, University of Dhaka, Dhaka 1000, Bangladesh

Abstract

The freshwater air-breathing swamp eel *Monopterus* spp. are native to the freshwater of Bangladesh and throughout the Indian subcontinent. To identify the different swamp eel species and to check the genetic diversity among them, a total of twelve swamp eel specimens were collected from four districts (Tangail, Bogura, Bagerhat and Sylhet) representing the four division of Bangladesh. The extracted DNA from twelve fish samples was amplified by the PCR technique for DNA barcoding and RAPD analysis. Among 12 specimens, 8 specimens showed a 95-100% similarity with *M. cuchia* species published in the NCBI GenBank database and BOLD system. The studied mct3 (collected from Tangail region), mcs1, mcs2 and mcs3 (collected from Sylhet region) specimens showed about 83% homology with *Ophisternon* sp. MFIV306-10 as per BLAST search; whereas BOLD private database showed 99% similarity with *Ophisternon bengalense* (Bengal eel). From the phylogenetic tree analysis, 8 samples were clustered with *M. cuchia* and 4 samples showed similarity with *Ophisternon* sp. MFIV306-10 and *Ophisternon bengalense* _ANGBF45828-19. In RAPD-PCR based analysis, it was found that the maximum genetic distance (1.6094) was observed between mcba2 and mcs3, while between mct1 and mct2, the minimum genetic distance was 0.000. A total of 192 bands, of which 35 were polymorphic with 17.88% polymorphisms among swamp eel species and the size of the amplified DNA fragments ranged from 250 to 1800 bp. The information on DNA barcoding and RAPD analysis help measure the genetic diversity among swamp eel species, ensure the reliability of the published taxonomic information, and initiate proper management programs to conserve these vulnerable species to meet future export demand.

Key words: DNA barcoding, RAPD, *Monopterus* spp., *Ophisternon* spp.

Introduction

The freshwater air-breathing swamp/mud eel *Monopterus* spp. belong to the family synbranchidae of the order synbranchiformes (Rosen and Greenwood 1976, Shafi and Quddus 1982). It commonly inhabits in the freshwater of Bangladesh and throughout India (Talwar and Jhingran 1991). The freshwater mud eel, *Monopterus cuchia* is a delicious, tasty, nutritional and economically valuable fish. In Bangladesh, only the tribal

*Corresponding author: <shamsur@du.ac.bd>.

people consume this fish but nowadays, it is a commercially exportable product because of its high demand worldwide (Begum *et al.* 2018). These hardy, pollution resistant species are carnivorous, nocturnal and like animal-based foods, such as small fishes, mollusks and worms (Nasar 1997). This species can also adapt to various adverse situations like low oxygen concentration, high temperature and shallow water (Rahman 2005). *Ophisternon* spp. is known as swamp eel belongs to the family of synbranchidae. Species of this family can breathe in the air, making them capable of surviving in low-oxygenated water and moving between ponds during the rainy season. Swamp eels are naturally distributed throughout India, Sri Lanka, Indonesia, Philippines and New Guinea (Roy *et al.* 2016). *Monopterus* are exported every year from Bangladesh to different countries like China, Japan, South Korea, Malaysia, Hong Kong, Thailand, Europe and earn 14 million US dollar in 2014 (DoF 2016). It is now over exploited from the natural water-bodies mainly for being exported (Begum *et al.* 2018). The swamp eel *viz. M. cuchia* and *O. bengalense* have been enlisted in the red list of threatened fishes of Bangladesh as a vulnerable (IUCN 2015). Therefore, it is very much important to identify the fish species accurately and unambiguously for conservation purposes. Genetic data are very important in assessing the gene flow between populations, which are also critical for maintaining genetic diversity. DNA based technologies have been recognized useful for their application in species identification (Palumbi and Cipriano 1998), monitoring fisheries (Menezes *et al.* 2006) and aquaculture (Liu *et al.* 1998). Genetic data can also be interpreted in such a way to set up conservation priorities. In many instances, genetics may be the best way to decide whether the species need conservation actions under the vulnerable category. Genetic data of threatened species can help to decide restocking of the species through translocation (Teske *et al.* 2003) by implementing a planned program based on quantitative genetics, life history data and DNA variation (Epifanio 2003). DNA barcoding is an identification tool in which a small fragment of mitochondrial genome acts as a 'DNA barcode' to identity an organism at its species level. DNA barcoding is suitable because the intra-species variations are lesser than inter-species variations (Peninal *et al.* 2017). DNA barcoding has the advantage of identifying the species from any sort of sample like whole fish, fillets, fins, juveniles, larvae, eggs, or tissue fragments. DNA barcoding can be a practical tool for the reliable identification of specimens for solving taxonomic problems and providing an in-depth analysis of gene flow (Smith *et al.* 2008).

Among DNA based systems, the RAPD-PCR procedure is simple and fast. Without prior information on DNA arrangements, it is conceivable to distinguish hereditary variety utilizing RAPD strategy. The technique, also known as arbitrary primer-polymerase chain reaction (AP-PCR), has been widely used for revealing the intraspecific variation (Basagoudanavar *et al.* 1998). We studied the genetic diversity and relationship among the swamp eel specimens collected from the four natural habitats of Bangladesh (Tangail, Bagerhat, Bogura and Sylhet). The present study was aimed at the molecular identification and characterization of swamp eel species lives in the natural water bodies of different regions of Bangladesh based on DNA barcoding and RAPD techniques.

Materials and Methods

Twelve specimens of swamp eel were collected from four districts representing four divisions of Bangladesh *viz*., Bogura of Rajshahi division, Tangail of Dhaka division, Sylhet of Sylhet division, Bagerhat of Khulna division and were subjected to molecular study. Fishes were examined while still fresh. Total length (TL) and weight were measured. The primary identification was made according to Shafi and Quddus (1982) and Talwar and Jhingran (1991).

Fresh muscle samples were collected and preserved at -20^{0}C. DNA from the muscle of 12 swamp eel specimens was extracted using a commercial kit (Maxwell 16 MDx Research Instrument, Promega, USA) according to manufacturer’s instruction. The absorbance of purified DNA by NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., USA) was checked at 260 nm. Extracted DNA used as a template for PCR amplification of a 650 bp fragment from the 5´ region of COI gene using the forward and reverse primer FishF1 (TCAACCAACCACAAAGACATTGGCAC) and FishR1 (TAGACTTCTGGGTGGCC AAAGAATCA) (Ward *et al.* 2005) and the PCR reaction mix for 25 µl contained GoTaq® G2 Hot Start Green Master Mix, 2X 12.5 µL, forward primer 1 µl, reverse primer 1 µl, DNA template 1 µl, nuclease free water 9.5 µl and thermal cycling conditions were followed as reported by Wong and Hanner (2008). Amplified DNA was then purified using Wizard PCR SV Gel and PCR Clean-Up System kit (Promega, USA) prior to sequencing. Sequencing of purified PCR products was performed through commercial service of the First Base laboratory, Malaysia.

COI sequences were identified by searching the GenBank database using the BLASTN algorithm (https://blast.ncbi.nlm.nih.gov) and by BOLD (http://www.boldsystems.org) identification engine to search DNA barcode records within BOLD (Ratnasingham and Herbert 2007). A Neighbor-Joining method (Saitou and Nei 1987) was used to generate tree in MEGA X (Kumar *et al*. 2018) software to observe the phylogeny among the studied specimens.

Among 10 primers tested for RAPD amplification, six primers [Operon Technologies Inc., USA (5) and University of British Columbia, Canada (1)] named OPA12 (TCGGCGATAG), OPC5 (GATGACCGCC), OPH4 (GGAAGTCGCC), OPG3 (AGTCGGCCCA), OPY7 (AGAGCCGTCA) and UBC4 (CCTGGGCTGG) exhibited good quality banding patterns and sufficient visibility. The bands of these six primers were further considered for the final RAPD analysis.

The PCR reaction mix for 25 μl contained Nuclease-Free Water 14, GoTaq® G2 Hot Start Green Master Mix 2X 7.5 μl, primer, 10 μM 1.5 μl, template DNA 2 μl. PCR amplification was done in an oil-free thermal cycle for 40 cycles after initial denaturation at 95^0C for 5 min, denaturation at 95^0C for 30 s, annealing at 34^0C for 30 s, extension at 72^0C for 1 min and a final extension at 72^0C for 5 min.

DNA bands were observed on UV-trans illuminator and photographed by a gel documentation system, after 1% agarose gel electrophoresis. The photographs were critically analyzed on the basis of presence (score 1) or absence (score 0) of the band, band size and overall polymorphisms of the bands (Kabir *et al.* 2017). The value of pairwise genetic distances was analyzed by using computer software "POPGENE 32" (version 1.31) among studied specimens of swamp eel of four geographically different regions from the data of six RAPD primers.

Results and Discussion

A total of twelve samples were collected from four different regions of Bangladesh, and the total length (TL) and the weight of the fish samples were measured (Table 1).

The extracted DNA from twelve fish samples was amplified by the PCR technique for Cytochrome c Oxidase subunit 1 (COI) gene through COI specific primers FishF1 and FishR1. All the samples showed a positive band at 650 bp (Fig 1).

The resulting PCR products were sequenced to generate full-length DNA barcodes of 678 bp in length, with no detectable insertions, deletions. Table 2 provides information about *viz.* maximum score, percentage of query coverage, E value, percentage identity, NCBI GenBank accession number, BOLD accession number of the matched sequences and the GenBank accession number of our submitted sequences of 12 swamp eel specimens.

Among 12 samples, 8 samples showed 95-100% homology with *M. cuchia* species that were already deposited in NCBI GenBank database. The studied mct3 (collected from Tangail region), mcs1, mcs2 and mcs3 (collected from Sylhet region) specimens showed 83% homology with *Ophisternon* spp. Four (mct3, mcs1, mcs2 and mcs3) were matched with *Ophisternon bengalense* ANGBF45828-19 under BOLD private database whereas mct1, mct2, mcbo1, mcbo2, mcbo3, mcba1, mcba2 and mcba3 were matched with *M. cuchia* BOLD accession number AAF8878. Finally, among 12 specimens, eight samples showed their genetic identity with *M. cuchia* known as freshwater mud eel and four samples showed similarity with *Ophisternon bengalense* known as Bengal eel or Asian swamp eel.

Table 1. Length-Weight measurements of swamp eel specimens collected from four different regions.

SI	Collection place	T.L (cm)	Weight (gm)
1-3	Tangail	41	60
		66	250
		56.5	350
4-6	Bogura	58.5	510
		61	520
		60	350
7-9	Bagerhat	51	490
		25	170
		56	500
10-12	Sylhet	45	250
		56	430
		53	360

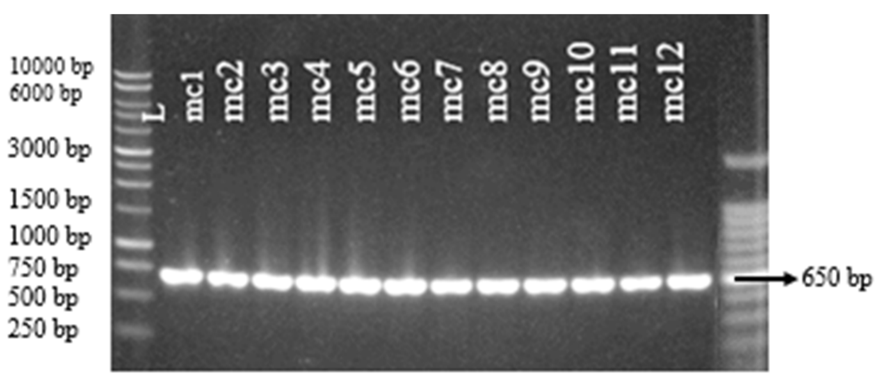


Fig. 1. PCR amplification of COI gene using FishF1 and FishR1 primers. L denotes 1 kb ladder.

COI gene sequence of mct1, mcbo1, mcba1 were compared with available sequences from the GenBank database and it has been found that these specimens were *M. cuchia*. After comparing these sequences with *M. cuchia* NBFGR: MC8069B_FJ4595, 37 (6.7%) out of 555 nucleotide bases found polymorphic (Fig. 2).

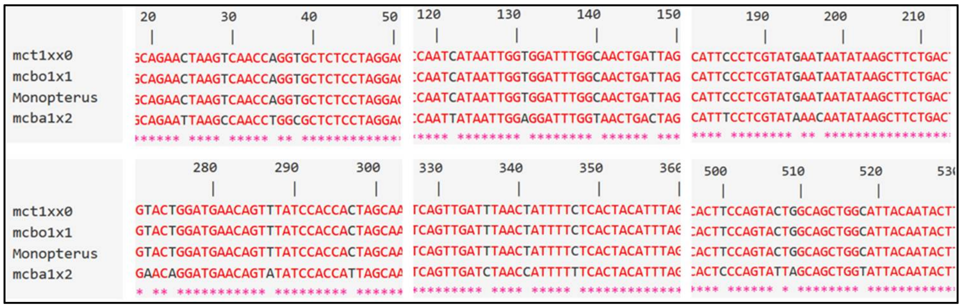


Fig. 2. Portion of Multiple Sequence Alignment (MSA) of COI gene fragment of swamp eel sample mct1, mcbo1, mcba1 and *M. cuchia*_NBFGR_MC8069B_FJ4595. Black nucleotides among the red indicate polymorphic sites.

A comparison of COI sequence of mct3, mcs1, mcs2 and mcs3 with available sequence from the GenBank database reflects that these specimens were under the genus *Ophisternon*. After comparing these sequences with *O. bengalense*_ANGBF45828-19, 74 (14.15%) out of 523 nucleotide bases found polymorphic (Fig. 3).

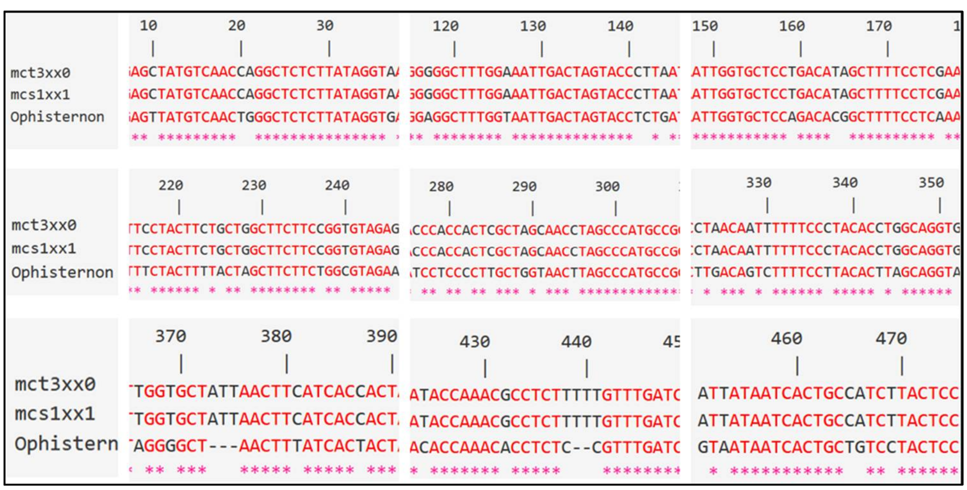


Fig. 3. Portion of Multiple Sequence Alignment (MSA) of COI gene fragment of swamp eel samples mct3, mcs1 and *O. bengalense*_ANGBF45828-19. Black nucleotides among the red indicate polymorphic sites.

Table 2. Identification of swamp eel specimens using the GenBank database and BOLD systems based on 678 base pairs of COI gene sequences.

Sl No.	Specimen ID	Study area	Description	Max Score	Query coverage (%)	E value	Identity (%)	GenBank Acc. no.	BOLD Accession	GenBank Acc. no. of studied sequence
1	mct1	Tangail	*M. cuchia* voucher M6	1026	100	0.0	100	MN520010.1	AAF8878	MT387298
2	mct2	Tangail	*M. cuchia* voucher M6	1026	100	0.0	100	MN520010.1	AAF8878	MT387299
3	mct3	Tangail	*Ophisternon bengalense* ANGBF45828-19	99.27% similarity as per private BOLD database				KP729589.1 unverified	ANGBF45828-19	MT387300
			Ophisternon sp. MFIV306-10	616	96	7e-172	83.72	JN313622.1	-	
4	mcbo1	Bogra	*M. cuchia* voucher M6	1026	100	0.0	100	MN520010.1	AAF8878	MT387301
5	mcbo2	Bogra	*M. cuchia* isolate B-3	1026	100	0.0	100	MN520004.1	AAF8878	MT387302
6	mcbo3	Bogra	*M. cuchia* isolate *B*-3	1026	100	0.0	100	MN520004.1	AAF8878	MT387303
7	mcba1	Bagerhat	*M. cuchia* IL15-0035	854	100	0.0	94.41	LC190133.1	AAF8878	MT387304
8	mcba2	Bagerhat	*M. cuchia* IL15-0035	854	100	0.0	94.59	LC190133.1	ADE0718	MT387305
9	mcba3	Bagerhat	*M. cuchia* NBFGR:MC8069	1026	100	0.0	100	FJ459510.1	AAF8878	MT387306
10	mcs1	Sylhet	*Ophisternon bengalense* ANGBF45828-19	99.45% similarity as per private BOLD database				KP729589.1 unverified	ANGBF45828-19	MT387307
			Ophisternon sp. MFIV306-10	621	96	2e-173	83.87	JN313622.1	-	
11	mcs2	Sylhet	*Ophisternon bengalense* ANGBF45828-19	99.45% similarity as per private BOLD database				KP729589.1 unverified	ANGBF45828-19	MT387308
			Ophisternon sp. MFIV306-10	621	96	2e-173	83.87	JN313622.1	-	
12	mcs3	Sylhet	*Ophisternon bengalense* ANGBF45828-19	99.27% similarity as per private BOLD database				KP729589.1 unverified	ANGBF45828-19	MT387309
			Ophisternon sp. MFIV306-10	616	96	7e-172	83.72	JN313622.1	-	

The phylogenetic tree (Fig. 4) indicates that mct1, mct2, mcbo1 are closely related to mcba3, mcbo2 and mcbo3 which confirming their similarity with the allocated species *M. cuchia.* On the other hand, the phylogenetic tree also supported the taxonomic position of mct3, mcs1, mcs2 and mcs3 as similar with the species under the genus *Ophisternon*; moreover, it can be said that these specimens are close to the species *Ophisternon bengalense*, but may be another species of the genus *Ophisternon* that is still not available in the GenBank or BOLD public database.

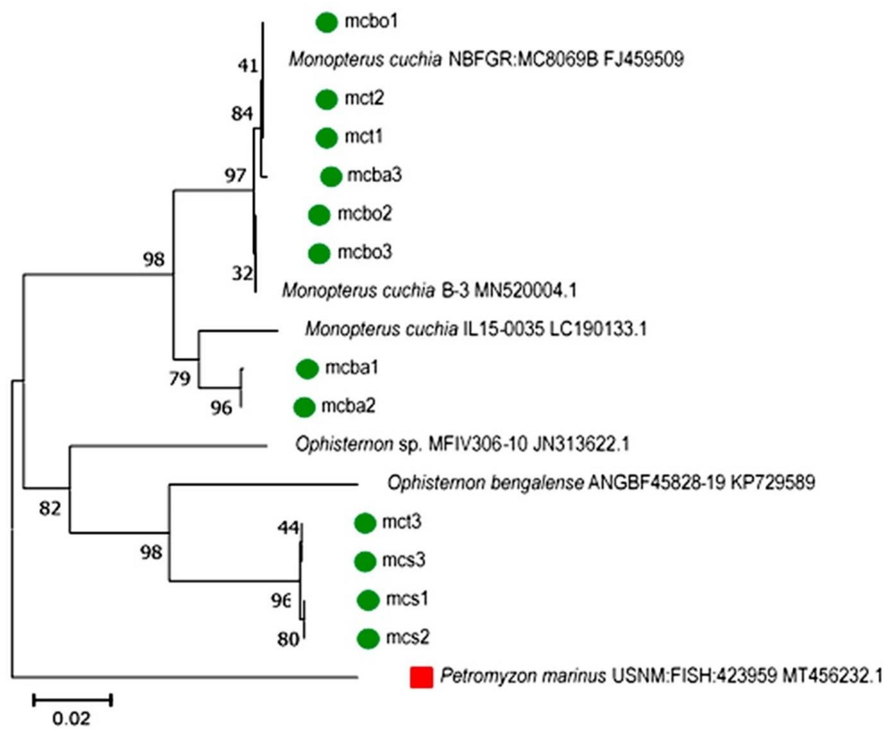


Fig. 4. Molecular Phylogenetic analysis by Neighbor-Joining method of studied 12 swamp eel sequences and downloaded 6 sequences from NCBI GenBank. *Petromyzon marinus* was used as an outgroup. The optimal tree with the sum of branch length =0.36912245 is shown. The analysis involved 18 nucleotide sequences. There were a total of 678 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018).

RAPD based genetic diversity: DNA profiling and data scoring were studied separately for six primers that were used for RAPD analysis. The bands of six primers were seen in different levels of the length of DNA ranged from 250 to 1800bp. A total of 192 bands, of which 35 were polymorphic with 17.88 average percentage of polymorphisms among species of swamp eel (Table 3). Primer OPH-04 gave the RAPD profile with the highest number of bands. The number and size of each unique band respective to each primer were shown in Table 3 and Fig. 5.

Table 3. Compilation of RAPD analysis data of swamp eel from four different locations of Bangladesh.

Primer code	Size ranges (bp)	Total bands	Number of polymorphic bands	Polymorphisms (%)	Average % polymorphism
OPA12	250-750	20	03	15.00	
OPY-07	375-1100	25	05	20.00	
OPG-03	300-1400	43	08	18.61	
OPH-04	250-1800	47	08	17.02	17.88
UBC-04	700-1000	12	02	16.67	
OPC-05	450-1100	45	09	20.00	
Grand total		192	35		17.88

The value of pair-wise genetic distances was analyzed by using computer software “POPGEME 32” (version 1.31). The genetic distances ranged between 0.0000 and 1.6094 (Below diagonal of Table 4) among 12 specimens of swamp eel. The maximum genetic distance (1.6094) was observed among mcs3 and mcba2, while between mct1 and mct2, the minimum genetic distance was 0.000.

After the analysis of RAPD fragments through computer software “POPGENE32” (version 1.31), the values of pair-wise genetic identity were ranged between 0.2000 and 1.000 (Above diagonal of Table 4). The highest genetic identity (1.000) was obtained between mct1 and mct2, while the lowest one (0.2000) was found between mcba2 and mcs3.

UPGMA based cluster analysis using linkage distance was done to reveal the phylogenetic relationships among twelve specimens of swamp eel genotypes examine under the current study (Fig. 6).

In the present study, UPGMA dendrogram showed that sample mct1 and mct2 were close to mcba3, whereas sample mcbo1, mcbo2 and mcbo3 were clustered together (Fig. 6). Specimen mcba1 and mcba2 were in the same cluster separated from other specimens. Sample mcs1, mct3, mcs2 and mcs3 formed a completely separate cluster away from others that is also supported by the DNA barcode data as these four specimens identified as *Ophisternon* spp.

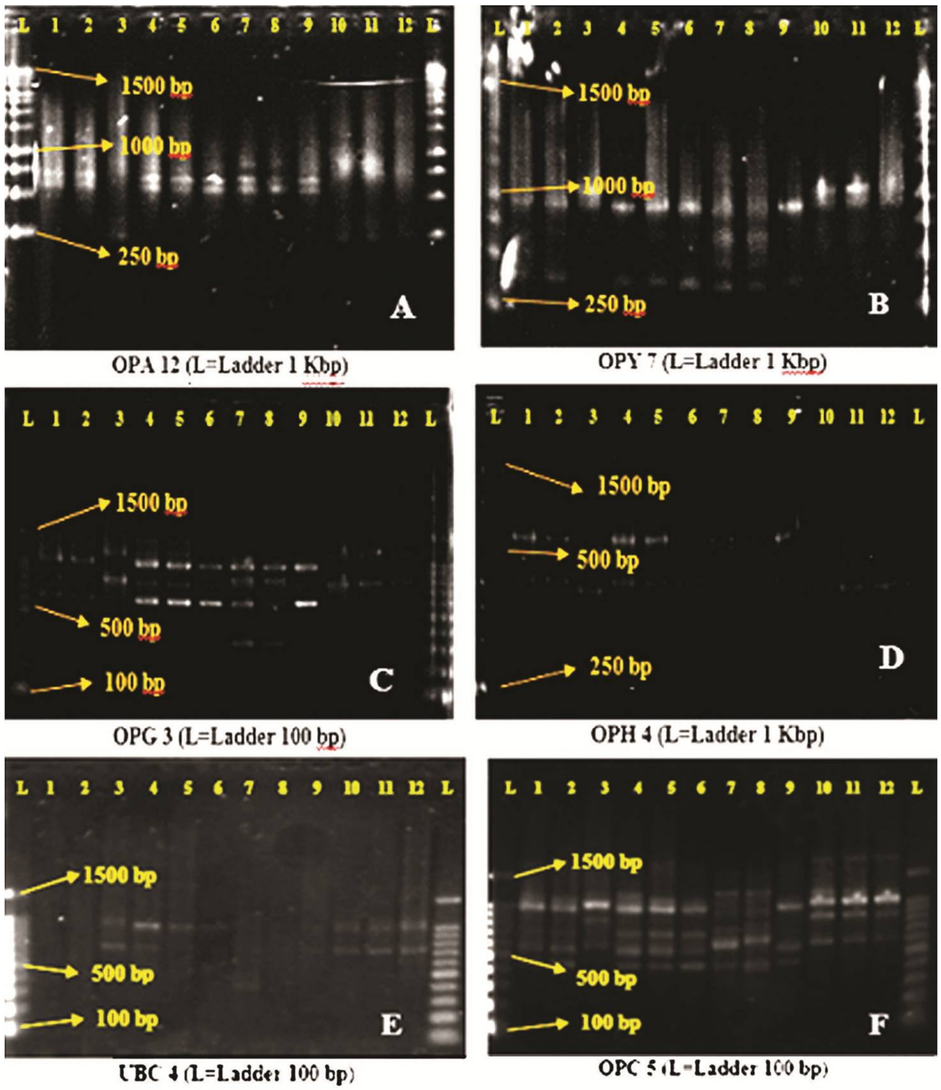


Fig. 5. RAPD analysis with six primers of twelve swamp eel samples. (Primers; A. OPA-12, B. OPY-07, C. OPG-03, D. OPH-04, E. UBC-04, F.OPC-05, where 1- 3=Tangail, 4-6=Bogura, 7- 9= Bagerhat and 10-12=Sylhet samples in order).

Table 4. Genetic distance and genetic identities among 12 samples of swamp eel collected from four different regions of Bangladesh.

POP ID	Sample ID	1	2	3	4	5	6	7	8	9	10	11	12
		mct1	mct2	mct3	mcbo1	mcbo2	mcbo3	mcba1	mcba2	mcba3	mcs1	mcs2	mcs3
1	mct1	***	1.0000	0.3429	0.7714	0.7714	0.8286	0.5143	0.5429	0.8286	0.3429	0.4000	0.3714
2	mct2	0.0000	***	0.3429	0.7714	0.7714	0.8286	0.5143	0.5429	0.8286	0.3429	0.4000	0.3714
3	mct3	1.0704	1.0704	***	0.4000	0.4000	0.4000	0.2571	0.2286	0.4000	0.8286	0.8857	0.9143
4	mcbo1	0.2595	0.2595	0.9163	***	0.9429	0.9429	0.5143	0.4857	0.7714	0.4000	0.4571	0.4286
5	mcbo2	0.2595	0.2595	0.9163	0.0588	***	0.9429	0.5143	0.4857	0.8286	0.4000	0.4571	0.4286
6	mcbo3	0.1881	0.1881	0.9163	0.0588	0.0588	***	0.5143	0.4857	0.8286	0.3429	0.4000	0.4286
7	mcba1	0.6650	0.6650	1.3581	0.6650	0.0588	0.6650	***	0.9714	0.5714	0.2571	0.2571	0.2286
8	mcba2	0.6109	0.6109	1.4759	0.7221	0.7221	0.7221	0.0290	***	0.6000	0.2286	0.2286	0.2000
9	mcba3	0.1881	0.1881	0.9163	0.2595	0.2595	0.1881	0.5596	0.5108	***	0.3429	0.4000	0.4286
10	mcs1	1.0704	1.0704	0.1881	0.9163	0.9163	1.0704	1.3581	1.4759	1.0704	***	0.8857	0.8571
11	mcs2	0.9163	0.9163	0.1214	0.7828	0.7828	0.9163	1.3581	1.4759	0.9163	0.1214	***	0.9143
12	mcs3	0.9904	0.9904	0.0896	0.8473	0.8473	0.8473	1.4759	1.6094	0.8473	0.1542	0.0896	***

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

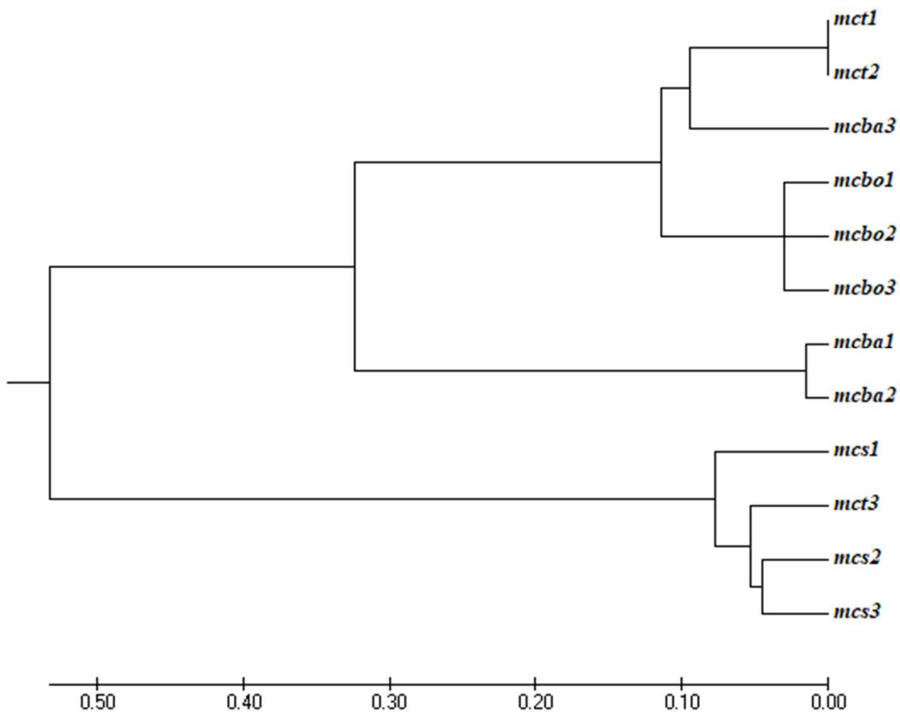


Fig. 6. UPGMA dendrogram based on RAPD analysis among 12 swamp eel samples. The dendrogram was constructed in MEGA7.

Bangladesh has vast water areas that are rich in fish and shellfish biodiversity (Rahman *et al.* 2016). Still many of our indigenous aquatic species are listed in the IUCN Red Book as endangered species. *Monopterus cuchia* and *Ophisternon bengalense* are red listed vulnerable species in Bangladesh (IUCN 2015). In the past, the identification of fish species was especially based on morpho-taxonomy (Kon *et al.* 2007). Expert taxonomists play an important role in the taxonomic classification of organisms and estimate species diversity, but this process is not enough to accurately identify the species. DNA barcoding provides rapid and accurate identification that can be used by the non-experts (Hebert *et al.* 2003). Lakra *et al.* (2016) worked on DNA barcoding of Indian freshwater fishes and observed the highest genetic distance (27.50%) between *Monopterus cuchia* and *Macrognathus pancalus*, which belongs to the order of Synbranchiformes. Java and Dasgupta (2007) worked on the morphometry of *M. cuchia* from the New Alluvial Zone of West Bengal. They concluded that the morphometric characters of *M. cuchia* showed a positive increase with the increase in the length of the fish. Matsumoto *et al.* (2010) used mitochondrial DNA (mtDNA) sequencing to see the phylogenetic relationships and genetic diversity of *M. albus*. They found three genetically distinct clades based on geographical populations [China–Japan (Honshu + Kyushu), Ryukyu Islands, and Southeast Asia clades].

In this study, molecular identification of swamp eel species was performed by DNA barcoding. A total 12 samples were sequenced, among them eight samples were genetically identified as *M. cuchia* and four samples showed similarity with *Ophisternon bengalense* known as swamp mud eel. Four sequences *viz.* mct3, mcs1, mcs2 and mcs3 could not be identified in the public BOLD species reference database. Steinke *et al.* (2009) found that analysis of COI gene from 391 species from 8 coral reef locations revealed 98% of these species exhibit distinct barcode clusters, allowing accurate identification.

In RAPD analyses specimens of swamp eel produced different banding pattern with six primer combinations. The average polymorphism was about 17.88% revealing a low range of polymorphisms among the two populations. Miah *et al.* (2013, 2016) used three RAPD primers to see the genetic diversity of freshwater mud eel, *M. cuchia* and found 100% intra-specific polymorphism among swamp eel. Yin *et al.* (2005) evaluated the genetic variation of the wild and cultured swamp eels *M. albus* using RAPD technique and found 44.79% and 36.5% polymorphism, respectively. Studies on RAPD fingerprinting of two eel-loaches, *Pangio piperata* and *Pangio filinaris* estimated 83% and 60% polymorphism, respectively in 82 bands generated from five RAPD primers (Ruzainah *et al.* 2003). Wei *et al.* (2006) worked on five populations of rice field eel, *M. albus* in China mainland and found a low level of polymorphism (ranged from 29.51 to 66.39%). However, two other populations showed a high proportion of polymorphic loci (82.79%).

The study was conducted on the genetic characterization of swamp eel of Bangladesh through DNA barcoding and RAPD techniques. The swamp eel can meet the increasing demand for animal source protein in Bangladesh and earn foreign currencies that can help our national economy. The information on DNA barcoding to identify freshwater mud eel can help to know the genetic diversity of them, ensure the reliability of the published taxonomic information, and initiate proper management programs to conserve this vulnerable species. The diversity of mud eel of Bangladesh can expand our export limit, which may help in employment generation in the production and marketing of fisheries and aquaculture sectors.

References

Basagoudanavar, S.H., J.R. Rao, R.K. Singh and G. Butchaiah. 1998. Random amplification of polymorphic DNA fingerprinting of *Trypanosoma evansi*. *Vet. Res. Commun.* **23**: 249-255.

Begum, N., M.M.H. Pramanik, M.G.S. Riar and Y. Mahmud. 2018. Effect of selected feeds, feeding frequency and density on growth and survival on mud eel *Monopterus cuchia* (Hamilton 1822) larvae. *Int. J. Fish. Aquat. Stud*. **6**(4): 562-569.

DoF. 2016. National Fish Week 2016. Department of fisheries, Ministry of Fisheries and Livestock, Bangladesh. pp. 76.

Epifanio, J.M. 2003. Genetic variation: nuclear DNA. In: Brown B.L., Hallerman E.J., editors. Genetic Principles and Practices for Fisheries Scientists. *American Fisheries Society;* Bethesda, Maryland: 101-126

Hebert, P.D.N., A. Cywinska, S.L. Ball and J.R. Waard. 2003. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond.* **270**: 313-321.

IUCN Bangladesh. 2015. Red List of Bangladesh Volume 5: Freshwater Fishes. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh, pp. 136.

Java, D. and M. Dasgupta. 2007. Morphometric study of the mud eel *Monopterus cuchia* (HAM.) From the new alluvial zone of West Bengal. *Rec. Zool. Surv. India.* **107**(2): 21-30.

Kabir, M., M.S. Rahman, M. Begum and M.H. Faruque. 2017. Genetic diversity by RAPD in four populations of Rohu *Labeo rohita*. *Croatian J. Fish.* **75**(1): 12-17.

Kon, T., T. Yoshino, T. Mukai and M. Nishida. 2007. DNA sequences identify numerous cryptic species of the vertebrate: A lesson from the gobioid fish *Schindleria*. *Mol. Phylogenet. Evol.* **44**(1): 53-62.

Kumar S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **35**: 1547-1549.

Lakra, W.S., M. Singh, M. Goswami, A. Gopalakrishnan, K.K. Lal, V. Mohindra, U.K. Sarkar, P.P. Punia, K.V. Singh, J.P. Bhatt and S. Ayyappan. 2016. DNA barcoding Indian freshwater fishes. *Mitochondrial DNA*. **27**(6): 4510-4517.

Liu, Z., P. Li, B.J. Argue and R.A. Dunham. 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their backcross hybrids. *Anim. Genet.* **2**(29): 58-62.

Menezes, G.M., M.F. Sigler, H.M. Silva and M.R. Pinho. 2006. Structure and zonation of demersal fish assemblages off the Azores archipelago (mid-Atlantic). *Mar. Ecol. Prog. Ser.* **324**: 241-260.

Miah, M.F., F. Haque, M.R. Mia, E. Jannat, H. Ali, M.M.A. Quddus and K. Ahmed. 2013. Molecular identification and sexual differentiation of freshwater mud eel (*M. cuchia*). *Univers. J. Agric. Res.* **1**(3): 54-58.

Miah, M.F., K.M.A. Zinnah, M.J. Raihan, H. Ali and M.N. Naser. 2016. Genetic diversity based population study of freshwater mud eel (*Monopterus cuchia*) in Bangladesh. *World. Acad. Sci. Eng. Technol.* **10**: 329-333.

Matsumoto, S., T. Kon, M. Yamaguchi, H. Takeshima, Y. Yamazaki, T. Mukai. and M. Nishida. 2010. Cryptic diversification of the swamp eel *Monopterus albus* in East and Southeast Asia, with special reference to the Ryukyuan populations. *Ichthyol. Res.* **57**(1): 71-77.

Nasar, S.S.T. 1997. Backyard Eel Culture: International Institute of Rural Reconstruction, Silang, Cavite, Philippines. pp. 88.

Palumbi, S.R. and F. Cipriano. 1998. Species identification using genetic tools: The value of nuclear and mitochondrial gene sequences in whale conservation. *J. Hered.* **89**(5): 459-464.

Peninal, S., J. Subramanian, A. Elavarasi and M. Kalaiselvam. 2017. Genetic identification of marine eels through DNA barcoding from Parangipettai coastal waters. *Genom. Data.* **11**: 81-84.

Rahman, A.K.A. 2005. *Freshwater fishes of Bangladesh,* (2nd ed.). Zoological Society of Bangladesh. University of Dhaka. Dhaka, Bangladesh. pp. 65

Rahman, M.M., M.B. Rahman, E. Okazaki, and M.N.A. Rithu. 2016. Assessment of species specificity of fishing gears and fish diversity status in the Andharmanik river of coastal Bangladesh. *J. Fish. Aquat. Sci.* **11**: 361-369.

Ratnasingham, S. and P.N.D. Hebert. 2007. BOLD: The barcode of life data system (www. Barcoding life. Org). *Mol. Ecol. Notes.* **7**: 355-364.

Rosen, D.E. and P.H. Greemwood. 1976. A fourth neotropical species of synbranchid eel and the phylogeny and systematic of Synbranchiformes fishes. *Bull. Am. Mus. Nat. Hist.* **157**: 5-69.

Roy, P., N. Lucky and M. Hossain. 2016. Morphological Characterization of Two Fresh Water Eels *Monopterus cuchia* (Hamilton, 1822) and *Ophisternon bengalense* (McClelland, 1844). *J. Environ. Sci. Nat. Resour*. **9**(1): 127-137.

Ruzainah, A., M.N.S. Azizah, I. Patimah and A. Amirrudin. 2003. RAPD fingerprinting of the eel-loaches *Pangio filinaris* and *Pangio piperata*: preliminary evaluation. *Aquacult. Res.* **34**: 959-965.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406-425.

Shafi, M. and M.M.A. Quddus. 1982. *Bangladesher Matshyo Sampad.* Bangla Academy, Dhaka. pp. 245-246.

Smith, P.J., D. Steinke, S.M. Mcveagh, A.L. Stewart, C.D. Struthers and C.D. Roberts. 2008. Molecular analysis of Southern Ocean skates (Bathyraja) reveals a new species of Antarctic skate. *J. Fish Biol.* **73**: 1170-1182.

Steinke, D., T.S. Zemlak and P.D.N. Hebert. 2009. Barcoding Nemo: DNA-Based Identifications for the Ornamental Fish Trade. *Plos One.* **4**(7): E6300.

Talwar, P.K. and A.G. Jhingran. 1991. *Inland Fishes of India and adjacent countries.* Oxford-IBH publishing Co. Pvt. Ltd. **2**: 776-777.

Teske, P.R., M.I. Cherry and M.I.C.A. Matthee. 2003. Population genetics of the endangered Knysna seahorse *Hippocampus capensis*. *Mol. Ecol.* **12**(7): 1703–1715.

Ward, R.D., T.S. Zemakak, B.H. Innes, P.R. Last and P.D.N. Homles. 2005. DNA barcoding Australia's fish species. *Phil. Trans. R. Soc. B.* **360**: 1847-1857.

Wei, R.B., G.F. Qiu and R. Song. 2006. Genetic diversity of rice field eel (*Monopterus albus*) in China based on RAPD analysis. *Asian Fish. Sci,* **19**: 61-68.

Wong, E.H.K. and R.H. Hanner. 2008. DNA barcoding detects market substitution in North American seafood. *Food Res. Int.* **41**: 828–837.

Yin, S., J. Li, G. Zhou and Y. Liu. 2005. Population genetic structure of rice field eel (*Monopterus albus*) with RAPD markers. *Chin. J. Appli. Environ. Biol.* **11**: 328-332.

(Revised copy received on 04.11.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 133-141, December 2020

TEMPORAL VARIABILITY, TRENDS OF CLIMATIC VARIABLES AND DROUGHT ANALYSIS OF RAJSHAHI AND SYLHET DISTRICT, BANGLADESH

FATAMATUJ SUNNY, MD. SELIM MIAH, MD. YOUNUS MIA*
AND RUKSANA HAQUE RIMI

Department of Environmental Science and Resource Management, Mawlana Bhashani Science and Technology University, Tanagil-1902, Bangladesh

Abstract

The study was conducted to quantify the change of selected climatic variables (rainfall, relative humidity, maximum and minimum temperature) over 50 years at Rajshahi and Sylhet districts in Bangladesh. Annual, seasonal, and monthly climatic data comparisons have been executed between 1968-1992 and 1993-2017 through trend analysis. The Mann-Kendall statistic and Sen's Slope model were used to reveal the trends and estimate the magnitude of change respectively. Prediction of the climatic variable of 10 years (2018-2027) was made based on the ARAR algorithm using MaxStat Pro software. Rainfall data were used to analyze drought by using climatic indices (De Mortone Aridity Index, IdM; Seleaninov Hydrothermic Index, IhS; Donciu Climate Index, IcD). Average rainfall was decreasing dramatically in monsoon season at Rajshahi and in both pre-monsoon and monsoon seasons at Sylhet. The negative change of average rainfall in the monsoon at Rajshahi from 1968-1992 to 1993-2017 was found 29.17 mm. The maximum temperature was increasing in all seasons in both Rajshahi and Sylhet. Annual Mann-kendall trend test and Sen's slope revealed that relative humidity was decreasing and maximum temperature was increasing significantly at Sylhet for the period 1993-2017. At Rajshahi, during 1968-1992, relative humidity was increasing by 0.247 % per year, and minimum temperature was decreasing 0.049°C per year. Rainfall was decreasing insignificantly in both time scales. ARAR algorithm predicted that average maximum temperature might become comparatively higher than the previous 50 years. 1992 and 2010 were identified as drought years from all climatic indices, and 1969, 1981, and 1997 as excessive wet years at Rajshahi. No drought events were identified during 1968-2017 at Sylhet and the year 2017 to be an excessively wet year. IhS predicted 2020, 2025, and 2027 as drought years and 2024 as an excessive wet year at Sylhet.

Key words: Climate change, Trend analysis, Rainfall, Temperature, Relative humidity, Drought analysis.

Introduction

The Inter-Governmental Panel on Climate Change (IPCC) characterizes environmental change as an adjustment in the condition of the atmosphere that can be recognized (e.g., using statistical tests) by changes in the mean and additionally the changeability of its

*Corresponding author: <mdmia1998@gmail.com>.

properties and that continues for an expanded period, ordinarily decades or more (Solomon *et al.* 2007). Changes in atmospheric condition and climate have critical effects on the society (Karl *et al.* 2008). Variability of temperature, rainfall, and relative humidity are significant influencing factors for studying climate variability and extremes (Fahad *et al.* 2015). Bangladesh, a South-Asian country, is one of the most climate vulnerable states in the world (Rajib *et al.* 2012). According to Bangladesh Meteorological Department (BMD) data, the average maximum temperature has an increasing trend at a rate of 0.29ºC per century but the rainfall is decreasing at a rate of 8.8 mm over the north-western region (Bhuyan *et al.* 2018)

Knowing the nature and extent of conceivable climate change at Sylhet and Rajshahi is crucially essential for individuals, stakeholders, and policymakers to adapt appropriate measures. For instance, accurately predicting rainfall trends can play a vital role in a country's potential economic development.

Time series analysis and forecasting have become a significant tool in numerous applications in meteorology and other environmental areas to understand different phenomena, like rainfall, humidity, temperature, draught, etc. (Nury *et al.* 2013). ARAR algorithm is one of the most widely used time series forecasting methods. The ARAR algorithm applies memory shortening transformation and fits the autoregressive model to the transformed data. It is used to predict future data from existing sequence data. This algorithm is the adaptation of the ARARMA algorithm and had functioned better in a wide range of real data sets (Miswan *et al.* 2016).

Globally many countries are vulnerable to drought due to climate change and other natural conditions (Wilhite 2000). The rainfall distribution throughout the region and year is non-uniform. This non-uniform rainfall very often causes droughts in every year (Keka *et al.* 2012). According to the NASA's Tropical Rainfall Measuring Mission (TRMM) data, the minimum rainfall zone was Rajshahi in summer monsoon during 2001-2010 (Bhuiyan *et al.* 2018). The temperature of this region is comparatively higher than other regions in Bangladesh. Thus, the Rajshahi region is vulnerable to drought conditions. On the other hand, Sylhet experiences the highest rainfall in Bangladesh and has susceptibility to heavy-rainfall and flash floods. Though the drought has attracted less scientific attraction than flood or cyclone, several authors found that the impact of drought can be more defenseless than flood and cyclone (Shahid and Behrawan 2008). For instance, the loss of 1978/79 drought was greater than the loss of flood in 1974 (Paul 1998). Rice production losses in the drought of 1982 were 50 percent more than the losses that occurred due to the flood in the same year (Selvaraju *et al.* 2006).

For ease of calculation, investigators frequently use the De Mortone Aridity Index (IdM) for identifying susceptibility to drought conditions (Botzan *et al.* 1998). We used different climatic indices; De Mortone Aridity Index (IdM), Seleaninov Hydrothermic Index (IhS) and Donciu Climatic Index (IcD) .

According to TRMM information, the minimum rainfall zone was Rajshahi in South Asia (Bhuiyan *et al*. 2018). On the other hand, Sylhet meets the highest rainfall in Bangladesh.

The study of drought trends is extremely critical as it is related to food security and the management of scarce water resources, which becomes critical in case of drought events. Our present study helps to identify the change of climatic variable (rainfall, temperature and relative humidity), understanding drought at Rajshahi and heavy rainfall at Sylhet using past rainfall performance. Further, the results may help in planning to take necessary actions to manage regional drought and reduce the adverse impacts of drought and heavy rainfall across Rajshahi and Sylhet districts of Bangladesh. The present study was aimed at assessing monthly, seasonal and annual climatic variables (rainfall, temperature and humidity) through trend analysis and prediction of the near future (10 years) changes in particular climatic variables.

Materials and Methods

Study area: This study was conducted at Rajshahi and Sylhet Districts. Geographically Rajshahi is situated within Barind Tract and lies 23 m (75 ft) above sea level. The city is located on the alluvial planes of the Padma River. Rajshahi sits between 24º 37′ north latitude and 88º 70′ east longitude. Sylhet district lies on 21 m above sea level in the north-eastern part of Bangladesh and sits between 24º 90′ north latitude and 91º 88′ east longitude on the River Surma bank. Fig. 1 shows the locations of these two districts.

Study materials and Data collection: The research was carried out on the basis of climatic data analysis. The climatic data were collected from the Bangladesh Meteorological Department (BMD). Daily maximum temperature (°C), minimum temperature (°C), relative humidity (%), and rainfall (mm) data for the period 1968-2017 (i.e., 50 years) were used in this study. To maintain the continuity the gaps were filled up by the long-term average values of the existing days of the month for each parameter. Secondary data and relevant information were collected from national and international journals, books, thesis and earlier research papers.

Data processing and analysis: After gathering the necessary data, all collected data were arranged, classified, and analyzed. MS Excel was used for statistical analysis of the data and making graphs. The map for location of the BMD stations was made by Arc GIS (version 10.3). The annual average, seasonal average, and monthly average of climatic parameters (rainfall, temperature, and relative humidity) of Sylhet and Rajshahi were calculated to analyze the variability of climatic parameters and compare at the change rate of the climatic variable of these districts. The software named MaxStat Pro (version 3.60) was used for the prediction utilizing the ARAR algorithm.

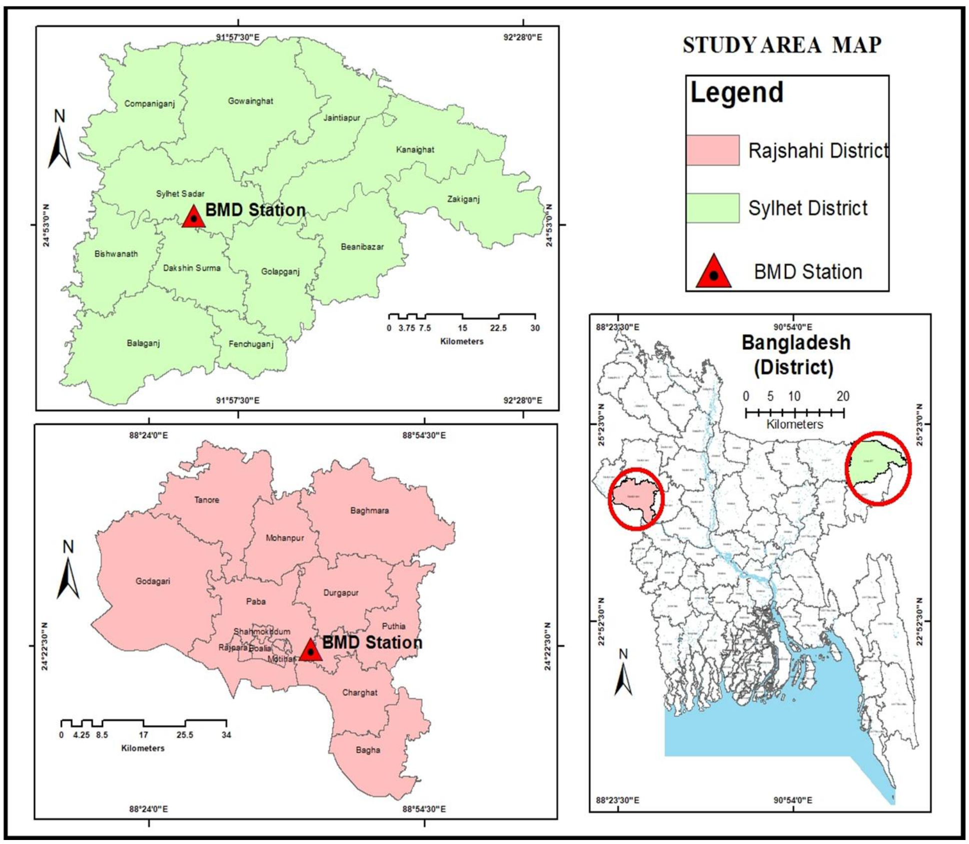


Fig. 1. Location of BMD stations of Rajshahi and Sylhet District.

Mann-kendall analysis: Trend analysis is the prediction of future outcome by using historical results. The increasing or decreasing trend of all the independent weather parameters (e.g., annual and seasonal temperature, rainfalls etc.) was statistically examined in two phases. The first one was the using of non-parametric Mann-Kendall test and the second one was the nonparametric Sen's slope estimator. The increasing or decreasing trend was tested based on normalized test statistics (Z) value. When Z is positive, the trend is said to be increasing, and when Z is negative, it is said to be decreasing. The trend's slope gives the annual rate and direction of change (Kendall 1995). The Mann-Kendall trend test is a non-parametric way of identifying trends in data collected over time series. Mann-Kendall Statistic (S) is given by,

$$S = \sum\sum sign(X_i - X_j) \quad (1)$$

here, i = 2, 3, … , n; j=1, 2, 3, …, i-1 and

$$sign(X_i - X_j) = \begin{cases} 1, if\ (X_i - X_j) > 0 \\ 0, if\ (X_i - X_j) = 0 \\ -1, if\ (X_i - X_j) < 0 \end{cases} \quad (2)$$

For a sample size > 10, a normal approximation to the Mann-Kendall test may be used. For this, a variance of S is obtained as,

$$V(s) = \frac{n(n-1)(2n+1) - \sum t_p(t_p - 1)(2t_p + 5)}{18} \quad (3)$$

Here, p= 1, 2, …., q

Where t_p is the number of ties for the p th value and q is the number of tied values. Then standardized statistical test is computed by:

$$sign(X_i - X_j) = \begin{cases} \frac{S-1}{\sqrt{V(s)}}, if\ S > 0 \\ 0\ , if\ S = 0 \\ \frac{S-1}{\sqrt{V(s)}}\ if\ S < 0 \end{cases} \quad (4)$$

The magnitude of the trend was estimated by Sen's slope method (Sen 1968), which was proceeded by calculating the slope as a change in measurement per change in time,

$$Q' = \frac{X_{t'} - X_t}{t' - t} \quad (5)$$

Where, Q' is the slope between data points $X_{t'}$ and X_t , $X_{t'}$ is the data measurement at time t' and X_t is the data measurement at time t. Sens slope estimator is simply given by the median slope,

$$B = \begin{cases} Q'_{\frac{N+1}{2}}, & N\ is\ odd \\ \frac{i}{2}\left(Q'_{\frac{N}{2}} + Q'_{\frac{N+2}{2}}\right), & N\ is\ even \end{cases} \quad (6)$$

Where, N is the number of calculated slopes. A positive value of B indicates an increasing trend, and a negative value indicates a decreasing trend in the time series. In this study, to represent the confidence level ***, **, * and + signs have been used to represent 100%, 99%, 95%, and 90% levels of confidence, respectively.

The Auto-regressive auto-regressive (ARAR) prediction method: The ARAR algorithm approach to modeling time series $\{S_t\}$ is described as one of the following three memory types: long memory, short memory, and no memory. A long-memory series is non-stationary due to trends or cycles. A short-memory series is a stationary and a no-memory series is a white noise. The memory-shortened series can be described as follows:

$$S_t = \upsilon_{0\,t} + \upsilon_{1\,t-1} + \cdots + \upsilon_{k\,t-k} \ldots\ldots \qquad (7)$$

Where, S is denoted as the sample means of $S_1, \ldots, S_T$ is the memory-shortened series, *t* is *1,…,T* and υ_0, $\upsilon_{1,}$ … υ_k is the coefficients of memory-shortening filter (Shitan and Ng., 2015). If the alternative long-memory or moderately long-memory is chosen, then the transformed series $\{S_t\}$ is again checked. If it is found to be long-memory or moderately long-memory, then a further transformation is performed. The process continues until the transformed series is classified as a short memory. The Autoregressive model is fitted using the model as below,

$$X_t = \lambda_1 X_{t-1} + \lambda_{l1} X_{t-l1} + \lambda_{l2} X_{t-l2} + \cdots + Z_t \qquad (8)$$

Where, $\{Z_t\}$~ WN $(0,\sigma^2)$, l_1, l_2, l_3 is lag values, λ_j is coefficient and σ^2 is white noise variance (Brockwell and Davis, 2016).

Yearly drought analysis by the climatic indices: To analyze drought, the meteorological drought analysis method was adopted. Yearly drought analysis was performed by four climatic indices. The climatic indices used are De Mortone Aridity Index, IdM; Seleaninov Hydrothermic Index, IhS; and Donciu Climate Index, IcD. With the help of annual rainfall assurance (A %), yearly drought analysis was performed by the climatic indices. The correlation established between the assured rainfall (A %) for years and climatic indices provide the polynomial expression, statistically very significant. It has quantified a very significant correlation between annual rainfall assurance (A %) and climatic indices using a logarithmic expression, which is shown in Table 1 (Keka *et al.* 2012).

Table 1 Climatic indices.

Climatic Index	Symbol	Mathematical Function
Mortone Aridity Index	IdM	IdM = – 7.3566 lnA + 58.037
Seleaninov Hydrotheremic Index	IhS	IhS = – 0.4006 lnA + 3.1001
Donciu Climate Index	IcD	IcD = – 24.269 lnA + 191.17

The data registered in the meteorological stations were used for the calculation of the climatic indices. The annual rainfall assurance (A %) was calculated in the following way: (i) the average rainfall of all years was determined (ii) percentage of rainfall for every year of average rainfall was determined (iii) deducting percentage value from 150 the annual rainfall assurance (A %) was found. By putting the value of A in each climatic index equation, each climatic index's value was determined. By comparing the value of climatic indices with Table 2, the yearly drought analysis was performed (Keka *et al.* 2012).

Table 2. Value of interpretation limits for climatic indices.

Characterization	Climate Index		
	IdM	IhS	IcD
Excessive rainy	> 40	> 2	> 110
Very rainy	33-40	1.6-2	100-110
Rainy	29-33	1.4-1.6	95-110
Medium	25-29	1.3-1.4	80-95
Droughty	24-25	1.1-1.3	70-80
Very droughty	23-24	1.0-1.1	65-70
Excessive droughty	< 23	< 1.0	< 65

Results and Discussion

The annual average maximum temperature was increasing with 0.005°C and 0.030°C per year in the trend of 1968-1992 and 1993-2017, respectively at Rajshahi. The trend of 1968-1992 and 1993-2017 had an increment rate with 0.024°C and 0.048°C per year respectively at Sylhet. According to Basak *et al.* 2013, the yearly average maximum temperature increased at the rate of 0.007°C in the north-western region and 0.022°C at eastern region of Bangladesh during the period of 1976-2008, which supports our findings. 2024 may be going to experience the highest average maximum temperature, 32.4°C, at Rajshahi over the last 60 years. Therefore, our findings suggest that the climate has become warmer both at Sylhet and Rajshahi in recent years, and warming will continue in the future too.

Rainfall trend in monsoon decreased at Rajshahi but increased in pre-monsoon. A decrease in monsoon rainfall may be a threat to agricultural production at Rajshahi. The annual rainfall of Rajshahi station had a significant negative trend in the study of Bari *et al*. 2016, which is analogous to the findings of this study. Overall trends of rainfall increased slightly, but a considerable decrease of monsoon and post-monsoon annual average rainfall was observed at Sylhet; Choudhury *et al*. 2013 reported decreasing monsoon rainfall for Sylhet region in recent time periods, which is not contradictory to this study. Heavy rainfall will occur in 2024 at Sylhet; this result encourages farmers to grow flood tolerant crops in 2024.

In recent years, relative humidity had increased more at Rajshahi than at Sylhet. So, the climate has changed in terms of relative humidity. Ferdous and Baten (2012) reported that the annual average relative humidity for the period 1961-2010 was found to increase over Rajshahi which supports this study's findings.

Instead of being in a drought prone zone, medium to the rainy condition of future drought analysis indicates no drought in the near future at Rajshahi. It is a relief for farmers of Rajshahi.

At Sylhet, the year 2020, 2025, and 2027 will be considered medium years in most climatic indices, but IhS identified those years as droughty. That means there is less indication for the probability of flood and higher dry conditions; therefore, farmers should be encouraged to plant drought-tolerant crops for those years.

References

Bari, S.H., M.T.U. Rahman, M.A. Hoque and M.M. Hussain. 2016. Analysis of seasonal and annual rainfall trends in the northern region of Bangladesh. *Atmospheric Res.* **176**: 148-158.

Basak, J.K., R.A.M. Titumir and N.C. Dey. 2013. Climate change in Bangladesh: A historical analysis of temperature and rainfall data. *J. Environ.* **2**(2): 41-46.

Bhuyan, M.D.I., M.M. Islam, and M.E.K. Bhuiyan. 2018. A Trend Analysis of Temperature and Rainfall to Predict Climate Change for North western Region of Bangladesh. *Amer. J. Clim. Change* **7**: 115-134.

Botzan, T.M., M.A. Mariño and A.I. Necula. 1998. Modified de Martonne aridity index: application to the Napa Basin, California. *Physical Geography* **19**(1): 55-70.

Brockwell, P.J. and R.A. Davis. 2016. Introduction to time series and forecasting. *Springer*, pp. 318-330.

Choudhury, S.A., T. Terao, F. Murata and T. Hayashi. 2013. Seasonal variations of temperature and rainfall characteristics in the northeastern part of Bangladesh around Sylhet. *J. Agrofor. Environ.* **6**.

Fahad, M., A. Islam, G. Islam and S.K. Bala, S.K. 2015.The Projection of Temperature and Precipitation over Bangladesh under RCP Scenarios using CMIP5 Multi-Model Ensemble. Proceedings of the International Conference on Recent Innovation in Civil Engineering for Sustainable Development, Gazipur, 11-13 December 2015, WRE-037.

Ferdous, M.G. and M.A. Baten. 2012. Climatic variables of 50 years and their trends over Rajshahi and Rangpur Division. *J. Environ. Sci. Nat. Resou.* **4**(2):147-150.

Karl, T.R., G.A. Meehl, C.D. Miller, S.J. Hassol, A.M. Waple and W.L. Murray. 2008. Weather and Climate Extremes in a Changing Climate. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Re-search, Department of Commerce, NOAAs National Climatic Data Center, Washington DC, pp. 164.

Keka I,A., I. Matin, M. Rahman and D.A. Banu 2012. Analysis of Drought in Eastern Part of Bangladesh, *J. Sci. Technol*.**7**: 1.

Kendall, W.S. 1995. A probabilistic model for the variance to mean power law in ecology. *Ecol. Modelling* **80**(2-3):293-297.

Miswan, N., N. Hussin, R. Said, K. Hamzah and E.Z. Ahmad. 2016. ARAR Algorithm in Forecasting Electricity Load Demand in Malaysia. *Global J. Pure and Appl. Math.* **12**(1): 361-367.

Nury, A.H., M. Koch and M.J.B. Alam. 2013. Time series analysis and forecasting of temperatures in Sylhet division of Bangladesh. In 4th International Conference on Environmental Aspects of Bangladesh (ICEAB), August, pp. 24-26.

Paul, B.K. 1998.Coping mechanisms practiced by drought victims (1994/5) in North Bengal, Bangladesh. *Applied Geography* **18**: 355-373.

Rajib, M.A., M.M. Rahman, Z.B. Rakib, M.T.H. Khondoker, S.M. Iskander, M.M. Hassan and M.R. Mortuza. 2012. Application of RCM-Based Climate Change Indices in Assessing Future Climate: Part I Temperature Extremes. *In:* World Environmental and Water Resources Congress 2012: Crossing Boundaries, *American Society of Civil Engineers*, Reston, 1-7.

Selvaraju, R., A.R. Subbiah, S. Baas and I. Juergens. 2006. Livelihood adaptation to climate variability and change in drought-prone areas of Bangladesh: Developing institutions and options, Asian Disaster Preparedness Center Food and Agriculture Organization of the United Nations, Rome.

Sen, P.K. 1968. Estimates of the regression coefficient based on Kendall's tau. *J. Amer. Statist. Assoc.* **63**(324):1379-1389.

Shahid, S. and H. Behrawan. 2008. Drought risk assessment in the western part of Bangladesh. *Nat. Hazards* **46**: 391-413.

Shitan, M. and Y.L. Ng. 2015. Forecasting the total fertility rate in Malaysia. *Pak. J. Statist.* **31**(5): 547-556.

Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller. 2007. Climate Change 2007: The Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC. *Cambridge University Press*, Cambridge, New York.

Wilhite, D.A. 2000. Drought as a natural hazard: concepts and definitions, in *Drought: A Global Assessment*, Routledge, London, UK, pp. 3-18.

(Revised copy received 10.11.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 143-153, December 2020

ANTAGONISTIC POTENTIALITY OF SOME SOIL FUNGI AGAINST SIX FUNGAL PATHOGENS ISOLATED FROM COTTON (*GOSSYPIUM HIRSUTUM* L.) SEEDS

AMINA KHATUN, SHAMIM SHAMSI* AND ABUL BASHAR
Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

The antagonistic potentials of four soil filamentous fungi *viz*., *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and *Trichoderma viride* Pers. against six pathogens isolated from 14 varieties of cotton (*Gossypium hirsutum* L.) were evaluated by "dual culture colony interaction", volatile and non-volatile metabolites. In dual culture colony interaction, out of four soil fungi, *T. viride* was found be the most effective to control the growth of the cotton pathogens. *T. viride* showed the highest growth inhibition against *Curvularia lunata*, *Fusarium moniliforme*, *Mucor* sp. and *Rhizoctonia solani*. On the other hand *A. niger* showed the highest growth inhibition against *Fusarium nivale* and *A. fumigatus* showed the maximum growth inhibition against *C. gloeosporioides*. The highest inhibition of radial growth of *C. lunata*, *F. nivale* and *F. moniliforme* was observed might be due to the secretion of volatile metabolites of *T. viride* whereas, the maximum inhibition of radial growth of *C. gloeosporioides* was observed because of the volatile metabolites of *A. flavus*. *Mucor* sp. and *R. solani* were inhibited due to the volatile metabolites of *A. fumigatus*. The greatest radial growth inhibition of *C. lunata* and *F. moniliforme* were noticed in case of *T. viride* owing to the effect of non-volatile metabolites. On the other hand, the radial growth of *C. gloeosporioides* and *F. nivale* were inhibited highest amount for the effect of non-volatile metabolites of *A. niger,* whereas highest growth inhibition of *Mucor* sp. and *R. solani* was observed due to the non-volatile effect of *A. fumigatus*. The present investigation suggests that the isolates of *Aspergillus* and *Trichoderma* may be further exploited as potential biocontrol agents against the fungal pathogens of cotton in field trial.

Key words: Antagonistic potentiality, Fungal antagonists, Cotton pathogens.

Introduction

Cotton is the most renowned, reliable fiber yielding crops as well as cash crops around the world including Bangladesh. It is the major textile fiber used by man in the world and playing a key role in the economic and social welfare (Munro 1994).

*Corresponding author: <prof.shamsi@gmail.com>.

Each year, cotton production is being reduced due to the presence of some harmful fungal pathogens. Majority of cotton diseases are seed-borne *viz*., *Alternaria* blight, bacterial blight, *Fusarium* wilt, *Myrothecium* blight, *Cercospora* blight, *Exserohilum* blight etc. A number of seed-borne fungal pathogens such as *Alternaria*, *Fusarium*, *Rhizopus* and *Aspergillus* are frequently identified in cotton seeds (Minton and Garber 1983). *Aspergillus flavus*, *A. niger* (Type-I), *Curvularia lunata*, *Fusarium moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for *Gossypium arboreum* in Bangladesh (Naznin and Shamsi 2018).

Application of antagonists to soil and seeds increased seed germination percentage and reduced the seed borne infection. Study of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology, because it has great potential to solve many agricultural and environmental problems. So, there is a need to screen newly available seed dressing molecules including bio-agents and botanicals for their efficacy in overcoming the seed-borne infections of fungi.

In Bangladesh, research on biological control of seed-borne fungi of cotton seeds through soil antagonists is very limited. So, for the sake of economy, more information on this regard is essential. Considering the importance of this valuable fiber, present investigation was undertaken to screen out the efficacy of antagonistic fungi against the fungal pathogens associated with cotton seeds.

Materials and Methods

Collection of samples: Seed samples of CB1-14 were collected from Cotton Research, Training and Seed multiplication Farm, Gazipur after harvesting and were kept in clean glass jars, labeled properly and preserved at room temperature for subsequent use. The experiment was conducted in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka.

Isolation and identification of fungi: The fungi were isolated from the collected seed samples following the “Tissue Planting Method” on PDA medium (CAB 1968), “Blotter method” and “Paper Towel Method” (ISTA 1996). Morphological identification of the isolates were determined following the standard literatures (Thom and Rapper 1945, Rapper and Thom 1949, Subramanian 1971, Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Sutton 1980) and molecular identification were done following Amer *et al.* 2011 with some modifications.

Antagonostic potentiality of fungi: Six pathogenic fungi, namely, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and

Rhizoctonia solani, isolated from 14 varieties of cotton seeds were selected as test pathogens against four antagonistic soil fungi.

Antagonistic fungi were isolated from rhizosphere soil of the host varieties following serial dilution method. Among the isolated fungi, *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride* were selected to test their antagonistic potentials against the pathogens following "dual culture technique" described by Bashar and Rai (1994). The parameter used for the assessment of the colony interaction and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976). Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied following the methods described by Bashar and Rai (1994).

Analysis of data: Data were evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Six fungal pathogens *viz., Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* were isolated from the seeds of 14 varieties of cotton seeds, which were found as virulent in the test of pathogenicity.

In colony interactions, antagonistic relationships among the soil fungi and test pathogens were grade 2 and 4. However, grade 2 was found to be the most commonly encountered type of colony interaction as 17 interactions were incorporated in this grade, which was followed by grade 4 (Table 1).

The intermingled zone between the soil fungi and test pathogens was very common. The maximum intermingled zone (0.3 cm) was observed in case of *A. fumigatus* and *T. viride* against *Mucor* sp. and *R. solani*. *T. viride* grew over the colony of the test pathogens but in case of *A. flavus, A. fumigatus* and *A. niger* inhibition zone was found and it was 0.2, 0.2 and 0.1, respectively.

All the tested soil fungi inhibited the growth of all the test pathogens to varied degrees in dual culture experiments on PDA plates. *A. fumigatus* showed the highest inhibition on radial growth of *C. gloeosporioides* (84.0%) followed by *A. niger* (80.0%), *A. flavus* (73.9%) and *T. viride* (63.1%). *T. viride* showed the highest (76.4%) growth inhibition on *Curvularia lunata* which was followed by *A. niger* (72.2%), *A. fumigatus* (68.4%) and *A. flavus* (59.0%). *T. viride* showed the highest growth inhibition on *F. moniliforme* (73.7%), which was followed by *A. flavus* (67.6%), *A. niger* (60.0%) and *A. fumigatus*

(53.8%). *A. niger* showed the highest growth inhibition (72.27%) on *F. nivale,* which was followed by *A. flavus* (68.1%), T. *viride* (66.6%) and *A. fumigatus* (45.4%). *T. viride* showed the maximum growth inhibition on *Mucor* sp. (60.0%) which was followed by *A. niger* (52.1%) *A. flavus* (50.0%), and *A. fumigatus* (44.1%). *T. viride* also showed the highest growth inhibition on *Rhizoctonia solani* (46.8%) which was followed by *A. niger* (30.2%) *A. flavus* (26.6%), and *A. fumigatus* (19.3%) (Table 1 and Fig. 1).

Table 1. Effects of dual culture between fungal antagonists and cotton pathogens.

Test pathogens		% inhibition of radial growth, intermingled and inhibition zone and type of reactions of the test pathogens			
		Aspergillus flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*
Colletotrichum gloeosporioides	% inhibition	73.9	84.0	80.0	63.1
	IMZ (cm)	0.1	0.2	0.2	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
Curvularia lunata	% inhibition	59.0	68.4	72.2	76.4
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.2	0.2	0.1	-
	Grade	4	4	4	2
Fusarium moniliforme	% inhibition	67.6	53.8	60.0	73.7
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.1	0.2	0.2	-
	Grade	4	4	4	2
Fusarium nivale	% inhibition	68.1	45.4	72.2	66.6
	IMZ (cm)	0.2	0.1	0.1	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
Mucor sp.	% inhibition	50.0	44.1	52.1	60.0
	IMZ (cm)	0.2	0.3	-	0.3
	IHZ (cm)	-	-	0.1	-
	Grade	2	2	4	2
Rhizoctonia solani	% inhibition	26.6	19.3	30.2	46.8
	IMZ (cm)	0.2	0.3	0.2	0.3
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2

IMZ = Intermingling zone, IHZ = Inhibition zone, and '-' = not applicable.

Similar observation was also noticed in the study of Akter *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019 where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

In dual culture technique, maximum growth inhibition was recorded for *Trichoderma* spp. against different pathogenic fungi in the research of Tapwal *et al*. 2015, Patel and Joshi 2001, Sunitha and Kurundkar 2007, Al-Ameen *et al*. 2017, Goswami and Islam 2002.

Grade 2 = Mutual intermingling growth where the growth of the fungus is ceased and being over growth by the opposed fungus. Grade 4 = Slight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm) based on Skidmore and Dickinson (1976).

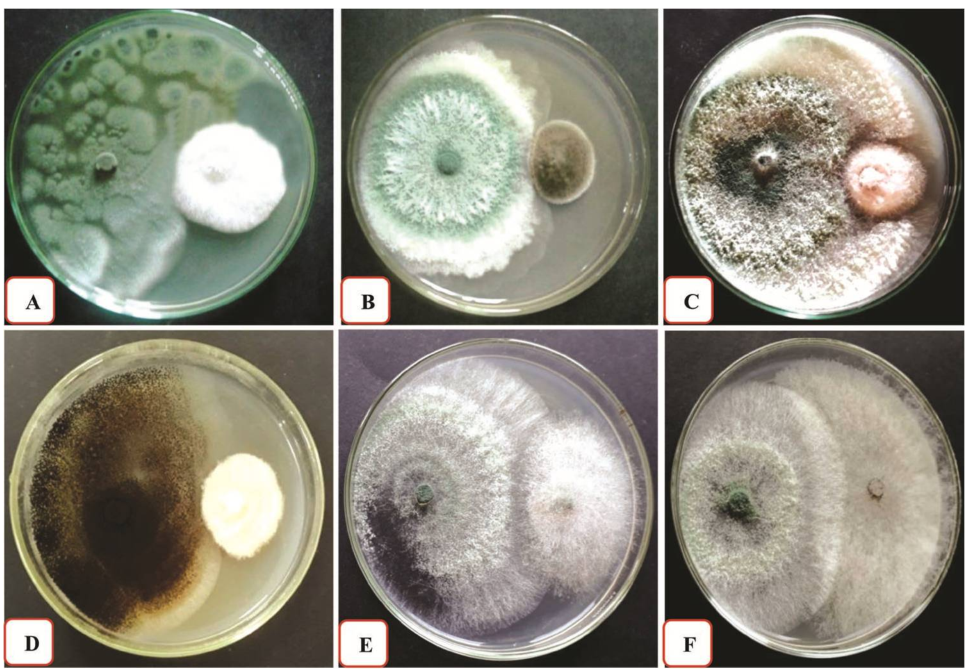


Fig. 1. Photographs showing colony interactions between pathogenic fungi and antagonists. A. *Colletotrichum gloeosporioides* and *Aspergillus fumigatus*; B. *Curvularia lunata* and *Trichoderma viride*; C. *Fusarium moniliforme* and *T. viride*; D. *Fusarium nivale* and *Aspergillus niger*; E. *Mucor* sp. and *T. viride*; F. *Rhizoctonia solani* and *T. viride.*

The effect of volatile metabolites of antagonistic fungi against cotton seed pathogens are presented in Table 2. The maximum inhibition of radial growth of *C. gloeosporioides* was observed in *A. flavus* (59%), which was followed by *A. niger* (48.7%), *A. fumigatus* (38.2%) and *T. viride* (35.9%) due to the volatile metabolites. The maximum inhibition of radial growth of *Curvularia lunata* was observed in *T. viride* (71.4%) followed by *A. fumigatus* (47.6%), *A. flavus* (38.1%) and *A. niger* (33.3%) owing to volatile metabolites. The maximum inhibition of radial growth of *Fusarium nivale* was also observed in *T. viride* (64.2%), which was followed by *A. niger* (54.7%), *A. fumigatus* (47.6%) and *A. flavus* (40.4%). Highest inhibition of radial growth of *F. moniliforme* was found by *T. viride* (51.1%) followed by *A. fumigatus* (42.2%), *A. niger* (40.0%) and *A. flavus* (35.5%). Whereas, *A. fumigatus* showed 58.2% inhibition of radial growth of *Mucor* sp. which was followed by *T. viride* (55.5%), *A. flavus* (47.2%) and *A. niger* (36.3%). At last, maximum inhibition of radial growth of *Rhizoctonia solani* was noticed in *A. fumigatus* (72.4%), which was followed by *T. viride* (55.5%), *A. niger* (53.3%) and *A. flavus* (52.2%) (Table 2 and Fig. 2).

Table 2. Percent inhibition of radial growth of the test pathogens owing to volatile metabolites of antagonistic fungi.

Antagonistic fungi	% inhibition of radial growth of the test pathogens					
	Colletotrichum gloeosporioides	*Curvularia lunata*	*Fusarium nivale*	*Fusarium moniliforme*	*Mucor* sp.	*Rhizoctonia solani*
Aspergillus flavus	58.9^{a}	38.1^{c}	40.4^{d}	35.5^{d}	47.2^{c}	52.2^{d}
A. fumigatus	38.4^{c}	47.6^{b}	47.6^{c}	42.2^{b}	58.1^{a}	72.4^{a}
A. niger	48.7^{b}	33.3^{d}	54.7^{b}	40.0^{c}	36.3^{d}	53.3^{c}
Trichoderma viride	35.9^{d}	71.4^{a}	64.2^{a}	51.1^{a}	55.5^{b}	55.5^{b}
CV%	0.0220	0.0210	0.0193	0.0237	0.0234	0.0171

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Similar observation was also noticed in the study of Aktar *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019, where *A. flavus*, *A. fumigatus*, *A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

Al-Ameen *et al.* (2017) reported that some volatile metabolites released from *T. viride* cultures might be responsible for extending the inhibitory activity against some pathogens such as *Colletotrichum* and *Fusarium* species isolated from banana (*Musa sepientum* L.)

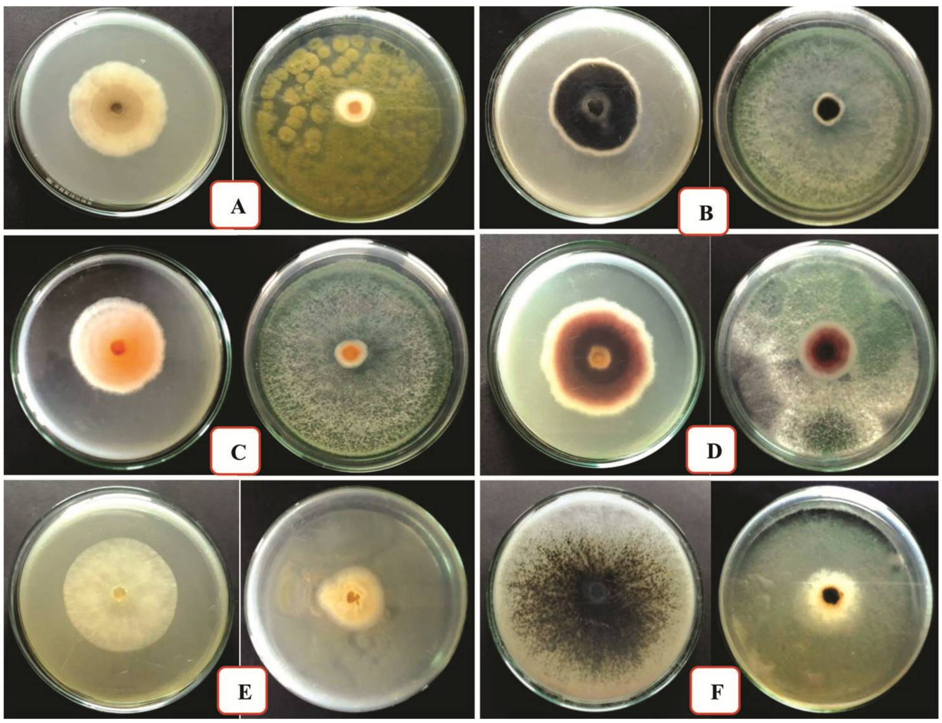


Fig. 2. Growth inhibition of pathogenic fungi owing to volatile metabolites of the antagonists. A. *A. flavus*: *Colletotrichum gloeosporioides*; B. *T. viride*: *Curvularia lunata*; C. *T. viride*: *Fusarium nivale*; D. *T. viride*: *Fusarium moniliforme*; E. *A. fumigatus*: *Mucor* sp. and F. *A. fumigatus*: *Rhizoctonia solani.*

Table 3 shows the effect of non-volatile metabolites on the growth of *C. gloeosporioides, C. lunata, F. nivale, F. moniliforme, Mucor* sp. and *R. solani*. The maximum inhibition of radial growth of *C. gloeosporioides* was observed with the culture filtrates of *A. niger* (76.3%), which was followed by *T. viride* (70.9%), *A. fumigatus* (58.4%) and A. flavus (57.7%) at 20% concentration. The maximum inhibition of radial growth of *Curvularia lunata* was observed with the culture filtrates of *Trichoderma viride* (78.2%) which was followed by *A. niger* (73.1%), *A. flavus* (72.3%), and *A. fumigatus* (57.4%) at 20% concentration. The highest inhibition of radial growth of *Fusarium moniliforme* was observed with the culture filtrates of *T. viride* (76.3%), which was followed by *A. niger* (74.0%), *A. fumigatus* (67.2%) and *A. flavus* (56.3%) at 20% concentration.

Table 3. Percent inhibition of radial growth of test pathogens by non-volatile metabolites of antagonistic fungi.

Test pathogens	Concen-tration (%)	% inhibition of radial growth of test pathogens by non-volatile metabolites owing to different antagonists				CV (%)
		Aspergillus flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*	
Colletotrichum gloeosporioides	5	28.9^{c}	27.6^{d}	47.2^{a}	41.8^{b}	0.0275
	10	48.8^{c}	42.5^{d}	58.1^{a}	49.0^{b}	0.0201
	15	55.5^{c}	51.0^{d}	66.0^{a}	65.4^{b}	0.0168
	20	57.7^{d}	58.4^{c}	76.3^{a}	70.9^{b}	0.0152
Curvularia lunata	5	36.1^{d}	38.1^{c}	56.3^{a}	45.6^{b}	0.0227
	10	48.9^{c}	44.6^{d}	65.4^{a}	65.2^{b}	0.0178
	15	63.8^{c}	53.1^{d}	72.7^{a}	69.5^{b}	0.0154
	20	72.3^{c}	57.4^{c}	73.1^{b}	78.2^{a}	0.0142
Fusarium moniliforme	5	30.9^{d}	38.1^{b}	33.3^{c}	43.6^{a}	0.0685
	10	43.6^{d}	52.7^{c}	55.5^{a}	54.5^{b}	0.0224
	15	50.9^{d}	58.1^{c}	62.9^{b}	65.4^{a}	0.0168
	20	56.3^{d}	67.2^{c}	74.0^{b}	76.3^{a}	0.0146
Fusarium nivale	5	33.3^{b}	30.9^{d}	32.7^{c}	50.0^{a}	0.0272
	10	40.4^{c}	38.1^{d}	50.9^{b}	64.0^{a}	0.0207
	15	47.6^{d}	50.0^{c}	67.2^{b}	68.1^{a}	0.0871
	20	64.2^{d}	71.4^{c}	81.8^{a}	74.0^{b}	0.0137
Mucor sp.	5	27.5^{d}	36.4^{b}	35.0^{c}	38.6^{a}	0.0290
	10	38.8^{d}	44.7^{a}	43.7^{c}	44.0^{b}	0.0234
	15	42.3^{d}	55.3^{a}	53.7^{c}	54.6^{b}	0.0194
	20	47.0^{d}	64.7^{a}	57.5^{c}	60.0^{b}	0.0581
Rhizoctonia solani	5	20.0^{a}	10.0^{d}	18.8^{b}	14.1^{c}	0.0634
	10	37.7^{b}	22.2d	31.1^{c}	54.1^{a}	0.0275
	15	45.5^{b}	30.0^{d}	37.7^{c}	58.8^{a}	0.0232
	20	55.5^{d}	72.2^{c}	58.8^{b}	62.8^{a}	0.0211

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

The highest inhibition of radial growth of *F. nivale* was observed with the culture filtrates of *A. niger* (81.8%), which was followed by *T. viride* (74.0%), *A. fumigatus* (71.4%) and *A. flavus* (64.2%) at 20% concentration. The maximum inhibition of radial growth of *Mucor* sp. was observed with the culture filtrates of *A. fumigatus* (64.7%)

which was followed by *T. viride* (60.0%), *A. niger* (57.5%) and *A. flavus* (47.0%) at 20% concentration. The maximum inhibition of radial growth of *Rhizoctonia solani* was observed with the culture filtrates of *A. fumigatus* (72.2%), which was followed by *T. viride* (62.8%), *A. niger* (58.8%) and *A. flavus* (55.5%) at 20% concentration (Table 3). Differences in percent inhibition with the present study might be due to the differences in organism strains involved in the interaction (Table 3 and Fig. 3).

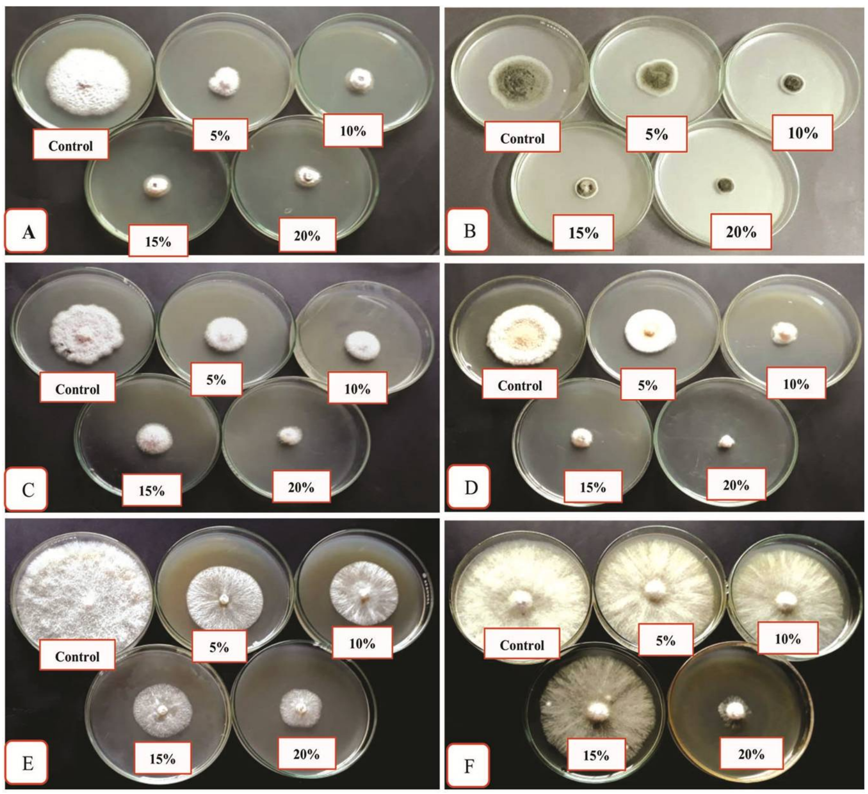


Fig. 3. Growth inhibition of pathogenic fungi owing to non-volatile metabolites of antagonists at 5, 10, 15 and 20% concentrations. A. *Colletotrichum gloeosporioides*: *Aspergillus niger*; B. *Curvularia lunata*: *Trichoderma viride*; C. *Fusarium moniliforme: T. viride*; D. *Fusarium nivale: A. niger*; E. *Mucor* sp.: *Aspergillus fumigatus* and F. *Rhizoctonia solani*: *A. fumigatus.*

Similar result was found in the research of of Akter *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019, where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

The non-volatile metabolites produced from the culture filtrates of *T. viride* and *A. niger* were responsible for maximum inhibition against different pathogenic species of *Colletotrichum* and *Fusarium* according to Al-Ameen *et al*. 2017 and Madhanraj *et al*. 2010.

Tapwal *et al.* (2015) also reported that, culture filtrates of *T. viride* showed major growth inhibition on *C. gloeosporioides.* A number of *Trichoderma* species are effective agents for the control of plant pathogenic fungi, such as *Fusarium* spp. (Sivan and Chet 1986), *Pythium* spp. (Naseby *et al.* 2000) and *Rhizoctonia* spp. (Lewis and Papavizas 1987).

The present investigation suggests that the isolates of *Aspergillus* and *Trichoderma* may be further exploited as potential biocontrol agents against the fungal pathogens of cotton in field trial.

Acknowledgements

The first author gratefully acknowledges to the "Ministry of Science and Technology", People's Republic of Bangladesh for providing financial support in her research through NST fellowship.

References

Aktar, M.T., K.S. Hossain and M.A. Bashar. 2014. Antagonistic potential of rhizosphere fungi against leaf spots and fruit rot pathogens of brinjal. *Bangladesh J. Bot.* **43**(2): 213-217.

Al-Ameen, M., S. Shamsi, S. Hosen and M.A. Bashar. 2017. Antagonistic potential of soil fungi against post-harvest pathogenic fungi of *Musa sapientum* L. *Bangladesh J. Bot.* **46**(2): 733-738.

Amer O.E., M.A. Mahmoud, A. El-Rahim and El-Samawaty. 2011. Nonliquid nitrogen-based-methodfor isolation of DNA from filamentous fungi. *Afr. J. Biotechnol.* **10**(65):14337-14341.

Barnett, H.L. and S.B. Hunter. 1972. *Illustrated Genera of Imperfect Fungi.* Burgess Publishing Company, Minneapolis. Third Edition, pp. 44-45.

Bashar, M.A. and M. Chakma. 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. *Dhaka Univ. J. Biol. Sci.* **23**(1): 53-60.

Bashar, M.A. and B. Rai. 1994. Antagonistic potential of root-region microflora of chickpea against *Fusarium oxysporum* f. sp. *ciceri*. *Bangladesh J. Bot.* **23**(1): 13-19.

Benoit, M.A. and S.B. Mathur. 1970. Identification of species *Curvularia* on rice seed. *Proc. Inst. Seed Test. Ass*. **35**(1): 1-23.

Booth, C. 1971. The Genus *Fusarium.* The Commonwealth Mycological Institute, Kew, England. pp. 267.

CAB (Commonwealth Agricultural Bureau). 1968. *Plant Pathologist Pocket Book*. 1st edn. The Commonwealth Mycological Institute, Kew. pp. 267.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. 1st edn. The Commonwealth Mycological Institute, Kew, Surrey, England. pp. 608.

Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, Kew. pp. 507.

Fokkema, N.J. 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. *In: Microbiology of Aerial Plant Surfaces* (eds. Dickinson CH and TF Preece), Academic Press, London, pp. 487-505.

Goswami, D. and M. Islam. 2002. *In vitro* study of antagonism of *Trichoderma* sp. and *Bacillus subtilis* against *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.). *J. Agril. Sci.* Society of North-East India. **17**: 24-26.

Helal, R.B. and S. Shamsi. 2019. Antagonistic potentials of some soil fungi against three post-harvest pathogenic fungi of C*arica papaya* L. *Dhaka Univ. J. Biol. Sci.* **28**(1): 1-7.

ISTA. 1996. International Rules of Seed Testing Association. *In*. Proc. Int. Seed Test. Assoc. pp. 19-41.

Lewis, J. A. and G.C. Papavizas. 1987. Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of *Rhizoctonia* damping-off. *Plant Pathol.* **36**: 438-446.

Madhanraj, P., V. Ambikapathy and A. Panneerselvam. 2010. Biological control of banana wilt caused by *Fusarium solani* (Mart.) Sacc. *Int. J. Appl. Biol. Pharm. Technol.* **1**(3): 1032-1039.

Minton, E.B. and Garber. 1983. Controlling the seedling disease complex of cotton. Plant Dis., **67**:115-118.

Munro, J.M. 1994. Cotton and its production, insect pests of cotton, CAB International, Wallingford. pp. 47-52.

Naznin, S. and S. Shamsi. 2018. Pathogenic potentiality of fungi isolated from seeds of three hill cotton varieties (G*ossypium arboreum* L.). *Dhaka Univ. J. Biol. Sci.* **28**(2): 187-193.

Naseby, D.C., J.A. Pascual and J.M. Lynch. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. *J. Appl. Microbiol.* **88**: 161-169.

Patel, K.D. and K.R. Joshi. 2001. Antogonistic effect of some bioagents *in vitro* against *Colletotrichum gloeosporioides* Penz. and Sacc. The causal agent of leaf spot of turmeric. *J. Mycol. Plant Path.* **31**: 126.

Raper, K. B. and C. Thom. 1949. A Manual of the Penicillia. Williams and Wilkins, Baltimore, MD. pp. 875.

Sivan, A. and I. Chet. 1986. Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum. J. Phytopathol.* **116**: 39-47.

Skidmore A.M. and Dickinson C.H. 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Brit. Mycol. Soc.* **66**: 57-64.

Subramanian, C.V. 1971. Hyphomycetes. Indian Council of Agriculture Research, New Delhi. pp. 930.

Sunitha, J. Waghmare and B.P. Kurundkar. 2007. Efficacy of local isolates of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *ciceri*. *J. Plant Dis. Sci.* **2**(1): 48-50.

Sutton, B.C. 1980. The *Coelomycetes*, Common Wealth Mycological Institute, Kew Surrey, England, pp. 1-696.

Tapwal, A., A. Tyagi, G. Thakur and S. Chandra. 2015. *In vitro* evaluation of *Trichoderma* species against seed borne pathogens. *IJCBS Research Paper* **1**(10).

Thom, C. and K.B. Raper. 1945. A Manual of the Aspergilli. Williams and Wilkins, Baltimore, MD. pp. 373.

(Revised copy received on 25.8.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 155-162, December 2020

EXTRACTION AND PROXIMATE STUDY OF *SANSEVIERIA TRIFASCIATA* L. AS FIBRE SOURCE FOR TEXTILE AND OTHER USES

A.B.M. ABDULLAH[1]*, MARUF ABONY[2], M.T. ISLAM[1], M.S. HASAN[1], M.A.K. OYON[1] AND MD. BOKHTIAR RAHMAN[3]*

[1]Departments of Textile Engineering, Primeasia University, Banani, Dhaka, Bangladesh.

[2]Departments of Microbiology, Primeasia University, Banani, Dhaka, Bangladesh.

[3]Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.

Abstract

Natural fibres are getting importance for their sustainable development in their uses in mitigation of climate change and ecological balance. A fibre extraction retting method is formulated and proximate chemical composition and various physical properties such as tensile strength, elongation, diameter along with fibre, cellulose, lignin and ash content were determined. This preliminary observation indicates its potential to be used as a source of fibre for textile and non-textile uses such as woven, nonwoven, composite, blanded and a good source of α-cellulose, microcrystalline cellulose, nano-cellulose and lignin-based products.

Key word: Ecology, Retting, S*ansevieria trifasciata* L., Microcrystal cellulose and lignin.

Introduction

The horizon of diversified uses of natural fibres is increasing for sustainable development strategy. Ecofriendly, biodegradability, recyclable and reusable properties of cellulosic and lignocellulosic fibres such as cotton, jute, kenaf, ramie, etc. are gaining focus and the marketing of their products are increasing (Kanimozhi 2011a, Kant and Alagh 2013, Wolela 2019). Cellulosic and lingo cellulosic fibres are bio-polymer and obtain from a large variety of plants and crops (Wolela 2019).

Recently, various studies are undertaken to find fibres from different non-conventional and indigenous sources such as banana, bamboo, water-hyacinth, etc. *Sansevieria trifasciata* L. is a long leafy plant (Fig.1). *Sansevieria* is a genus of 70 species with great variation within the genus such as from succulent S*ansevieria pinguicula* to the leafy tropical plant, such as *Sansevieria trifasciata* L. (Wolela 2019). They are native to India,

*Corresponding authors: <abm.abdullah45@yahoo.com and bokhtiarbdj@gmail.com>.

Indonesia and Africa (Kanimozhi 2011a, Wolela 2019). It is an evergreen and perennial plant. Its leaves grow vertically from the Basal rosette. Its leaves are a stiff, sword-shaped, band with yellow on either slightly band center. But matured leaf is deep green with light gray-green cross bending usually 70-90 cms long, 5-6 cms wide. Indigenously, it is called in different names as “snake plant, mother in laws tongue”, Saint George’s sword, bowstring hemp, in our locality it is called “Baghachokor”. It is a thicked leaves fibres laminated with waxy membrane type of smooth surface (Abral and Kenedy 2015, Kanimozhi 2011b, Kumar *et al.* 2011, Wolela 2019).

Presently, this plant is urbanized as a decorative garden plant for their air purification characteristics property and converting CO_2 into O_2 at night (Kant and Alagh 2013). Some parts of the plant are medicinally important in the traditional system of medicine in India and other countries as fodder, edible and mosquito repellant (Rose *et al.* 2017).

In the present study, matured *Sansevieria trifasciata L.* leaves were collected from Phokir-Bari garden, Kapasia, Gazipur. The primary objective of this study is to identify fibre extraction method and proximate studies such as fibre content, chemical composition and other physical properties such as strength, diameter, colour, elongation, moisture content, etc.

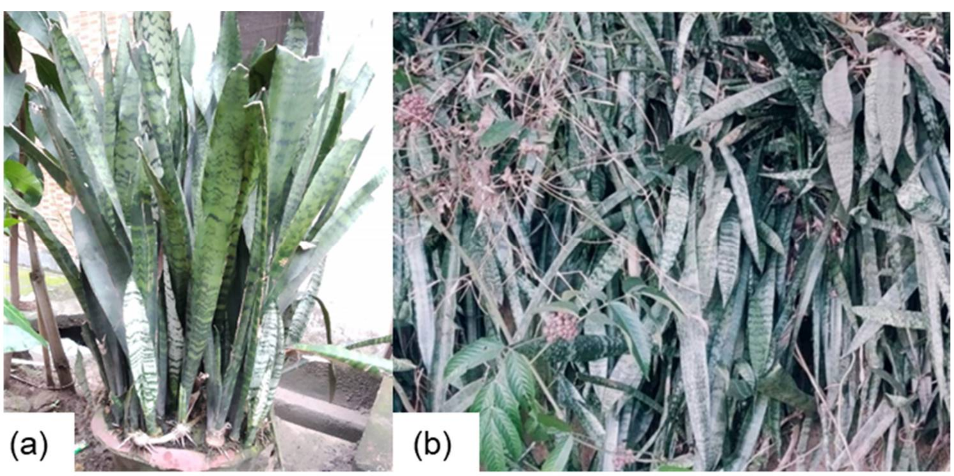


Fig. 1. *Sansevieria trifasciata* plants (a) in pot culture and (b) in bush.

Extraction can be made manually by hand, water retting, dew retting, chemical retting decortication, etc. (Murthy and Karthikeyan 2015). Retting is a complex biochemical process where bacteria and enzymes play a complex role in separating fibre from the leaf.

In this study, a composite Chemi-Biochemi-Mechanical method has tried to accelerate the retting process with minimal water and time of retting.

Materials and Methods

In this study, a Chemi-Biochemi-Mechanical retting method was formulated for accelerated fibre extraction from *Sansevieria trifasciata* L. collected from the local bush. In this process, clean, gray-white, shining and bright fibres were obtained in 48-72 hrs whereas in mechanical, chemical and water retting extraction of similar fibre were obtain having longer time (3 weeks) involving higher temperature (70^{o}C) and a greater amount of chemicals (5 % NaOH) (Kant and Alagh 2013, Wolela 2019).

Green leaves were cut down into 2-3 inches pieces after gently hammering it. A number of experiments were undertaken with the variation of retting time, pH, liquor ratio, the concentration of alkali, urea, molasses and observed fibre yield by occasional examination/test and color/ordor of retting water. All experiments were carried out at room temperature (25 - 29^{o}C) and washed with tap water in the dyeing laboratory of Primeasia University and the following recipe was determined:

Sansevieria trifasciata L. leaves- 50 gms, NaOH- 1-2 % on the weight of material (O.W.M), Urea- 2-3% (O.W.M.), Molasses- 1 % (O.W.M.), pH- 9-11, L.R.- 1 : 20, Temperature- 25-29 ^{o}C, Times- 2-3 days, Stirring- occasionally, Solution colour- green and Odor- pungent and eye sensitive.

After retting, fibre was thoroughly washed with tap water until it was freed from colour and waxy materials, and finaly, it was washed with distilled water. Hydro-extracted fibres were dried at room temperature in an open atmosphere.

Hammered leaves (500 gms) were retted in a big bucket by following the modified method of Abdullah *et al.* (1987) and fibres were obtained and washed and dried as before and obtained results are shown in Table 1.

Similarly, chemical composition and physical properties were determined with standard laboratory methods and conditions at dyeing and microbiology laboratories, Primeasia University (PAU), Banani, Dhaka. The following results were obtained and shown in Tables 2 and 3.

Results and Discussion

Properties of fibre extracted with this method and compared with the results from other sources are shown in Table 4.

Table 1. Crude fibre properties.

Content	Amount	%
Fibre yield	16 to 17 gm	3
Waxy material	1-5 gm	1.06
Residue	-	
Liquid color	Green solution	
Fibre color	Gray white (slight yellowish)	
Fibre shape	Smooth, distinct, less branching	
Crimpiness	Lack of crims	
Brightness	Silky and shiny	
Diameter	Cylindrical and Uniform	

Table 2. Chemical composition.

Component	%
Cellulose content	70-75
Lignin Content	16-18
Ash content	2-3
Fatty materials	10-12

Table 3. Physical properties.

Fibre properties	
Tensile strength	5.97 – 6.0 gm/denin
Diameter	40-50 micron
Elongation at break in %	3.12-3.80
Breaking strength in gm	110-112
Moisture content in %	12-13
Color change after exposure to sunlight in normal days light 10 am to 3 pm (100 hours exposure)	Color change yellow to dark brown

Table 4. Comparison of extracted fibre properties with that of other sources.

Fibre properties	Observed	Other sources	
		Value	Reference
Average number of fibre/ single leaf	680-720	772	(Wolela 2019)
Fibre yield in %	2-3	3-4	(Wolela 2019)
Fibre length in cm	60-85	90	(Wolela 2019)
Diameter in micron	45-48	50.76	(Kant and Alagh 2013)
Breaking strength in gms	110-112	114.99	(Kant and Alagh 2013)
Elongation at the break in %	2.9-3.1	3.27	(Kant and Alagh 2013)
Moisture content in %	12-13	13.1/13.9	(Wolela 2019)
Tensile strength gm/diner	12-14	15.54	(Wolela 2019)
Colour	Grey white	Grey white	(Wolela 2019)
Colour change in sunlight after exposer in normal sun light for 10 am to 3 pm (100 hours)	Colour change to yellow to brown	-	-
Cellulose content in %	70-75	-	-
Lignin content in %	16-18	-	-
Ash content in %	2-3	-	-
Fatty materials in %	8-10	-	-
STPLR (fibre content) in %	2-3	-	-

Sansevieria trifasciata leaf fibre (STLF) is a lingo cellulosic fibre like a jute, kenaf, and ramie though they are bast fibre, the retting method, which is adopted here, is a slight modification of bast fibre and coconut fibre retting (Abdullah *et al.* 1987). As depictured above, comparative physical properties indicated that the retting recipe and method is a positive improvement of traditional and chemical extraction methods practice in fibre extraction methods from STLF. This is an experimental laboratory study, further and critical studies and pilot-plant experimentation will be needed to find and postulate optimal commercial adaptation methods.

Preliminary experimentation indicates that this fibre can be scoured, bleached and can be dyed in commercial dyes (Fig. 2) through a slight modification of existing facilities (Abdullah *et al.* 2019). Sustainable and ecofriendly development needs renewable biomass and energy. In addition to traditional natural fibre, new indigenous sources of fibres are highly needed to protect the environment and climate change induced disaster.

STLF is natural wild fibres and its origin is in India, Indonesia and Africa. They grow anywhere in full sunlight, shade and even in the dark but thrive in a moist, fertile land with high organic content with minimum or no agronomic care (Kant and Alagh 2013).

Presently, as a decorative garden plant and air purification, roof-agriculture has better potentiality (Kant and Alagh 2013). Moreover, leaf fibre plants have a higher degree of fibre yielding capacities. A comprehensive socio-economic study is highly needed for taking any commercial ventures.

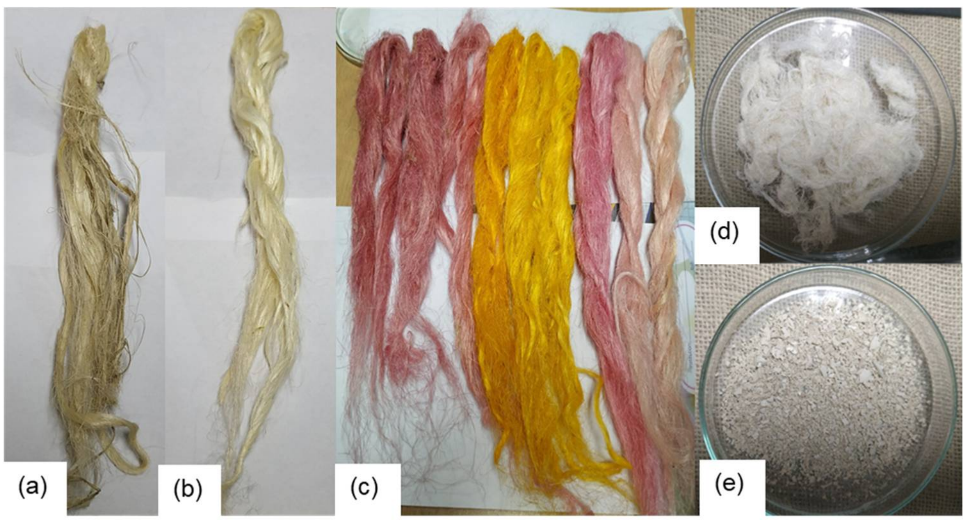


Fig. 2. Preliminary observation of STLF and products. (a) Untreated fibres, (b) bleached fibres, (c) dyed fibres, (d) cellulose and (e) crude microcrystalline cellulose.

Though it is leafy fibres still like other bast fibres the color change in sunlight occurred due to lignin.

From the observation, in the above-compared Table 4, it is showed that the fibre content here is 2-3% whereas, in jute, flax and banana fibre content are 2, 27 and 2.6%, respectively (Whewell 1948). In comparison, it is better than jute but lower in flax. On the other hand, banana fibre is coarser and shorter fibre length with less cellulose content.

Total cellulose content indicates it has a potentiality of α-cellulose, microcrystalline cellulose and other cellulose derivatives and pulp and paper. Other properties, like the number of fibres single leaf, breaking strength, diameter, and moisture content, are its positive side. But, poor crispiness indicates its spainablity will be different from present coton/jute spinning, but there is enough potential to be used in handicraft as a string, cord, decorative materials in boutique production and decoration of Jamdani and Katan products (Abdullah 2019). Murthy and Karthikeyan (2015) reported that its roots have the potential for the production of various nano products along with some medicinal values. The synthesis of nanoparticles has received much attention for its wide range of applications. The aqueous root and bark extracts of STLF act both as a reducing and capping agent (Rose *et al.* 2017). Due to the presence of flavonoids, it can be used as an antibacterial and antioxidant agent as well as an inhibitor of xanthine oxidase enzyme, which is the main culprit of hyperuricemia (Yumna *et al.* 2018).

Sansevieria trifasciata L. is a wild leafy fibres plant. It is a source of strong white fibre. They are commonly used as rope, fishing lines/net, cordage, bowstring and clothing materials. From the results of the above study and information, it can be inferred that this fibre and other parts of this plant have the potential for wide-scale used and diversified product development for the use as a raw material in different industries such as textile and garments, pulp and paper, pharmaceuticals and cosmetics along with various decorative furnishing industries. Renewable and ecologically natural fibres are in great demand for a sustainable and pollution-free planet. *Sansevieria trifasciata L.* is leafy and perennial fibre. Climatic and agro-ecological conditions reflect the possibility of wide-scale production in our non-agricultural and bushy barrel land along with newly practices urbanized roof-agriculture and environmental mitigation.

References

Abral, H. and E. Kenedy. 2015. Thermal degradation and tensile strength of *Sansevieria trifasciata*-polypropylene composites. *Paper presented at the IOP Conference Series: Materials Science and Engineering*, **87**. doi:10.1088/1757-899X/87/1/012011

Abdullah, A.B.M. 2019. Personal Communication with Bibi Russel, renowned fashion designer. Mid-september.

Abdullah, A.B.M., M.T. Islam, M.S. Hasan and M. A. Oyon. 2019. A study of dyeability properties of newly extracted fibres STET of local source. *Accepted for publication to Journal of Primeasia Univ*. **2**(1): 34-44.

Abdullah, A.B.M., M.K. Kabir, B. Rahman, F. Uddin, N.N. Khan and H.U. Ahmed. 1987. Production of jute/coir blended yarn from low grade jute and coir fibres. *Bangladesh J. Jute and Fibre Res.* **12**(1&2): 27-30.

Kanimozhi, M. 2011a. Investigating the physical characteristics of *Sansevieria trifasciata* fibre. *Intl. J. Sci. Res. Pub.* **1**(1): 1-4.

Kanimozhi, M. 2011b. Extraction, fabrication and evaluation of *Sansevieria trifascicata* fiber. *Ind. J. Appl. Res.* **1**: 97-98.

Kant, R. and P. Alagh. 2013. Extraction of fiber from *Sansevieria trifasciata* plant and its properties. *Intl. J. Sci. Res,* **4**(7): 2547-2549.

Kumar, M.A., G.R. Reddy, G.H. Reddy, N.S. Reddy, K.H. Reddy and Y. Reddy. 2011. Mechanical properties of randomly oriented short *Sansevieria trifasciata* fibre/epoxy composites. *J. Metallurgy and Materials Sci.* **53**: 85-95.

Murthy, V.N. and N. Karthikeyan. 2015. Development of *Sansevieria trifasciata*-carbon fiber reinforced polymer hybrid nanocomposites. *ILVPA* **50**: 179-187.

Rose, A.L., F.J. Priya and S. Vidhya. 2017. Comparative study on the synergistic action of differentially synthesized copper nanoparticles with *Escherichia coli* and *Staphylococcus aureus*. *Intl. Res. J. Pharm.* **8**(11): 85-90.

Whewell, C. 1948. Matthews' Textile fibers: their physical, microscopical and chemical properties. *Nature,* **161**(4094): 581.

Wolela, A.D. 2019. Extraction and characterization of natural cellulose fibers from *Sanseveria trifasciata* plant. *TTEFT* **5**(2): 630-634.

Yumna, M., R. Arbianti, T.S. Utami, H. Hermansyah and S. Ningsih. 2018. Flavonoid isolation and identification of mother-in-law's tongue leaves (*Sansevieria trifasciata*) and the inhibitory activities to xanthine oxidase enzyme. *E3S Web of Conferences,* **67**: 1-6. https://doi. org/ 10.1051/e3sconf/20186703011

(Revised copy received on 08.9.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 163-178, December 2020

ACCESSIBILITY ANALYSIS OF CYCLONE SHELTERS - A CASE STUDY FOR ATULIA UNION, SATKHIRA, BANGLADESH

TANZIM HAYAT[1], A.S.M. MAKSUD KAMAL[1], MD. SHAKHAWAT HOSSAIN[1*], SAIYEBA ZAMAN[1], B.M. RABBY HOSSAIN[1] AND TAPAS RANJAN CHAKRABORTY[2]

[1]Department of Disaster Science and Management, University of Dhaka, Dhaka-1000, Bangladesh

[2]Disaster Risk Financing Coordinator, Oxfam

Abstract

Cyclone shelters are considered as a solution to reduce cyclone risk in coastal districts of Bangladesh. The location of a shelter plays a crucial part in a potential user's decision-making process. If the perception is that the shelter is too far away, the user may decide not to use it. On the other hand, it would not be financially feasible to construct shelters near every settlement cluster. Therefore, network analysis using GIS has been applied to reveal the optimal location. Apart from distance, there are some other factors (like space, presence of gender segregated rooms and toilets, ramped access way, availability of drinking water, etc.), which affect a user's affinity to evacuate to a specific shelter. All the shelters in Atulia Union from Satkhira District of Bangladesh were visited to identify these characteristics. Finally, an index was developed to determine the preference of each shelter to its potential users. It was found that there is inadequate number of shelters in the study area and two new shelter locations were recommended.

Key words: Cyclone, Shelter, Accessibility, Optimal Location

Introduction

The Bay of Bengal is a hotspot for forming tropical low-pressure systems (i.e., the first stage of cyclone formation) (Paul 2009). About 10 percent of all the tropical cyclones in the world form here (Ali 1996). On average, a severe cyclone strikes Bangladesh every 3 years (Government of Bangladesh 2009). The country has experienced over 70 severe cyclones (associated with storm surges) from 1797 to 2007 (Khan 1995). In 1970, Cyclone Bhola, the deadliest recorded cyclone in human history, took about 300,000 lives and caused widespread damage to its infrastructure and economy (Hossain *et al.* 2008). However, recent studies suggest that the number of deaths resulting from storm surge has decreased like; Cyclone Sidr (2007) claimed the lives of 3,363 people despite being of the same category as cyclone Bhola (1970) due to improved state of preparedness and better infrastructure (Jia 2010).

*Corresponding author: <shakhawat.dsm@du.ac.bd>.

Consequently, the combination of multiple factors is responsible for the frequent occurrence of cyclones and their immense damages. The damages are primarily due to the associated surges rather than the cyclones themselves. Firstly, it is the country's location, being just beside the Bay of Bengal and its funnel shaped coastline. Secondly, it is a low-lying delta incised by a multitude of rivers. The third factor is the volume of the population residing along the coasts of Bangladesh (that amounts to about 48 million or around 30 percent of the total population) and their general socio-economic status and prevalence of poverty. Fourthly, it is the presence (or rather the absence) of quality infrastructures like roads, polders, shelters and various other structures (Paul 2009, Jia 2010, BBS 2011).

Moreover, climate change is likely to exacerbate the frequency and severity of cyclones. Intergovernmental Panel on Climate Change (IPCC) suggests that the rise of sea surface temperature between 2 to 4°C can correlate with an increase in tropical cyclone frequency (IPCC 2014). Furthermore, any rise in Sea Surface Temperature could "fuel" the weaker depressions/cyclones, increasing their intensities and consequences (Ali 1996, Huq *et al.* 2010, IPCC 2014).

As was stated previously, there is overwhelming evidence suggesting cyclones are a frequent occurrence in coastal parts of Bangladesh. These cyclones are quite devastating as well. Approximately 53 percent of the cyclones with 5,000 or more casualties have occurred in Bangladesh and 49 percent of the world's cyclone related casualties were incurred in Bangladesh (Ali 1999, Government of Bangladesh 2008, Paul and Routray 2013). Therefore, it is not surprising that the 2011 Global Assessment Report of the UNISDR ranks Bangladesh sixth out of 89 countries regarding the number of people exposed to a cyclone (United Nations International Strategy for Disaster Reduction 2011).

World Bank (2010) shows that a significant portion of the population in Shyamnagar (an Upazila of Satkhira, located in the southwestern part of Bangladesh) transitions around the poverty line. Since Atulia Union is situated in Shyamnagar, it is true for its residents as well. The lack of sustainable income sources throughout the year substantiates their vulnerability (Fenton *et al.* 2017). This translates as large portion of the people being unable to afford resilient housing and end up living in Kacha or Semi-Pucca houses (Islam *et al.* 2015). These houses are not strong enough to resist the winds and surges associated with cyclones, thus exposing them to greater risks. This was one of the foremost reasons behind the high number of lives lost in the 1970 and 1991 Cyclones. Although resilient housing for everyone would dramatically reduce risks, it was not financially possible. Therefore, the government decided to build cyclone shelters (CS) to reduce the consequences of cyclone disaster. Despite the success of such a measure, there are other factors that motivate people actually to use shelters like cyclone forecasting ability, the ability to generate trustworthy, accurate warning signals, dissemination of such information to the vulnerable communities, distance and ‘access’ to cyclone shelters (Paul 2009). Paul and Routray (2013), along with Roy *et al.* (2015) showed that coastal inhabitants are aware of the risks resulting from cyclones but “choose” not to respond because of poor road networks, long distances between home and shelters, low capacity of shelters to accommodate everyone and fear of household goods being stolen.

The objective of this paper is to investigate the current location of the shelters in Atulia Union and how accessible they are, given the crucial role they play in the disaster preparedness phase. Since the accessibility of shelters depends not only on distance but also on the available facilities, the paper will provide an overview of the facilities within each individual cyclone shelter.

Materials and Methods

Study area: The study was conducted in the Atulia Union (one of the smallest administrative divisions of Bangladesh) of Shyamnagar Upazila of Satkhira District, situated in the southeastern corner of Bangladesh (Fig. 1) in the month of February, 2018. The total population of the Atulia Union is about 42,000, with area of 4,475,823.2 square meter. Around 25% of families survive below the poverty line (Atulia Union Parishad 2018). Atulia is situated beside the Kholpetua River, which flows out to the Bay of Bengal. Storm surges travel through the river unimpeded and the relative height of the surges is greater due to its proximity from the Bay. This provides motivation to determine the distance to be traversed by people to their nearest shelter since they are amongst the first population who experience the surge and every minute is crucial. In addition, the study determines whether the facilities within the shelters are suitable. Moreover, the level of poverty in the study area also means that there are many people who need to use the shelters, thus it is essential to figure out whether they may be able to access the shelters when the time comes. The study is even more relevant since, in previous times, many lives were lost due to inadequate preparedness. In 1991 and 2009, the Cyclones Gorky and Aila had caused widespread damages and casualties to this region (IFRC 2002, 2009).

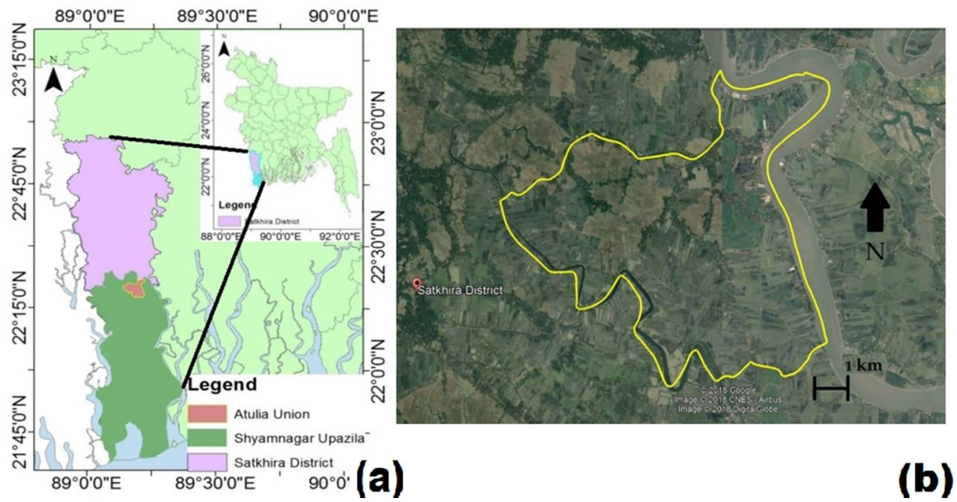


Fig. 1 (a) Location of the Atulia Union in reference to Satkhira (Local Government Engineering Department 2018), (b) The Satellite image by Google Earth (2021) of the Atulia union is shown with a yellow border.

Methodology: Initially, literature review and expert opinions helped to illustrate the overall situation as well as to identify the potential gap and research objectives, i.e., to carry out accessibility analysis, which would become a useful resource for future infrastructure development and maintenance. The experts included an NGO professional, a University Professor and researchers who worked on cyclone disaster risk. In addition, a field reconnaissance survey, Focus Group Discussions (FGD) and Key Informant Interviews (KII) were conducted to collect primary data, like locations of shelter, settlements, roads, different shelter facilities and walking speeds. These were combined with secondary data that included population, satellite images (to digitize road networks) and settlement clusters. Finally, the travel times to each shelter was computed using Network Analysis. Afterward, an index was created to condense the travel time data with individual shelter facilities to understand the current accessibility of each shelter. The term accessibility used here means the ease of a potential user of the shelter to use it. Finally, two new optimal shelter locations were suggested. The methodological framework adopted for this study is given in Fig. 2.

Initially, three FGDs were conducted to identify the overall distribution and location of existing CS in the study area, along with road conditions and the hindrances people face when accessing them. Most FGDs had around 10 members (Focus Group Discussion 2020). The later FGDs and 20 KIIs were with people who have already used the shelters in crisis times or were potential users. While FGDs revealed broad problems, like lack of nearby shelters, or low quality of access roads, KIIs helped to shed light on details of the facilities within the shelters. For instance, a shelter may have good access roads, but a portion of it becomes very slippery during the monsoon. The data obtained from KII helped to determine how long it takes for people to walk a certain distance on a normal day compared to if they use a van. The walking time for elders and pregnant women were also determined. Furthermore, it helped to find the shelter capacity and various factors included in Table 1, 2 and 3. This formed the core data in the research. In addition, KIIs were conducted with the Chairman (Union Parishad), Disaster Management Committee members, members of the Cyclone Shelter Management Committee (including teachers/Principals of the School/College which are used as CS and various users of the shelters (including disadvantaged people like the elderly and women). The purpose was to identify the qualities of specific shelters that affected their affinity to evacuate to that shelter.

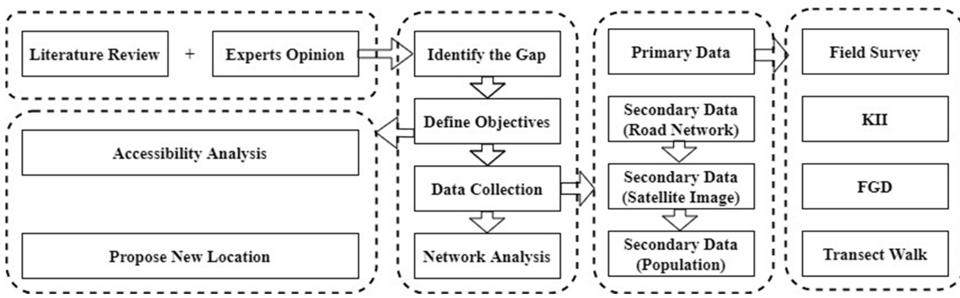


Fig. 2. Methodological Framework.

Only public buildings with more than or equal to two story were considered as shelters, because the surge heights can reach about 3 to 5 meters in height, which would easily drown single story buildings. Furthermore, an individual inspection of each cyclone shelter was conducted, and their near-exact Geolocations was recorded with a Calibrated Garmin GPS and Android device. During those inspections, a checklist was used to record the conditions of the structure and various facilities of the shelter (Table 2). Moreover, the checklists contained fields for location information; access road's condition; the number of floors, rooms and floor size; actual and recommended capacity; the presence of ramps; domestic animal storage space; the number of toilets and their height above ground; water source and storage; the presence of solar based fan, lights; and presence of visible structural defects. Such information was then cross matched during the KIIs with the users. The conditions and types of roads were also recorded from field observation.

The recorded locations of the shelters, settlements, and roads were translated into shapefiles. Digitized roads and settlement cluster locations were re-examined in Google Earth to ensure that their identification, condition and extent were accurate. Whereas, the number of inhabitants of the settlements was interpolated based on the area (i.e. size) and density of each cluster. To find the shortest distance from settlement to shelters and to identify the closest shelter for each settlement cluster, the ArcGIS Network Analyst tool was utilized (ArcGIS Network Analyst Overview 2020).

Results and Discussions

Both KIIs and FGDs revealed that the population who were currently living in two-story concrete housings were using their houses as shelters rather than going to the CS. It is obvious that those who live in substandard housing require better shelters during an emergency. However, the population will willingly choose not to go to one when the need arises. Their reason for choosing such action varies, as has been studied by Roy *et al.* (2015). One of the demotivating factors is the distance to the shelters. The distance data is calculated using the Closest Facility tool. This is placed under 'Road Length max.' (Table 1). The term means a shelter's distance from the farthest settlement, from which people will actually travel to the shelter. The second column of the Table 1 (Walk time normal) shows the time taken to walk (the maximum road length) for a normal person on a normal day. The third column represents the walking time of the most vulnerable groups (such as elders, pregnant women, children, etc.) on rainy days. The fourth column represents the time it would take to travel on a van. Access roads denote the type of road (Kacha- bare soil, Pucca- asphalt).

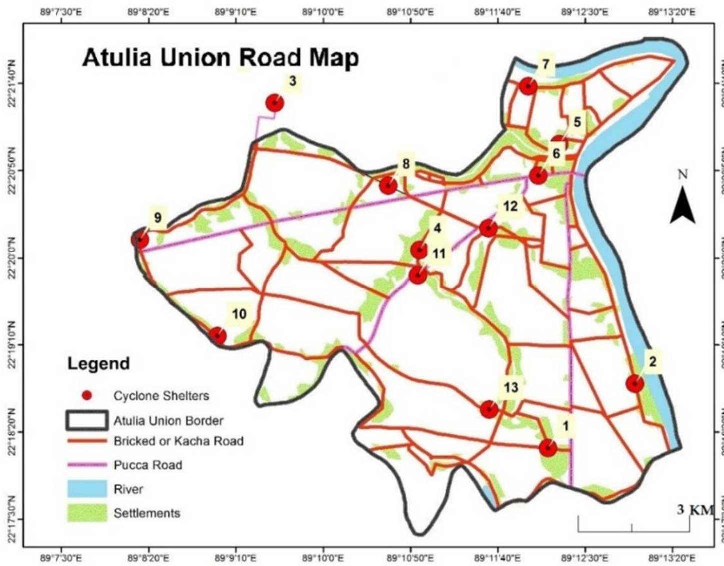
In the study area, nine shelters are located more than 1500 m away from settlements, which is deviatory from the recommendations of Cyclone Shelter Construction, Maintenance and Management Policy 2011. Shelters are usually placed near the largest settlement catchments (owing to the multipurpose use of the structures as educational-institutions). This, on the other hand, shows that remote settlement catchments do not have shelters nearer to them. In terms of distance, it may seem that accessing CS 2 and 6 are similar, but further review reveals that their access roads have different conditions. For CS 2 the entire connecting road is bricked or even Kacha. This means that it takes longer to travel compared to CS 6 Pucca roads. This is reflected in the second column (Table 1- 'Walk time normal' column), which is not simply a function of their average walking speeds; rather, the value is based on the experience of the local people. The road length is the result of network analysis and the different times were based on information obtained from locals during KIIs and FGDs. When the factor of rain is included (in the third column), roads which are Kacha or Bricked (Fig. 3) pose a greater risk for the evacuees, especially risky for pregnant women, thus needs more time to travel. Pink lines in Fig. 3 indicate Pucca roads (good quality roads that are concrete or ashphalt). Orange Lines show roads that either has craters or are not constructed with Pucca road materials, making them less friendly to pedestrians or traffic. Both secondary data (Local Government Engineering Department 2018) and primary observations have been used to generate this map. This accounts for the conditions that the people might face during an actual evacuation, when heavy torrential rains are common during cyclones. The fourth column represents the time it would take to travel on a van. Given that, people only start to evacuate in the "eleventh hour" the travel time forms an important element in determining the accessibility of the shelters.

Table 1. Maximum length (metres) of roads and the time (minutes) taken to traverse them.

Shelter name	Road length (max.) (m)	Walk time normal (min)	Walk time slowest (min)	Van time (min)	Access road
1- Atulia CS1	1582	25	56	16	Pucca
2-Boro Kupot Primary (81)	2409	40	96	25	Bricked
3-Kashimari Primary	880	22	53	8	Pucca
4-Rabeya Dakhil Madrasa	617	13	30	3	Pucca
5-Beralakkhi Madrasa	1331	22	53	15	Bricked
6-Noabeki Mahabiddyaloy	2417	34	59	7	Pucca
7-Beralakkhi Primary (146)	1474	24	58	17	Bricked
8-Kachari Bridge School	2235	25	60	12	Pucca
9-Abdul Kader High School	2657	34	82	20	Pucca
10-Atulia Primary (40)	2707	45	108	28	Kacha
11-Choto Kupot Primary(41)	2073	40	96	25	Kacha
12-Choto Kupot Primary(89)	1979	40	97	20	Bricked
13-Henchi High School	2913	57	137	32	Bricked

Source: Field survey, 2018.

Apart from the distance and difficulty of evacuation, there needs to be a consideration of the facilities within the shelter itself. A compilation of such facilities has been made from observation in Table 2. Significant among those, is access to water and a sanitation system that remains functional during the emergency. Access to fresh drinking water was seen to be ensured by installations of large capacity tanks (>5,000 litres) for all the shelters except for CS 9. The tube well was not raised but was at ground level, which meant it would be drowned by surges. The presence of solar lights ensures that even when the main electricity is disrupted, there is a source of light in the shelter.



Source: Field survey, 2018.

Fig. 3. Road map of Atulia, with the classification of its types.

Even though all the structures (Table 2) have two stories or more, many of the older school buildings (like CS 11 & 12) only had toilets on the ground floor, which would be rendered unusable after surges. Fresh water source is an important factor for evacuees, so the presence of water tanks to store groundwater (GW) or from rain harvesting (RWH) was seen in all but 1 cyclone shelter. Ramp entry helps those who cannot climb stairs to reach higher stories in the shelter. Also, if toilets are not raised, but are at ground level, then during surge induced flooding, they cannot be used. According to locals, the surving water can take two days or more to recede, so the availability of a toilet is crucial in determining the shelter's usability. On a positive note, all the toilets were gender-segregated, conducive to the local customs.

The holding capacity is determined by the size of the building and the number of floors. The data has been collected from observation and KIIs. The settlement field indicates the total population each shelter is meant to serve. The difference shows the population of people who cannot take shelter in the designated building. CS 6 and CS2 have the highest difference meaning their size and capacity differ a lot. In contrast, CS 4 has the lowest difference, which means that their sizes are more appropriate according to the size of the population they are meant to serve.

None of the shelters had ramped access ways or cattle storage space except three (CS 6, 7 and 10). This means people have to climb stairs, which can prove to be difficult for the elderly and pregnant women. Most of the shelters had solar panels for lighting. All the shelters were originally supplied with panels, but some were no longer functional and are yet to be replaced. Solar powered lights are considered a useful utility as the presence of lights at night can provide a sense of security.

Major structural damages were noticed in CS 2 (Figure 4) and 10, where load-bearing columns had fractures and concrete spalling. These demand immediate repair before they can be used as shelters. In fact, these shelters have not been repaired since they incurred damage in Cyclone Aila. The saline surge water exposure has possibly increased the process of concrete deterioration, according to the opinion of locals.

Table 2. Summary of shelter conditions.

Shelter name	Water source	Solar lights	Ramp entry way	No. of toilets	Structural damage
1- Atulia CS1	Tank(GW)	Yes	No	2 (Ground)	Not found
2-Boro Kupot Primary (81)	Tank(RWH)	No	No	2 (Ground)	Major
3-Kashimari Primary	Tank(RWH)	Yes	No	2 (Ground)	Not found
4-Rabeya Dakhil Madrasa	Tank(GW)	Yes	No	6 (Raised)	Not found
5-Beralakkhi Madrasa	Tank(GW)	No	No	6 (Raised)	Negligible
6-Noabeki Mahabiddyaloy	Tank(RWH)	Yes	Yes	4 (Raised)	Not found
7-Beralakkhi Primary (146)	Tank(RWH)	Yes	Yes	4 (Raised)	Not found
8-Kachari Bridge School	Tank(RWH)	No	No	2 (Ground)	Negligible
9-Abdul Kader High School	Tube well	Yes	No	6 (Ground)	Not found
10-Atulia Primary (40)	Tank(RWH)	No	Yes	2 (Ground)	Major
11-Choto Kupot Primary(41)	Tank(RWH)	Yes	No	2 (Ground)	Not found
12-Choto Kupot Primary(89)	Tank(RWH)	No	No	2 (Ground)	Not found
13-Henchi High School	Tank(RWH)	Yes	No	2 (Ground)	Negligible

Source: Field survey, 2018.

Apart from these shelters, there are about 350 double story structures in the union. The figure is an estimate obtained from locals during FGDs and KIIs. Most of those structures are being used as residents. These serve as impromptu/transitional shelters. People have reported that the owners of such structures allow others to take shelter without much discrimination. However, having relatives accelerates the process and may offer the advantage of having space “reserved” for them. People, who are not familiar with the owners of such structures, may have to visit many buildings before they find space. This is because often times the closest structures are “reserved” or already occupied by people familiar with their owners. This factor also serves as a crucial stepping-stone in the decision-making process of potential evacuees. Some fear the rejection and decide not to evacuate at all.

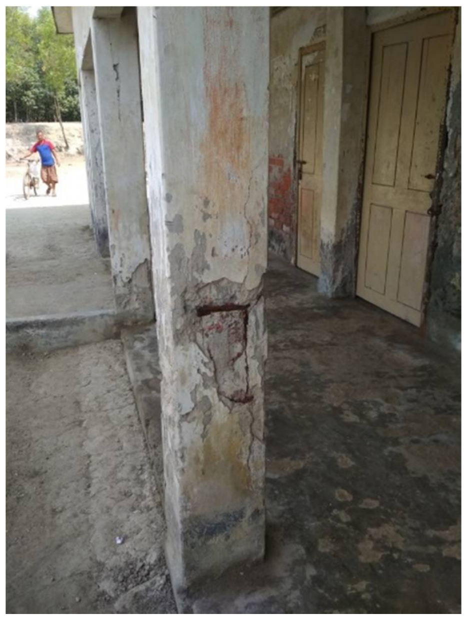


Fig. 4. The extent of structural damage of CS 2 Boro Kupot Primary (81) can be seen to be extreme. The column, which had the load bearing responsibility, has become fragile with the concrete spalling. Moreover, the rods are exposed to air and is rusting away, substantially decreasing their strength.

These structures can accommodate up to 60 people on average. Simple arithmetic shows that about 21,000 evacuees (in addition to the owners) can take refuge there. However, the location of these structures is not uniformly distributed. In addition, people have predominantly reported (contrary to the norms reported in the 1991 Gorky Cyclone evacuation) that they send the most vulnerable members (elders, women, children, etc.) first, with the male household head evacuating in the impending minutes. It is often seen that the population is threefold or more than the capacity of the shelters. It is this population who cannot access the shelters that travel to privately owned structures.

Table 3. Summary of shelter capacity.

Shelter Name	Capacity	Settlement	Difference
1- Atulia CS1	800	2600	1800
2-Boro Kupot Primary (81)	800	3800	3000
3-Kashimari Primary	700	2600	1900
4-Rabeya Dakhil Madrasa	1800	2700	900
5-Beralakkhi Madrasa	1500	3600	2100
6-Noabeki Mahabiddyaloy	1600	5400	3800
7-Beralakkhi Primary (146)	1600	3400	1800
8-Kachari Bridge School	1200	2400	1200
9-Abdul Kader High School	1600	2900	1300
10-Atulia Primary (40)	1000	3600	2600
11-Choto Kupot Primary (41)	800	2700	1900
12-Choto Kupot Primary (89)	800	2800	2000
13-Henchi High School	1000	3300	2300

It is clear that accessibility depends on distance, road quality, and facilities within the CS. So to gain an overall idea of the accessibility of each shelter, an index has been developed (Table 4). The slowest walk time is the same indicator in the 3rd column of Table 1. It means the time taken to walk to the CS by older people on a rainy day. Population difference is the population greater than the shelter capacity. Water source indicates the presence of fresh drinking water in the shelters. To be considered unsustainable, there has to be an absence of storage tanks. The factor “Solar power” indicates the existence of working solar panels and lights. The presence of structural damage has been observed. The index translates the importance of each factor in the decision-making process of the evacuee using “Weights”. The weights have been found by rank ordering technique during FGDs. The “Values” represent each factor’s current condition. Like, if the distance to the shelter is too long, then the walk time increases. The greater the walk time, the higher is the value, which in turn indicates less accessibility. Similarly, the corresponding facilities of each shelter enable a value to be found, representing the accessibility of that shelter. This is compiled in Table 5. The higher the value, the less affinity it has to potential users.

As shown in Table 5, the greater the shelter score, the less attractive it is to its user. A score of >25 means it requires immediate attention, and that of around 15 means regular maintenance is due. This threshold value is based on local opinions. The score of CS 2 is the highest and therefore indicates to be the least desirable. The most impoverished road condition and major structural damages played a significant role in that score. Also, the longer distances in such roads also create difficulties for locals aiming to seek shelter elsewhere. This region had the least amount of privately owned double-story buildings, which meant the unaccommodated population had to travel long distances to find safety.

CS 10's relatively high score is contributed by its structural damages and low access road (Figure 5). The only access road leading to the shelter is simply a narrow pile of bare soil, reported being notoriously slippery during rains. Figure 5 shows the route of each settlement cluster to their corresponding cyclone shelter.

In Fig. 5, it can be seen that there are multiple roads leading to each shelter. For instance, about 9 roads connected to CS13. The length of these roads is not the same. The roads coming from the west are much longer than the roads on the east. The longest road has been considered when calculating the accessibility. This is because the accessibility of the people who live the farthest away from the shelter is prioritized to represent the worst-case scenario.

Table 4. The Table describes the weighting criteria for index scoring.

Weight	Factor	Values		
2	Slowest walk time	1 if <60minutes	2 if >60minutes	
4	Population difference	1 if <2000	2 if <2500	3 if >2500
1	Water Source	0 if Sustainable	1 otherwise	
1	Toilets	0 if Present	1 otherwise	
1	Solar Power	0 if Sustainable	1 otherwise	
5	Structural Damage	0 if Absent	1 if present	
6	Road Condition	0 if Pucca	1 if moderate	2 if severely damaged

Table 5. Accessibility Score of each shelter.

Name	Index score	Name	Index score
1- Atulia CS1	7	8-Kachari Bridge School	10
2-Boro Kupot Primary (81)	35	9-Abdul Kader High School	10
3-Kashimari Primary	7	10-Atulia Primary (40)	31
4-Rabeya Dakhil Madrasa	6	11-Choto Kupot Primary(41)	15
5-Beralakkhi Madrasa	7	12-Choto Kupot Primary(89)	16
6-Noabeki Mahabiddyaloy	6	13-Henchi High School	13
7-Beralakkhi Primary (146)	12		

The index takes into account the holding capacity of people in the shelter, and, for CS 6, which has an unaccommodated population of 3,800 people, many of them actually live in their own double-story buildings and therefore do not go to the shelters. This is why its scores have gone down. Both CS 11 and 12 have roads with moderate damage, which is the reason behind their score. The rest of the shelters are in overall good condition but still require regular monitoring, especially before each cyclone season gives a better idea of why longer distances make shelters like CS 2 and CS 13 provide high scores even though they had adequate facilities within the shelters.

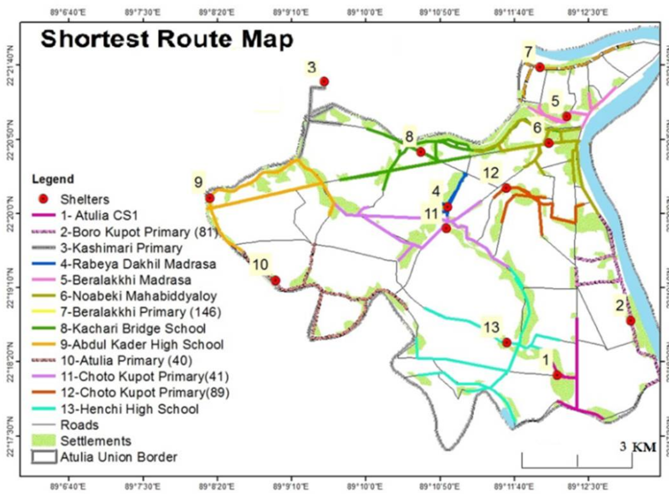


Fig. 5. Map of the shortest route.

The union has a current population of about 42,000, but shelters (both conventional and transitional) have a capacity of 38,000 (Table 3). Even if all the roads and shelters are fixed, 4,000 people still do not have any shelter. Therefore, it is logical to build newer shelters (Fig. 6) to benefit the maximum number of people in the union. Based on the shortest route calculation, the suggested location (14) will serve the greatest number of people by accommodating those in the CS 2 region who currently have the least desirable shelter, poor roads, and live far away from 2 story buildings. The capacity could be around 3,000. Given that, this community is directly beside the river, which brings in the surge, having a shelter nearby could be lifesaving. It will also benefit those, that have to travel the longest distances in the region of CS 12 and 13, by cutting their travel times by as much as half. The road conditions and shelter need to be fixed immediately for CS 10 to increase its desirability. As for the region south of CS 13 there existed no shelters at the time of the field visit. So, a suggestion could be to build a shelter of around 3,000 capacity (location 15). This will drastically reduce the pressure on CS 13. Many of the 2,100 unaccommodated people can then take shelter in Location 15 instead. If the access roads for CS 10 are not fixed, a portion of that population could travel to location 15.

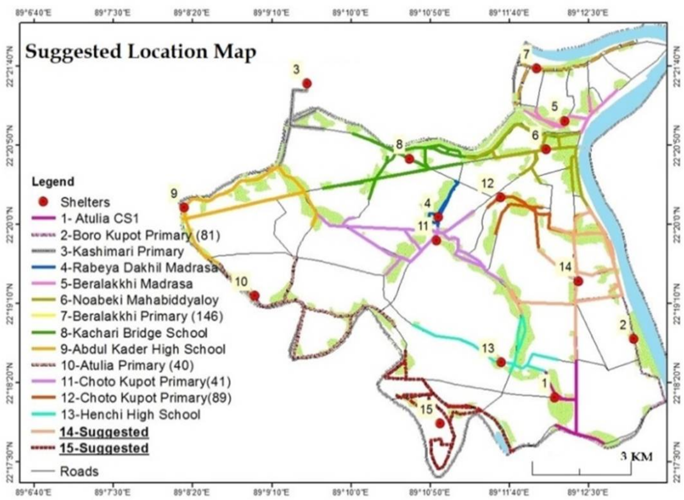


Fig. 6. Map showing suggested locations (14 & 15) for new construction.

There is no doubt that Bangladesh had significant progress in terms of Cyclone Preparedness. This is not only reflected in the infrastructure setting but also in the level of awareness of people. The manifold reduced casualties in Cyclone Aila compared to Cyclone Gorky stand testament to this fact. However, there are still credible risks, and not to mention gaps and challenges in Preparedness. When Atulia is taken as an example, it was seen that although 13 traditional shelters exist, they are by no measure enough. Even the capacity of those shelters quoted here is beyond the recommended thresholds (3.75 m^2 per person) of the Government of Bangladesh Cyclone Shelter Management Policy. When infrastructure support proves inefficient, it is the social cohesion of the rural communities that enables them to survive. This cohesion alone is not sufficient. The research reveals that there are still about 4,000 people who would not have any shelter within the union.

The research suggests the two best possible sites to construct new shelters. The generated shortest route map can also be used for training or awareness raising purposes. It could serve as a guide for Cyclone Preparedness Programme Volunteers who would prefer to take the shortest routes during dissemination. The research also advocates the local administration to introduce awareness and training programs for resilient construction practices to ensure that shelters are safe and capable of withstanding surge impacts. In addition, older shelters are often not maintained, and they pose a risk of collapse or partial structural failure.

In the context of rural Bangladesh, conventional Multipurpose Cyclone Shelters are still considered the best form of infrastructural support for Cyclone Preparedness (Amin *et al.* 2016). The research shows that through appropriate locational analysis, the selection of shelter construction plots could enhance preparedness. If that is done by following the opinions of stakeholders (especially the locals), then a truly sustainable solution can be expected.

Acknowledgments

We thank Oxfam for funding the field visits. Shahidul Islam provided part of the Road network shapefile. Nafis Sazeed has provided support during field surveys. We also thank Ashraful Islam, Assistant Professor, Department of Geology, DU, for his guidance in structuring the paper.

References

Ali, A. 1996. Vulnerability of Bangladesh to climate change and sea level rise through tropical cyclones and storm surges. *In:* Water, Air, and Soil Pollution (Vol. 92, pp. 171-179). Kluwer Academic Publishers. https://doi.org/10.1007/978-94-017-1053-4_16

Ali, A. 1999. Climate change impacts and adaptation assessment in Bangladesh. *Climate Research*, 12:109-116. https://doi.org/10.3354/cr012109

Amin, M., S. Shil and M. Hasan. 2016. Status of cyclone shelter facilities in south central Bangladesh. *Journal of Environmental Science and Natural Resources*, *9*(1), 75. https://doi.org/10.3329/jesnr.v9i1.30295

Atulia Union Parishad 2018. Budget Allocation Report', Ministry of Local Government, Rural Development and Co-operatives.

ArcGIS Network Analyst Overview 2020. Retrieved from https://www.esri.com/en-us/arcgis/products/ArcGIS-network-analyst/overview (Accessed: 23 September 2020),

Bangladesh Bureau of Statistics 2011. *Population & Housing Census*.

Fenton, A., J. Paavola and A. Tallontire 2017. The Role of Microfinance in Household Livelihood Adaptation in Satkhira District, Southwest Bangladesh, World Development. Pergamon, 92, pp. 192–202. Doi: 10.1016/J.WORLDDEV. 2016.12.004.

Focus Group Discussion 2020. ODI Available at: https://www. odi.org/publications/5695-research-tools-focus-group-discussion (Accessed 01 September 2020).

Google Earth 2018. Digital Satellite Imagery of Atulia union, Satkhira. Google Earth. Retrieved from: https://earth.google.com/web/@22.322474,89.15974176,1.01564195a,6926.17277109d,35y,0h,0t,0r (Accessed 04 January 2021)

Government of Bangladesh 2008. Cyclone Sidr in Bangladesh: Damage, loss, and needs assessment for disaster recovery and reconstruction - Bangladesh | *ReliefWeb*. Available at: https://reliefweb.int/report/bangladesh/cyclone-sidr-bangladesh-damage-loss-and-needs-assessment-disaster-recovery-and (Accessed: 2 May 2018).

Government of Bangladesh 2009. Bangladesh Climate Change Strategy and Action Plan. Available at: https://www.iucn.org/content/bangladesh-climate-change-strategy-and-action-plan-2009 (Accessed: 2 May 2018).

Hossain, M.Z., M.T. Islam, T. Sakai and M. Ishida 2008. Impact of Tropical Cyclones on Rural Infrastructures in Bangladesh. *Invited Overview.* Retrieved from http://www. cigrjournal.org/index.php/Ejounral/article/viewFile/1036/1029

Huq, M., M.F. Khan, K. Pandey, M.M.Z. Ahmed, Z.H. Khan, S. Dasgupta and N. Mukherjee 2010. *Vulnerability Of Bangladesh To Cyclones In A Changing Climate : Potential Damages And Adaptation Cost.* The World Bank. https://doi.org/10.1596/1813-9450-5280

International Federation of Red Cross and Red Crescent Societies 2002. World Disasters Report 2002. Damage Assessment Report, Dhaka, Bangladesh: International Federation of Red Cross and Red Crescent Societies.

International Federation of Red Cross and Red Crescent Societies 2009. Cyclone Aila Report. Damage Assessment Report, Dhaka, Bangladesh: International Federation of Red Cross and Red Crescent Societies.

IPCC 2014. Fifth Assessment Report (AR5): Synthesis Report. Intergovernmental Panel on Climate Change, *Geneva*, 2014. Available at: http://www.ipcc.ch/ (Accessed: 28 September 2017).

Islam, D., S.M. Bhuiyan and A.L. Ramanathan 2015. Climate change impacts and vulnerability assessment in coastal region of Bangladesh: a case study on Shyamnagar upazila of Satkhira District. *Journal of Climate Change* **1,2**:37-45.

Jia, Z. 2010. *Cyclone Shelters and Cyclone Resilient Design in Coastal Areas of Bangladesh.* Thesis, Department of Urban Studies and Planning, Tsinghua University.

Khan, S.R. 1995. Cyclone Hazard in Bangladesh, Case Study in Coastal Hazard Assessment. ADPC. Available at: http://www.adpc.net/casita/Case_studies/Coastal hazard assessment/ modelling cyclone hazard in Bangladesh/ Background_information_on_ the storm_ surge_modelling.pdf (Accessed: 1 May 2018).

Local Government Engineering Department 2018. Administrative Boundaries. Available at: http://gis.lged.gov.bd/Map/Public/published-map (Accessed 23 September 2020)

Paul, B.K. 2009. Why relatively fewer people died? The case of Bangladesh's Cyclone Sidr. *Natural Hazards*, **50**(2), 289–304. https://doi.org/10.1007/s11069-008-9340-5

Paul, S.K., and Routray, J.K. 2013. An Analysis of the Causes of Non-Responses to Cyclone Warnings. In *Climate Change Management, Springer*. https://doi.org/10.1007/978-3-642-31110-9_2

Roy, C., S. Sarkar, J. Åberg and R. Kovordanyi 2015. The current cyclone early warning system in Bangladesh: Providers' and receivers' views. *International Journal of Disaster Risk Reduction, 12*, 285–299. https://doi.org/10.1016/J.IJDRR.2015.02.004

United Nations International Strategy for Disaster Reduction 2011. *Global assessment report on disaster risk reduction (2011).* Retrieved from: https://www.unisdr.org/ we/inform/ publications/19846 (Accessed 01 September 2020).

World Bank 2010. *Poverty maps.* http://documents.worldbank.org/curated/en/ 9167614 68211 763695/pdf/904870WP0WB0Po00Box385319B00PUBLIC0.pdf (Accessed 01 September 2020).

(Revised copy received 27.o9.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 179-189, December 2020

BREEDING BIOLOGY OF THE RED-WHISKERED BULBUL, *PYCNONOTUS JOCOSUS* (LINNAEUS, 1758) IN MADHUPUR NATIONAL PARK, BANGLADESH

SHAWKAT IMAM KHAN[1] AND HABIBON NAHER[2]

[1]*Department of Natural History, Bangladesh National Museum, Dhaka-1000, Bangladesh*

[2]*Department of Zoology, Jagannath University, Dhaka-1100, Bangladesh*

Abstract

Breeding biology of the Red-whiskered Bulbul, *Pycnonotus jocosus* (Linnaeus, 1758) in Madhupur National Park (MNP), Bangladesh, was carried out from January to June in 2009. The breeding season lasted from February to May. Both the parents built a hollow cup-shaped nest, the weight, length, and depth of which were respectively, 9.9-12 g (10.98±0.78 g), 8-10 cm (8.98±0.67 cm), and 4.1-5 cm (4.62±0.312 cm). The outer and inner diameter of the nest opening was 8-9.7 cm (8.92±0.54 cm) and 6-7.5 cm (6.72±0.49 cm), respectively. The height of the nest from the ground ranged from 0.6-2.1 m (1.41±0.55 m). They took 14-16 days (15±0.85 days) in nest building and laid 2-4 (2.74±0.81) whitish with brown blotched eggs. The egg size was 2.10×1.45 cm. The hatchlings hatched out after 12-15 days (13.14±1.17 days) of incubation period and fledged between 13-18 days (14.85±1.77 days) after hatching. The breeding success was 81.82% in relation to nestlings hatched and 51.92% in relation to eggs laid. Infertility (47.36%) was the leading cause of egg loss and stolen (66.67%) for nestlings.

Key words: Breeding season, Nestling, Fledging, Red-whiskered Bulbul

Introduction

Red-whiskered Bulbul (*Picnonotus jocosus*) is a common resident bird of Bangladesh (IUCN Bangladesh 2015). It belongs to the Family Pycnonotidae and Order Passeriformes. Its global range extends through South, East, and South-east Asia (IUCN Bangladesh 2015). In Bangladesh, it occurs in different habitat types (IUCN Bangladesh 2015). It inhabits forests, gardens, orchards, and bushy areas, including villages and towns (Siddiqui *et al.* 2008). It is seen in pairs or loose feeding flocks in lightly wooded areas and open country with bushes and shrubs and farmland (IUCN Bangladesh 2015). It feeds mainly on drupes, berries, figs, flower-buds, nectar, soft bodied insects such as

[1]Author for correspondence: <shawkat194@gmail.com>.

caterpillars, ants, and spiders (Siddiqui *et al.* 2008). It is one of the most common and important seed dispersal agents in anthropogenic open habitats in tropical Asia (Corlett 1998). However, information on the breeding biology of this species is limited in Bangladesh. Information on breeding ecology is found at Lucknow in India (Mazumdar and Kumar 2007) and south-west China (Li *et al.* 2015). Hence, we aimed to collect detailed information on the breeding biology of this species in a deciduous forest, which may be the baseline information in Bangladesh.

Material and Methods

Study area: The study was carried out at Madhupur National Park (MNP) (24°36' to 24°42' N, 90°00' to 90°06' E), Tangail, Bangladesh (Fig. 1) from January to June in 2009. The total area of the park is 8,430 ha (Khan and Ahsan 2011). This is situated in the northern part of Bhawal-Madhupur *Sal* (*Shorea robusta*) forest tract, 50 km south of the Garo Hills of the Meghalaya State of India, and about 151 km north of Dhaka (Khan and Ahsan 2015), the capital of Bangladesh. The park's altitude is about 20 m above the mean sea level (Monirujjaman and Khan 2018).

The park is within the administrative District of Tangail except for a portion of the southeast corner that falls in the Mymensingh District. The park forms a slightly elevated tract of approximately 1-2 m in height over the surrounding plains. There are numerous depressions with gentle slopes intercepting the ridges, which runs north to south, forming the irregular masses of high lands with gentle slopes (Ismail and Mia 1973). In the rainy season, the low-lying depressions in the park accumulate water and become marshy. These marshy places dry up in summer and winter seasons, but they expand into broad, shallow areas in the rainy season. The forest is partly dense, partly thin, and there are scrub jungles also (Khan and Ahsan 2015). The climate is moderate with warm weather from March to October; the maximum temperature is 37.6°C in April. The cold weather lasts from November to February with a minimum temperature of 7.8°C. The annual average rainfall is 2,091 mm. Maximum humidity varies from 97 to 100% and a minimum of 20 to 58%. Madhupur National Park is dominated by Sal (*Shorea robusta*) trees associated with other deciduous plant species (Khan and Ahsan 2015).

To find out the nest and nesting trees, the area was surveyed through a transect line with the help of local field assistance. A total of 28 km transects were covered to find out the nest (Table 1). A total of 19 nests were identified during the nest building and egg laying period. While a nest was located, the GPS location was recorded. Nest characteristics were noted on unused nests (nesting materials, nest height from the ground, the outer and inner diameter of the nest opening, depth of the nest cup) after fledging. Altogether, 15 nests were followed to record the nest building period. The nest was visited a week thrice to record the egg-laying time, clutch size, egg measurements, incubation period, hatching time, hatchling number, hatchling measurements, fledgling number, and fledging date. Fourteen nests were examined to find out the incubation period and 27 nestlings were monitored for fledgling period. A total of 52 eggs and 33 hatchlings were measured to find out the egg and hatchlings size respectively. Food items provisioned to the nestlings by the parents were also recorded. Stolen of nestling by local urchins' and any sign of nest destruction was also recorded.

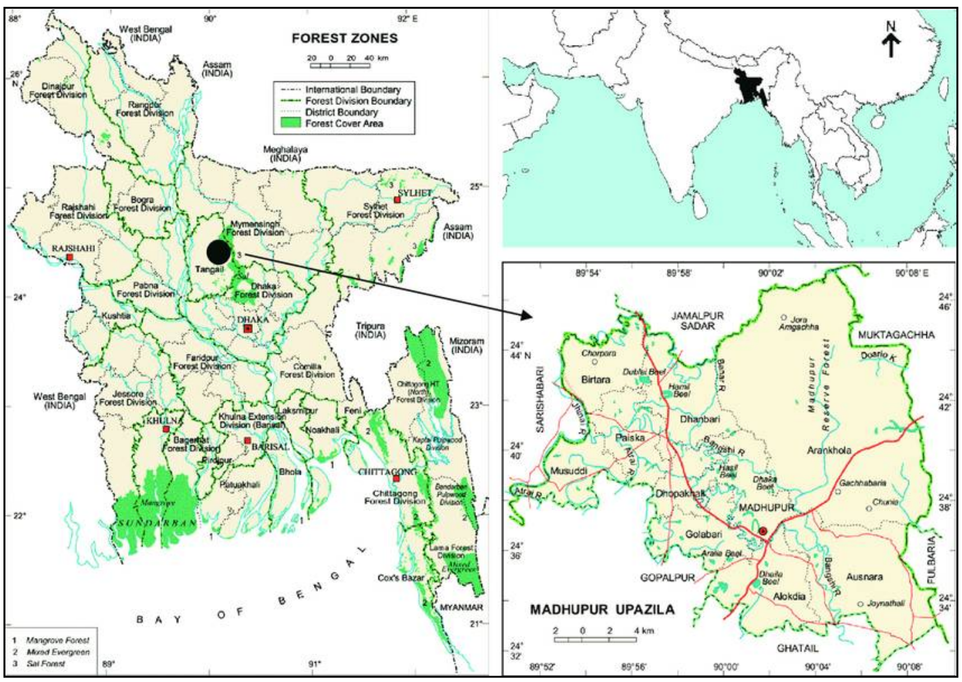


Fig. 1. Location of the Madhupur National Park (Islam *et al.* 2014).

A digital electronic scale (500 g×0.1 g) was used to weigh the nestling, fledgling, and unused nest after fledging, a slide caliper to take egg and nestling measurement, meter tape (1 cm to 50 m) to measure the nest height from the ground, binoculars (Bushnell 10×42) to monitor the nest and behaviour of the parents from a safe distance and GPS

(Garmin *etrex* 8) machine to take GPS of the nest site and to measure transect length during the study period.

Table 1. Length of transects used during the study period.

Transect number	Transect site	Transect length (km)	GPS location	
			Start Point	End Point
1	National park main gate, Rasulpur to Dokhola beat forest gate	8	24°41'20.16"N 90° 8'21.25"E	24°42'34.18"N 90° 3'58.44"E
2	Dokhola beat forest gate to Pochish mail bazar	9	24°42'34.18"N 90° 3'58.44"E	24°38'44.55"N 90° 4'41.62"E
3	Rasulpur Mazar to Pochish mail bazar	9	24°41'20.16"N 90° 8'21.25"E	24°38'44.55"N 90° 4'41.62"E
4	Bangladesh Air Force Firing range road	1	24°40'40.50"N 90° 7'38.70"E	24°40'06.92"N 90°07'42.05"E
5	Rasulpur to Kathalia village	1	24°41'20.16"N 90° 08'21.25"E	24°40'28.16"N 90°08'41.96"E

Windows spreadsheet program Excel was used to do all the computations and numerical analyses. All the mean values are provided with standard deviations. The breeding success was calculated by using the following formulae:

Hatching success (%) = (No. of eggs hatched / total no. of eggs laid) × 100

Fledging success (%) = (No. of nestlings fledged / total no. of nestlings hatched) × 100

Breeding success (%) = (No. of eggs laid / No. of nestlings fledged) × 100

Result and Discussion

Breeding season: The breeding season was recorded from February to May in the Madhupur National Park. It was somewhat later (March to September) in Bangladesh compared to Siddiqui *et al*. (2008). The breeding season varied in different countries of the world. It was mid-February to May in Florida (Florida Fish and Wildlife Conservation Commission 2003), March to July in the north of India, December to June in south India with the second period in September after the monsoon (Riper *et al.* 1979), August to March in Australia (Australian Museum 2020), February to August at Xishuangbanna Tropical Botanical Garden in southwest China (Li *et al.* 2015). Ali and Ripley (1971) stated that they breed in March, chiefly April in India.

*Nesting tree and nest site***:** Nests were built on low forked branches of available shrubs or small trees in bushes. Low tree fork was also reported as a nest site in Sydney (Australian Museum 2020). Virtually shrubs, hedges, or small trees within suburbs were used as a nesting site in Florida (Carleton and Owre 1975). Shrubs or small trees were reported as a nesting site in Florida (Florida Fish and Wildlife Conservation Commission 2003). Nests in low bushes or creepers were observed by Siddiqui *et al.* (2008).

In MNP, this bird was noted to prefer to build its nest at roadside bushes of the forest apart from human habitation (Table 2). Baker (1922) mentioned that this species selected garden and cultivated lands for its abode; breeding excursions into jungle and forest are rare.

Table 2. GPS of the nesting sites.

Nest no	GPS	
	N	E
1	24°41'49.47"	90°07'41.59"
2	24°41'17.93"	90°08'29.31"
3	24°40'41.03"	90°07'37.77"
4	24°41'23.70"	90°08'03.39"
5	24°41'41.37"	90°06'35.88"
6	24°42'07.74"	90°04'57.48"
7	24°40'21.56"	90°07'57.48"
8	24°40'21.86"	90°06'44.54"
9	24°41'11.14"	90°08'07.22"
10	24°41'22.28"	90°08'03.01"
11	24°41'43.11"	90°04'55.54"
12	24°40'20.14"	90°07'44.79"
13	24°41'04.62"	90°07'55.34"
14	24°40'58.36"	90°08'31.30"
15	24°39'40.13"	90°05'20.11"
16	24°40'01.43"	90°05'46.97"
17	24°39'19.29"	90°05'04.82"
18	24°39'06.77"	90°04'36.39"
19	24°42'25.09"	90°04'15.45"

Nest building: Both the partners took part in the nest building. During nest building, when one partner was busy to place the nesting materials, the other partner flew away to collect nesting materials. They used their beak to place nesting materials in a crisscross way and rubbing their breast and belly inside the nest to make it cup shape. They put cobweb around the rim to bind the materials tightly and placed the soft, thin fiber inside the nest's cup. Sharing nest building by both partners was also reported by Mazumdar and Kumar (2007). Sharing nest building was also reported in other birds (Naher *et al.* 2009, Naher 2012).

Morphometric of nest: Nests were cup shaped, deeply hollow at the center (Fig. 2). Shallow cup shaped nest was also described by others in different countries (Mazumdar and Kumar 2007, Siddiqui *et al*. 2008 and Li *et al.* 2015).

The vertical length of the outer layer of the nest was 8-10 cm (8.98±0.67 cm). The present study was very close to Riper *et al.* (1979), who found 8 cm length in Hawaii. The outer diameter of the nest opening in MNP was 8-9.7 cm (8.92±0.54 cm) which was very close to the study of Riper *et al.* (1979) study (8-10 cm). The inner diameter of the nest opening was 6-7.5 cm (6.72±0.49 cm), which was the same as the findings of Riper *et al.* (1979). The cup depth was 4.1-5 cm (4.62±0.312 cm) in the present study, but Riper *et al.* (1979) stated a little bit larger (5.5 cm) depth. The weight of dry, unused nest was 9.9-12 g (10.98±0.78 g), but Riper *et al.* (1979) recorded a little bit lower (9.9 g) weight in Hawaii.

Fig. 2. Nest of a Red-whiskered Bulbul.

Nesting materials: They used the materials to build the nests which were available surrounding the nesting sites. The nest was built with leaves, soft fibers of bamboo, twigs, cobwebs, small grasses, and different small wild plant's fibers. Various nesting materials were reported by many researchers in other study sites of the world. Siddiqui *et al*. (2008) reported that the nest was built up of twigs and dead leaves bound with cobwebs, especially around the rim, and lined with roots, grasses, and hairs. Florida Fish and Wildlife Conservation Commission (2003) reported that the nest is constructed of rootlets grass and the Australian pine needles and are usually embellished with bits of dried leaves, paper, plastic, snake skin, or the bark of the cajeput tree in Florida. Australian Museum (2020) noted that the nest is built up of rootlets bark, and leaves, lined with soft fiber in Sydney. According to Riper *et al.* (1979), the nest had a leaf base with coarse grass and flexible stems of small shrubs woven together to make a bowl, there was no cup lining tissue paper, and a strand of bark adorned the outside of the cup in Hawaii. Carleton and Owre (1975) found rootlets and grasses in the nest in Florida .

Nest height from the ground: The height of the nest from the ground ranged from 0.6-2.1 m (1.41±0.55 m) in MNP. A very close finding (0.6-2.4 m) was reported in Florida by Carleton and Owre (1975). But in Hawaii, the nest was built at 1.5-3.6 m above the ground (Riper *et al.* 1979).

Territory: During the breeding season, the male bird maintained a territory around its nesting site by chasing the intruder and by territorial song at morning and afternoon. This species uttered 'territorial song' to ensure the territory boundary at Songkhala Province in the south of Thailand (Sotthibandhu 2003). Red-whiskered Bulbuls are among the most range restricted breeding birds in Florida (Pranty 2010).

Nest building time: Both sexes took part in nest building. They took 14-16 days (15±0.85 days) to build a nest.

Clutch size: They laid one egg each day. The clutch size ranged from 2-4 (2.74 ± 0.81) in MNP. Same clutches (2-4 eggs) were found in Florida (Florida Fish and Wildlife Conservation Commission 2003), Japan (Eguchi and Amano 2004), and Australia (Australian Museum 2020). Two to three clutches were also reported in different sites (Mazumdar and Kumar 2007, Siddiqui *et al.* 2008, Li *et al.* 2015).

Egg characteristics: Egg colour was whitish with brown blotched or spotted in MNP. Brown blotched with white background eggs were also reported by Riper *et al.* (1979) in Hawaii. The whitish colour egg was noted in Florida (Florida Fish and Wildlife Conservation Commission 2003) but in Australia, pale pink eggs streaked and spotted with shades of red were reported (Australian Museum 2020).

The length of eggs varied from 2-2.2 cm (2.10±0.08 cm) and width 1.4-1.5 cm (1.45±0.05 cm) in MNP. Siddiqui *et al.* (2008) recorded the egg size as 2.2 × 1.6 cm.

Incubation and incubation period: Both the partner took part in incubation in MNP. Sharing in incubation was also presumed by Ali and Ripley (1971) in this species and Naher *et al*. (2009) and Naher (2012) in other species. When one bird incubated, the other stayed at the nearby branches of the nesting tree and guarded the nest. If an intruder came near the nest, the guarder made the threat call while the bird engaged in incubating escaped from the nest immediately to a hiding place.

The incubation period was 12-15 days (13.14±1.17 days) in MNP, a little bit shorter (11 days) in Hawaii (Riper *et al.* 1979) but almost same (12-13 days) in Florida (Florida Fish and Wildlife Conservation Commission 2003) and in Bangladesh(12 days) (Siddiqui *et al.* 2008).

Hatchling: The newly born hatchling was naked with reddish body colour and the eyes were closed. The naked hatchling was also reported in Hawaii (Riper *et al*. 1979). The weight of the one-day hatchling in MNP was 2-4 g (2.7±0.7 g), which was similar to Carleton and Owre (1975) reported in Florida. In MNP, the total body length of the hatchling was 41-44 mm (41.8±0.8 mm). The length of the beak of the hatchling was 3-4 mm (3.4±0.5 mm), and the head was 11-13 mm (11.9±0.8 mm).

Hatching success: The hatching success was 63.46% in relation to eggs laid, which was higher than Li *et al*. (2015) findings (53.78%) in southwest China.

Causes of eggs loss: A total of 19 eggs (36.5%) were lost due to different causes (Fig. 3). Infertility was the prime factor of egg loss, followed by a heavy storm, predation, and stealing by local urchins. Local urchins used to steal the eggs for fun. Infertility, storms, parent desertion, and predation were causes of egg failure to hatch in Xishuangbanna Tropical Botanical Garden, southwest China (Li *et al*. 2015).

Nestling food and parental care: Both partners took care of nestling, fed, and cleaned the feces of nestlings. They fed the young, providing caterpillars, small insects, berries, and crushed fruits in MNP. Mazumdar and Kumar (2007) stated that both parents were involved in feeding the young and provided them regurgitated food in India. Li *et al.* (2015) stated that both invertebrate and plant food were provided to the nestlings in southwest China.

Fledging period: In MNP, the young fledged between 13-18 days (14.85±1.77 days) after hatching which was shorter (12 days) in Hawaii (Riper *et al.* 1979) but longer (16 days) in Florida (Florida Fish and Wildlife Conservation Commission 2003). The weight of the fledgling was 17.94-20.98 g (19.21±1.07 g) with a total body length of 79.10-84.30 mm (81.18±1.77 mm). In MNP, the tail length was 10.96-12.88 mm (11.99±0.58 mm), beak length was 15.45-16.05 mm (15.74±0.19 mm), tarsus length was 19.90-20.80 mm (20.31±0.29 mm), and wing length was 47.13-51.10 mm (48.97±1.36 mm).

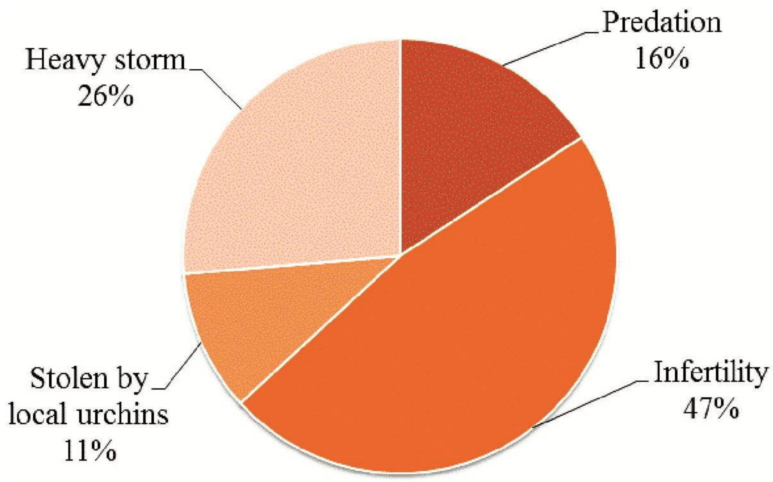


Fig. 3. Causes of egg loss during the nesting period of Red Whiskered Bulbul in Madhupur National Park.

Causes of nestling loss: A total of 6 nestlings were lost due to stolen (66.7%) by local urchins' and nest parasites (33.3%). The local urchins' collected fledglings for fun, which was the prime factor in decreasing breeding success. Nestlings died due to nest parasites, storms, and predation in Xishuangbanna Tropical Botanical Garden, southwest China, while predators are the main factor in nest failure (Li *et al.* 2015). Predators, diseases, size of nests, and chances of nestlings falling off their nests were the factors to death or mortality of fledglings in Lucknow, India (Mazumdar and Kumar 2007). Carleton and Owre (1975) mentioned that the common potential predators of the bulbuls are snakes, rats, cats and certain birds in Florida.

Breeding success: The fledging success was 51.92% in relation to eggs laid and 81.82% in relation to nestlings hatched. The fledging success was 63.63% in southwest China (Li *et al.* 2015), and the overall nest success was 34.22%. Mazumdar and Kumar (2007) reported that the nesting success was 72.2% in the Lucknow city center, but 80.5% among the periphery of the Lucknow city of India.

The Red-whiskered Bulbul builds their nest in roadside bushes of the forest. Clearing of the forest is a regular phenomenon of our country, a threat to their nesting sites. Furthermore, cutting trees by the people to expand agricultural land and firewood collection also creates hazards for breeding habitats of the Red-whiskered Bulbul. Moreover, the regular practice of destruction of eggs and nestlings for fun by local urchins increases the mortality rates of this species. Therefore, an awareness campaign among the local people should be the best approach to conserve the habitat of the Red-whiskered Bulbul.

Acknowledgements

The authors are grateful to local field guide Mr. Nuru and other people of Madhupur National Park for their help during data collection.

References

Ali, S. and S.D. Ripley. 1971. *Handbook of the birds of India and Pakistan*. Vol. 6. Bombay, Oxford University Press.

Australian Museum. 2020. Red-whiskered Bulbul. http://australian.museum/learn/ animals/birds/ red-whiskered-bulbul-pycnonotus-jocosus/. Date accessed 4 July 2020.

Baker, E.C.S. 1922. *The fauna of British India, including Ceylon and Burma*. Vol. **1**, second ed. London, Taylor and Francis.

Carleton, A.R. and O.T. Owre. 1975. The Red-whiskered Bulbul in Florida: 1960-71. *The Auk*. **92**: 40-57.

Corlett, R.T. 1998. Frugivory and seed dispersal by birds in Hong Kong shrubland. *Forktail*. **13**: 23-27.

Eguchi, K. and H.E. Amano. 2004. Invasive birds in Japan. *Global Envtal. Res.* **8**(1): 29-39.

Florida Fish and Wildlife Conservation Commission. 2003. Florida's breeding bird atlas: A collaborative study of Florida's birdlife. http://www.myfwc.com/bba/. Date accessed 4 July 2020.

Islam, M.K., S. Saha, I. Mahmud, K. Mohamad, K. Awang, S.J. Uddin, M.M. Rahman and J.A. Shilpi. 2014. An ethnobotanical study of medicinal plants used by tribal and native people of Madhupur forest area, Bangladesh. *J. Ethnophar*. **151**(2): 921-930.

Ismail, M. and M.M. Mia. 1973. *Studies on some deciduous sal forests of Bangladesh; Ecology of Bangladesh Vegetation.* Botanical Survey of Bangladesh, University of Dhaka.

IUCN Bangladesh. 2015. *Red List of Bangladesh Volume 3: Birds*. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh. pp. xvi+676.

Khan, S.I. and M.F. Ahsan. 2011. Birds of the Madhupur National Park, Bangladesh. *Bangladesh J. Zool*. **39**(1): 49-63.

Khan, S.I. and M.F. Ahsan. 2015. Frugivorous birds and fruit plants in a deciduous forest in Bangladesh: a case study in the Madhupur National Park. *Bangladesh J. Zool*. **43**(2): 173-187.

Li, H., M.X. Zhang, X.J. Yang, L.W. Cui and R.C. Quan. 2015. The breeding biology of Red-Whiskered Bulbul (*Pycnonotus jocosus*) in Xishuangbanna, southwest China. *Zool. Res.* **36**(4): 233-240.

Mazumdar, A. and P. Kumar. 2007. Nesting ecology of the Red Whiskered Bulbul at city center and periphery in Lucknow, Northern India. *Том 16 Випуск*: 98-102.

Monirujjaman and M.M.H. Khan. 2018.Wildlife Diversity of Madhupur National Park, Bangladesh. *Jahangirnagar Univ. J. Biol. Sci.* **7**(1): 1-13.

Naher, H. 2012. *Food habit, breeding biology and conservation issues of Kingfishers, Alcedo atthis and Halcyon smyrnensis.* Ph.D. thesis. University of Dhaka. pp. xiii+228.

Naher, H., N.J. Sarker, M.K. Rahman and S.I. Khan. 2009. Breeding biology of the Little Cormorant *Phalacrocorax niger* (Pelecaniformes: Phalacrocoracidae) in Bangladesh. *J. Threatened Taxa*. **1**(4): 221-225.

Pranty, B. 2010. Status and current range of Red-whiskered Bulbuls (*Pycnonotus jocosus*) in Florida. *Florida Fie. Nat.* **38**(4): 146-149.

Riper, C.V.III, S.G.V. Riper and A. Berger. 1979. The Red-whiskered Bulbul in Hawaii. *Wilson Bull.* **91**(2): 323-328.

Siddiqui, K.U., M.A. Islam, S.M.H. Kabir, M. Ahmad, A.T.A. Ahmed, A.K.A. Rahman, E.U. Haque, Z.U. Ahmed, Z.N.T. Begum, M.A. Hassan, M. Khondker and M.M. Rahman. (eds.). 2008. *Encyclopedia of Flora and Fauna of Bangladesh,* Vol. 26. *Birds.* Asiatic Society of Bangladesh, Dhaka. pp. 662.

Sotthibandhu, S. 2003. Territorial defense of the Red-whiskered Bulbul, *Pycnonotus jocosus* (Pycnonotidae), in a semi-wild habitat of the bird farm. *Songklanakarin J. Sci. Technol.* **25**(5): 553-563.

(Revised copy received on 6.12.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 191-200, December 2020

IDENTIFICATION AND QUANTIFICATION OF SOIL PESTICIDES IN COASTAL LAKSHMIPUR DISTRICT OF BANGLADESH

M. AMIN UDDIN[1], NASIDULABEDIN CHOWDHURY[2], MD. ABDUR RAHMAN[1], Md. HARUNUR RASHID[1], M. ALAMGIR ZAMAN CHOWDHURY[1] AND ZEENATH FARDOUS[1*]

[1]Agrochemical and Environmental Research Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy commission, Ganakbari, savar, Dhaka, Bangladesh.
[2]Department of Biochemistry and Molecular Biology, Bangladesh National University (Tejgaon College), Dhaka-1215, Bangladesh.

Abstract

This study was carried out to determine the presence and quantity of some selected pesticides from soil sediments collected from some ponds and canals located in the Lakshmipur district of Bangladesh. The high performance liquid chromatography (HPLC) technique was used to determine the concentration of pesticide residues. Some soil samples were found to be contaminated with carbamate (carbofuran and carbaryl) and organophosphorus (diazinon) pesticides. The concentration of carbofuran pesticide ranged from 0.303 μg/kg to 1.851 μg/kg. The highest concentration of carbofuran pesticide was found in SSP_6 (1.851 μg/kg) and the lowest concentration was found in SSP_9 (0.303 μg/kg). Carbaryl pesticide was found to be present in the sediment of only one pond, the concentration being 1.047 μg/kg. Organophosphorus (diazinon) pesticide was found in soil samples and the concentrations ranged from 0.147 μg/kg to 0.759 μg/kg, which were higher than the EEC-recommended limit of 0.1 μg/kg.

Key words: Carbaryl, Carbofuran, Diazinon, HPLC, Soil

Introduction

Bangladesh is predominantly an agricultural country with an area of 1, 47,570 sq. km. It has only 0.31 percent of the world's total agricultural land, but 2.0 percent of the globe's total population (Hossain *et al.* 2015, Rasul and Thapa 2004). To feed 160 million people, different agrochemicals in the form of pesticides and fertilizers have been used in this limited agricultural land over the last few decades. This practice has led to the build-up of pesticide residues in the products, the destruction of beneficial insects, and pest resurgence. Pesticides have also been associated with environmental pollution (Rashid *et al.* 2015). Exposure of farm workers to pesticides has been causing various types of

*Author for correspondence: <zafardousbace2gmail.com>.

cancer. These pesticides are also causing health hazards for humans such as headache, body aches, skin rashes, poor concentration, feelings of weakness, circulatory problems, dizziness, nausea, vomiting, excessive sweating, impaired vision, tremors, cramps, psychomotor dysfunction, depression, Parkinson's disease and, in severe cases, coma (Fardous *et al.* 2015, Hancock *et al.* 2008). Barring a few, humans have no remedies for the diseases caused by pesticides. Therefore, it is necessary to determine if pesticide residues are present in environmental matrices like sediments.

The World Health Organization (WHO) estimates that there are 3 million cases of pesticide poisoning each year and up to 220,000 deaths, primarily in developing countries (WHO 2001). The application of pesticides is often not very precise, and unintended exposures occur to other organisms in an area where pesticides are applied. Children and many young and developing organisms are particularly vulnerable to the harmful effects of pesticides. Even low levels of exposure during development may have adverse health effects (Sarwar 2015).

In the past, pesticides have contributed significantly to improve the yield of crops, which ensured food security for the global population. The widely cultivated high yielding variety is highly vulnerable to pests and diseases. Thus, the use of pesticides are now an inherent part of agriculture for pest control (Bagchi *et al.* 2008). Pests pose a serious problem because of their high reproduction potential and rapid turnover of generations. Farmers use large quantities of chemical insecticides for effective control of the pest larvae. Therefore, the aim of this study was to investigate the occurrence and amount of pesticides in the soil of a coastal region of Bangladesh.

Materials and Methods

*Collection and pretreatment of samples***:** Twenty-five soil samples (15 soil samples from different ponds and 10 soil samples from different canals) were collected from Sadar Upazila of Lakshmipur district near the coastal area. Bags, permanent markers, spoons, boxes, etc., were used to collect soil samples. Before the collection of soil samples, bags were cleaned with detergent. Bags were marked by a permanent markers.After collection, the samples were brought to the lab at the Agrochemical and Environmental Research Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka. The samples were immediately preserved in a deep fridge (-20ºC) to prevent the loss of the pesticide residues. The samples were collected in August, 2016, which belongs to the rainy season in the country. Heavy rainfall washes out the topsoil and brings pesticide residues into water bodies.

Selection of the study area: Lakshmipur is a small southeastern district of Bangladesh. Chandpur District borders it to the north, Bay of Bengal and Noakhali district to the south, Noakhali District to the east, and Meghna river to the west. About 47.51% people are engaged in agriculture for their occupation in Lakshmipur district. The main crops in this district are paddy, wheat, potato, pulse, sugarcane, etc. But paddy is the most cultivated crop. Our study area is Lakshmipur Sadar Upazila, which is located at 22.95°N and 90.82°E (Fig. 1). In the Sadar Upazila, paddy and vegetables, especially cucumber are cultivated the most. 'Basudin', an organophosphorus pesticide, is used by the farmers which contains 'Diazinon'. Another brand of pesticide, which contains mostly 'Carbaryl' and 'Carbofuran' of 'Carbamate' is also used by the farmers. Most of the paddy fields are located near ponds, and most of the time, the drainage water of those paddy fields flows to the nearest ponds. Some ponds are lying, and their banks are too low to prevent the incoming water from the nearby paddy fields in rainy seasons. The vegetable fields are located near the canals and the drainage water flows to the canals.

Sample processing in laboratory: Fifty (50) gm soil sample was taken in a conical flask (250 ml) and 100 mL of solvent (a mixture of hexane and acetone at 1:1) was added to the conical flask. The conical flask was then shaken for 6-7 hrs using a mechanical shaker and the contents were allowed to settle down. Finally, the extract (hexane: acetone mixture) was collected from the conical flask. Similarly, the extract mixture was collected two more times with 25 ml solvent by hand shaking for 5 min. Ten gm of anhydrous sodium sulphate Na_2SO_4 was added to the combined extract and the contents were allowed to settle. The solvent was then decanted and subsequently evaporated by a rotary vacuum evaporator to dryness. Two (2) mL of HPLC-grade acetonitrile was added in three portions and the sample extracts were collected into a vial for clean-up. The extract was subjected to clean-up using florisil column chromatography, where necessary. The top 1.5 cm of the florisil column was packed with anhydrous Na_2SO_4. 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-stoppered test tubes. The solvent was completely removed under mild nitrogen flow. The evaporated sample was dissolved in acetonitrile and the volume was made up to 1 ml in a volumetric flask for analysis in high performance liquid chromatography (HPLC) (Uddin *et al.* 2016, 2018).

Identification and quantification procedures: The analysis was conducted by an HPLC (Shimadzu, Japan) LC-10ADvp, equipped with an SPD-M 10 Avp attached to a photodiode array detector (Shimadzu SPD-M 10 Avp, 200-800 nm). A C18 Reverse Phase Alltech (250 × 4.6 mm, 5 µm) was used as the analytical column, and the column temperature was maintained at 30^0C. Acetonitrile in distilled water (70:30) was used as the mobile phase at a flow rate of 1.0 ml/min. Prior to HPLC analysis, the samples were filtered through 0.45 µm nylon (Alltech Associates, IL, USA) syringe filters. The chromatograms were obtained following manual injection (20 µl) of both standard and sample solution. The suspected pesticides were identified based on the retention times of the respective standard pesticide preparation.

For the preparation of the calibration curve, equal volumes of several different concentrations of standard solutions were injected into the HPLC machine. Tentative identification of the suspected pesticides was carried out in relation to the retention time (RT) of the pure analytical standards. Quantification was performed according to the calibration method described by Bhattacharjee *et al.,* (2012). For this purpose, the injection of equal volumes of differently concentrated standard solutions into HPLC prepared calibration curve for each pesticide. To determine the residual levels of pesticides, the following equation was used:

$$R' = \frac{H_A V_{END} W_{ST}}{H_{ST} V_i G}$$

Where,

R' = mg/l for water and mg/Kg for soil

G = Sample weight (l or Kg)

V_{END} = Terminal volume of the sample solution (mL)

V_i = Portion of volume V_{END} injected into HPLC (µL) column

W_{ST} = Amount of standard pesticides injected with standard solvent (µg)

H_A = Peak area obtained from V_i (mm^2)

H_{ST} = Peak area obtained from W_{ST} (mm^2)

Extraction efficiency/recovery: The validation of the analytical method was performed according to the European Commission (EC) guidelines in terms of the accuracy, precision, and limit of quantification (LOQ) (DG SANCO, 2010). Accuracy was calculated by analyzing the samples of known concentration (n =3) and comparing the estimated values with the actual values. Within our experimental limit, the mean recovery for accuracy should be within 70-120%. For accuracy experiments, soil (50 g) was utilized as a matrix after homogenization and the addition of an appropriate amount of pesticide standards at two different fortification levels (0.02 and 0.20 ppm). Control samples were processed along with spiked ones. Both sample and standard preparation were allowed to stand for one hour to permit equilibration. Equilibration was followed by the extraction and clean up process, as described above. Percentage recovery was calculated by the following equation:

Percentage recovery = [CE/CM × 100]

Where CE is the experimental concentration determined from the calibration curve and CM is the spiked concentration. The precision was estimated by monitoring the repeated (n=6) peak response and expressed by the relative standard deviation (RSD). The acceptance criterion for precision is RSD $\leq$ 20%. Analytical procedures employed are found to be satisfactory and average recoveries between 72% and 95% were obtained for Carbofuran, Carbaryl and Diazinon pesticides from the soil samples (the fortifications were made in the range 0.02-0.2 ppm level), indicating the suitability of the methodology. The LOQ and LOD were evaluated as signal-to-noise ratios (S/N) of 10:1 and 3:1, respectively. In the present study, the LOD and LOQ were 0.01 mg/kg and 0.05 mg/kg, respectively.

Results and Discussion

Toxic pesticides are widely used in agricultural lands for prevention of valuable crop losses by pests. Pesticides improve yield as well as the quality of the product. However, pesticides pose serious health risks to farmers exposed to pesticides when they mix and apply for pesticides or work in the pesticides-treated fields. People in general also get exposed to the pesticide residues in food and drinking water. In this investigation, Sadar Upazila of Lakshmipur district was taken as the study area. The outer part of Sadar Upazila belongs to "lotic" ecosystem because of the presence of the Meghna River, while the inner part of it belongs to "lentic" ecosystem because of the presence of many man-made ponds and some natural water sources such as pools, canals, and lakes. Most of the man-made ponds and canals are in close proximity to these agricultural fields. Therefore, when soils of agricultural fields are contaminated by pesticides, the nearby water bodies are also contaminated. Pesticide residues find their way to these water bodies through surface run-off during precipitation and after irrigation.

Tables 1 and 2 show the average concentration of pesticide residues in soil samples collected from different ponds and canals of Sadar Upazilla, Lakshmipur. The retention time and area for carbofuran, carbaryl, and diazinon are presented in Tables 3-5. Fifteen soil samples were collected from ponds and ten soil samples were collected from canals of the area. Six soil samples representing ponds and canals were found to be contaminated with residues of carbofuran. Carbofuran was present in SSP_6, SSP_7, SSP_9 and SSC_6, SSC_7, SSC_{10}. The concentration of carbofuran ranged from 0.303 to 1.851 µg/kg. The highest concentration (1.851 µg/kg) was found in SSP_6 and the lowest concentration (0.303 µg/kg) was in SSP_9. Carbaryl residue was observed only in the SSP_8 sample with a concentration of 1.047 µg/kg. Table 1 and Table 2 also show the soil samples that were found to be contaminated with diazinon. Four soil samples, namely SSC_9, SSP_1, SSP_{13}, and SSP_{15}, were contaminated with diazinon. The highest concentration (0.759 µg/kg) was found in SSP_{15}, and the lowest concentration (0.147 µg/kg) was found in SSP_1.Data of the present study indicate the contamination of soil with an organophosphate. Organophosphorus pesticides have been widely used in Bangladesh since 1990 because organochlorine insecticides were banned due to their persistence as well as acute toxicity in the environment (Chowdhury *et al.* 2012). The use of organophosphorus and carbamate pesticides such as chlorpyrifos, diazinon, malathion, carbofuran, and carbaryl has greatly increased because of their less detrimental effects on the environment (Chowdhury *et al.* 2012). Many studies reported very high concentration of organophosphate and carbamate residues in the soil and water of Bangladesh (Bhattacharjee *et al.* 2012, Chowdhury *et al.* 2012, Chowdhury *et al.* 2013, Shammi *et al.* 2014). In our previous study, carbofuran was detected at a higher concentration of 3.21 µg/kg, carbaryl at 2.52 µg/kg and diazinon at 0.235 µg/kg in samples collected from vegetable and paddy field in the coastal district Feni of Bangladesh (Uddin *et al.* 2018). Carbofuran and carbaryl concentrations in the present study were lower than those of the previous study. On the other hand, diazinon concentration was found to be at a higher level than our previous study. However, the contamination level of organophosphorus and carbamate was relatively low compared to the IAEA/FAO/Codex Alimentarius Guideline values.

Carbamate pesticides were observed in soils of both pond and canal near the paddy and vegetable fields of Sadar Upazila. Among the carbamate and organophosphate pesticides, diazinon, carbofuran and carbaryl are frequently used in Bangladesh. When the soil samples were collected, farmers said, they had been using diazinon, carbofuran, and carbaryl. Therefore, the study was carried out to determine the presence and quantity of diazinon, carbofuran, and carbaryl pesticides. A better understanding about degradation of these pesticides in soil and the factors affecting the degradation process may help in the judicious application of these pesticides and the consequent mitigation of environmental pollution (Chowdhury *et al.*, 2002). There is no data on pesticide pollution in the studied area. Therefore, this study provides some baseline data to help future investigation on pesticide pollution in the area.

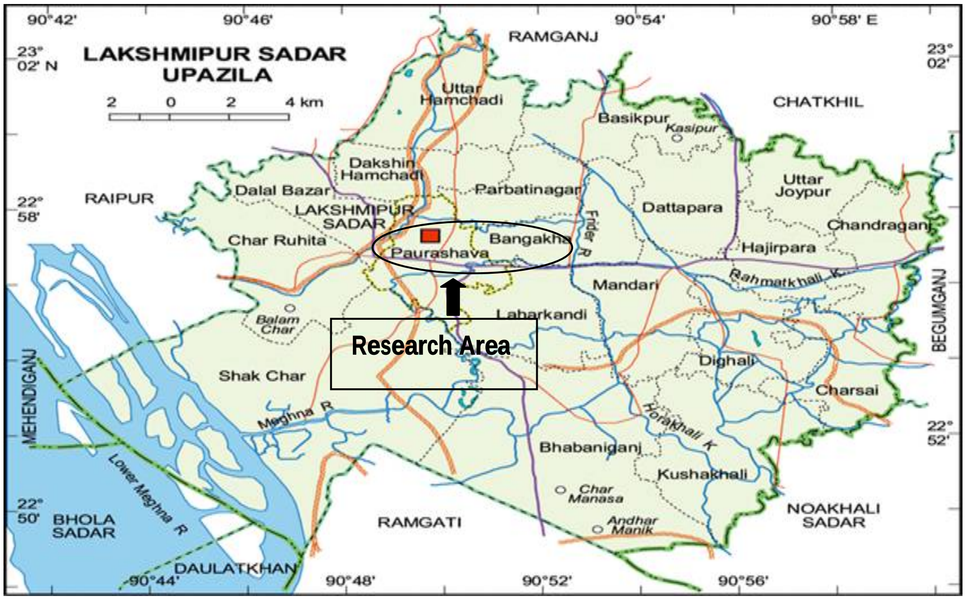


Fig. 1. Map of Sadar Upazila showing the sampling locations.

Table 1. The average concentration of pesticide residues in soil samples which were collected from different ponds of Sadar Upazila, Lakshmipur.

Sample ID	Sampling location	Carbofuran (μg/kg)	Carbaryl (μg/kg)	Diazinon (μg/kg)
SSP$_1$	Kachari Bari pond	BDL	BDL	0.147
SSP$_2$	Lakshmipur Markaz Mosque Pond	BDL	BDL	BDL
SSP$_3$	Monir Uddin Patwary Bari Pond	BDL	BDL	BDL
SSP$_4$	Tomehar Bazar Pond	BDL	BDL	BDL
SSP$_5$	Tomehar Bazar Mosque Pond	BDL	BDL	BDL
SSP$_6$	West Shayedpur Mosque Pond	1.851	BDL	BDL
SSP$_7$	Pearapur Bazar Pond	0.167	BDL	BDL
SSP$_8$	Vobanigonj Bazar Pond	BDL	1.047	BDL
SSP$_9$	Mia Bari Big Pond	0.303	BDL	BDL
SSP$_{10}$	Chourasta Bazar Pond	BDL	BDL	BDL
SSP$_{11}$	Jakshin Road Point Pond	BDL	BDL	BDL
SSP$_{12}$	Vobachat Bazar Pond	BDL	BDL	BDL
SSP$_{13}$	Torabgonj Borobari Pond	BDL	BDL	0.395
SSP$_{14}$	Torabgonj Bazar Pond	BDL	BDL	BDL
SSP$_{15}$	Koroitola Bazar Pond	BDL	BDL	0.759

Note: SSP = Soil Sample of Pond, BDL = Below Detection Limit and μg/kg = microgram per kilogram.

Table 2. The average concentration of pesticides residues in soil samples which were collected from different canals of Sadar Upazila, Lakshmipur.

Sample ID	Sample Sources	Carbofuran (μg/kg)	Carbaryl (μg/kg)	Diazinon (μg/kg)
SSC_1	Meghna River	BDL	BDL	BDL
SSC_2	Wapdar Khal West Point	BDL	BDL	BDL
SSC_3	Wapdar Khal Middle Point near Voberhat	BDL	BDL	BDL
SSC_4	Wapdar Khal North Point near Torabgonj	BDL	BDL	BDL
SSC_5	Wapdar Khal near Lakshmipur Bas Terminal	BDL	BDL	BDL
SSC_6	Rahmat Khali Khal near Meghna River	1.732	BDL	BDL
SSC_7	Rahmat Khali Khal near West Point	1.657	BDL	BDL
SSC_8	Rahmat Khali Khal near Cucumber field (Koroitola)	BDL	BDL	BDL
SSC_9	Rahmat Khali Khal north Point	BDL	BDL	0.222
SSC_{10}	Lakshmipur Khal near Ramgong Highway Road	1.069	BDL	BDL

Note: SSC = Soil Sample of Canal, BDL = Below Detection Limit and μg/kg = microgram per kilogram.

Table 3. Sample Analysis Report of Carbofuran.

Sample No	Retention Time	Area	Concentration (μg/kg)
SSC_6	3.334	332133	1.732
SSC_7	3.351	317882	1.657
SSC_{10}	3.367	205149	1.069
SSP_6	3.357	355086	1.851
SSP_7	3.304	32159	0.167
SSP_9	3.344	58121	0.303

Table 4. Sample Analysis Report of Carbaryl.

Sample No	Retention Time	Area	Concentration (μg/kg)
SSP_8	3.549	604601	1.047

Table 5. Sample Analysis Report of Diazinon.

Sample No	Retention Time	Area	Concentration (μg/kg)
SSC_9	3.329	76875	0.222
SSP_1	3.296	51027	0.147
SSP_{13}	3.296	136634	0.395
SSP_{15}	3.284	262320	0.759

[SSC=Soil Sample of Canal; SSP= Soil Sample of Pond].

The wide use of pesticides in Bangladesh causes major health and environmental problems. It is necessary to learn more about the problems caused by exposure to pesticides with respect to safety, health and the environment. A guideline should be provided for farmers on pesticide usage, safety instructions, preparation, application and disposal, and washing of equipment. As a result, the health hazards of pesticides may be reduced to a great extent. The environment and ecosystems will also be protected from the scourge of pesticide pollution. The present study has implications considering the health hazard associated with these pesticides. A further exhaustive investigation needs to be carried out to assess the status of these pesticides in foodstuff and in the food chain.

Acknowledgements

The authors would like to thank the Agrochemical and Environmental Research Division authorities, Bangladesh Atomic Energy Commission, for providing laboratory facilities to carry out these experimental studies. Also, thanks to Md. Alamgir Kabir, Abdul Hamid, and Gopal Mojumder for helping in the sample extraction process.

References

Bagchi S., A.K. Azad, M. Alamgir, Z. Chowdhury, M. Amin Uddin, Sharif M. Al-Reza and A. Rahman. 2008. Quantitative Analysis of Pesticide Residues in some Pond water samples of Bangladesh. *Asian J. Water, Environ. Pollut.* **6**(4): 27-30.

Bhattacharjee, S., Fakhruddin, A.N., Chowdhury, M.A.Z., Rahman, M.A., Alam, M.K. 2012. Monitoring of selected pesticides residue levels in water samples of paddy fields and removal of cypermethrinand chlorpyrifos residues from waterusing rice bran. *Bull. Environ. Contam. Toxicol.* **89**(2): 348-353.

Chowdhury, M.A.Z., Islam, M.N., Moniruzzaman, M., Gan, S.H. and Alam, M.K. 2013. Organochlorine insecticide residues are found in surface, irrigated water samples from several districts in Bangladesh. *Bull. Environ. Contam. Toxicol.* **90**(2): 149-154.

Chowdhury, M.A.Z., Jahan, S.A., Islam, M.N., Moniruzzaman, M., Alam, M.K., Zaman, M.A., Karim, N., Gan, S.H., 2012. Occurrence of organophosphorus and carbamate pesticide residues in surface water samples from the Rangpur district of Bangladesh. *Bull. Environ. Contam. Toxicol.* **89**(1): 202-207.

Chowdhury, N., M. A. Malek, S. M. Ullah and M. M. Rahman., 2002. Fate of [^{14}C]-carbofuran pesticide studied in a sandy loam soil in laboratory conditions. *J. Asiat. Soc. Bangladesh, Sci.* **28**(1): 19-26.

DG SANCO, 2010. Method validation and quality control procedures for pesticide residues analysis in food and feed, Document SANCO/10684/2009, 2010, http://www.crl-pesticides.eu/library/docs/allcrl/Aqc Guidance Sanco 2009 10684.pdf.

Fardous Z., Rashid M.H., Chowdhury M.A.Z, Alam M.K., Md Rahman A, Uddin M.A. and Bari M.L., 2015. Assessment of heavy metals, minerals and pesticides in different branded drinking bottled water of Bangladesh and their impact on human health. *Asia-Pacific J. Food Safety and Security* **1**(1): 27-41.

Hancock, D.B., E.R. Martin, G.M. Mayhew, J.M. Stajich, R. Jewett, M.A. Stacy, B.L. Scott, J.M. Vance and W.K. Scott. 2008. Pesticide exposure and risk of Parkinson‘s disease: A family-based case-control study. *BMC Neurology* **8**(6): 1-12.

Hossain S, M. Alamgir Zaman Chowdhury, Md. Mashihul Alam, Nazrul Islam, Md. Harunur Rashid and Israt Jahan. 2015. Determination of Pesticide Residues in Brinjal, Cucumber and Tomato using Gas Chromatography and Mass Spectrophotometry (GC-MS). *Adv. Biochem. Biotechnol.* **1**(1): 1-16.

Rashid,M.H., D. Sikdar, I. Jahan and S. Mojumder. 2015. Characterization of Total Cellulase and endo-β-1,4-glucanase and their Application in Biofuels Production as well as Protection of Crops from Damaging by Insects. *Canadian Chem. Trans.* **3**(3): 275-284.

Rasul G and G.B. Thapa. 2004. Sustainability of ecological and conventional agricultural systems in Bangladesh: An assessment based on environmental, economic and social perspectives. *Agricult. System* **79**(3): 327-351.

Sarwar M. 2015. The Dangers of Pesticides Associated with Public Health and Preventing of the Risks. *Intl. J. Bioinformatics and Biomedical Engin.* **1**(2): 130-136.

Shammi, M., M.A.Z. Chowdhury, N . Ferdous, M.A. Rahman, M.N. Islam, A.K.M. A. Sultana and M.K. Alam. 2014. Pesticide contamination of rice field agro-ecosystem: hazard perspectives of surface water quality and aquatic livesfrom Gazipur District, Bangladesh. *JU Environ. Bull.* **3**: 11-22.

Uddin M.A, M.H. Rahman , M. Nesha, M.A.Z. Choudhury, Z. Fardous and M.A. Rahman. 2018. Studies Pesticide Residues in Soils of Some Selected Spots of Coastal Region of Bangladesh. *Nuclear Sci. Appl.* **27**(1&2): 13-17.

Uddin M.A,M. Alamgir Z. Chowdhury, Z. Fardous and M. Hasanuzzaman. 2016. Quantification of Pesticide Residues in some Soils of Narshindi Area 2016. *Bangladesh J. Sci. Res.* **29**(1): 85-88.

World Health Organization. 2001. International code of conduct on the distribution and use of pesticides by Food and Agricultural Organization of the United Nations, Rome.

(Revised copy received on 29.11.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 201-207, December 2020 **- Short communication**

HOST PLANTS OF MEALYBUGS WITH THEIR DAMAGE EXTENT ON SOME SELECTED PLANTS

M.M.H. KHAN*

Department of Entomology, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh

There are several species of mealybugs that can be pests of greenhouse, nursery, and landscape plants. Nearly 246 families of various plants on which 5000 species of mealybug feed have been reported all over the world (Ben-Dov 1994). According to Afzal *et al*. (2009) and Aheer *et al.* (2009), mealybugs feed on nearly 149 plant species, suck plant sap and cause leaves to distort and fall. Mealybug infestations appear on plants as tiny, soft-bodied insects surrounded by a fuzzy, white mass around the stems and leaf nodes (VanZile 2013). The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is a small polyphagous sucking insect with pest status. It has a wide host range and great potential to damage economically important tropical fruits, vegetables and ornamental plants (Cham *et al.* 2011). Immature and adult stages of *P. marginatus* suck the sap of the plant and weaken it. The leaves become crinkled, yellowish and wither. The honeydew excreted by the bug and the associated black sooty mold formation impairs the photosynthetic efficiency of the affected plants. The nymphs and females of mango mealybugs suck plant sap from inflorescences, tender leaves, shoots and fruit peduncles. As a result, the infested inflorescences dry up, affects the fruit set, causing fruit drop. These bugs also exude honeydew over the mango tree leaves, on which sooty mold fungus develops, reducing the photosynthetic efficiency of the tree (Meena *et al*. 2014). However, so far, there are no records of host plants infested by mealybug species and their damage extent in Bangladesh. Therefore, the present study was undertaken to record the host plants infested by different mealybug species and determine the extent of damage caused by mealybug on brinjal, papaya, pigeon pea and mango.

The survey was carried out on different host plants in campus of Patuakhali Science and Technology University (PSTU), located in the southern coastal region of Bangladesh, from March to June 2014. Different plant species of fruits, vegetables and ornamental plants are grown on roadsides were selected for the study. Infested plants were examined with a magnifying glass. Samples of vegetable crops, fruits and ornamental plants were collected at weekly intervals throughout the period of study. Mealy bugs found on twigs,

*E-mail: mohasin1965@pstu.ac.bd

leaves, branches, stems, flowers, inflorescences and roots were assorted and recorded. The mealybugs were kept in 70% alcohol. Total adults and nymphs of mealybug were counted. Some plant samples with mealybugs were preserved in a glass jar containing 70% alcohol.

Six mealybug species *viz.*, *Ferrisia virgata* Ckll, *Paracoccus marginatus* Williams and Granara De Willink, *Drosicha mangiferae, Pseudococcus longispinus, Phenacoccus solani* and *Maconellicoccus hirsutus* were observed in different host plants. *Ferrisia virgata* was found on *P. guajava, Solanum melongena, Annona* sp. and *Carica papaya.* Long tailed mealybug was observed on *Jatropha glandulifera* and *P. guajava.* Mango mealybug, *Rastrococcus* spp. was found on *Mangifera indica. Paracoccus marginatus* were recorded on *P. guajava, S. melongena, C. papaya, Cajanus cajan, M. indica, Corchorus olitorius* and *Hibiscus rosa-sinensis. Phenacoccus solani* was observed in *S. melongena,* and *P. guajava. Maconellicoccus hirsutus* was recorded on 11 host plant species, namely, *Codiaeum variegatum, Acalypha hispida, A. wilkesiana, Mussaenda philippica, Hibiscus rosa-sinensis* (Hibiscus), *Ixora grandiflora, Punica granatum, P. guajava, M. indica, Lycopersicon esculentum* and *H. rosa-sinensis* (Table 1). Mealybugs were found to cause serious damage to succulent plants (Fig. 1a-m).

The number of *P. solani* on various plant parts of brinjal is presented in Fig. 2. It has been illustrated that the highest number of mealybug was found on a mature leaf (141.4) while the highest percent area covered by mealybug was found on a young leaf (74%) (Fig. 3). Fig. 4 revealed that the highest number of 1st instar per leaf followed by 3rd and 2nd instar nymphs was recorded in brinjal. The ventral surface of foliage and fruit, along with the calyx of brinjal, was damaged by *P. solani* shown in Fig. 1d. The variation in the number of mealybug on different aged leaves might be due to the nutritional variation of brinjal leaves. It was reported by Evans (1984) that monophagous and oligophagous herbivores often show strong preferences for the more nutritious, younger leaf tissues that are high in toxins, where polyphagous herbivores demonstrate a strong preference for the less nutritious, mature leaf tissues. As mealybug is a polyphagous pest, it might have a preference for mature leaves to young leaves.

Fig. 5 illustrated that the number of mealybug per colony was maximum (67) on the leaf stalk. Likewise, the percentage of leaf stalk infestation per plant was 86.42%, while the area of a leaf covered by mealybug colonies was 41.75%. On highly infested plants, papaya mealybug looked like small pieces of cotton attached to the plant. On heavily infested plants, the mealybug colonies formed cotton-like masses on the aerial parts (Fig. 1b).

Table 1. Host plants of six mealybug species at PSTU campus.

Common name of mealybug	Scientific name of mealybug	Common name of host plant	Scientific name of host plant
Guava mealybug (Fig. 1a)	*Ferrisia virgata* Ckll	Guava	*Psidium guajava*
		Custard apple	*Annona* sp.
		Sharifa	*Annona squamosa*
		Brinjal	*Solanum melongena*
		Papaya	*Carica papaya* L.
Long tailed mealybug (Fig. 1a)	*Pseudococcus longispinus*	Jatropha	Jatropha glandulifera
		Guava	*Psidium guajava*
Papaya mealybug (Fig. 1b & e)	*Paracoccus marginatus* Williams and Granara De Willink	Papaya	*Carica papaya* L.
		Guava	*Psidium guajava*
		Brinjal	*Solanum melongena* L.
		Pigeon pea	*Cajanus cajan* (Linn.)
		Mango	*Mangifera indica* L.
		Jute	*Corchorus olitorius* L.
		China rose	*Hibiscus rosa-sinensis* L
Mango mealybug (Fig. 1c)	*Drosicha mangiferae*	Mango	*Mangifera indica* L.
Brinjal mealybug (Fig. 1d)	*Phenacoccus solani*	Brinjal	*Solanum melongena*
		Guava	*Psidium guajava*
Pink hibiscus mealybug (Fig. 1f-m)	*Maconellicoccus hirsutus*	Maite patabahar	*Codiaeum variegatum*
		Lal hatisur	*Acalypha hispida*
		Khat Patabahar/ Copper leaf	*Acalypha wilkesiana* 'Dwarf'
		White double mussaenda	*Mussaenda philippica* 'Aurorae'
		Rangan	*Ixora grandiflora*
		China rose	*Hibiscus rosa-sinensis* L
		Pomegranate	*Punica granatum* L.
		Guava	*Psidium guajava*
		Mango	*Mangifera indica* L.
		Tomato	*Lycopersicon esculentum* Mill.
		Daffodil (Hallod jaba)	*Hibiscus rosa-sinensis* L

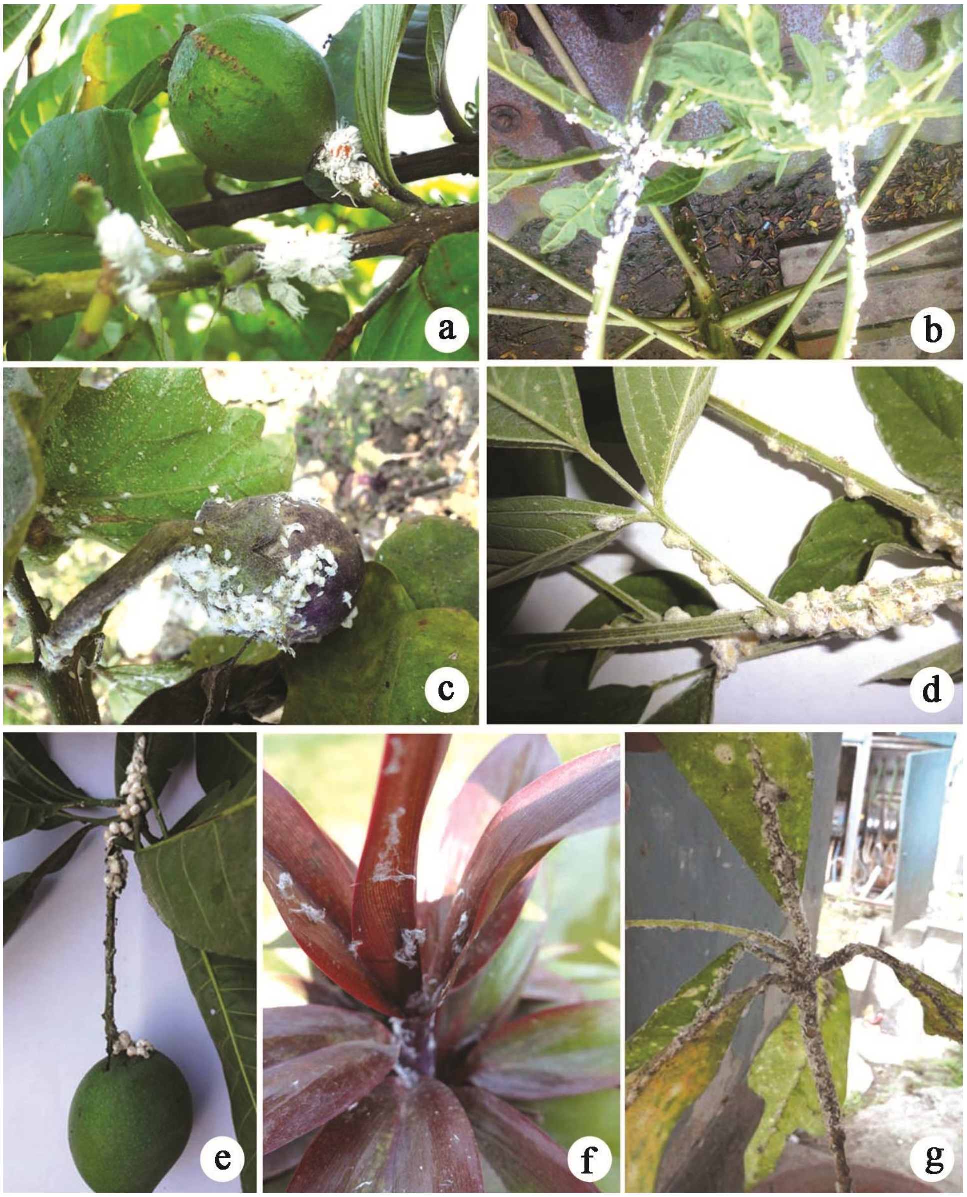


Fig.1(a-g): (a). ***Psidium guajava.*** **(b).** ***Carica papaya*****, (c).** ***Solanum melongena,*** **(d).** ***Cajanus cajan*****, (e),** ***Mangifera indica*****, (f).** ***Cordyline terminalis,*** **(g).** ***Codiaeum variegatum*** **Elite.**

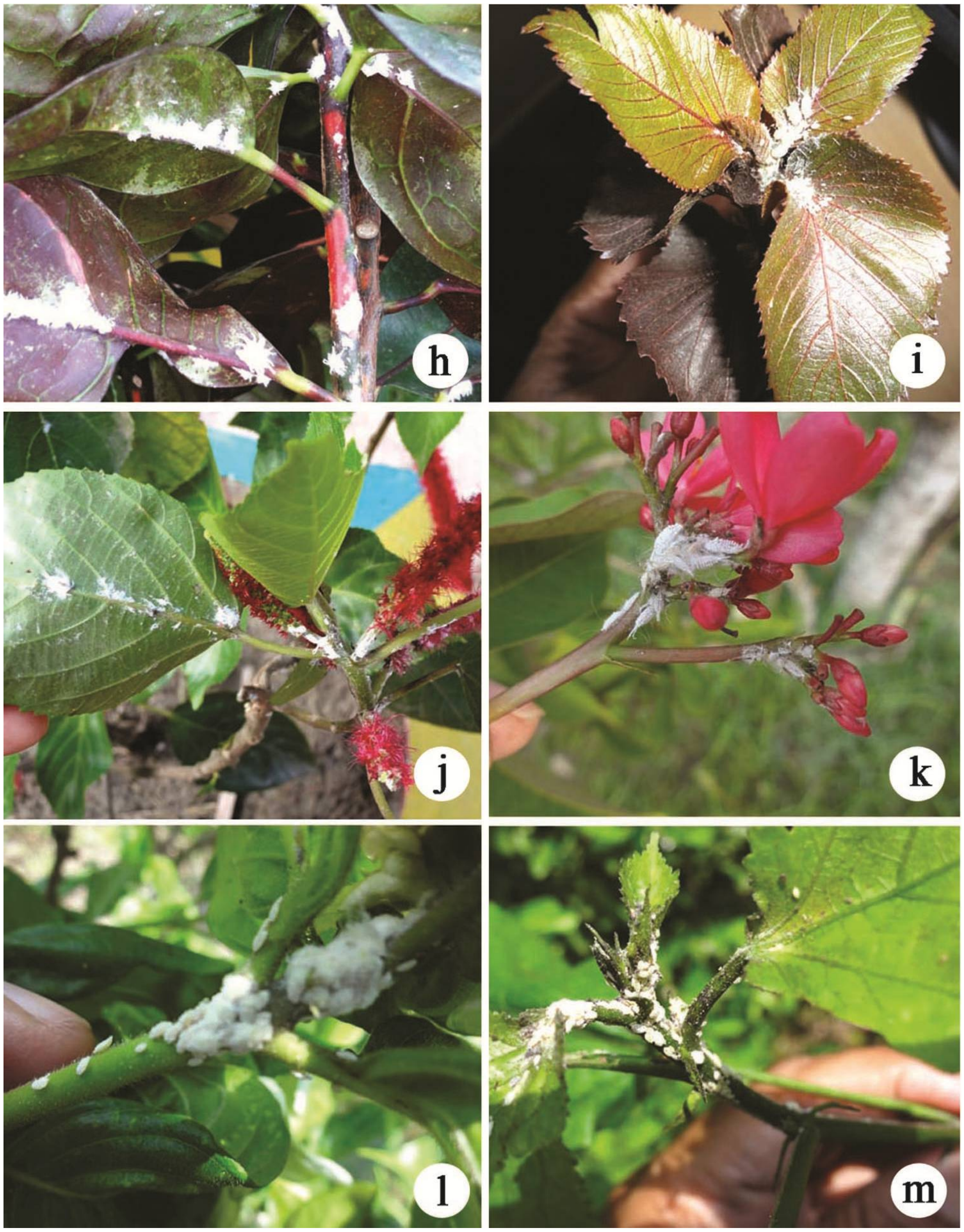


Fig. 1(h-m): (h). ***Codiaeum variegatum*** **Elite, (i).** ***Acalypha wilkesiana,*** **(j).** ***Acalypha hispida,*** **(k).** ***Jatropha glandulifera*** **, (l).** ***Mussaenda philippica,*** **(m).** ***Hibiscus rosa-sinensis.***

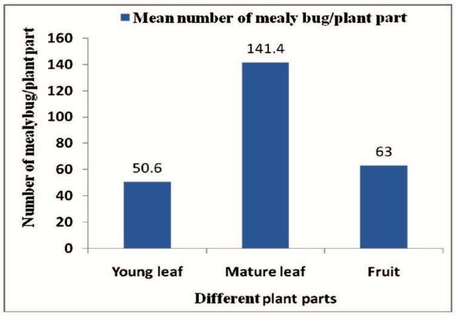


Fig. 2. Number of *Phenacoccus solani* on leaf and fruit of brinjal.

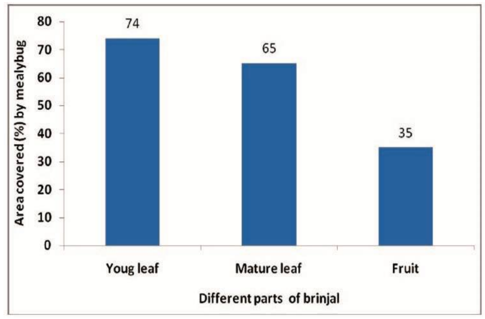


Fig. 3. Percentage of the area covered by *Phenacoccus solani* on leaf and fruit of brinjal.

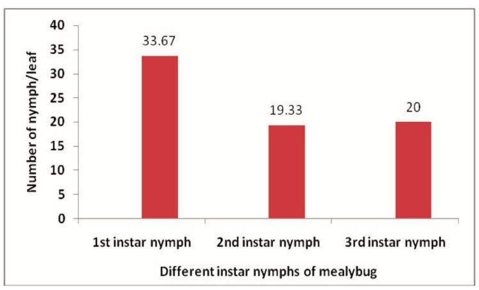


Fig. 4. Different instar nymphs of *Phenacoccus solani* per leaf of brinjal.

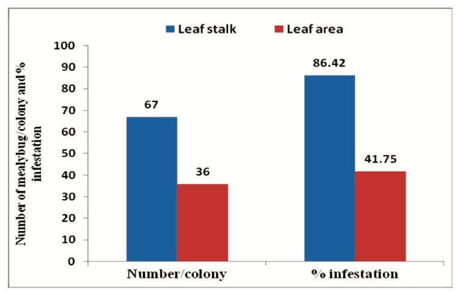


Fig. 5. Percentage of leaf stalk infestation per plant and leaf area covered by *Paracoccus marginatus.*

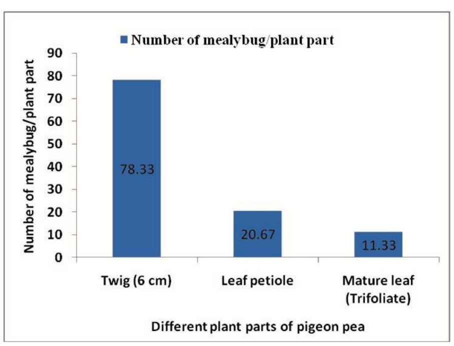


Fig. 6. Number of *Paracoccus marginatus* on plant parts of Pigeon pea.

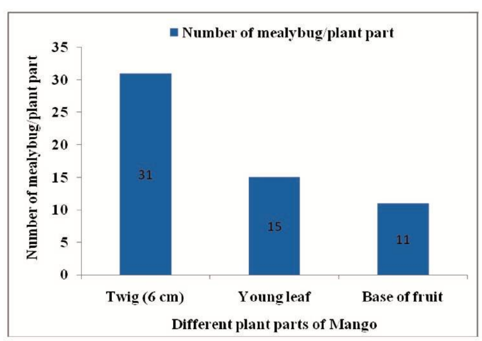


Fig. 7. Number of *Drosicha mangiferae* plant parts of Mango.

These damage symptoms resembled those caused by papaya mealybug described by Walker *et al*. (2003) and Hue *et al*. (2007).

Fig. 6 revealed that the highest number of mealybug was found on 6 cm twig (78.33), followed by leaf petiole (20.67), while the lowest was in trifoliate mature leaf (11.33).

Fig. 7 revealed that the highest number of *Drosicha mangiferae* was found on 6 cm twig (31), followed by young leaf (15), while the lowest number was in the base of fruit (11).

In this study, six mealybug species were enlisted, which caused damage to several host plant species. The author is grateful to the authority of Patuakhali Science and Technology University for financial assistance to carry out the research work.

References

Afzal, M., S.U. Rahman and M.T. Siddiqui. 2009. Appearance and management of a new devastating pest of cotton, *Phenacoccus solenopsis* Tinsley, in Pakistan. Belt-wide Cotton Conference, San Antonio, Texas, pp. 5-8.

G.M. Aheer, Z. Shah and M. Saeed. 2009. Seasonal history and biology of cotton mealy bug, *Phenacoccus solenopsis* Tinsley. *J. Agric. Res.* **47**(4): 423-431.

Ben-Dov, Y. 1994. A systematic catalogue of the mealybugs of the world, Intercept Limited, UK, p. 686.

Cham, D., H. Davis, D. Obeng-Ofori and E. Owusu. 2011. Host range of the newly invasion mealybug species *Paracoccus marginatus* Williams and Granara De Willink (Hemiptera: Pseudococcidae) in two ecological zones of Ghana. *Res. Zool*. **1**(1): 1-7.

H.E. Evans. 1984. Insect Biology: A textbook of Entomology. Addison-Wesley Publishing Company, Massachusetts-California-London-Amsterdam-Ontario-Sydney. p. 436.

Heu, R.A., M.T. Fukada and P. Conant. 2007. Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococ-cidae). State of Hawaii New Pest Advisory. Department of Agriculture No. 04-03 March 2007.

Meena, B.M., A. Chattopadhyay, S.C. Meena and S.S. Rathore. 2014. Package and Practices for Integrated Pest Management of Mango (*Mangifera indica*). Popular Kheti. **2**(3): 100-108.

VanZile, J. 2013. Mealybugs- How to Control Mealy bugs.

Walker A., M. Hoy and D.E. Meyerdirk. 2003 Papaya Mealybug. Univ. Florida Featured Creatures.http://creatures.ifas.ufl.edu/fruit/mealybugs/papaya_mealybug.htm (Accessed on 29 Sept 2008)

(Revised copy received on 22.9.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 209-214, December 2020 **- Short communication**

GROWTH AND YIELD OF *AVICENNIA OFFICINALIS* (BAEN) L. AND *HERITIERA FOMES* (SUNDRI) BUCH. HAM. PLANTATIONS ESTABLISHED FROM PLUS TREE SEEDS IN THE WESTERN COASTAL BELT OF BANGLADESH

MD. ABDUL QUDDUS MIAH* AND MD. GOLAM MOULA
Plantation Trial Unit Division, Bangladesh Forest Research Institute, Barishal, Bangladesh

The mangrove forest is distributed in the inter-tidal region between the sea and the land in the world's tropical and subtropical regions between approximately 30^0 N and 30^0S latitude (Alongi 2009). Mangrove is among the most productive and biologically important ecosystems of the world because they provide important and unique ecosystems, goods and services to humans, society and coastal and marine systems (Giri *et al*. 2010). It also performs some important ecological, biophysical and socio-economic functions, including serving as a natural barrier against storms, typhoons, and tsunamis, thus protecting coastal habitats (Latief and Hadi 2007). The forests help stabilize shorelines and reduce the devastating impact of natural calamities like tidal wave surge, cyclones and tsunamis. They also provide breeding and nursing grounds for marine and aquatic species and foods, medicine, fuel and building materials for local communities. Mangrove plantations in the shoreline area leads to rapid accretion because the mangrove roots and pneumatophores effectively slow water movement and act as efficient sediment trappers (Woodroffe 1992) and also could be beneficial for long term coastal protection both to continuous erosion and severe natural hazards. Mangrove species are also good candidates to change the coastal environments, social and ecological situation (Nandy and Ahammad 2012).

Over the last two decades, mangrove forest plantation areas have been increasing in Bangladesh. A total of 0.192 million hectares of accreted land were afforested with mangrove species in the coastal regions till 2013 (Hasan 2013). Plantations are increasing day by day to cover the barren forest land and newly accreted land. Bangladesh is one of the leading countries of coastal afforestation programs with different mangrove species. Afforestation program along the coastal belt was initiated in 1966 with the primary objective to protect the lives and properties of coastal communities from the cyclone and tidal surges by creating mangrove forest cover or green belt in the exposed 710 km coastal belt (Das and Siddiqi 1985). Subsequently, the objectives of coastal afforestation

*Corresponding author: <maqmiah2009@yahoo.com>.

were expanded to reclamation and stabilization of newly accreted land and production of timber for fuelwood (Saenger 1987). At the first time of plantation, most commercial mangrove species were tried on newly accreted land periodically inundated by tidal water. Among them, only *Sonneratia apetala* Buch.-Ham is the most successful and *Avicennia officinalis* (baen) L. is the second most successful planted species of the coastal mangrove plantations (Siddiqi 2001a). Among the cultivated mangrove species, *Avicennia officinalis* is about 5% of the total mangrove plantations (Siddiqi 2001b).

At present, the coastal mangrove plantations are under severe threat due to geomorphic changes, species succession and insect infestation to the *S. apetala* (Siddiqi 2001a). In most old plantation sites, the forest floor rises up due to heavy sedimentation and resulted less inundation during the full moon. Large scales stem borer attack in *S. apetala* plantations were observed for a long time (Baksha 1996). The mortality of planted seedlings of this species in the coastal areas is also high (Siddiqi and Das 1988). As a result, gaps are created inside *S. apetala* plantations. Moreover, insufficient regeneration appeared under these plantations due to the rising forest floor, compactness of soil and scarcity of other mangrove species' seedlings. The life cycle of *S. apetala* is short and the rotation period of felling is about 12-15 years. After harvesting of matured *S. apetala* trees, there will be no second rotation forests for the sustainability of this coastal forest. Thick and dense vegetation can diminish the height of tides and thus reduces the magnitude of the devastation. The dense forest along the coastline can protect human habitation, lives, properties and crops from extreme climate changes (Islam and Rahman 2015). Tropical mangrove forests have been shown highly productive. Still, the growth and yield of planted mangrove species in Bangladesh are lower than in other South Asian countries such as Thailand's, Indonesia, Malaysia and the Philippines (Hawlader 1999). The main reasons for the low productivity of mangrove species were the use of poor quality seeds and planting stocks (Nandy *et al*. 2004). The use of improved seed source will always remain an essential factor for achieving maximum yield. Intensive forest management activities will never maximize yields unless supplemented with the use of genetically superior trees and their improved seed sources (Zobel and Talbert 1983). Therefore, the present study was undertaken to assess the growth and yield of *Avicennia officinalis* and *Heritiera fomes* plantations from plus tree seed sources with high quality seeds from phenotypically superior trees.

The experiment was laid out in two places of maderbunia char lands under Rangabali islands of Patuakhali districts in 2010. One place was newly accreted (char) lands and another medium raised land. Rangabali is located at latitude 21^0 92´ N and longitude $90^0$45´ E. Soil of the sites is silt-clay-loam. In monsoon, water salinity ranged 3-27 ppt, while in the dry season, it ranged from 10-33 ppt (Siddiqi and Khan 1990). Soil salinity also varies remarkably between the monsoon and dry seasons. Soil salinity ranged from 0.3-4.2 dS/m in December and reach its peak in April-May, when average salinity is as high as 9 dS/m (Hasan 1987). Soil salinity in the monsoon and dry season changed remarkably between 1.5 and 4.0 dS/m. Soil pH was slightly alkaline and varying between 7.5 and 8.0 (Siddiqi and Khan 2000). The climate is humid. Temperatures ranges between 18ºC and 32°C (Siddiqi 2002). Annual rainfall varies from 2500 to 3000 mm (Siddiqi 2002). The Bangladesh Forest Research Institute established 50 plus trees of *A. officinalis* species at Rangabali and Char Kukri-Mukri Island for good quality seed sources in the pure stand of *Avicennia officinalis* experimental plantations. The plus tree of *Avicennia officinalis* (baen) species was selected from different char lands of Rangabali and Char Kukri-mukri in the year 2000. The plus trees were evaluated by providing the highest score 10 for diameter at breast height (dbh), 15 for individual tree height, 20 for bole form, 15 for branch angle, 10 for branch diameter, 10 for apical dominance, 5 for forking and 10 for individual tree health and 30 for wood properties (Nandy 1989). The score range of selected plus tree of *A. officinalis* species was from 91 to 106 at char Kukri-Mukri, when the stand was 17 years old. The score range of selected plus tree of *A. officinalis* at Rangabali sites was between 104 and 122 and the stand was seven years old. Another 45 plus tree of *Heritiera fomes* (sundri) species established in 2000 at Terabaka, bogi, under shoronkhola range of Sundarbans from natural stands and score was between 96 and 114.

Seeds of *Avicennia officinalis* and *Heritiera fomes* were collected from different selected plus tree seed sources. The seeds were collected during August-September. Seedlings were raised in polybags of size 25 cm × 15 cm filled with powdered soil and cow dung mixture at a ratio of 3:1. Seedlings were maintained in the nursery for about 10 months. Eight to ten month old polybag seedlings were planted at 1.5 m × 1.5 m spacing. In each plot, 100 (10 × 10) seedlings were planted. The experiment was laid out in Randomized Complete Block Design (RCBD) with 3 replications. The seedlings of *Avicennia officinalis* species were planted in newly accreted land and *Heritiera fomes* seedlings planted in under keora forest as an under planting at char maderbunia of Rangabali island. Experimental data on tree survival, height, diameter at breast height (dbh), bole height, and crown diameter, and branch number were collected in August 2018 when the plantation stands seven years old. The wood volume of standing trees was calculated following *Avicennia officinalis* volume table (Latif *et al.* 1994). All these data were analyzed using Minitab statistical package. Analysis of variance (ANOVA) was used to determine the effect of plantations with plus tree seed sources on tree height, diameter and wood volume.

The growth performance of *Avicennia officinalis* and *Heritiera fomes* planted in 2010 in the western coastal belt of Bangladesh are shown in Table 1. The highest survival was found 46 % and 40 % for plus tree seed sources and mass collection seed sources of *Avicennia officinalis*, respectively. The highest height, the diameter of breast height, crown diameter and bole height was found 12.75 m, 15.45 cm, 7.39 m and 6.80 m for plus tree and 9.29 m, 9.96 cm, 4.00 m, and 3.65 m for plus tree seed and mass collection seed sources, respectively. Great wood volume was found 7.78 m^3/ha/yr and 1.80 m^3/ha/yr for plus tree seed sources and mass collection seed sources, respectively, in *Avicennia officinalis* species (Table 1).

Table 1. Growth performance of *Avicennia officinalis* and *Heritiera fomes* planted in 2010 at Rangabali islands from different seed sources.

Name of species	Seed sources	Survival (%)	Height (m)	Dbh (cm)	Mean annual height increment (m)	Crown Dia (m)	Bole height (m)	Wood volume (m^3/ha/yr)
Avicennia officinalis	Plus tree seeds	35.00a	12.75a	15.45a	1.83	7.39a	6.80a	7.78a
	Mass collection seeds	28.50b	9.29b	9.96b	1.33	4.00b	3.65b	1.80b
Heritiera fomes	Plus tree seeds	46.00	2.56	0.17	0.36m	-	1.72	-
	Mass collection seeds	40.00	1.21	-	0.17m	-	0.58	-

The highest survival, height, and dbh were found 46 %, 2.56 m and 0.17 cm for plus tree seeds sources and 40 % and 1.21 m was found for mass collection seed sources of *Heritiera fomes*, respectively (Table 1). In the present study, the result significantly greater in plus tree seed sources than mass collection seed sources of *Heritiera fomes* species when the stand was seven years old.

In comparison with other experimental plantations conducted by Bangladesh Forest Research Institute, growth of *Avicennia officinalis* in 19 years old highest height was 13.52 m, dbh 32.16 cm, bole height 4.71 m, crown diameter 4.74 m and highest wood volume found 10.04 m^3/ha/yr (Miah *et al*. 2014). Another plantation conducted by Forest Department at char kashem under the Rangabali island height was 13.15 m, dbh 28.96 cm, bole height 4.71 m, crown diameter 4.59 m and wood volume 5.73 m^3/ha/yr found in the age of 21 years old *Avicennia officinalis* stand (Miah *et al*. 2014). In the present study, the height, dbh, bole height, and crown diameter of *Avicennia officinalis* for plus tree seeds were found significantly greater, followed by mass collection seeds. Wood production was also significantly much higher for plus tree seeds, followed by mass collection seeds. The growth increment and yield were much higher for plus tree seeds of *Avicennia officinalis* at the age of seven years in coastal forest of Bangladesh.

In the present study growth of *H. fomes* species was found satisfactory in comparison to other established plantation in the coastal belts of Bangladesh. Siddiqi and Khan (1990) was found mean annual height increment from 0.27 m to 0.70 m in the 11-14 years old plantations of *H. fomes* plantations and present study mean annual height increment was found 0.36 m. The result revealed that seed sources are an important factor for tree growth and their yield. So, the use of improved seed sources from established plus tree provide significant growth and yield with the ability to withstand extreme climatic conditions in the coastal belt.

Avicennia officinalis and *Heritiera fomes* species are the important mangrove species for coastal plantation. They play a significant role in the established accreted char land, moderately raised char land and enrich forest vegetation of the man-made coastal forest of Bangladesh. These two species are planted using plus tree seed sources for getting maximum growth and timber production. The improved seed sources can enhance these species growth performance and their adaptability to vulnerable coastal areas. Therefore, now practice to plantations with plus tree seed sources can be raised using these types of seed sources for achieving significant gain from the coastal mangrove ecosystem. This, however, an interim report, and the comprehensive one will be available after a few years.

References

Alongi, D.M. 2009. Introduction of in the energetic of mangrove forest. *Springer Science and Business Media* BV. New York. p. 177.

Baksha, M.W. 1996. Behole borer infestation in coastal plantations in Bangladesh and possible management options, *Wallacena* **77**: 17-20.

Das, S. and N.A. Siddiqi. 1985. *The Mangroves and Mangrove Forests of Bangladesh*. Mangrove Silviculture Division. Bulletin No. 2, Chittagong: Bangladesh Forest Research Institute, p. 142.

Giri, C., E. Ochieng, L.L. Tieszen, Z. Zhu, A. Singh, T. Loveland, J. Masek and N. Duke. 2010. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecology and Biogeography, (Global Ecol. Biogeogr.), pp. 1-6 .

Hassan, D.Z. 2013. *Plants in Mangroves and Coastal Afforestation in Bangladesh*. Dewan House Publisher, Ukilpara, Naogaon. pp. 164.

Hasan, M.M. 1987. Preliminary report on coastal afforestation sites. *In*: Drigo R, Latif MA, Chowdhury JA, Shaheduzzaman M (eds.), *The Maturing Mangrove Plantations of the Coastal Afforestation Project*. FAO/UNDP Project, BGD/85/085. Field Document No. 2, Mohakhali, Dhaka: Bangladesh Forest Department, pp. 64-66.

Hawlader, N.I. 1999. Forest Resources Management Project. Mid-Term Review, Ministry of Environment and Forest, Dhaka. pp. 103.

Islam, S.A. and M.M. Rahman. 2015. Coastal afforestation in Bangladesh to combat climate change induced hazards. *Journal of Science Technology Environment Informatics*, vol. 14. No. 1, pp. 13-25.

Latif, M.A., S. Das, M.F. Rahman and J.A. Chowdhury. 1994. *Tree Volume Tables for Baen (Avicennia officinalis) in the Coastal Plantations of Bangladesh*. Bulletine 8, Forest Inventory Division, Chittagong: Bangladesh Forest Research Institute, pp. 21-32.

Latief, H. and S. Hadi. 2007. The role of forests and trees in protecting coastal areas against tsunamis, paper presented at the Regional Technical Workshop on Coastal Protection in the Aftermath of the Indian Ocean Tsunami What Role for Forests Trees, Proceeding of the Regional TechnicalWorkshop, Khao Lak, Thailand , 28-31 August 2006. pp 5-24.

Miah, M.A.Q., S.A. Islam, M.A. Habib and M.G. Moula. 2014. Growth performance of *Avicennia officinalis* L. and the effect of spacing on growth and yield of trees planted in the Western coastal belt of Bangladesh. *J. For. Res.* **25**(4): 835-838.

Nandy, P. 1989. *Inn*. Proc. First National Symposium, June 5-7, 1989. Plant Breeding in Bangladesh. pp. 230-252.

Nandy, P., M.J. Alam and M.R. Haider. 2004. Establishment of mangrove seed production area for *Sonneratia apetala*. *J. Trop. For. Sci.* **16**(3): 363-368.

Nandy, P., Ahammad, R. 2012. Navigating mangrove resilience through the ecosystem-based adaption approach : lessons from Bangladesh, Sharing Lessons on Mangrove Restoration, Vol. **1**, pp. 243-254.

Saenger, P. 1987. *Bangladesh Mangrove Afforestation Project*. Shedden Pacific Pty, Limited, Melborns, Australia. pp. 62.

Siddiqi, N.A. and M.A.S. Khan. 1990. Growth performance of mangrove trees along the coastal belt of Bangladesh. *Mangrove Ecosystems Occasional Papers*. No. 8. UNDP/UNESCO REGIONAL MANGROVES PROJECT RAS/86/120. Thomson Press, Delhi. 5-14 pp.

Siddiqi, N.A. and S. Das. 1988. Effect of time and method of storage on the survival of uprooted keora (*Soneratia apetala* Buch-Ham) seedlings. *Ind. J. Forest.* **11**(4): 326-330.

Siddiqi, N.A. 2001a. *Mangrove Forestry in Bangladesh*. Institute of Forestry & Environmental Science: University of Chittagong, pp. 201.

Siddiqi, N.A. 2001b. Creating second rotation crops in the existing keora (*Sonneratia apetala*) plantations. *In*: Siddiqi NA, Baksha M W (eds), *Mangrove Research and Development*. Chittagong: Bangladesh Forest Research Institute, pp. 102-107.

Siddiqi, N.A. and M.A.S. Khan. 2000. Raising plantations of *Phoenix paludosa* – a mangrove palm in the coastal areas of Bangladesh. *J. Asiat. Soc. Bangladesh (Sci.)* **26**(2): 259-264.

Siddiqi, N.A. 2002. Development and sustainable management of coastal plantations in Bangladesh. *J. Asiat. Soc. Bangladesh (Sci.)* **28**(2): 144-166.

Woodroffe. 1992. Mangrove sediments and geomorphology" in *Tropical mangrove ecosystems. Coastal and Estuarine Studies* 41, A.I.Robertson and D. M. Alongi, Eds, pp. 7-41, American Geophysical Union, Wash, USA, 1992.

Zobel, B. and J. Talbert. 1983. Applied Forest Tree Improvement. Wiley, New York. pp. 505.

(Revised copy received on 20.9.2020)

J. Asiat. Soc. Bangladesh, Sci. **46**(2): 215-220, December 2020 **- Short communication**

COMPARATIVE KARYOMORPHOLOGICAL ANALYSIS OF FOUR BARI RELEASED GERMPLASMS OF GARLIC

ROKSANA FERDOUSI, SUSMITA SAHA AND KAZI NAHIDA BEGUM*
Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

Several garlic varieties (*Allium sativum* L.) have been released by Bangladesh Agricultural Research Institute (BARI), namely BARI Rosun-1, BARI Rosun-2, BARI Rosun-3 and BARI Rosun-4, along with some lines which are experimentally cultivated under the breeding program, such as ASGAZ001, ASGAZ002, ASGAZ003, ASGAZ004, ASGAZ005, ASGAZ006, ASGAZ007, ASGAZ008 and ASGAZ009. The morphological features are used to characterize these varieties, and many lines that often create complications as phenotypic characteristics are not always authentic. Thus, classical cytogenetics will be helpful for numerical and structural features of chromosome set, including karyotype characteristics for authentic characterization and further molecular investigations and conservation (Saha *et al.* 2020). Many previous studies have been reported on somatic chromosome numbers (Manzum *et al.* 2014, Awe and Akpan 2017), but there is no previous report on cytogenetical analysis of BARI released garlic germplasms.

For the present analysis, the four germplasms of *Allium sativum* L. (BARI Rosun-1, LINE ASGAZ002, LINE ASGAZ003, and LINE ASGAZ005) were collected from the Regional Spices Research Centre (RSRC) of Bangladesh Agricultural Research Institute (BARI) and then maintained in the Botanical garden of Jagannath University. Young, healthy roots were cut 2 cm away from the root tips of *Allium sativum* by a fine forceps and then gently washed with water for proper cleaning. Afterwards, the cleaned root tips (RTs) were soaked on a filter paper to remove surface water and pretreated with PDB (Para-dichloro Benzene) for 5.30 hours at room temperature (28-30ºC). For fixation of RTs, 45% acetic acid was used at 4ºC for 15 min and preserved in 70% alcohol for further use. A mixture of 1N HCl and 45% acetic-acid (2:1) was used to hydrolyze the pretreated RTs for 5 min at 60ºC. Then the hydrolyzed RTs were taken on a clean slide and soaked by a filter paper. With a fine blade, the meristematic region of RTs about 0.5 cm was cut and a drop of 1% aceto-orcein was added to keep it in an acetic acid chamber to stain for 1 hour and then a clean cover glass was placed on the material. Gently, the

*Corresponding author: <kazinahida@yahoo.com>.

materials were tapped by a tooth pick and then squashed by placing thumbs. After all, XSZ-107BN, namely binocular microscope was used with 100× magnification for slides observation and the microphotographs were captured by Euromex CMEX-10 digital USB camera (Holland).

Based on the staining nature of interphase nuclei and prophase chromosomes the germplasms were differentiated into ‘Diffuse Type’ and ‘Continuous Type’. All germplasms had $2n = 2x = 16$ chromosomes in which the highest TCL was found in line ASGAZ005 (208.16 ± 4.96 µm) and lowest in ASGAZ002 (145.57 ± 3.17 µm) (Table 1). Line ASGAZ002, and line ASGAZ005 were placed under 2A karyotype, but karyotype 1B and 2B were found in line ASGAZ003 and BARI Rosun-1. On the basis of symmetry-asymmetry indices, AI vs CV_{CI} graph was used to reveal the karyotypic nature of the germplasms.

Twenty to twenty-five interphases and prophases were used to examine their nature based on Tanaka's (1971) classification, whereas finely scattered three metaphases were used for karyomorphological analysis. At first, the karyotype and a haloid idiogram were established regarding the decreasing chromosomal length to classify the chromosomes following the nomenclature of Levan *et al.* (1964). Using Karyo Type Software, different parameters of karyomorphological characteristics were estimated (Altinordu *et al.* 2016). Nature of interphase nuclei and prophase chromosomes, somatic chromosome number, karyotype, idiogram have been presented in Figure 1 and karyomorphological features in Table 1.

On the basis of orcein-staining properties, the interphase nuclei of analyzed four *Allium sativum* L. germplasms *i.e.* BARI Rosun-1, LINE ASGAZ002, LINE ASGAZ003, LINE ASGAZ005 were found as the uniformly stained nucleus (Figs 1A-D), in which heterochromatin blocks were homogeneously distributed throughout the nucleus and categorized as ‘Diffuse Type’ by Tanaka (1971). In the interphase of analyzed germplasms, prominent nucleolus was found but varied in number (Figs 1A-D, arrow) but no prophase nuclei was found. A prominent nucleolus was observed both in LINE ASGAZ003 and LINE ASGAZ005 (Figs 1C-D, arrow), whereas two and three nucleoli were found in BARI Rosun-1 and LINE ASGAZ002, respectively (Figs 1A-B, arrow). After orcein-staining, the prophase chromosomes of analyzed four germplasms of *A. sativum* had to possess homogenous nature within the inter-length, which was counted as ‘Continuous Type’ according to the classification of Tanaka (1971) (Figs 1E-H). Tanaka’s classification (1971) revealed that the specimens who possessed ‘Diffuse type’ in interphase nuclei were generally displayed ‘Continuous Type’ in prophase chromosomes. Thus, the present finding correlates with Tanaka's classification, which also indicates constitutive heterochromatin in all the analyzed germplasms.

The four *Allium sativum* L. germplasms had 2n = 16 chromosomes in a somatic cell (Figs 1, I-P and Table 1) and basic number x = 8 (Figs 1 Q-T and Table 1). These findings are similar to previous reports (Manzum *et al.* 2014, Awe and Akpan 2017). However, several researchers reported 2n =11, 12, 18, 22 and 32 chromosomes in garlic. This difference might be due to the study materials belonging to different aneuploid series or different cytotypes of garlic.

Table 1. Comparative karyomorphological analysis of four *Allium sativum* L. germplasms.

Features	BARI Rosun-1	Line ASGAZ002	Line ASGAZ003	Line ASGAZ005
2n	2n = 2x = 16	2n = 2x = 16	2n = 2x = 16	2n = 2x = 16
CF	12m + 4sm	14m + 2sm	16m	14m + 2st
TCL (µm)	184.96 ± 2.56	145.57 ± 3.17	195.30 ± 4.12	208.16 ± 4.96
ACL (µm)	11.56	9.10	12.21	13.01
CV_{CI}	14.98	13.63	8.44	18.36
CV_{CL}	14.87	15.95	17.37	16.31
M_{CA}	15.33	12.97	6.74	12.22
AsK %	57.67	56.26	53.23	55.76
TF %	42.33	43.74	46.77	44.24
Syi %	73.41	77.74	87.88	79.34
Rec %	74.44	72.22	79.78	80.18
A_1	0.25	0.21	0.12	0.19
A_2	0.15	0.16	0.17	0.16
A	0.15	0.13	0.07	0.12
AI	2.23	2.17	1.47	2.99
DI	6.22	7.48	7.89	7.77
Category	2B	2A	1B	2A

2n = Somatic chromosome number, x = Basic chromosome number, CF = Centromeric formula, TCL = Total chromosome length (µm), ACL = Average chromosome length (µm), CV_{CI} = Coefficient of variation of centromeric index, CV_{CL} = Coefficient of variation of chromosome length, M_{CA} = Mean centromeric asymmetry, AsK % = Karyotype asymmetry index (%), TF % = Total form value (%), Syi % = Karyotype symmetry index (%), Rec % = The index of chromosomal size resemblance, A_1 =

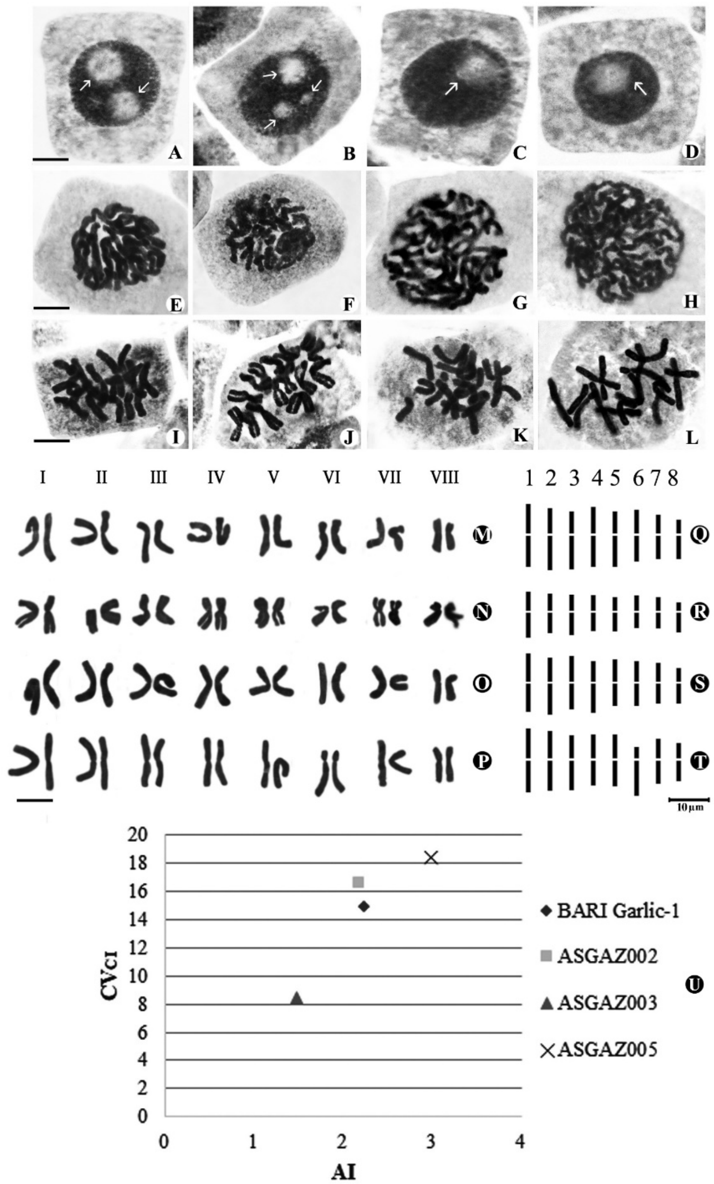


Fig. 1. Orcein-stained mitotic interphase nuclei, prophase chromosomes, metaphase chromosomes, karyotypes and idiograms of four germplasms of *Allium sativum* L. (A, E, I, M, Q) BARI Rosun-1, (B, F, J, N, R) line ASGAZ002, (C, G, K, O, S) line ASGAZ003, (D, H, L, P, T) line ASGAZ005, Arrows indicate the presence of nucleolus, except idiogram all Scale Bar = 5 µm and (U) AI vs CV_{CI} of four BARI germplasms of *Allium sativum* L.

Intrachromosomal asymmetry index, A_2 = Interchromosomal asymmetry index, A = Degree of asymmetry of karyotypes, AI = The asymmetry index, DI = The dispersion index, m = metacentric chromosome, sm = sub-metacentric chromosome, st = acrocentric chromosome.

The four germplasms of *Allium sativum* L. had dominance of metacentric chromosomes. The highest number of sub-metacentric chromosomes was found in BARI Rosun-1 with twelve metacentric chromosomes (12m + 4sm), whereas line ASGAZ003 possessed sixteen metacentric chromosomes (16m) (Figs. 1I, 1K, 1M, 1O and Table 1). A pair of the sub-metacentric chromosome was found in line ASGAZ002 along with fourteen metacentric chromosomes (14m + 2sm) (Figs. 1J, 1N, and Table 1). Only inline ASGAZ005, a pair of the acrocentric chromosome was found where remaining chromosomes were metacentric (14m + 2st) (Figs. 1L, 1P, and Table 1). Among these four garlic germplasms, the highest total chromosome length (TCL) was 208.16 ± 4.96 µm observed in line ASGAZ005 with an average chromosome length (ACL) of 13.01µm and line ASGAZ002 had been exhibited the lowest value of (ACL) which was 9.10 µm along to total chromosome length (TCL) of 145.57 ± 3.17 µm that was also lowest as value. The total length of diploid chromosome complement and average chromosome length were reported as 184.96 ± 2.56 µm and 11.56 µm in BARI Rosun-1, whereas 195.30±4.12 µm, and 12.21µm line ASGAZ003, consecutively (Table 1). No secondary constriction was observed in any of the analyzed garlic germplasms. Based on the classification of Stebbins (1971), line ASGAZ002 and line ASGAZ005 both were categorized as 2A, whereas BARI Rosun-1 and line ASGAZ003 were placed to 2B and 1B, respectively (Table 1).

Karyosystematic analysis is widely used in the study of divergence and genetic relationships among species. In the present analysis, values of CV_{CI} and CV_{CL} showed a wide variation, ranging from 8.44 to 18.36 for $CV_{CI,}$ whereas 14.87 to 17.37 for CV_{CL} (Table 1). Higher chromosomal homogeneity was found in line ASGAZ003 along with CV_{CI} and CV_{CL} values of 8.44 and 17.37, respectively (Table 1). In LINE ASGAZ005 slightly higher CV_{CI} (13.01) and CV_{CL} (18.36) were found, which is of comparatively lower chromosomal homogeneity (Table 1).

Now-a-days, CV_{CI} and AI are considered as two reliable indices that exhibit the inter-relationships and asymmetry of karyotype. As such, these two asymmetry indexes AI and CV_{CI} are put on the X- axis and vertical (Y) axis to draw a bi-directional scattered plot where line ASGAZ003 located at the bottom with lowest AI (1.47) and CV_{CI} (8.44) indicating primitive type in nature meanwhile with highest AI (2.99) and CV_{CI} (18.36)

LINE ASGAZ005 was found on the top with relatively advanced features (Fig. 1U). Each of the analyzed germplasms displayed an adequate variation ranging from 0.12 to 0.25 A_1 and 0.15 to 0.17 in the case of A_2 (Table 1). The total form value (TF%) showed a proportional relationship with Karyotype symmetry index (Syi%) and index of chromosomal size resemblance (Rec%) but formed an opposite connection with Karyotype asymmetry index (AsK%) whereas Dispersion index (DI) was negatively correlated to Mean centromeric asymmetry (M_{CA}) (Table 1).

The present involving karyomorphological analysis with asymmetrical and symmetrical indices of BARI released four *Allium sativum* L. germplasms indicate rearrangement of the chromosome as well as reveals different steps in evolution which may be useful in breeding studies. Thus, the outcome of this research may help in research with these *A. sativum* germplasms as an aid to identification based on for performing the present analysis.

References

Altınordu, F., L. Peruzzi, Y. Yu and X. He. 2016. A tool for the analysis of chromosomes: KaryoType. *Taxon.* **65**(3): 586-592.

Awe, E.T. and U.U. Akpan. 2017. Cytological study of *Allium cepa* and *Allium sativum*. *Acta Satech* **9**: 113-120.

Levan, A., K. Fredga and A.A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas.* **52**: 201-220.

Manzum, A.A., S.S. Sultana, A.A. Warasy, R. Begum and Sk.S. Alam. 2014. Characterization of four specimens of *Allium sativum* L. by different karyotype and RAPD analysis. *Cytologia.* **79**: 419-426.

Saha, S., M.S. Pinky, S. Akter and K.N. Begum. 2020. Karyotype diversity among twelve varieties of *Brassica* L. (Brassicaceae) from Bangladesh. *Tropical Plant Res.* **7**(2): 476-483.

Stebbins, G.L. 1971. Chromosomal Evolution in Higher Plants. University Park Press, Baltimore. pp. 208.

Tanaka, R. 1971. Type of resting nuclei in Orchidaceae. *Bot. Mag. Tokyo.* **84**: 118-122.

(Revised copy received on 10.12.2020)