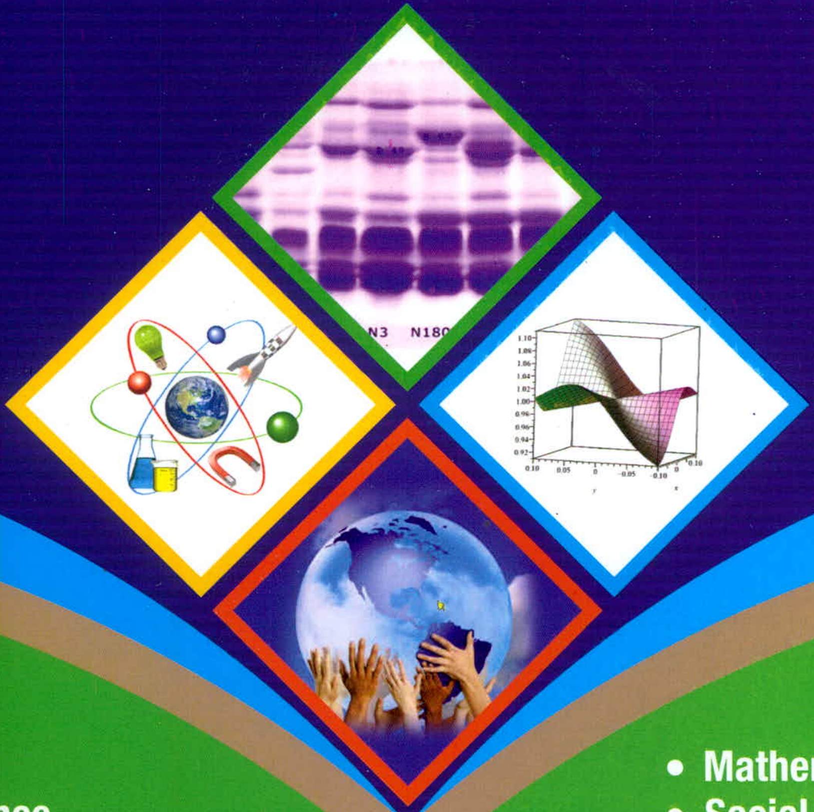
গণপ্রজাতন্ত্রী বাংলাদেশ সরকার

Volume 3

Report on Advanced Research

2015-2016



- Life Science
- Physical Science
- Mathematical Science
- Social Science

Grants for Advanced Research in Education

Secondary and Higher Education Division
Ministry of Education

গণপ্রজাতন্ত্রী বাংলাদেশ সরকার

Ministry of Education

Report on Advanced Research
2015 - 2016

Grants for Advanced Research in Education (GARE)

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بسم الله الرحمن الرحيم

PRIME MINISTER
GOVERNMENT OF THE PEOPLE'S REPUBLIC OF
BANGLADESH

Message

I am happy to learn that Ministry of Education is going to publish a compilation of researches carried out under the programme 'Grants for Advanced Research in Education (GARE)' during 2015-2016.

We believe that research is essential branches for promotion of knowledge innovations and sustainable solutions. Higher education can achieve desired quality and added value if research is properly stressed and undertaken. Research in all branches of knowledge can help create an enlightened society. So, our government has been allocating increased resources over the years to ensure greater access to research opportunities. We have also undertaken measures so that the institutions concerned can have state of the art facilities to conduct researches in order to increase our productivity in all sectors of our national life.

I hope that the Ministry of Education will continue to do its best to nourish and assist research endeavours through higher educational institutions and research organisations of the country.

I wish the publication a total success.

Joi Bangla, Joi Bangabandhu
May Bangladesh Live Forever.

Sheikh Hasina

Dr. Dipu Moni M.P.
Minister
Ministry of Education
Government of the People's
Republic of Bangladesh

Message

I am delighted that Ministry of Education is going to publish the research progress report of the completed research projects under Grants for Advanced Research in Education (GARE) during the period of 2015-2016. This report includes 35 research papers.

Pursuing research is a challenging and rewarding experience which enables a researcher to conduct an in-depth investigation on a topic of interest. Well planned and scholastic research is vital to the success of national, regional and global endeavors. Research draws its power from the fact that it is empirical, rather than merely theorizing about what might be effective or what could work. The systematic, controlled, empirical and critical investigation by researchers on social, cultural, industrial and economic propositions is necessary to contribute effectively to the productive outputs of the country.

We shall get authentic data from researchers on which we may base our policy decision. In many developed countries, university academics and researchers are playing vital role through their innovations in high-tech industries and industrial development. So, we expect our researchers with funding from GARE to enhance their knowledge of how to address national problems and improve our economy. We are living in a decade when the world is actively replacing the human resources with the automations. As a result, the capital-intensive industries are on the driving seat. So, there is an ever urgent need to hunt down specific skills that can co-exist with automation and robotics of the future.

The achievement and performance of our researchers will be demonstrated in this report through their publications which ultimately will make our research known to national and international community.

I hope, this progress report will be a useful source of information, especially for those who are interested in conducting research in future and contribute significantly in our national and industrial development.

Dipu Moni

Dr. Dipu Moni, MP

Mohibul Hassan Chowdhoury
Deputy Minister
Ministry of Education
Government of the people's
Republic of Bangladesh

Message

The necessity of building competitive higher education system inspired the government to initiate a program of Grants for Advanced Research in Education (GARE). The objective of GARE is to develop a research culture in the country so that we can address our problems, find remedies and hence contribute to the economic and social growth of the country. This edition of the publication - a third in the process contains the research findings and results for the benefit of the nation. These may bring about useful application and may also invite more and more researches for fruitful utilization to the benefit of the country.

Bangladesh economy is doing well despite frequent global crises, climate change induced disasters, fast population growth, runaway urbanization and increasing environmental degradation. These phenomena put up fresh challenges to the country which must seek out ways collectively to mitigate the problems and thrust upon the universities and research institutions a historic task of generating knowledge, new ideas and technologies that would contribute towards achieving the nation's development goals. The GARE research should also address issues connected with the government's Delta plan 2100 for the future generation.

We are confident, more and more researchers will come forward with innovative proposals and take research grants for enhancing the quality of higher education and quality of our lives and living.

I take this opportunity to thank the officials involved with organizing and managing this project and for contributing to build a strong higher education system in Bangladesh.

Mohibul Hassan Chowdhoury

Md. Sohorab Hossain
Senior Secretary
Secondary and Higher Education Division
Ministry of Education
Government of the People's
Republic of Bangladesh

Message

I am pleased to know that the Ministry of Education (MoE) is going to publish the third progress report on the completed research projects funded under the Grants for Advanced Research in Education (GARE) program during the period 2015 – 2016. This fund is available for pensive academics in higher education for conducting research to enhance their capacity for creating new knowledge and developing products and processes for commercial exploitations. We are tempted to know that research in higher education generating results which are examinable by peers, methodologies and those can be replicated and technologies would be applied for solving national problems and improving our economy.

The concept of research is as old as science. The ultimate relationship between research and subsequent scientific development is now well recognized. The need for sharing knowledge between researchers and industries has become increasingly evident in recent years. In fact, researchers are perceived as source of new ideas and industry offers a natural route to maximize the use of these ideas. It is imperative that researchers play a more active role in their relationship with the industries in order to maximize the use of these research results. We believe, to meet the challenges in future development of Bangladesh successfully, higher education and research should progress simultaneously.

I feel proud that our talented researchers are working relentlessly for investigating specific problems identified by them in order to establish relationship between and among the variables. Every research project has specific objectives and researchers seek to achieve these objectives without ambiguity. However, our research in higher education should be need based, demand led and designed to benefit the students and the industries rather than the researchers themselves. Our research should be of high quality in order to create knowledge that is applicable outside of the research setting. Furthermore, the results of the research should have implications for policy making and national projects implementation.

I express my gratitude to all researchers, reviewers, experts and different committee members for their immense contribution and supports in GARE program. I am also thankful to the officials of Secondary and Higher Secondary Education Division and BANBEIS for offering their valuable efforts in preparing this progress report.

সোহরাব

Md. Sohorab Hossain

Mesbahuddin Ahmed
President
Editorial committee
Grants for Advanced Research in Education (GARE)

Preface

It is my pleasure to find that third volume of the report on Grants for Advanced Research in Education (GARE) is coming out. This volume contains reports of the projects completed between the period 2015-16. I am also very happy to see that this volume contains some very important research findings which are likely to attract attention of a wider section of people and possibly will find useful applications.

We know that a wide range of skilled individuals is essential for the development of research and hence we, not only support research of teachers of the universities and research organizations, but also put in effort to develop skilled manpower by giving support for M.Phil, Ph.D students with the research projects. The program of GARE is growing from strength to strength as more and more researchers are applying for funds. The government of the People's Republic of Bangladesh has taken the laudable stand of offering support to all applicants considered suitable for funding by the reviewer's committee. This is simply a big achievement for all of us to realize that the country can make progress in social and economic sectors only through utilizing results of research. Therefore, developing a research culture should be our top priority and this GARE project is just doing that.

I wish this volume also will receive due attention of our community.

M Ahmed

Mesbahuddin Ahmed

Md. Fashiullah
Member Secretary
Editorial Commrttee &
Director General
BANBEIS

Foreward

This is a great pleasure for me that we are going to publish the Third Report on completed Advanced Research Project, supported by Ministry of Education under the Grants for Advanced Research in Education (GARE). Bangladesh Government has continued strong emphasis on advanced research, because for the proper development of a country, there is no alternative to research. For providing the country in its success, the undeveloped sectors should be developed and for the identification of that gap, research is very much important. Now, the Government mandate is to reach middle income status by 2021 and developed status by 2041. For that, the Government has taken several programmes, advanced research is one of them. Recently, the Government of Bangladesh is allocating enhanced budget for research in education. As a consequence, we may hope that research works in Bangladesh will be able to achieve its goal.

I express my gratitude and thank to all concerned for their important assistance and co-operation in this regard. I hope, the published report will be a great asset for the researchers and will help to build a resilient nation.

Md. Fashiullah

Executive Summary

The Government of Bangladesh is offering the most priority to promote intellectual capital to solve national problems, improve the quality of individual's life and ensure economic growth through research and innovations. The Ministry of Education (MoE) initiated the Grants for Advanced Research in Education (GARE) program in 2008 and subsequently started functioning in the Fiscal Year 2009 – 2010. In response to the circular issued by the MoE, the learned academics of the universities and institutions of higher learning submitted prospective and potential proposals targeting to solving important national and international problems. The received proposals were initially scrutinized by the primary selection committee members and then evaluated by at least two experts in the relevant field. Nature of the proposal, objectives and activities, involvement of post graduate research students in the studies, experimental costs, cost of minor equipments are analyzed before approving the project and finalizing the budget of the grants. In case of any ambiguity, committee invites the researcher for presentation of the proposal and discussion for further clarification.

The MoE is funding research proposals through GARE on competitive basis for conducting research in different public and private Universities, Post-Graduate Colleges of National and Open Universities, Public Medical Colleges and Universities and Engineering Universities in the areas of Mathematical Sciences, Life Sciences, Physical Sciences, Social Sciences, ICT, Marine Sciences and Sustainable Development Goals and Research to Achieve 7th Five Years Plan targets. The aims and objectives of this research program are:

- Acquisition, generation and dissemination of improved knowledge and technologies;
- Development of necessary critical manpower;
- Reducing the total production cost and enhancing GDP through application of country's own resources and intelligence;
- Capacity building to meet the challenges of climate change, create favorable condition for production and mainstream as a part of development actions;
- Taking active participation in global initiatives;
- Ensuring priority to both fundamental and applied research; and
- Fostering and maintaining research culture in higher education.

The MoE is very much delighted to welcome more researchers with innovative ideas to submit Project Concept Notes (PCNs) as well as Completed Proposal (CPs) in the designated areas for GARE funds. The eligibility for research grants are:

- The applicants should have sufficient and relevant research papers published in reputed journals.
- The research team/individual researcher should have experience on presenting scientific papers in International/National Seminar/Symposium/Workshop home and abroad.
- On-going number of research programs of the researchers and physical facilities and international relationship of receiving departments/institutions should be satisfactory.
- The topic of the research should reflect the national need and demand and technically feasible for its application in the country.
- Priority is given to the researchers who already have notable achievement in research in his/her field of specialization.

This publication includes a total of 35 Project Completion Reports (PCR) submitted by Principal Investigator of the projects and covers different areas of research under the purview of the Grants for Advanced Research in Education. There are 21 PCR from life science, 08 from physical science, 05 from medical science and 01 from mathematical science in this publication. The reports are of satisfactory standard and based on original work of the researchers. The learned and reputed Principal Investigators summarized their studies in brief indicating the outcomes/findings of their valuable research.

The academics in universities and higher education institutions are supposed to create knowledge through research and innovations. The research involves creative thinking and experimentation. Through research individuals and professionals know what works and why. Thus the action of teachers having research experience lead to improve their students performance, increase motivation, commitment, better behavior and so on. So, the MoE and the GoB are generous to make more funds available for creative and need based research. Since academics are accountable, their performances need to be demonstrated through their publications. We trust our talented researchers conducting research with GARE funds will publish their valuable research findings in renowned journals home and abroad and highlight Bangladesh. We hope some of their innovations might get IPRs and be used by the country's developing industries and thus contribute in economic development of Bangladesh.

Editorial Committee

CONTENTS

FUNDING YEAR (2015-2016)

CONTENTS

FUNDING YEAR (2015-2016)

Funding Year
2015-2016

DNA Barcoding of Small Indigenous Species (SIS) from Tanguar and Hakaluki Haor

Md Sagir Ahmed and Mohammad Mamun Chowdhury

Location: Department of Zoology, University of Dhaka
Duration: Two years (2014-2016)
Expenditure of the project: Tk. 2800000.00

Introduction

Small indigenous fish are a vital contribution to the diet of the rural poor in Bangladesh, where more than 30,000 children go blind every year from vitamin A deficiency and 70% of women and children are iron-deficient (Thilsted *et al.,* 1997). Small fish are a rich source of animal protein, fatty acids and essential vitamins and minerals. As many small fish are eaten whole, with head, organs and bones, they are a particularly good source of calcium, and some are also rich in vitamin A, iron and zinc (Roos *et al.,* 2007). Some important mentionable small indigenous fishes are mola, dhela, Kachki, punti, and chela.

A global DNA-based barcode identification system that is applicable to all animal species will provide a simple, universal tool for the identification of fish species and products. The barcode system is based on sequence diversity in a single gene region (a section of the mitochondrial DNA cytochrome c oxidase I gene, COI). When the reference sequence library is in place, new specimens and products can be identified by comparing their DNA barcode sequences against this barcode reference library (Hubert *et al.,* 2003). Many countries of the world (Australia, Brazil, Canada, China, Cuba, Germany, Indonesia and Indian) have already barcoded all species of their freshwater and marine fishes (Ward *et al.* 2005; Lakra *et al.* 2011, 2015, Hubert *et al.,* 2008, 2015, Steinke *et al.,* 2009, Knebelsberger *et al.,* 2014).

The country now needs to maintain the genetic identity as well as integrity of species in their natural habitats. So, it is of prime importance to study the genetic diversity of SIS which would give direction for the future conservation genetics. Considering the above mentioned facts, it is crucial to establish a DNA barcode based database of SIS for accurate identification and base reference for future conservation genetics.

Objectives

- To know the status and abundance of small indigenous fish species (SIS) from two Ramsar sites, Tanguar and Hakaluki haor in Bangladesh;
- To document the genetic diversity of SIS from the above mentioned water bodies;
- Resolving the potential cryptic variation as well as conflicting patterns of divergence with respect to current taxonomic designations and
- To determine the diversity within and among geographical populations of SIS from the above mentioned water bodies.

Methodologies

Sample fish species have been collected from Tanguar and Hakaluki haor. Digital photographs of all the fishes were taken immediately and taxonomically identified following Siddiqui *et al.* (2007), Talwar and Jhingran (1991) and Rahman (2005), Shafi and Quddus (1982). Immediately after the collecting the specimens, tissue samples were excised and stored in 70% ethanol.

DNA barcoding: Genomic DNA was extracted from the muscle tissue samples by the standard Proteinase-K/Phenol-Chloroform-isoamyl alcohol method (Sambrook *et al.,* 1989; Chowdhury *et al.,* 2016). The quality and quantity of the extracted DNA was measured using NanodropTM spectrophotometer. Approximately 655 bp was amplified from the 5′ region of the cox1 gene from mitochondrial DNA using the F1-R1, F2-R2 fish primers. PCR products were visualized on 1% agarose gels. The PCR products were purified using PureLinkTM PCR purification kit and sequence from First BASE Laboratories, Malaysia. Sequences were checked and aligned using Sequencher v5.4, and submitted to GenBank with referred accession numbers. Bioinformatics analyses of the sequences were performed using CLC Workbench v7.7.1, Mega v5.05, Clustal Omega, and T-Coffee. Genetic distance and sequence divergences were calculated using the Kimura two parameter (K2P) distance model (Kimura 1980). Neighbour-joining (NJ) tree of K2P distances was created to provide a graphic representation of the patterning of divergence between species (Saitou and Nei 1987). All the statistical calculations were performed in Microsoft Excel. All the data including taxonomic characteristics and GenBank/BOLD accession number are tagged with the voucher specimen are preserved at the museum of Department of Zoology, University of Dhaka.

Results

An attempt has been made to genetic identification of SIS species through DNA barcoding, a molecular method that uses a short standardized DNA sequence as a species identification tool. The standard 652 base-pair region of the mitochondrial cytochrome oxidase subunit I gene (COI) was sequenced in freshwater fish specimens from Taguar Haor. A total of 83 species have been sampled during the present survey and the primers used amplified the target region of all, but six species: *Gudusia chapra, Xenontodon cancila, Trichogaster fascita, T. chuna, Pethia ticto and Hyporhamphus limbatus.* Thus, a total of 94 COI barcodes of approximate 658-bp have been obtained for 77 species belong 52 genera and 23 families and six orders. Our results reveal that COI barcoding will permit the unambiguous identification of the vast majority of fish species (Ahmed *et al.,* 2015). The high efficiency of species identification was demonstrated in the present study. The average GC content of the 77 barcoded SIS species was 45.14%. The highest GC content (47.14 %) was observed in the Order Perciformes. Among the 12 Families of SIS highest (48.98%) and lowest (43.48%) GC content was observed in Family Channidae and Schilbeidae, respectively (Fig. 1). The average genetic distance within species, genus, family, order, and class was 0.40%, 9.60%, 13.10%, 17.16%, and 18.30%, respectively. The overall average genetic distance among all the species was 23.90%. The Neighbor Joining (K2P) of 77 barcoded SIS species revealed that the similar groups (Order, Family) of fish clustered in a same clade (Fig. 2).

Conclusion

Tanguar Haor is Bangladesh's most important freshwater wetland, designated an Ecologically Critical Area (ECA). It is also a protected Ramsar site of international importance for the conservation and sustainable utilization of wetlands. The present study is the first to assess the resolution of barcoding for freshwater fish species from Bangladesh waters. We have effectively used partial COI genes as DNA barcode in 77 freshwater fish species from Bangladesh waters representing six orders (Cypriniformes, Siluriformes, Perciformes, Synbranchiformes, and Osteoglossiformes) and 23 families. This study has strongly validated the efficacy of COI barcodes for identifying fish species. Among the 77 species 26 species (34%) have been found threatened as per IUCN red list (2015). The Threatened fishes comprise one as critically endangered (CR), 11 endangered (EN) and 02 species as vulnerable (VU). Twelve species of fish were near threatened (NT), 39 species were least concern (LC) and the rest 05 species were data deficient (DD). We reported six species as new records from Bangladesh. All individual SIS species have been assigned NCBI GenBank Accession No. (KT762359-KT762387, KX455892- KX455912, etc). which confirms the authentic molecular identification of these species and patent ship of these species from Bangladesh waters. Scientists, researchers, fishery managers and policy makers any time can retrieve the sequence data from GenBank for further use.

This DNA barcode resource will enhance capacity in many areas of fish conservation biology that can benefit from improved knowledge of genetic provenance. These include captive breeding and restocking programs, life history studies and ecological research into the interactions between populations of native and exotic species.

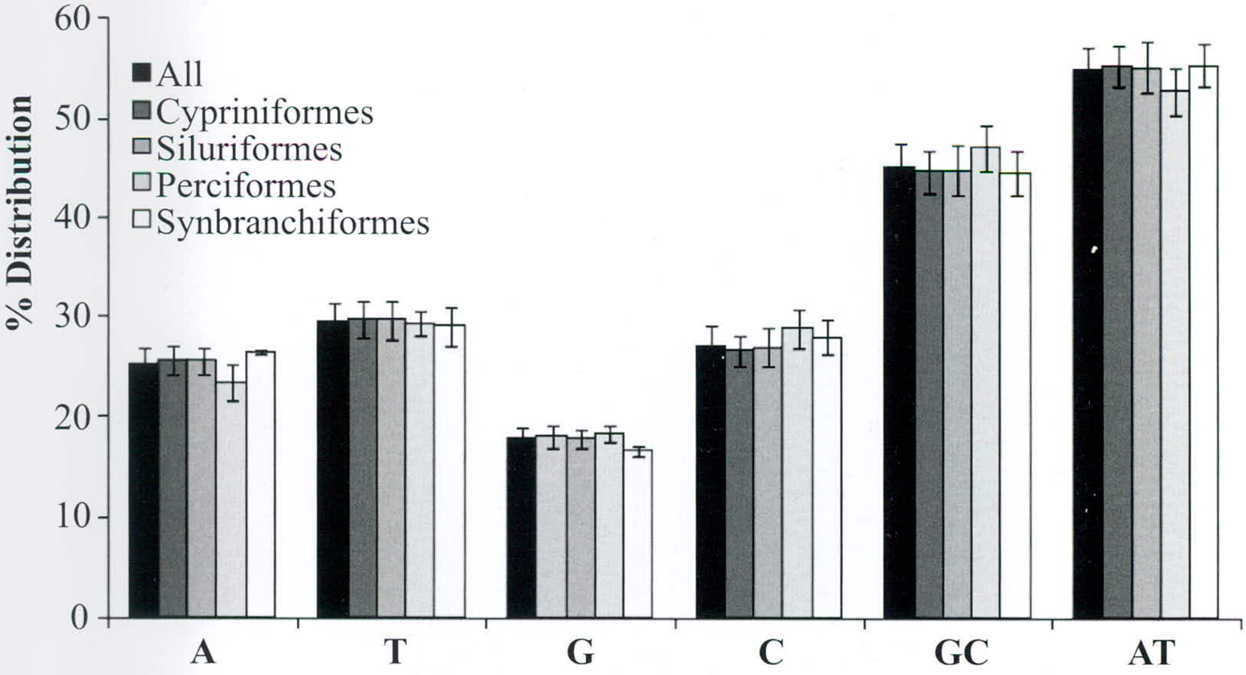

Fig. 1: Composition of the nucleotides in the sequenced COI region of small indigenous fishes (N=77) of Bangladesh.

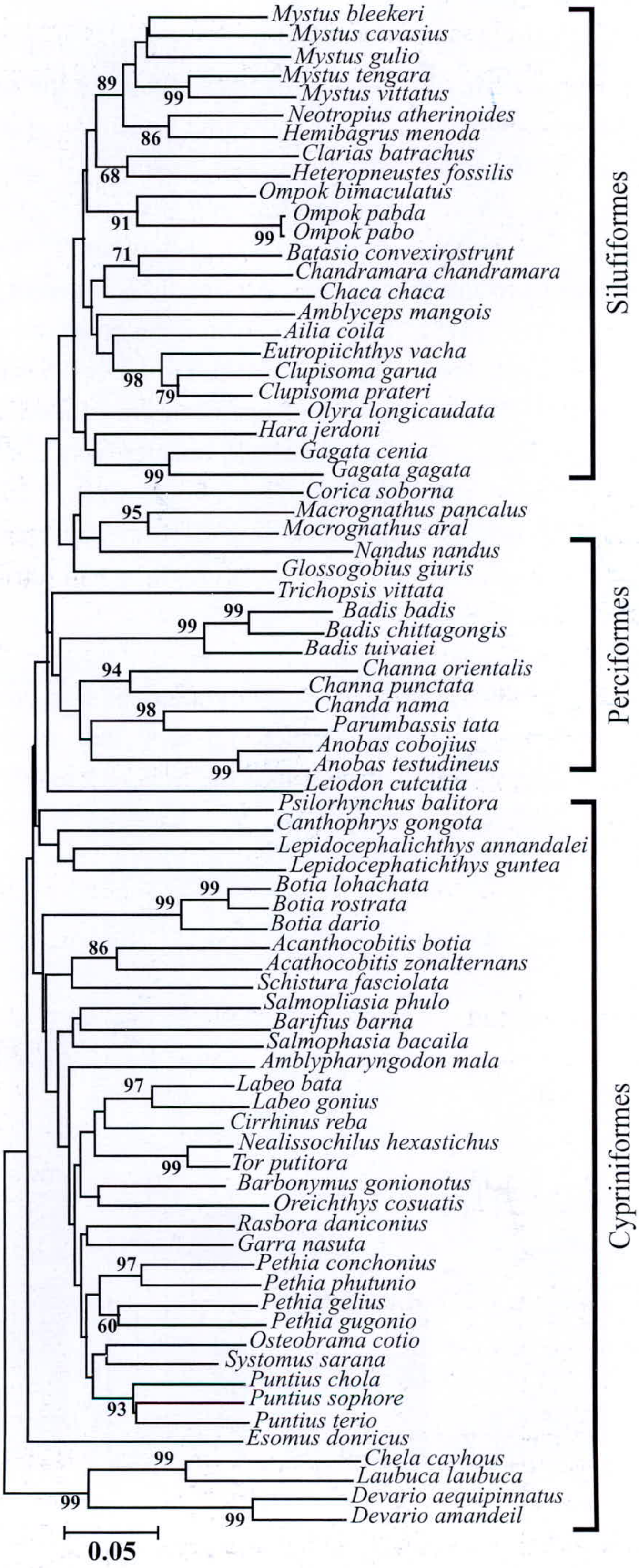

Fig. 2: Neighbour-joining (NJ) phylogenetic tree of the sequenced data based on K2P distances.

Publication from this Research

Ahmed M.S., Chowdhury M.M., Rahman M., Nahar L. 2015. DNA Barcoding of small indigenous fish species (SIS) from Tanguar Haor. *Genome 58: 186.* dx.doi.org/10.1139/gen-2015-0094.

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Genetic Variation, Population Structure and Identification of Different Strains of the Small Indigenous Species, Mola (*Amblypharyngodon mola,* Hamilton 1822) in Bangladesh Using PCR-RAPD Marker: Implication in Sustainable Culture

Md Shahidul Islam and Mohd Golam Quader Khan

Location: Department of Biotechnology, Bangladesh Agricultural University, Mymensingh
Duration: Two years (2014-2016)
Expenditure of the project: Tk. 2300000.00

Introduction

Mola *(Amblypharyngodon mola)* belonging to the family cyprinidae is considered the most potential small indigenous species (SIS) of fish in Bangladesh. It is found in ponds, rice fields and floodplain areas of Bangladesh and in many other South and Southeast Asian countries. The species is nutrient-dense. Roos *et al.* (2002) showed that 100 g of raw edible mola contains 2680 μg retinol equivalents (RE) whereas rui, L. rohita contains very little vitamin A (<30 μg RE). Mola is therefore, be considered 'a living capsule of vitamin A'. With its benefits for nutrition and health, mola has become a high-priced SIS in the country. Frustratingly, anthropogenic activities such as improper use of pesticides in agricultural lands, drainage of hazardous chemicals from nearby industries, construction of dams and overfishing result in decrease in production of the mola from open waterbodies (Belton *et al.,* 2014). To combat the situation, the species is being considered for pond culture (Thilsted and Wahab, 2014).

A population of mola that is lack of substantial level of genetic diversity could have potential negative consequences for its growth, nutritional contents, adaptive response to global climate change and survival (Pauls *et al.,* 2013). Use of this population as the parental stock for culture could potentially lead to the negative consequences. Baseline information on population genetic structure of mola is, therefore, very important to be known for its sustainable culture, management and conservation. In the study, phenotypic and randomly amplified polymorphic DNA (RAPD) analyses were carried out to determine population structure of the species.

Objectives

- To estimate morphometric-based fitness of mola;
- To define whether RAPD markers are useful in estimation of genetic variability in mola;
- To estimate intra-population genetic variation; and
- To determine inter-population genetic variation of the species.

Methodologies

Collections of fish

Fish were collected from 8 different populations viz. Dhulia beel in Nilphamari district (Nilphamari), a gher in Khulna district (Khulna), jolmohol of the Shuma river in Sunamgonj district (Sunamgonj), Karimpur canal from Noakhali district (Noakhali), a pond from Mymensingh district (Mymensingh), bukvora baor from Jessore district (Jessore), a pond from Rangpur district (Rangpur) and Kaptai lake.

Phenotypic analysis

Both meristics and land mark based morphometric analyses of 7 populations of mola mentioned above except Mymensingh were carried out to detect variation in fishes from different populations using KLONK image measurement software (version 13.1.2.1), microsoft excel and SPSS version 20 for windows (Armonk, NY: IBM Corp.).

RAPD analysis

RAPD analysis was performed for seven populations of mola viz., Nilphamari, Khulna, Sunamgonj, Mymensingh, Noakhali, Jessore and Rangpur. Genomic DNA from 280 fishes (40 from each population) was isolated, confirmed and quantified following Islam and Alam (2004). In primer test, initially 50 decamer random primers were used to amplify DNA samples of 2 fishes from 2 different populations. Twelve out of the 50 primers that revealed sufficient level of genetic polymorphism and generated reproducible banding patterns were selected to screen whole sample set. PCR amplification and agarose gel electrophoresis were performed following protocols described by Islam *et al.* (2004). RAPD data were analyzed using AlphaEaseFCTM Version 4.0 (Alpha Innotech Corporation), POPEGENE (version 1.31) (Yeh *et al.* 1999) and TFPGA (Miller, 1997) computer programs.

Results

Phenotypic analysis

Significant differences were observed in 4 (dorsal fin rays, caudal fin rays, anal fin rays and pectoral fin rays,) of 5 meristic counts, 8 (standard length, head length, eye diameter, pre-dorsal length, post-dorsal length, caudal peduncle length, maximum body depth and head depth) out of 9 morphometric measurements and in 27 landmark distances or truss measurements. Canonical discriminant functions (DF) analysis based on both landmark distances and morphometric measurements separated 7 populations from each other (Fig. 1).

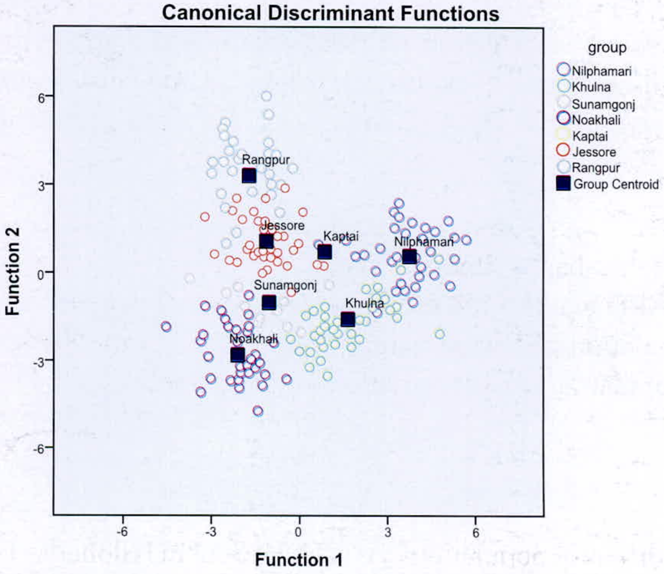

Fig. 1. Sample centroids of the discriminant function scores based on morphometric measurements and landmark distances.

RAPD analysis

RAPD profiles of different populations of mola using the 12 primers are shown in Fig. 2. The number of loci amplified by the 12 primers was varied between 3 to 11 (Table 1). A total of 80 loci were amplified using 12 primers. The size range of the loci varied between 232 to 2373 bp. Of the 80 loci amplified, 49 (61.25%) were found to be polymorphic (occurring in or absent in less than 95% of all individuals) in nature.

Table 1: Loci amplified by RAPD primers from genomic DNA of *A.mola*

Primer code	Sequence (5'-3')	No. of total loci scored	Size range (bp)	No. of polymorphic loci	% polymorphic loci
AO9	GGGTAACGCC	3	608-1283	2	66.67
S1136	GTGTCGAGTC	8	290-1102	7	87.50
OPAB14	AAGTGCGACC	8	387-1304	4	50.00
OPA18	AGGTGACCGT	4	463-817	4	100
OPB08	GTCCACACGG	5	621-2373	5	100
S1063	GGTCCTACCA	10	368-1962	7	70.00
OPAB06	GTGGCTTGGA	5	343-1860	4	80.00
OPH19	CTGACCAGCC	11	314-1421	8	72.73
S1234	TCGCAGCGTT	6	232-724	3	50.00
OPAB10	TTCCCTCCCA	7	465-2184	1	14.29
OPB12	CCTTGACGCA	8	423-1569	3	37.50
GO3	GAGCCCTCCA	5	262-873	1	20.00
Overall		80	232-2373	49	61.25

Estimation of intra-population genetic variation is shown in the Table 2. The number and percentage of polymorphic loci found to be the highest (30 and 37.50%) in the Rangpur population whereas the values for the Jessore was found to be the lowest (23 and 28.75%). Nei's (1973) gene diversity and Shannon's information index were found to be the highest (0.141 and 0.208) in Rangpur population and the values were found to be lower (0.106 and 0.160) in Mymensingh population. Two private or population specific alleles were found in Rangpur population whereas both Jessore and Mymensingh populations contained 1 private allele.

Table2: Intra-population genetic variation in A. *mola:* polymorphic loci and gene diversity

Populations	Parameters				
	No. of polymorphic loci	% polymorphic loci	Nei's gene diversity	Shannon's Information index	No. of private allele
Nilphamari	29	36.25	0.138	0.203	0
Khulna	29	36.25	0.140	0.207	0
Sunamgonj	26	32.50	0.112	0.168	0
Noakhali	27	33.75	0.116	0.174	0
Mymensingh	26	32.50	0.106	0.160	1
Jessore	23	28.75	0.110	0.162	1
Rangpur	30	37.50	0.141	0.208	2
Overall	49	61.25	0.144	0.228	4

Inter-population genetic variation was also estimated. Pair wise band sharing based similarity indices (Sij) and gene flow values (Nm) were found to be higher in Sunamgonj vs Noakhali (94.81 and 10.242) and Khulna vs Jessore (94.26 and 8.760) populations whereas the lower level of similarity and gene flow values were found in, Khulna vs Mymensingh (93.63 and 3.448), Khulna vs Sunamgonj (93.42 and 3.582), Khulna vs Noakhali (93.42 and 4.294) and Khulna vs Rangpur (92.19 and 5.252) population pairs. Pair-wise genetic distance (GD) values ranged from 0.010 to 0.038. Cluster analysis based on the GD showed that the Sunamgonj vs Noakhali and Khulna vs Jessore populations are closure each other with lower level of GD (0.010 and 0.013) whereas Khulna vs Sunamgonj and Khulna vs Mymensingh population pairs are comparatively distantly related with the highest GD (0.038) (Fig. 3).

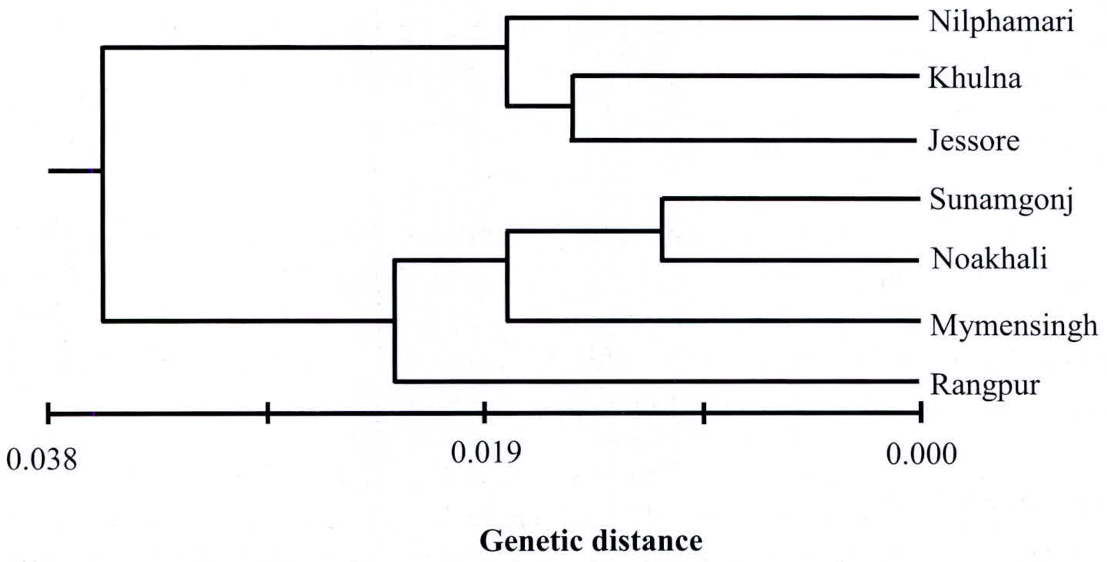

Fig. 2. Clustering of different populations of A. *mola,* using the Unweighted Pair Group Method of Arithmetic Mean (UPGMA) algorithm, which was constructed on the basis of Nei's genetic distances summarizing differentiation according to the RAPD profiles.

Conclusion

One of the important objectives of the study was to investigate phenotypic variation in different populations of mola. Significant differences in some meristic counts, morphometric measurements and truss measurements or landmark distances caused separation of different populations of mola. As the morphometric variation of an organism is induced by the environment where it lives, the variation that causes separation from each other might be due to physicochemical properties of the habitats. During collection of samples data on water temperature, salinity, current, habitat size, geographical position etc were collected. Variation in these parameters in different populations could a play important role in the separation of mola populations.

Another major objective was to use RAPD markers for analysis of both intra- and inter-population genetic variation in mola. RAPD analysis found to be effective in detecting genetic variation in mola. Though the study found lower level of genetic variation in each of the populations, overall polymorphism or genetic diversity was found to be higher in the species. Interestingly, 2 alleles (S1136512 and S1136449) at a higher frequency (0.152) were found in Rangpur population whereas they were absent in other populations indicating that mola of Rangpur population is different from other populations. These two alleles could be used as diagnostic markers for Rangpur population and the mola having these markers could be a potential strain. One private allele in Mymensingh and another one in Jessore populations were also found but their frequencies were lower (0.020).

Significant genetic variation was found between different pairs of mola populations. Out of 49 polymorphic loci, 32 played significant role in separation of populations from each other. The dendgrogram based on the genetic distances according to the RAPD profiles separated populations into 2 major clusters where Nilphamari, Khulna and Jessore populations were in one cluster and Sunamgonj, Noakhali, Mymensingh and Rangpur populations made another cluster.

From the above discussion, it can be concluded that substantial level of genetic variation exists in the mola of Bangladesh. Since overall genetic diversity is higher than that found in each of the population, it could be a good practice that fish from different populations should be mixed before being used as parental fish for wetland ranching and pond culture or for induced breeding in future. This could help in sustainable culture, management and conservation of this important SIS in Bangladesh.

Publication from this Research

Islam M.S., Khan, K., Sutradhar, P. and Sutupa, S.S. 2017. Genetic variation, population structure and identification of different strains of the small indigenous species, mola *(Amblypharyngodon mola* Hamilon 1822) in Bangladesh using PCR-RAPD marker: implication in sustainable culture. In: Proceedings of the workshop of BAU research progress. 13-14 May 2017, Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh, Bangladesh Vol. 27, pp 105-106.

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Microbial Arsenotrophy: A Possible Genetic Control on Arsenic Mobilization

Munawar Sultana and M Anwar Hossain

Location: Department of Microbiology, University of Dhaka
Duration: One year (2015-2016)
Expenditure of the project: Tk. 1500000.00

Introduction

Arsenic in soil and aqueous environments is considered as one of the prominent environmental causes of cancer mortality in the World, especially in Bangladesh, India and China. Alarming situation in Bangladesh is that the irrigation of soil with As contaminated groundwater is threatening the quality of the human food supply, which could affect millions more people.

Arsenite (III) is considered to be 100 times more toxic than arsenate (V). Bio-detoxification of arsenic contaminated soils and groundwater shows a great potential for future developments due to its environmental compatibility and possible cost-effectiveness. Bacteria can cope with arsenic either by detoxification or by gaining energy from inorganic arsenic. Arsenite can be oxidized to arsenate either by chemolithoautotrophic bacteria, for which arsenite serves as the electron donor as part of their energy generation process (Santini *et al.,* 2000; Oremland *et al.,* 2002) or it is oxidized by chemoorganotrophic bacteria (Gihring & Banfield, 2001) as part of their detoxification process. The transformation processes in microorganisms are conferred by specific genes such as arsenite oxidase (*aro*A and *aox*B) and arsenate reductase (*arr*A). Therefore, isolation of such arsenic transforming species and analysis of respective genes is necessary to discern the microbial involvement of natural arsenic transformation.

Objectives

- Isolation and Characterization of As-metabolizing microbiomes collected fromAs-contaminated aquifers, sediments and agriculture lands.
- Analysis of different geochemical compositions of arsenic-contaminated environments in Bangladeshand microbial (arsenotrophic) associations occurring at that area.
- Investigation of As-metabolism efficiency of the isolated organisms and characterization of -their functional genes involved in As-metabolism.
- Determination of biotransformation potentialityof arsenite oxidizing bacteria.

Methodologies

Five water and 7 soil samples were collected at various locations of arsenic prone area of Bogura and Manikganj districts of Bangladesh. Chemical parameters of the waterand soil samples including arsenic concentration were determined according to the standard methods.

Groundwater and soil samples were enriched in 60 mL of minimal salt medium (MSM) (Santini *et al.,* 2000) and heterotrophic enrichment medium (Gihring *et al.,* 2001) containing 2 mM of $NaAsO_2$ (Merck, Germany) and plated onto the corresponding solid media.

Denaturing Gradient Gel Electrophoresis (DGGE) was performed with bacterial 16S rRNA gene fragments that were initially amplified from DNA extracted from groundwater samples and their enrichments.

As(III) tolerantand phenotypically (KMnO4 screening)(Sanyal et al. 2016)As(III) oxidizing isolateswere selected for their plasmid pattern and genotyping by both Amplified Ribosomal DNA Restriction Analysis (ARDRA) and Randomly Amplified Polymorphic DNA analysis (RAPD). Selected isolateswere screenedfor specific genes (*aio*A, *ars*B) by PCR.According to ARDRA genotyping pattern, representative heterotrophic water and soil isolates including 5aioA positive isolateswere selected for sequence analysis and phylogenetic tree was constructed.

Minimum inhibitory concentration of As(III) was determined for all of the aio A gene positive isolates. The arsenite oxidation efficiency was determined using the molybdene blue method (Lenoble *et al.,* 2003). The enzymatic activity of As (III) oxidizing bacteriawas examined byimmobilizing it in Ca-alginate beads whichwas incubated in 100mL deionized water containing 3mM As(III) at 30 and subjected to periodic As(III) conversion test using $KMnO_4$.

Result

Major hydrological and geochemical parameters of the samples

The sampling sites were initially selected on the basis of the reference data from the Department of Public Health Engineering (DPHE). Major hydrological and geochemical parameters are displayed in Table-1 and 2.

Table-1: Hydrological parameters of ground water samples

Sample ID	Sampling Site	Depth (m)	pH	DO (mg/L)	TDS (mg/L)	TS (mg/L)	TSS (mg/L)	Alakalinity as CaCO 3 (mg/L)	As Conc (mg/L).
BSW-1	Shibganj	10.66	6.76	13.4	222	8	230	200	0.1
BSW-2	Shibganj	47.24	6.75	7	174	44	218	325	0.07
BCW-3(c)	Gabtali	56.08	6.47	10.5	152	96	248	225	0.01
BCW-4	Gabtali	27.43	6.65	8.5	182	14	196	275	0.5
BCW-5	Gabtali	18.28	6.7	8.5	139	11	150	275	0.3

Table-2: Chemical parameters of ground water and soil samples (Anions) by Ionic chromatograph

Sample ID	Sampling site	Chloride (mg/L)	Floride (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Phosphate (mg/L)	Sulphate (mg/L)	Bromide (mg/L)
BHW -1	Shibganj	17.526	0.698	<1.5	3.301	<3	<4	<1
BHW -2	Shibganj	3.216	0.168	<1.5	1.228	<3	<4	<1
BHW -3	Gabtali	2.576	0.358	<1.5	2.174	<3	6.146	<1
BHW -4	Gabtali	15.012	<0.5	<1.5	<3	<3	<4	<1
BHW -5	Gabtali	4.481	0.289	<1.5	1.541	<3	5.163	<1
BHS -1	Shibganj	24.439	ND	<1.5	5.557	<3	7.757	<1
BHS -2	Shibganj	182.501	ND	<1.5	33.969	<3	50.325	<1
BHS -3	Gabtali	8.779	1.302	<1.5	2.311	<3	1.891	<1
BHS -4	Gabtali	2.570	0.426	<1.5	ND	<3	2.091	<1
BHS -5	Gabtali	59.241	ND	<1.5	ND	ND	36.139	<1

Isolation of arsenite resistant bacteria

A total of 42 heterotrophic water and 60 heterotrophic soil bacteria were isolated from the five water and seven soil sample. These isolates were selected according to their distinct colony morphology.

Culture-independent bacterial community of As affected groundwater and enrichments

To assess the bacterial population thriving in As contaminated groundwater, 16S rRNA gene PCR products of the water samples and respective enrichments were purified and was used for DGGE analysis. DGGE yielded a total of 56 bands (Fig.S1). PCR amplification of 23 prominent DGGE bands and sequencing revealed diverse microbial presence (Table 3).

PCR of 16S rRNA and arsenotrophic genes

All heterotrophic enriched isolates showed characteristic's band at approximate 1500 bp when it was amplified using 16S rRNA gene specific primers in PCR (Figure-1). 5 *aio*A (1100 bp) positive water isolate (BHW-15, BHW-17, BHW-19, BHW-21, BHW-23)were found from BSW-2 and BSW-3 sampleand 5 *aio*A (1100 bp) positive soil isolate from BSS-1 and BSS-2sample **(Fig. 2).**

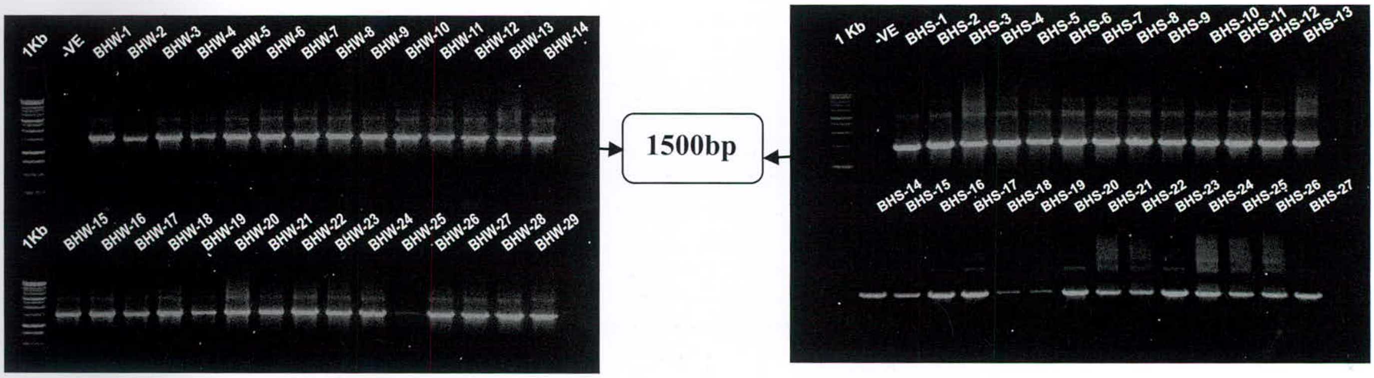

Fig. 1: Agarose gel electrophoresis of 16S rRNA gene PCR of arsenotrophic water and soil isolates

Table 3: DGGE bands of bacterial 16S rRNA gene amplified from arsenic contaminated groundwater samples and respective enrichments (SNGW: DNA extracted from original sample, AESNGW: DNA extracted from autotrophic enrichment of the sample, HESNGW: DNA extracted from heterotrophic enrichment of the sample)

BAND ID	**Sample Source**	**Close similarity to (accession numbers)**	**Maximum Identity (BLAST)**
MS1	SNGW -1	*Pseudomonas* sp. SMX321 (HF572913.1)	99%
MS5	SNGW -2	*Porphyrobacter* sp. clone (JQ701408.1)	96%
MS 18	HESNGW -1	*Acinetobacter* sp. F601 (FJ827775.1)	95%
MS19	HESNGW -2	*Chryseobacterium* sp. TV93Nov (KJ482906.1)	98%
MS 20		*Chryseobacterium hispanicum* NW120 (JF915333.1)	99%
MS 24		*Pseudomonas indica* strain NR4_009 (KF843864.1)	100%
MS 25		*Acinetobacter junii* strain: BN -22 (AB777646.1)	100%
MS 28	HESNGW -3	*Pseudomonas plecoglossicida* strain SR7 (KC634234.1)	99%
MS30		*Flavobacterium cucumis* strain S10 (KF261012.1)	99%
MS 31		*Aquabacterium* sp. R2A (KC424519.1)	100%
MS 32	AESNGW -1	*Pseudomonas* sp. MNL -2-2 (AB812782.1)	98%
MS33	AESNGW -1	*Pseudomonas* sp. Zj5 (GQ859170.1)	100%
MS35		*Aeromonas* sp. SH1 (KF835810.1)	100%
MS41	AESNGW -3	*Acinetobacter* sp. 114 (KC494698.1)	98%
MS49		*Aeromonas* sp. BTSQ7 (JQ964244.1)	100%
MS53	SNGW -4	*Acinetobacter* sp. *ERR551 (KC412139.1)*	95%
MS55		*Acidovorax* sp. CPO 4.0046 (KF923837.1)	100%
MS56		*Azospira* sp.(KC866209.1)	97%

Fig. 2: Agarose gel electrophoresis of PCR specific amplicon of arsenite oxidizing gene (*aio*A) of heterotrophic (a) water and (b) soilisolates.

Molecular profiling of arsB gene positive isolates

Sixty seven*ars*B positive isolates were retrieved from water and soil samples (Figure-3). Among them 6 from BSS-1, 10 from BSS-2, 5 from BCW-3,7 from BCW-4, 4 from BCW-5, 12 from BSS-1, 3 from BSS-2, 1 from BCW-3, 13 from BCW-4, 6 from BCW-5.

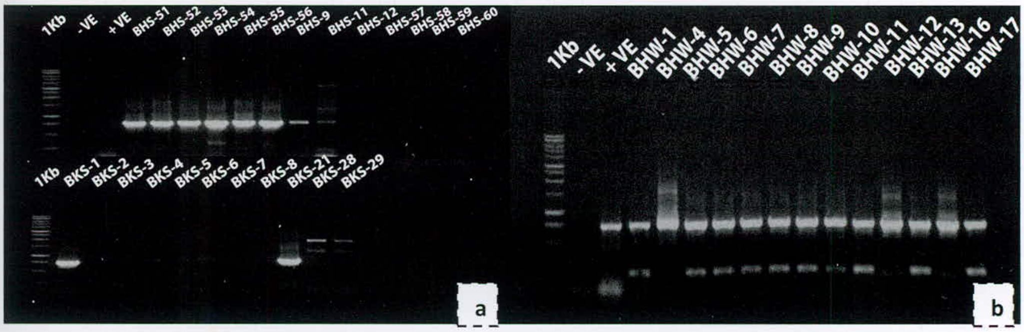

Fig. 3: Agarose gel electrophoresis of PCR specific amplicon of arsenite resistance gene (*ars*B) of heterotrophic (a) water and (b) soil isolates.

ARDRA of the heterotrophic water isolates

Restriction digestion with *Alu*1enzyme of the approximate 1500 bp amplified fragment of 16S rRNA gene showed 14 different ARDRA groups (Figure-4) among heterotrophic water isolates (G-1:14,16; G-2:15,17,19,21,23; G-3:18,26; G-4:20; G-5:22; G-6: 1,4,5,6,7,8,9,10,11,12,24,27,28,29,30,31,35,36, 42; G-7: 2; G-8: 32; G-9: 34; G-10: 37; G-11: 40; G-12: 42; G-13: 3; G-14: 13). According to the restriction pattern, all the 5 *aio*Aheterotrophic isolates from water samples of Bogura (BSW-2 and BSW-3) were in same group.

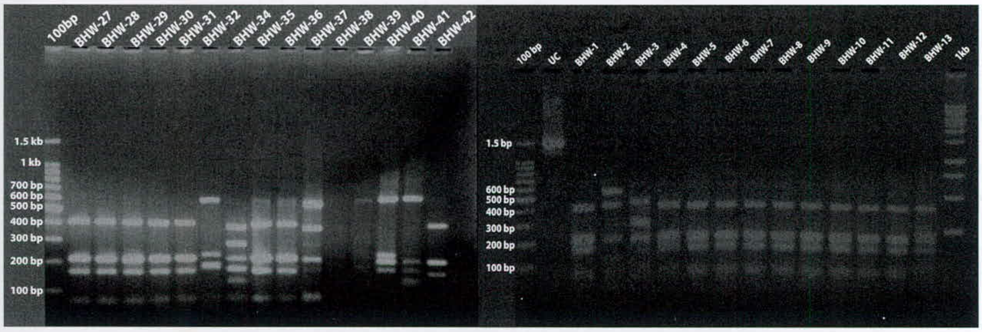

Fig. 4: Amplified restriction digestion analysis of heterotrophic water isolates of Bogra.

Sequencing and Construction of Phylogenetic Tree

16S rRNA gene of representative isolates (BHW-1,2,3,13,14,20,22,26,32,34,35,37,40) from different ARDRA group were sequenced. They showed close similarity with different genera belonging to *Pseudomonas putida* (94%), *Delftia* sp. (99%), *Stenotrophomonas* sp. (100%), *Lysinibacillus* sp. (99%), *Bacillus toyonesis*. (99%), *Microbacterium* sp. (99%), *Brevundimonas diminuta* (90%). *Bacillus* sp. (98%) from gene bank database for 16S rRNA gene (Figure-5).

Partial *aio*A gene of BHW-15, BHS-15 and BHS-25 from different ARDRA groups was sequenced usingprimers which amplified around 1100 bp of arsenite oxidase gene. *Achromobacter* sp. BHW-15 (water isolate) and *Pseudomonas* sp. BHS-25 (soil) showed 99% identity with *Achromobacter* (WP00622190.1) arsenite oxidase protein sequence (Figure-6). Another soil isolate *Enterobacter* sp. BHS-15 showed 98% identity with *Alcaligenessp.* S46 (ADF47192.1).

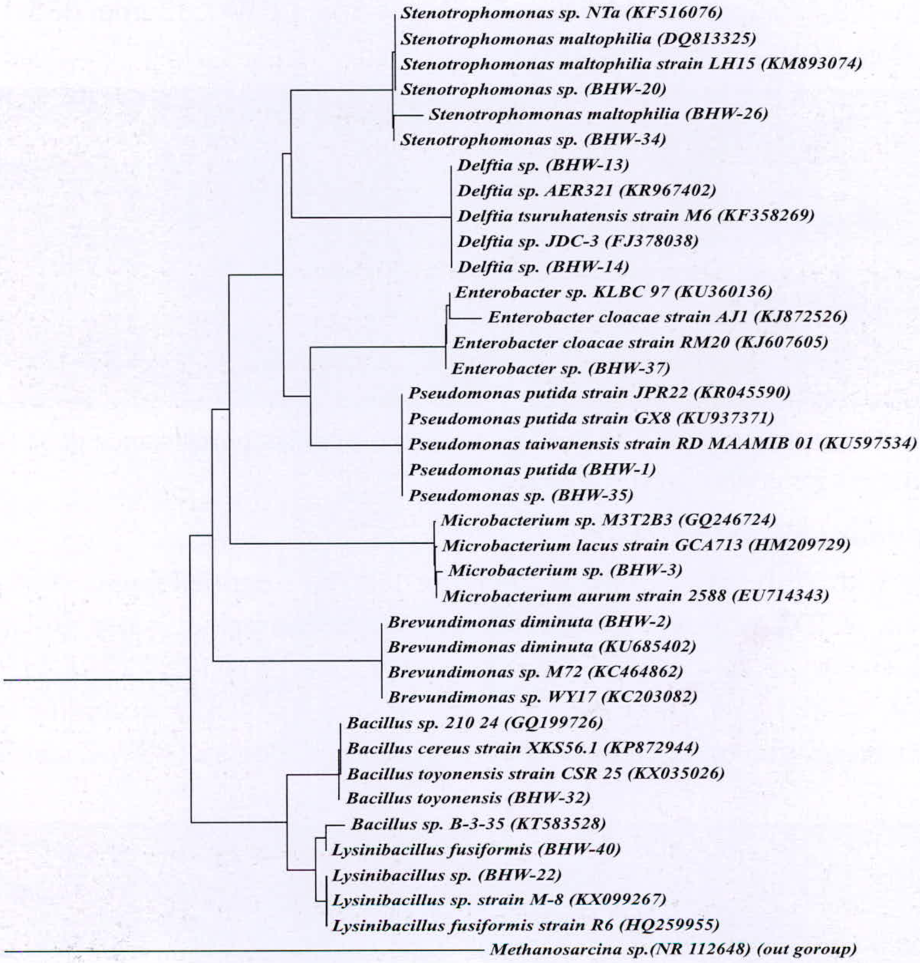

Fig. 5: Phylogenetic tree of 16S rRNA gene sequences of Bogura heterotrophic water isolates and close relative reference isolates retrieved from database.The tree was generated in program MEGA7 using the Neighbour-Joining algorithm with *Methanosarcina sp.* sequence serving as out-group. Bootstrap values (n=1000 replicates) are shown at branch nodes and the scale bar represents the number of changes per nucleotide position.

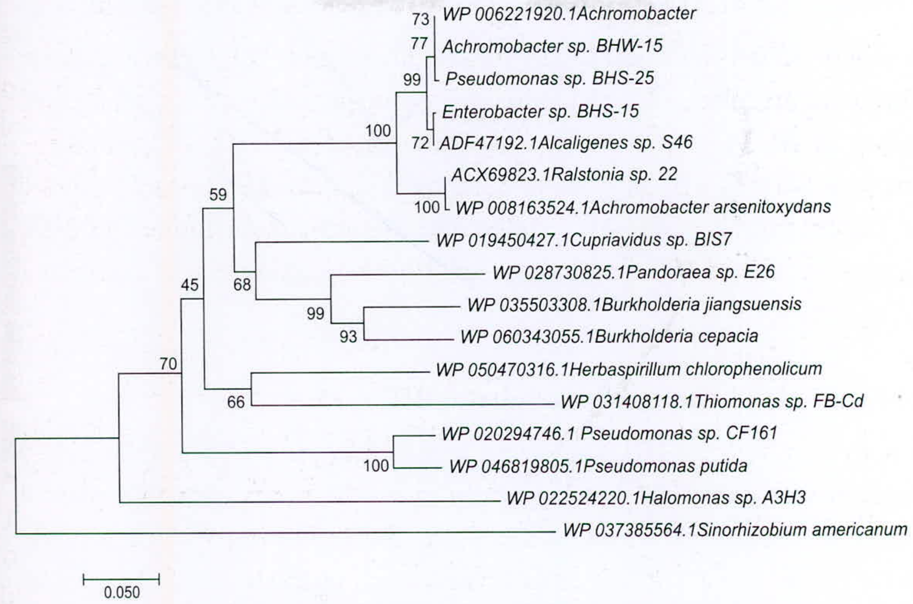

Fig. 6: Phylogenetic tree of arsenite oxidase amino acid sequences from threerepresentative genotypic group. The tree was calculated from deduced amino acid sequencesaligned in program ClaustalX The tree was generated in program MEGA7 using theNeighbour-Joining algorithm. Bootstrap value (n = 1000 replicates).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of all 10 of the arsenite oxidizing isolates were in the range of 4 to 11mM. Although some arsenite oxidizing groundwater isolates belonged to the same genotypic group, they differ in their MIC As(III) pattern **(Fig. 7).**

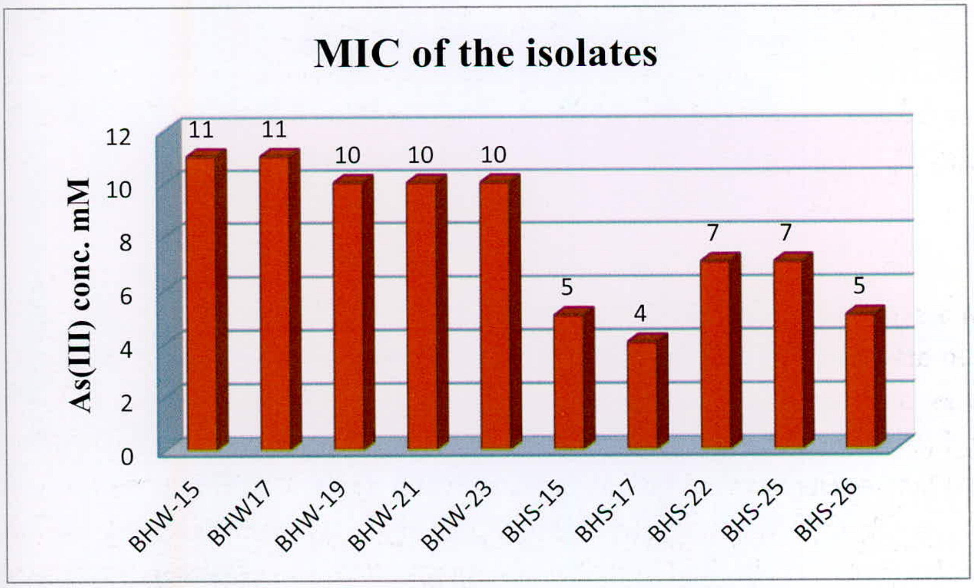

Fig. 7: Minimum Inhibitory Concentration of arsenite of all arsenite oxidizing isolates.

Quantitative Determination of Arsenite Oxidation by Potential Water Isolate

Achromobactersp. BHW-15 was selected for its maximum arsenite tolerance and presence of *aio*A geneto determine the quantitative arsenite oxidation in heterotrophic growth medium along with their growth curve analysis. It was grown in 40µM sodium arsenite and incubated at 30oC, 120rpm. Lag phase was 12 hours and generation time was 6 hours. By molybdene blue method concentrations of arsenite and arsenate were measured during different phases of growth. The isolate started converting arsenite efficiently at 14 hours after inoculation and took around 17 hours to convert 40µM of arsenite into arsenate after the logarithmic phase started.

Enzymatic immobilizations of *aio*A positive isolate, BHW-15

Ca-alginate beads with immobilized BHW-15 were kept deionized water supplemented with 3mM sodium arsenite and conversion was checked phenotypically every 24 hours. Positive phenotypic detection was observed after 3 days of incubation and continued for the following two days with intense pink color formation. The same beads were subsequently transferred to fresh 3mM As(III) solution 3 times and conversion of As(III) was observed for the first 2 times (Figure-9). In the third transfer after 18th days, no positive phenotypic arsenite conversion was observed. The immobilized cells could convert arsenite at detectable level within 3 days. On the other hand, free cells in 3 mM heterotrophic media took 5 days to convert arsenite at detectable level.

After 6 days **After 12 days** **After 18 days** **After 24 days**

Fig. 9: Qualitative estimation of arsenite oxidase activity of immobilized BHW-15.

Conclusion

The presence of arsenic in the soil and water is frequently reported. The best approach to remove arsenite is to oxidize it into arsenate; this last form is less soluble and much more easily to removed. The present investigation was an attempt to isolate potential arsenite oxidizing bacteria from local Bangladesh environment and to delineate its arsenite transformation capacity with genetic basis of biotransformation. Physicochemical heterogeneity of investigated groundwater and soil of Bogra district was independent of variable arsenic concentrations ranging from 0.01 – 0.5 mg/L. Arsenite oxidizing isolates were retrieved belonging to *Achromobacter* sp. and soil isolates represented *Pseudomonas* sp. and *Enterobacter* sp. Although arsenite oxidation has been reported within these genera, their potentiality of As transformation and complete genome structure of arsenite oxidase has not much been explored.

The Minimum inhibitory concentration (MIC) showed that water isolates possess a higher arsenic tolerance ability. Arsenite transformation efficiency of potential immobilized *Achromobacter* sp. BHW-15 in calcium alginate showed arsenite conversion rate of 11.8µM/hour. The potentiality of the selected strain necessitates the in depth assay of in situ bioremediation strategy. Results of this study will provide valuable information on microbial species with arsenic redox metabolisms that may contribute to the arsenic abnormality of groundwater in our country and their functional gene analysis may be useful for development of a sustainable bioremediation model of arsenic contaminated groundwater.

Publication from this Research

Sultana M., Mou T.J., Sanyal S.K., Diba F., Mahmud Z.H., Parvez A.K., Hossain M.A. 2017. Investigation of Arsenotrophic Microbiome in Arsenic-Affected Bangladesh Groundwater, Ground Water. 2017 Apr 18. doi: 10.1111/gwat.12520

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Effects of Heavy Metals in Brain Development of Mice

Mahmud Hossain and Obaidur Rahman

Location: Department of Biochemistry and Molecular Biology, University of Dhaka
Duration: One year (2015-2016)
Expenditure of the project: Tk. 1600000.00

Introduction

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers; and they are toxic in different ways. Considering a heavy metal as a metallic element with a specific gravity greater than 5, there are several numbers of heavy metal present, including Chromium, Copper, Zinc, Arsenic, Cadmium, Lead, Radium, Titanium. There are several types of industries developed in Bangladesh which play vital roles to fulfill our domestic necessity as well as to earn foreign currency. The leather industry is one of them. But unfortunately the chemicals (e.g. Chromium, Sulphuric acid, Magnesium oxide, Chrome syntans, Acrylic resins, Aldehyde tanning agents, Caustic soda, etc.) they are using very toxic. Chromium also use in alloys such as stainless steel to give the steel a polished silvery mirror coating and corrosion resistance. The main human activities that increase chromium concentrations are chemical, leather, electro-painting and other chromium applications in the industry. These applications will mainly increase concentrations of chromium in water. Through coal combustion chromium will also end up in the air and through waste disposal chromium will end up in soils. Chromium is a danger to human health, mainly for people who work in the above mentioned industry.

Objectives

In the present study among the list of heavy metals, we have selected chromium as a candidate as it is used in several industries in Bangladesh. Precise strategy and planning was followed to examine the effect of chromium on the brain and biochemical parameters using swiss-albino mice as an experimental model. The aims and objectives were:

- Measurement and comparison of the mean body weight of the control and different doses of chromium contaminated drinking water to understand the effect of chromium on body weight.
- Observation of the effects of chromium contaminated drinking water on the littre survival rate of the healthy female control and chromium treated mice to understand the effects chromium on the brain as well as the ultimate development of the fetus.
- Measurement and comparison of brain phospholipids of healthy control and different doses of chromium treated mice through drinking water to find out the effects of chromium on the brain.
- Measurement and comparison of the plasma glucose concentration of the healthy control and different doses of chromium treated mice through drinking water to understand the effect of chromium on the glucose level as it is the main energy source for the brain.

- Measurement and comparison of cardiovascular parameters and ALT (Alanine aminotrasferase) activity of the healthy control and different doses of chromium treated mice to understand the effect of chromium on hepatocellular tissues.
- Observation of the effects of chromium contaminated drinking water on DNA damage.

Methodologies

Swiss Albino mice were bought from Animal Resource Branch of ICDDR,B (International Centre for Diarrheal Disease Research, Bangladesh). Animals were acclimated for 2 months before use. After the acclimation, the mice were divided into several groups. In each subgroup there were 5-6 mice. Different doses (0.025, 0.5, 2.0 ppm) of chromium solution were prepared and treated the mice orally for 16 weeks. Anthropometric analysis was done in regular interval. Completion of the treatment blood samples was collected by cardiac puncture for different parameter analyses and mice were deeply anesthetized with diethyl ether. Four percent (4%) para-formaldehyde (PFA) heart perfusion was performed followed by decapitation. Part of hind brain was used for brain phospholipid estimation and rest of the brain was further processed (20% sucrose solution). For histopathological analyses processed brain went through block preparation using O.C.T compound. Nissl/cresyl violet staining and DNA damage was performed.

Results

Anthropometric data

We made 3 different dilutions of the chromium contaminated water and used this water as the drinking water as a part of the exposure of chromium to the mice (Fig. 1). We provided fresh drinking water to the control mice. The Individual body weight of healthy control and chromium treated mice was measured at the start of the treatment in every week.

Body Weight Analysis of Group 1 Mice (Control)

All the male mice which belonged to group 1, received fresh drinking water and considered as control mice. We measured their body weight in every week and continued up to 16 weeks (Fig. 2). We observed that the body weight of the control mice was gradually increased significantly in every four weeks.

Body Weight Analysis of Group 2

Mice were treated with 0.025 ppm chromium containing water and measured the body weight in every week. We found that the body weight of the individual mouse was either similar (before starting the chromium containing water) or gradually a decreasing trend. We observed that the weight was sharply decreased after 5 weeks and continued to 8 weeks of treatment. Later, the weight increased, but did not return to the initial weight (Fig. 2).

Body Weight Analysis of Group 3

Mice were treated with 0.5 ppm chromium containing water and measured the body weight in every week. We observed that the body weight of the individual mouse gradually decreased. We found that the weight sharply decreased after 3 weeks and continued to 5 weeks of treatment. Later, the weight increased up to 8 weeks and even it crossed initial body weight before the treatment started and again decreased the body weight from week 9 and continued until the mice were sacrificed for further analysis (Fig. 2).

Body Weight Analysis of Group 4

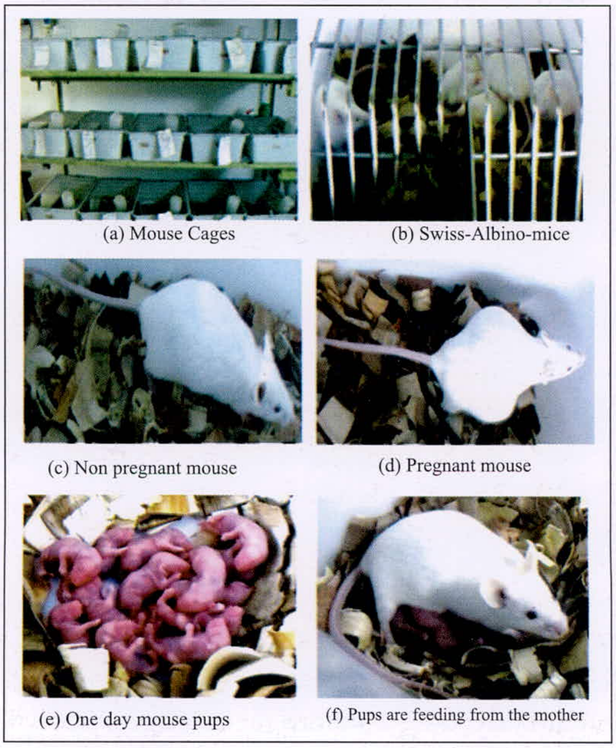
Male mice were treated with 2.0 ppm chromium containing water and measured the body weight in every week. We observed that the body weight of the individual mouse sharply decreased from the first week of the treatment and continued it up to 5 weeks of the treatment. Later, the weight increased up to 8 weeks and reached almost their initial weight and again decreased the body weight from week 9 and continued until the mice were sacrificed for further analysis (Fig. 2).

Pups Survival Rate

We found that the survival rate of the pups of the healthy control mice was higher (81.7%) whereas in case of Cr treated female the survival rate of the pups was only 33.7%, which is much lower than that of control that indicates the adverse effects of chromium on mouse brain as well as the ultimate development of the fetus.

Estimations of Brain Phospholipids

We treated the mice by Cr contaminated water with different concentrations (0.025ppm, 0.5ppm and 2.0ppm) for 16 weeks and sacrifice the mice followed by brain isolation. We then measured the amount of phospholipid in the brain. We prepared a standard curve of phosphates that followed the Beer-Lambert's law and the absorbance was taken at 660nm which is optimal for phosphates determination. We found that the mean value was gradually decreased with relatively higher concentration of chromium treated mice in compared to that of control mice. In the Pearson's correlation coefficient test, it was found that the brain phospholipids level of the different groups of mice was negatively correlated with the increasing dose of chromium



(a) Mouse Cages (b) Swiss-Albino-mice

(c) Non pregnant mouse (d) Pregnant mouse

(e) One day mouse pups (f) Pups are feeding from the mother

Fig. 1: Different stage of animal house (a)-(f)

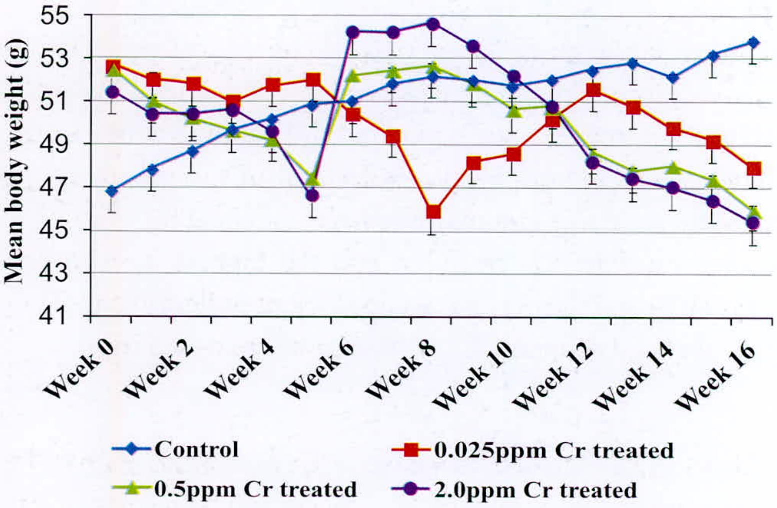


Fig. 2: Line graph for comparison of the mean body weight of Cr exposed mice Group-2, Group-3 and Group-4 with healthy control

where the healthy control was assumed to have administered with chromium concentration of 0 ppm and the highest dose to be 2.0 ppm. At the 95% confidence interval level, P =.363 and the correlation coefficient indicated a weak negative correlation between brain phospholipids content and different doses of chromium (r= -.263).

Estimation of Plasma Glucose

We measured random plasma glucose of mice by the GOD-PAP method. We have collected the mouse blood during decapitation followed by plasma preparation. As soon as possible of the sample collection, we measured the plasma glucose level. From the experimental analysis, we observed that the level of the glucose was gradually decreased non-significantly in the plasma of mice in 0.025ppm and 0.5 ppm Cr treated groups compared to that of control. However, we found that the plasma glucose was increased in the mice, those who were treated with 2.0 ppm Cr contaminated water in compared to that group of control.

Measurement of ALT

The liver is one of the vital organs which play important roles in heavy metal metabolism. To address the issue of liver function after chronic oral exposure of different doses of Cr containing water to the mice, the ALT enzyme activity in plasma was measured. ALT activity was measured by decreasing kinetic activity of the enzyme. We analyzed the level of ALT and found that the activity had been gradually increased in plasma of chromium treated mice compared to that of healthy controls.

Triglycerides

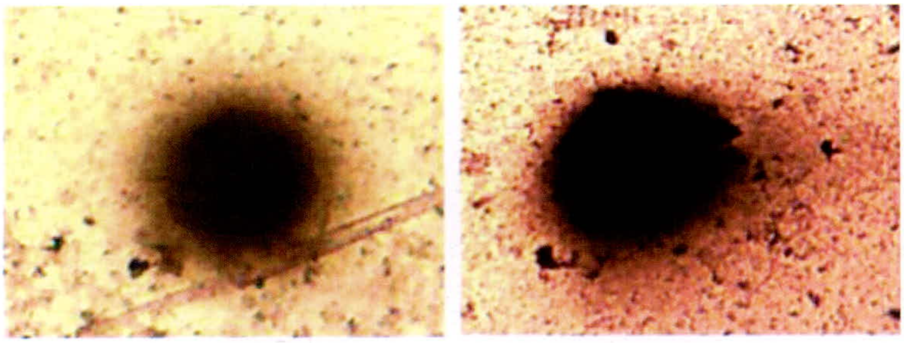
For the triglycerides content in the mouse, total plasma triglycerides were measured by the GPO-PAP method which is an enzymatic colorimetric test. We collected the plasma and analyzed the level of triglycerides. From the experimental analysis, we observed that there was no significant increase of the triglycerides content in the Cr treated groups (0.025ppm, 0.5ppm and 2.0ppm) compared to that of the control mice.

Total Plasma Cholesterol

For the total cholesterol content in the mouse, total plasma cholesterol was measured by the CHOD-PAP method which is an enzymatic colorimetric test. It has been found that total plasma cholesterol content was higher in the plasma of chromium treated mice compared to healthy control. In the Pearson's correlation coefficient test, it was found that the total plasma cholesterol of the groups of mouse was significantly correlated with the increasing dose of chromium where the healthy control was assumed to have administered with chromium concentration of 0ppm and the highest dose to be 2.0 ppm. At the 95% confidence interval level, P=0.029 and the correlation coefficient indicated a moderate correlation between the total plasma cholesterol level and different doses of chromium, r= 0.536.

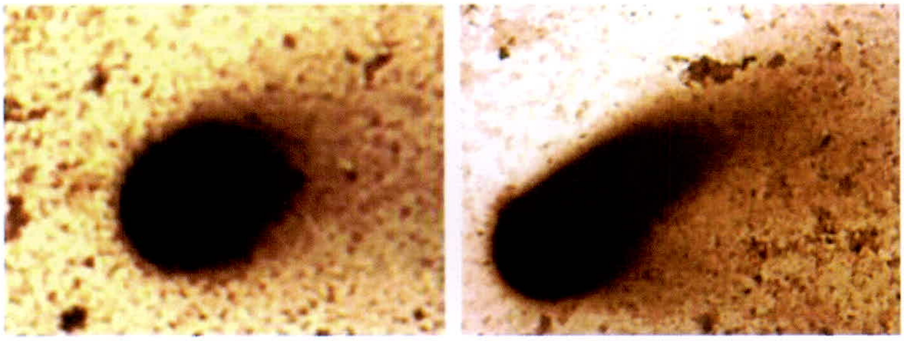
DNA Damage Analysis

Chromium is a genotoxic heavy metal. It can damage the DNA by binding with it. To measure and compare the DNA damage in chromium treated mice and the healthy control comet assay was done using the mouse blood sample collected. The comet assay was established in our laboratory through trial and error method. By cardiac puncture, mouse blood was collected in EDTA containing eppendorf tube. The blood sample was loaded on normal melting point (NMP) agarose coated slides and then sandwiched with low melting point (LMP) agarose and fixed at 00C. The fixed slides were addressed to lysis buffer



(a) Control cell with intact DNA

(b) Mildly damaged DNA of 0.025ppm Cr treated cells



(c) Moderately damaged DNA of 0.5ppm Cr treated cells

(d) Highly damaged DNA of 2.0ppm Cr treated cells

Fig. 3: Representative Comet images of control and chromium treated mice DNA

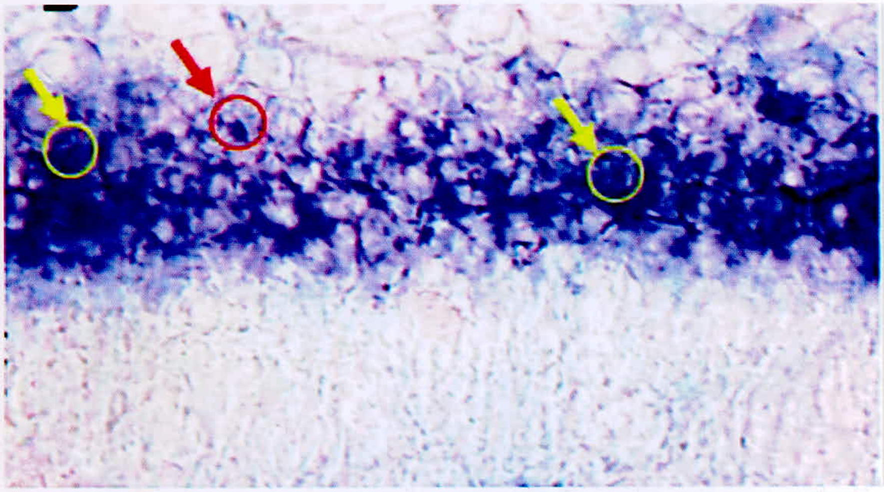


Fig. 4: Nissl/ cresyl violet staining of mouse brain shows the damaged cell (red circle)

to lyse the cells and then electrophoresis was done in alkaline buffer (pH>13). The slides were then neutralized (pH 7.4) and dried overnight at room temperature. The dried slides were then fixed using fixative solution and silver staining was done. The stained slides were dried at room temperature and observed under microscope. In the observation, the intact DNA looked like spherical shape and the damaged DNA took the shape of a comet. The length of the comet tail was proportional to the extent of the DNA damage. In the study, the DNA damage was also found to be proportional to the increasing dose of chromium administered to the mice. The 2.0 Cr treated DNA showed highest comet tail, therefore, was highly damaged, 0.5ppm Cr treated DNA was moderately damaged and the 0.025ppm Cr was mildly damaged with the shortest comet tail. The control slides showed no damaged DNA. The images were captured and then analyzed to measure the head and tail area of the comet and to compare their ratio (Fig. 3). The 8-OH guanosine analysis also gives the similar results of the commet assay i.e. more DNA damage occurs in the chromium treated mice compared to the control mice. The mice brain block was further cryo-sectioned and performed the Nissl staining and we found similar observation (cell death is higher in the chromium treated mice) (Fig. 4).

Conclusion

We have tried our best to finish our experiments within the stipulated time and found some very interesting data which will lead us to continue some experiments in different dimensions in the future. We are preparing a manuscript to submit these interesting data in a good journal. Finally, I would like to express my gratefulness to the Ministry of Education, Government of Bangladesh; honorable reviewers; and the teachers of my department for supporting me to conduct this project using the mouse as an experimental model.

Effect of Slow Breathing Exercise on Cardiac Autonomic Nerve Function in Migraine Patients

Sultana Ferdousi and Taskina Ali

Location: Dept of Physiology, Bangabandhu Sheikh Mujib Medical University, BSMMU

Duration: One year (2015-2016)

Expenditure of the project: Tk. 650000.00

Introduction

Migraine is a primary headache disorder with hypersensitivity to sensory stimuli (Headache classification sub-committee of IHS 2004). Presence of autonomic symptoms during migraine attack suggest autonomic dysfunction in migraine (Aygül *et al.* 2006).

Controversial and inconclusive report on autonomic impairment and its character in migraine have been published using Ewing's cardiovascular reflex tests (vijayalaksmi *et al.* 2010; Samir *et al.* 2006; Aygül *et al.* 2006). But recent studies recommended Heart Rate Variability (HRV) analysis to assess cardiac autonomic tone by Time and Frequency domain method (Task Force 1996). There is lack of published report on Autonomic Function in migraine in Bangladeshi population which stresses the importance of this research. Besides symptomatic relief by drug, no specific therapy for autonomic dysfunction has been advocated. Recent studies reported success of slow breathing exercise for improvement of cardiovascular, pulmonary diseases (Jerath *et al.* 2010) as well as autonomic function in healthy volunteers (Pal, Velkumary and Madanmohan 2004).

Though the benefit of SBE on autonomic function has been tested in healthy subjects,but the information about the effect of SBE in migraine patients is lacking. Therefore, has been designed to investigate the effect of SBE on the cardiac autonomic status in migraine patients during their headache free period.

Objectives

General Objective

To observe the effect of slow breathing exercise on cardiac autonomic nerve function status in newly diagnosed migraine patients by analysis of heart rate variability.

Specific objectives

- To observe resting pulse rate and blood pressure in newly diagnosed migraine patients in order to detect any deviation from normal.
- To record the HRV measures by frequency domain and time domain method in these patients before onset of medication or SBE in order to observe their autonomic nerve function status at baseline.
- To record HRV data 3 months after regular practicing slow breathing exercise and/or medication to observe the difference between pre and post intervention value.
- To compare post intervention data between SBE and only medicated group to isolate the effect of SBE on autonomic change in migraine patients.
- To assess all these HRV measures in age, sex and BMI adjusted healthy controls for comparison.

Methodologies

This intervention study was carried out in the department of Physiology at Bangabandhu Sheikh Mujib Medical University (BSMMU) during 2013. The protocol was approved by the Institutional Review Board. Sixty new migraine patients (group B) aged 15-30 years of both sex were enrolled by purposive sampling from the neurology of BSMMU. Thirty healthy control (Group A) also participated. Informed written consent was taken. All subjects were free from cardiovascular, neurological disorders, diabetes, smoking thyroid, renal or hepatic dysfunction and Psychiatric illness. Migraine patients performed slow breathing exercise (SBE), twice a day for 3 months and continued their medication for migraine.

Patients were sub-divided in 2 groups. 30 patients with medication only (group B1) and 30 patients with SBE and medication (group B2). They were designated according to period of data collection.

Group B1b -After 3 months follow up with medication only.
Group B2a – At baseline.
Group B2b - After 3 months of SBE with medication.

The HRV was measured by Polyrite D (Version-2.2), India.

Parameters

1.Resting pulse rate & BP 2. R-R, HR,.SDNN, RMSSD 3.LF nu ,HF nu,LF / HF.

Statistical analysis

Data were expressed as mean ± SE and analyzed by SPSS version 16.0. For significance “unpaired t” test ANOVA& paired t test was used as applicable. P value <0.05 was accepted as level of significance.

Results

Age and BMI

Subjects of all groups are similar in respect of age and BMI.(Table- I)

Table –I: Age and BMI in different groups (n=90)

Groups	Age (years)	BMI (Kg/m^2)
A (n=30)	22.2±0.70 (15 -29)	23.79±0.50 (18.9 -28.4)
B1 a (n=30)	20.4±0.70 (15 -28)	24.32±0.50 (20.4 -29.2)
B2 a (n=30)	21±0.67 (15 -29)	24.80±0.49 (19.5 -29.8)

BMI= Body Mass Index

A : Control, B1a : Patients before medication. B2a : Patients before SBE with medication.

Resting pulse rate and BP

The resting pulse rate was significantly higher in both patient groups than control at baseline but the resting BP were not significantly different in patients versus control. After 3 months resting pulse rate significantly decreased in patients under SBE whereas it increased in patients without SBE. This value was significantly lower in SBE patients when compared to only medicated patients after 3 months follow up. So, SBE significantly improved resting pulse rate in Migraine patients. (Table-II)

Table-II: Pre and post intervention values of pulse rate and BP in different groups.(n=90)

Groups	Pulse (beat/min)	SBP (mm of Hg)	DBP (mm of Hg)
B1$_a$ (n=30)	79.2±1.55 (64-90)	110.17±1.47 (100-120)	68±1.35 (60-80)
B1$_b$ (n=30)	82.5±1.05 (65-91)	110.5±1.18 (100-120)	69.83±1.05 (60-85)
B2$_a$ (n=30)	80.06±1.02 (70-95)	112.33±1.23 (100-120)	68.5±1.34 (60-80)
B2$_b$ (n=30)	70.3±0.90 (62-82)	112.67±1.06 (100-120)	69.67±1.07 (60-80)
A (n=30)	68.37±0.80 (60-76)	109.67±1.42 (100-120)	67.33±1.04 (60-80)

Statistical analysis

Groups	p value		
$B1_a$ vs. $B1_b^{\Omega}$	0.001^{**}	0.801	0.094^{s}
$B2_a$ vs. $B2_b^{\Omega}$	0.000^{***}	0.722^{s}	0.109^{s}
A vs. $B1_b^{\varphi}$	0.000^{***}	0.653	0.097
A vs. $B2_b^{\varphi}$	0.114	0.096	0.123
$B1_b$ vs. $B2_b^{\varphi}$	0.000^{***}	0.179	0.912

Data were expressed as Mean ± SE. Figures in parentheses indicate ranges.
Statistical analysis were done by Independent sample t-testφ and Paired sample t-test Ω
DBP=Diastolic blood pressure SBP= Systolic blood pressure.
Group A : Apparently healthy control
Group B1a : Patients before medication
Group B1b : Patients after medication
Group B2a :Patients before SBE with medication
Group B2b :Patients after SBE with medication
*** = $p<0.001$ ** = $p<0.01$ n = number of subjects

Mean RR interval, Mean HR,SDNN &RMSSD:

The mean values of mean RR interval, SDNN and RMSSD were significantly lower and mean HR was significantly higher in migraine patients before intervention than those of control. After 3 months these values improved in SBE patients but these were further aggravated in patients without SBE. (Table-III and IV)

Table-III: Pre (Baseline) and post intervention values of Mean heart rate and Mean R-R interval in migraine patients (n=60)

Groups	Mean heart rate (beat/min)	Mean R-R interval (sec)
$B1_a$ (n=30)	85.2±1.35 (68-95)	0.71±0.02 (0.56-0.88)
$B1_b$	87.9±2.67 (52-117)	0.71±0.03 (0.35-1.16)
$B2_a$ (n=30)	87.20±1.88 (68-108)	0.70±0.02 (0.56-0.91)
$B2_b$	72.63±1.19 (61-92)	0.84±0.01 (0.65-0.99)
A (n=30)	69.13±1.22 (60-82)	0.87±0.02 (0.70-1.0)

Statistical analysis

Groups		p value
$B1_a$ vs. $B1_b^{\Omega}$	0.467	0.976
$B2_a$ vs. $B2_b^{\Omega}$	0.000^{***}	0.000^{***}
A vs. $B1_b^{\varphi}$	0.000^{***}	0.000^{***}
A vs. $B2_b^{\varphi}$	0.045^{*}	0.157
$B1_b$ vs. $B2_b^{\varphi}$	0.000^{***}	0.000^{***}

Data were expressed as Mean ± SE. Figures in parentheses indicate ranges.
Statistical analysis were done by Independent sample t-testφ and Paired sample t-test Ω
*** = $p<0.001$ * = $p<0.05$

Table-IV: Pre and post intervention values of SDNN and RMSSD in different groups (n=60)

Groups	SDNN (ms)	RMSSD (ms)
$B1_a$ (n=30)	47.16±1.46 (38.28-68.50)	35.97±0.90 (25.66-48.21)
$B1_b$	42.57±2.34 (19-64.64)	34.84±2.17 (23.27-70.06)
$B2_a$ (n=30)	47.16±2.22 (24.64-78.46)	37.88±1.38 (28.33-55.97)
$B2_b$	71.74±2.78 (38.60-108.36)	41.55±5.25 (23.93-160.57)
A (n=30)	161.95±6.51 (100.54-209.29)	41.81±1.11 (31.25-54.19)

Statistical analysis

Groups	p value	
$B1_a$ vs. $B1_b^{\Omega}$	0.163	0.000^{***}
$B2_a$ vs. $B2_b^{\Omega}$	0.000^{***}	0.419
A vs. $B1_b^{\varphi}$	0.000^{***}	0.000^{***}
A vs. $B2_b^{\varphi}$	0.000^{***}	0.485
$B1_b$ vs. $B2_b^{\varphi}$	0.000^{***}	0.000^{***}

Data were expressed as Mean ± SE. Figures in parentheses indicate ranges.
Statistical analysis were done by Independent sample t-testφ and Paired sample t-test Ω
*** = $p<0.001$

HF, LF norm &LF/HF ratio

The mean values of mean HF norm were significantly lower and mean LF norm and LF/HF ratio were significantly higher in migraine patients before intervention than those of control. After 3 months follow up these values were improved in SBE patients but these were further aggravated in patients without SBE. (Table-V)

Table-V: Pre and post intervention values HF, LF norm and LF/HF ratio in different groups (n=90)

Groups	HF norm (nu)	LF norm (nu)	LF/HF ratio
$B1_a$ (n=30)	29.69±1.54 (14.8-40.6)	70.64±1.52 (59.4-85.2)	2.69±0.12 (1.72-4.28)
$B1_b$	25.39±1.51 (13.9-39.9)	74.81±1.45 (61.7-86.1)	3.72±0.19 (1.99-6.30)
$B2_a$ (n=30)	25.86±1.15 (12.7-44.3)	74.14±1.15 (55.7-87.3)	2.97±0.14 (1.85-4.88)
$B2_b$	37.60±0.90 (26.8-47.2)	62.40±0.90 (52.8-73.2)	2.03±0.03 (1.63-2.31)
A (n=30)	41.1±0.70 (34.5-47.3)	58.9±0.70 (52.7-65.5)	1.52±0.07 (1.11-2.72)

Statistical analysis

Groups	p value		
$B1_a$ vs. $B1_b^{\Omega}$	0.000^{***}	0.006^{**}	0.000^{***}
$B2_a$ vs. $B2_b^{\Omega}$	0.000^{***}	0.000^{***}	0.000^{***}
A vs. $B1_b^{\varphi}$	0.000^{***}	0.000^{***}	0.000^{***}
A vs. $B2_b^{\varphi}$	0.003^{**}	0.067^{ns}	0.000^{***}
$B1_b$ vs. $B2_b^{\varphi}$	0.000^{***}	0.000^{***}	0.000^{***}

Data were expressed as Mean ± SE. Figures in parentheses indicate ranges.
Statistical analysis were done by Independent sample t-test φ And Paired sample t-test Ω
*** = p<0.001 ** = p<0.01

Conclusion

From the results of this study it can be concluded that there wass autonomic dysfunction characterized by suppressed parasympathetic while increased sympathetic activity in the newly diagnosed migraine patients. In addition, the autonomic balance was shifted to strong sympathetic dominance even in resting state .

The results of migraine patients who were treated with drug only suggest further detoriation of autonomic derangement with further increased sympathetic and decreased parasympathetic function in them. The overall variability reduced to greater extent.

However the results of autonomic parameters in migraine patients who performed regular SBE concluded that both sympathetic and parasympathetic component of cardiac autonomic function improved as well as normal autonomic balance was restored by regular slow breathing exercise.

The above facts suggest that if an appropriate measure against cardiac autonomic dysfunction is not adopted in migraine patients, this dysregulation may be aggravated silently with time without apparent cardiovascular symptoms.

Thus migraine patients though they are free of migraine headache because of the cardiac autonomic dysfunction they become more vulnerable with the risk of adverse cardio vascular event.

Finally it has been observed that regular practice of SBE improved the autonomic balance in migraine patients which could protect them from cardiovascular risk. Thus it can be concluded that regular performance of SBE is an effective measure to reduce the risk of cardiovascular disease in migraine patients.

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Audio-Visual Design in Respect to the Subjective Evaluation for a Standard Lecture Room

Kazi Saifuddin

Location: Department of Psychology, Jagannath University, Dhaka

Duration: One year (2015-2016)

Expenditure of the project: Tk.500000.00

Introduction

The quality of education has been discussed and debated in many concern Government organizations and NGOs from the Liberation of Bangladesh in 1971. The discussions almost took places on syllabus, about teacher's quality and their salary, method of teaching, student number in classroom, and so on. But no body as far as known, raise voice about the visual-acoustic environment of the classroom which is very much concern to ensure effective communications between the teacher and students. There are some researches relating to this issue conducted in western countries (ANSI-S3.5 1997, and Hopkins 2004) which are not applicable in this country because of mainly different human and cultural factors. Therefore, a research has been designed by which new standards can be organized audio-visually. As per western study the measured and investigated parameters should be:

i. Reverberation time (EDT, T20,T30) according to ISO 3382-1
ii. Speech clarity (C50) according to ISO 3382-1
iii. Sound strength (G) according to ISO 3382-1
iv. Speech intelligibility STI, RASTI according to IEC 60268
v. Sound reduction
vi. Inter-aural cross correlation IACC
vii. New acoustic measures, such as "room gain" and "voice support"

But in this study standard size and number have been measured on the basis of the subjective preference teats within a certain visual conditions in relation to the perceived time.

Objective

The main objectives of this study are:

- To find out the standard room size where the listeners (students) preferred best to learn.
- The audio-visual positions in the room are to be measured is this preferred conditions.
- To determine the factors of autocorrelation function (ACF) the subjective time will be measured at different geometrical positions in the room.

Methodologies

Audio-visual lecturer in the classroom will be justified by the subjective evaluation of the students as listener. The evaluation will be taken on the subjective judgments of the students where they prefer the loudness of the speech and also the perception of time duration of the lecture. Acoustic factors within certain visual conditions and certain structural conditions as available in this country will be measured. Subjective time will be measured at different geometrical positions in the classroom. Such subjective time (time perception) is a common parameter of the four factors of the Autocorrelation function (ACF) which is expressed mathematically as below (Ando *et al.,* 1977, 1998, 2000; Saifuddin *et al.,* 2001a, 2001b, 2016).

$$\phi(\tau)=\frac{1}{2T}\int_{-T}^{+T} p(t)p(t+\tau)dt \quad (1.1)$$

Where, $p(t)$ is the stimulus at the entrance of the ears is the delay time, and $2T$ is the integration interval. In such ACF analysis, there are four parameters, namely-

(a) the energy represented at the origin of the delay, $\Phi(0)$;
(b) the amplitude, $\phi 1$, between the first peak and the zero crossing number;
(c) the structure including the time delay of the first peak, $\tau 1$; and
(d) the effective duration of the envelope of the normalized ACF, τe, which is defined by ten percentile delay or at which the envelop of the ACF becomes -10dB

Results

In this study perceived time was measured in respect to a) time duration, b) acoustic amplitude (Table 1.1) were the base of two experiments which results by the expression of psychometric distribution (Figure 1.1) as example. The theoretical aspects of these experiments as per ACF method (Equation 1.1) also explained (Saifuddin, K., 2016). On the basis of the design, subjective evaluation of the four important positions (common setting in a room) in the room is found in the Figure 1.2 as results of four positions. Here, 75%, 50% and 25% data of the thresholds in the psychometric functions (Figure 1.1) by paired-comparion subjective tests for

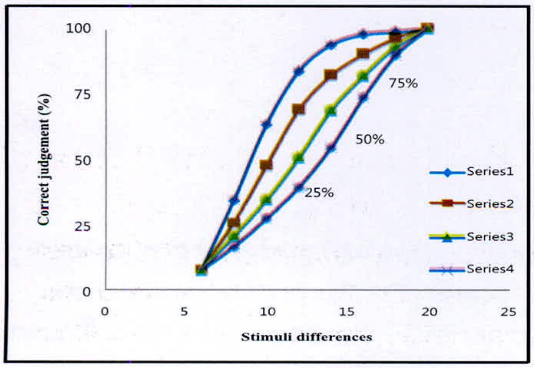

Fig. 1.1: Psychometric functions obtained from paired comparison subjective tests.

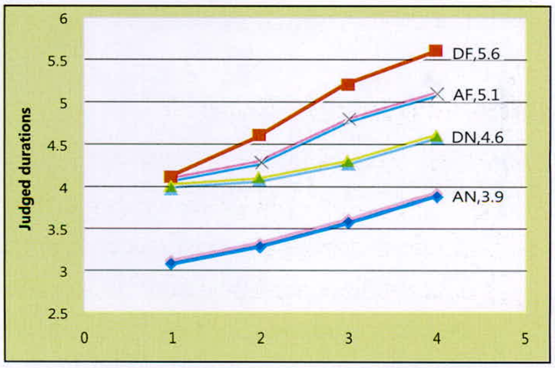

Fig. 1.2: Data obtained by threshold points of the psychometric functions.

Here, in the Figure 1.2, DN = Duration (time) Judged for duration at near point = A and B; DF = Duration Judged for duration at far point = C and D; AN = Duration Judged for amplitude at near point = A and B; AF Duration (time) Judged for amplitude at far point = A and B. Thus the time perception was measured as per signal duration and signal amplitude (power) at different points of setting positions in a lecture room to justify the standards of audio-visual point of views for the class room listeners.

Conclusion

This study aimed was to understand how learners perceive lecture of the teacher in the class room in different positions by which effective audio-visual design can be made for a standard lecture room. The subjective time which, is very much concern to understand the lecture for the listeners was measured from the different positions of the class room. White noises were propagated from the conventional lecture point in the room and subjects have to judge between the two pair sound stimuli. Pared comparison tests were conducted maintaining psychophysical method. The 75%, 50% and 25% levels of subjective judgments of time were averaged finally in the psychometric functions according to the parameters. Those parameters were expressed in the five different psychometric distributions. Such subjective time is a common parameter of the four factors of the ACF as a signal processor which is a electronic devise is expressed mathematically in the Equation 1.1.

The results revealed simply that the subjective time duration found by experiment different between the frontier two positions (A & B) and backward two positions (C & D). For the positions A and B the subjective time was found longer against duration measurement than that of the amplitude. Another difference was found for the C and D positions than the forward positions A and B. So the C and D positions are better for lecture understanding than A and B positions. It means the four factors of ACF in the audio-visual conditions of lecture in the class room is responsible parameters to make the listeners more capable for lecture understanding. That is why the results on this study in much significant to design a classroom well functions.

Publication from this Research

Saifuddin K. 2016. Auditory Temporal Perception of the Stimuli as Factors of Signal Processor. The 32nd Annual Symposium of the *International Society for Psychophysics* (Fechner Day Celebration, 12-20 August), and *Russian Academy of Science,* Moscow State University, Moscow, Russia.

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Development of Novel Bridged Nucleic Acid Analogues with Cationic Functional Moiety for Application in Various Genomic Technologies

S M Abdur Rahman and Sajal Kumar Saha

Location: Department of Clinical Pharmacy and Pharmacology, University of Dhaka
Duration: One year (2015-2016)
Expenditure of the project: Tk.1300000.00

Introduction

Since the approval of first antisense drug Vitravene (ISIS-2922) in 1998 by FDA (Uhlmann, 1998), the use of chemically modified oligodeoxynucleotides (ODNs) in biomedical and molecular biological research fields has intensified many folds. Modified ODNs are increasingly utilized in various gene silencing technologies such as antisense, antigene and RNA interference etc. in nucleic acid nanotechnology and DNA-based diagnostics (Yan, 2004) One of the promising strategies of developing highly efficient ODNs is the preorganization of sugar puckering of nucleotides via conformational restriction. Preorganization of sugar conformation has improved the properties of nucleic acid greatly. In this project, we want to develop very efficient modified nucleic acid derivative based on locking the sugar conformation of nucleic acid via bridging. We called it Bridged Nucleic Acid (BNA) (Rahman, *et al.* 2008, 2012). In addition to bridging the sugar moiety, we want to incorporate a cationic amino side chain on the phosphate moiety (Figure 1: structure A and B) because cationic groups are shown to improve target binding affinity via an electrostatic interaction with the phosphate anion of target oligonucleotides. (Michael, 2003). The length of the cationic moiety will be adjusted for obtaining optimum efficacy. After the synthesis, the modified ONs will be investigated for their hybridizing ability against target DNA and RNA strands. Their nuclease resistance against endo- and exonucleases will be evaluated and compared with the natural and BNA modified nucleic acid without cationic moiety.

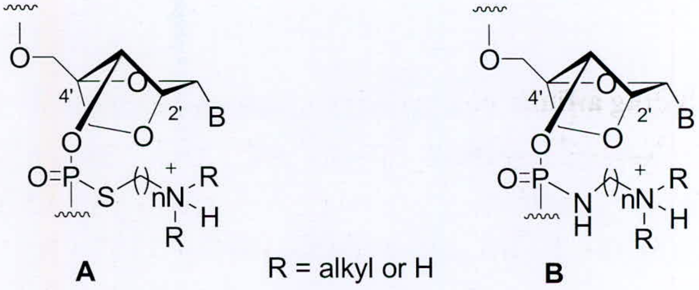


Fig. 1: Cationic Bridged Nucleic Acids

Objectives

The main objective of the proposed research is to develop smart bridged nucleic acid analogues which can be highly efficient in antisense, antigene and other gene-silencing technologies. As described in the summary section of this proposal, some nucleic acid analogues having conformationally favorable bridged structure with suitable backbone architectures will be designed. Nucleic acid analogues with the general structure of A and B with varying length of cationic linker will be synthesized. After synthesizing the monomers, several ODNs comprising these monomers will be synthesized by automated DNA synthesizer in the laboratory of my collaborator (Prof. Satoshi Obika, Osaka university, Japan). Their affinity to target RNA and DNA strands will be evaluated by UV melting experiments. Nuclease resistance power will be examined by exposing the ODNs to exonuclease and endonuclease. The results will be compared with those obtained with the existing analogues. Finally, the nucleic acid analogues showing promising properties will be tested for their antisense, antigene and RNAi activities In addition the synthesis intermediates will be screened for their bioactivities.

Methodologies

All the reagents, chemicals and materials used in this research were either purchased or prepared in the laboratory. They re purified by standard methods. Some Procedures applied in the research are described below:

1. Synthesis of aminoalkylated PS-ODNs: Each aminoalkylation reagent (1.0 M, 2μL, 2 μmol) in DMF or H_2O was added to a solution of-5’d(CCCTTTsTTTCCC)-30 (ODN 1) (10 nmol) in 22 μM HEPES buffer (18 μL, pH 6.5) and the reaction mixture was incubated at 45 ºC for 24 h. After completing the reaction, ODN was precipitated by adding ethanol (100 μL). The mixture was kept at 0 ºC for 15 min, centrifuged at 13,200 rpm for 15 min at 4 ºC, and the resulting supernatant solution was removed.The obtained ODN was purified by RP-HPLC and characterized by MALDI-TOF mass spectrometry.

2. Assessment of target binding affinity against ssDNA, ssRNA and dsDNA: Target binding affinity was evaluated using the synthesized artificial DNA and RNA Targets (Targets: ssDNA: 5’-d (AGGAAAAAAGGG)-30; ssRNA: 5’-r (AGGAAAAAAGGG)-3’ dsDNA: 5’-d(GCAGCGGGAAAAAAGGAGCAGC)-3’;/3’-d(CGTCGCCCTTTTTTCCTCGTCG)-5’). This experiment was performed by measuring UV-melting temperature (Tm) using standard methods.

3. Evaluation of Nuclease resistance ability: Nuclease resistance ability of the modified oligonucleotides was examined by using Snake Venom Phosphodiesterase (SVPDE). at appropriate concentration for several time period. The stability of the parent oligonucleorides will be measured by HPLC.

Results

Various stereochemically pure cationic phosphorthioate oligonucleotides based on amino alkyl moiety were synthesized in 24-55% yield (Scheme 1) and their Target binding affinity or duplex- and triplex-forming ability against single-stranded DNA (ssDNA), single-stranded RNA (ssRNA) and double-stranded DNA (dsDNA) respectively were evaluated by UV melting experiment. The cationic Rp stereoisomer showed enhanced duplex-forming ability against ssDNA and triplex forming ability against dsDNA (Table 1). Duplex formation against ssRNA was discouraged. 2-(3-Aminopropyl)aminoethyl conjugate was found to produce maximum stabilizing effect for duplex and triplex formation. Nuclease rtesistance study was also accomplished and the synthesized derivatives showed better stability against snakae venom phosphodiesterae (Rahman *et.al* 2012).

Scheme 1:

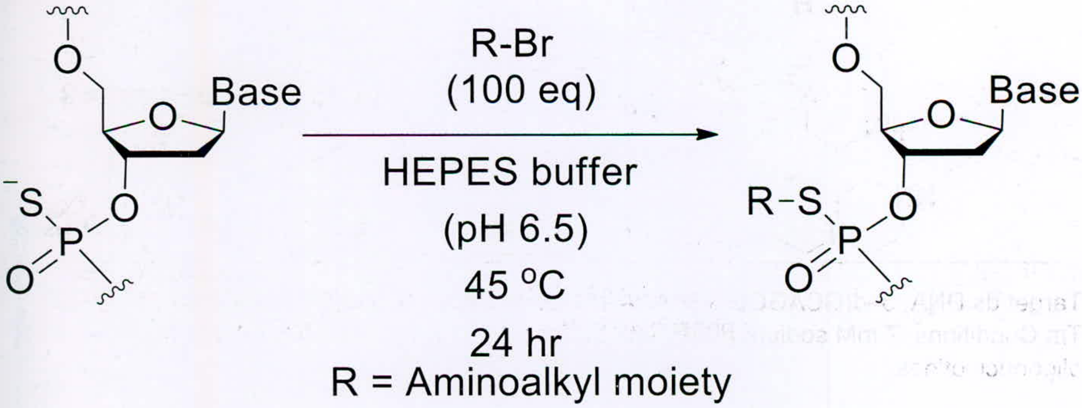
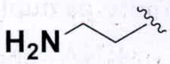
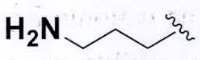
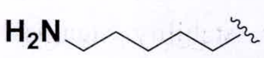
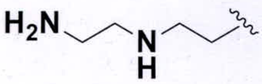
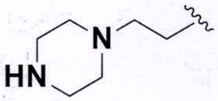
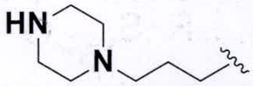


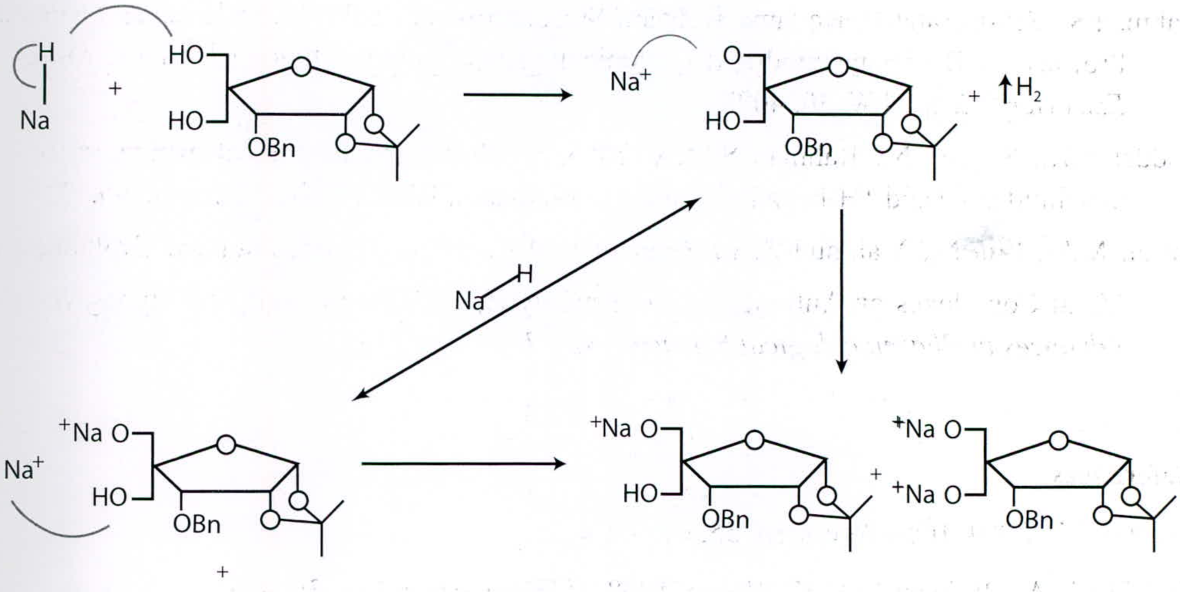
Table 1:

Oligonucleotide	Sp T_m	Sp ΔT_m	Rp T_m	Rp ΔT_m
5'-d(ccc tt1 ttt cct)-3'	27	-	26	-
5'-d(ccc tt2 ttt cct)-3'				
R =  (a)	24	-3	29	+3
 (b)	23	-4	29	+3
 (c)	23	-4	27	+1
 (d)	24	-3	27	+1
 (e)	22	-5	26	0
 (f)	25	-2	30	+4
 (g)	25	-2	31	+5
 (h)	24	-3	28	+2
 (i)	24	-3	27	+1

Target ds DNA; 5'-d(GCAGCGGGAAAAAAGGAGCAGC)-3'/3'-d(CGTCGCCCTTTTTTCCTCGTCG)-5'
Tm Conditions; 7 mM sodium phosphate buffer (pH 5.5), 140 mM KCl, 10 mM $MgCl_2$, 1.5 μM oligonucleotides

In another study, 3-O-Benzyl-4-C-(hydroxymethyl)-1,2-O-isopropylidene-α-D-ribofuranose (Koshkin *et al.* 1998) was used as starting material for the synthesis of modified derivatives of α-D-Ribofuranose. Here in this study, four derivatives of α-D-Ribofuranose have been synthesized via benzylatin, tosylation and acetylation reactions as shown below (Scheme 2). Further modification will be accomplished to synthesize nucleoside, nucleotides and finally cationic moiety will be introduced (Fahad, 2016).

Scheme 2



Conclusion

In conclusion, we have successfully introduced various aminoalkyl moieties to the sulfur group of stereodefined PS-ODNs. The aminoalkyl conjugated Rp-isomers of phosphorothioate exhibited increased stabilization of DNA duplexes while the Sp-conformers destabilized the duplexes. Both the cationic Rp- and Sp-isomers showed decreased affinity towards RNA. Triplex formation was enhanced by all the aminoalkyl functionalized Rp-isomers. The most significant triplex stabilization was observed with 2-(3-aminopropyl) aminoethyl and 2-(2-aminoethyl)aminoethyl linked Rp-PSODNs. We also revealed that the alkylation of PS-ODNs is effective to enhance the nuclease stability and increase of nuclease stability is more pronounced for Rp isomers. Considering all the results we assume that cationic R*p*-PS-ODNs might be an interesting candidate for DNA based technologies such as DNA microarray, DNA nanostructures and antigene technologies.

Apart from the above research result we also synthesized some modified ribofuranose starting from a commercially available diol 3-O-Benzyl-4-C-(hydroxymethyl)-1,2-O-isopropylidene-α-D-ribofuranose which will be used for the synthesis of new class of cationic nucleic acids.

In addition, under the project, we also synthesized some important organic compounds such as benzimidazoles and metal complexes which showed significant biological activities. (Podder *et al.* 2016) and Hasan *et al.* 2016). Research activities in these directions are now underway in our laboratory. We are grateful to the Ministry of Education and BANBEIS for their support for this research.

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Off Farm Commercial Culture of Fish Live Food Tubificid Worms and Their Genetic Characterization

Mahmud Hasan and Mohammad Shamsur Rahman

Location: Department of Fisheries, University of Dhaka
Duration: One year (2015-2016)
Expenditure of the project: Tk. 2700000.00

Introduction

Farming of catfishes requires live food in the nursing of spawn to fry and rearing of fry to fingerling. Sustainable production of catfishes requires reliable supply of good quality fish seed which in turn depends upon the reliable supply of fish live food tubificid worms that is currently not farmed in Bangladesh.

Mollah *et al.* (2012) have found higher production of tubificid worms by using the mixture of 35% MOC, 20% WB, 25% CD and 20% sand. Mariom and Mollah (2012) suggested to use the mixture of 20% MOC, 20% WB, 30% SBM, 20% CD and 10% sand as the best media to culture tubificid worms. Similar findings have also been confirmed by Hossain *et al.* (2012). However, all these above mentioned studies have been undertaken are on station and cattle blood has never been used as wet medium.

Molecular phylogeny of tubificid Oligocheates by using 16S rDNA technique has been developed by Beauchamp *et al.* (2001). Phylogenetic relationships of tubificidae worms have been detected by using mitochondrial 16S rDNA and nuclear 18S rDNA sequence data (Sjolin *et al.* 2005). Viven *et al.* (2015) found COI barcode very effective in identifying aquatic Oligocheates. However, tubificid worms found in Bangladesh have never been identified at species level by using several molecular techniques.

Objectives

The overall objective of this study was to test the possibility of commercial culture of tubificid worms at farmer level. The specific objectives were to:

- Determine the effects of wet mediums in the yield of tubificid worms;
- Detect the effects of media ingredients and wet mediums on proximate and amino acid composition of tubificid worms; and
- Identify the tubificid wroms at species level through molecular techniques.

Methodologies

Wild harvest tubificid worms were bought from the collectors and used as the study animals. Worms were cultured at the newly constructed culture system of Maa Fatema Fish hatchery, Dalmil Jessore in 2016. Molecular identification of worms through COI and 16S rDNA gene was done in the Advance Research Laboratory, Department of Fisheries, University of Dhaka. The sequencing was performed in the First BASE Laboratories SdnBhd, Malaysia.

Newly constructed nine cemented culture raceway were (72 x 80 x 10 cm) used as culture system. Each culture unit was given continuous water flow through 1 inch diameter porous uPVC spray bar.
Mixture of 30% SM, 40% MOC and 30% mud were used as media ingredients in the present study. Locally available SM and MOC were bought from Jessore, Bangladesh. Blood was harvested from the slaughter house and rice gruel was drawn from restaurant at free of cost.

Each culture unit (5760 cm^2) was given 1440 g media (250 mg cm-2) upon wetting in blood, rice gruel and water for 7 days as suggested by Mariom and Mollah (2012) and Hossain et al. (2012). SBM (576 g) and MOC (432 g) were mixed and wetted in three plastic bowls with blood and held for 7 days. Similar quantities of media ingredients were also wetted in six plastic bowls with rice gruel and water. Mud was applied 1 day later of inoculation. Each culture unit was filled with water until spill out.

Batch culture technology was applied. The system was inoculated once and the total worms were harvested after 42 days culture duration. The culture system was inoculated with wild harvest worms at a density of 50 mg cm-2 determined through a series of experiments.

Each culture unit was renewed by 7 day wetted culture media once at 50 mg cm2 for the first 2 weeks and twice for the next 4 weeks. Water flow was stopped before application of the media and resumed 10 minutes later.

Moisture, ash, protein, fat, fiber and available carbohydrate of the media ingredients, wetted media in blood, rice gruel and water, and cultured worms in three wet mediums were measured by following the method described by AOAC (2000).

Amino acid profiles of the media ingredients wetted in water, rice gruel and cattle blood and wild and cultured worms were determined by an automatic Amino Acid Analyzer (HitachiL-8800, Tokyo, Japan). DNA was extracted from mixed worms sample by Maxwell® 16 automated nucleic acid extraction system (Promega, Madison, WI, USA). The concentration and purity of the extracted DNA were measured by NanoDropTM 2000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The extracted DNA was directly amplified by adding GoTaq® Hot Start Colorless Master Mix (Promega, USA) with two set of primers (Folmer *et al.* 1994). The time and temperature profile of the thermocycler (Biometra) was at 95ºC for 60 seconds, 48ºC for 30 seconds and 72ºC for 45 seconds. The amplified products were run on 1 % agarose gel. After sequencing the results were analyzed by using different bioinformatics tools.

Data were analyzed by 1-way anova followed by Tukey's HSD post hoc for multiple comparisons. SPSS version 20.0 was used to analyze the data with the level of significance at $p<0.05$.

Results

Yield of tubificid worms

The highest yield of tubificid worms (683.68 ± 3.86 mg cm-2) was harvested from the treatment where media ingredients were wetted in cattle blood while the lowest yield (584. 38 ± 1.41 mg cm-2) was found in the treatment in which the media ingredients were wetted in subsurface clean water (Figure 1). Rice gruel wetted media resulted in the yield of worms (615.63 ± 3.66 mg cm-2) that was significantly different from other two treatments.

Proximate composition of the worms

Worms raised in the media wetted in blood had nearly 58% protein followed by rice gruel (45%) and water (43%). Similarly highest level of fat (13%) was detected in the worms produced in the media wetted in blood followed by rice gruel (12%) and water (11%).

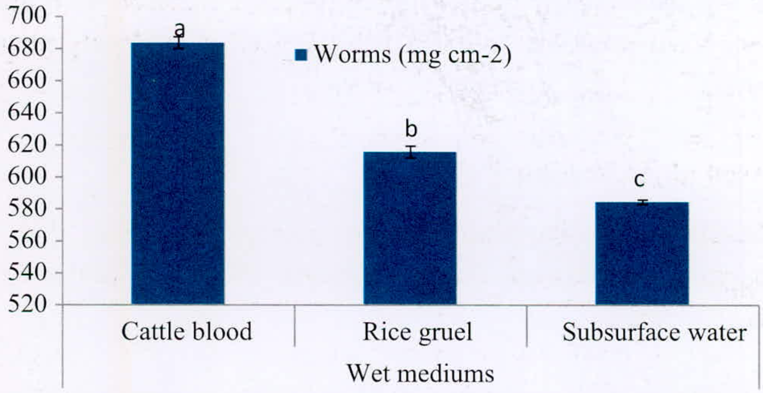

Fig. 1: Yield of tubificid worms (mg cm-2) harvested from the media wetted by cattle blood, rice gruel and subsurface water after 42 day culture duration in cemented culture raceway of 0.567 m^2 at farmer level. Bars (mean ± 1 SEM) with different letters indicate significant differences (ANOVA, HSD; p<0.05).

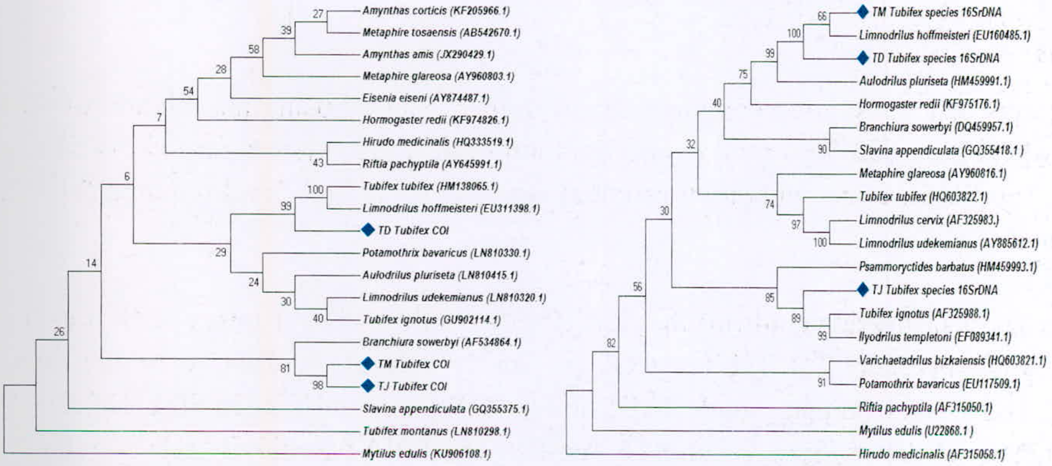

Fig. 2: The neighbor-joining Phylogenetic tree based on partial COI and 16S rDNA gene sequences. The evolutionary distances were compared by Maximum Composite Likelihood method. Number in tree are bootstrap values.

Amino acid profiles of the wild and cultured worms

Some 14 amino acids were detected due to the hydrolysis of the samples in HCl. All 14 amino acids were found at significantly higher level in the worms raised in the media wetted in blood than in the wild, rice gruel and water. Of 8 essential amino acids detected in the worms, lysine, arginine and leucine were found highest level in the worms raised in the media ingredients wetted in blood followed by water and rice gruel.

COI sequence based identification

Phylogenetic analysis based on COI gene sequences of the extracted DNA from 3 sample tubificid worms by using neighbor joining (Figure 2) confirmed the taxonomic position of the worms. Sample TD_Tubifex_COI was 87% similar to *Tubifex tubifex,* TM_Tubifex_COI was 81% similar to *Tubifex tubifex* and TJ_Tubifex_COI (cultured) was 87% similar to *Branchiura sowerbyi.*

Phylogenetic tree (Figure 2) have clearly demonstrated that TM_Tubifex_COI and TJ_Tubifex_COI was closely related with *Branchiura sowerbyi* and TD_Tubifex_COI was closely related with *Tubifex tubifex* and *Limnodrilus hoffmeisteri.*

16S rDNA sequence based identification

Sample TD_Tubifex_16SrDNA was 98% similar to *Limnodrilus hoffmeisteri,* TM_Tubifex_16SrDNA was 99% similar to *Limnodrilus hoffmeisteri* and TJ_Tubifex_16SrDNA (cultured) was 93% similar to *Limnodrilu shoffmeisteri.*

The neighbor-joining phylogenetic tree (Figure 2) confirmed the taxonomic positions of the worms. Phylogenetic tree have clearly demonstrated that TM_Tubifex_16SrDNA and TD_Tubifex_16SrDNA were closely related with *Limnodrilus hoffmeisteri* and TJ_Tubifex_16SrDNA was closely related with *Tubifex ignotus* and *Ilyodrilus templetoni.*

Conclusions

Tubificid worms can be cultured commercially at farmer level by using the mixture of 40% mustard oil cake, 30% wheat bran and 30% mud over a duration of 42 days. System renewal is 50 mg cm-2 media ingredients. Tubificid worms contain all essential amino acids at high level required for fishes need high protein in food.

COI gene based identification confirms that TM_Tubifex_COI and TJ_Tubifex_ COI are closely related to *Branchiura sowerbyi* and TD_Tubifex_COI is closely related to *Tubifex tubifex* and *Limnodrilus hoffmeisteri.* 16SrDNA tells that sample TM_Tubifex_16SrDNA and TD_Tubifex_16SrDNA were closely related with *Limnodrilus hoffmeisteri* and TJ_Tubifex_16SrDNA was closely related with *Tubifex ignotus* **and** *Ilyodrilus templetoni.*

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Improvement of Tide-surge Forecasting Model Including Offshore Islands Along the Coastal Belt of Bangladesh

Muhammad Mizanur Rahman and Mohammad Sayful Islam

Location: Shahjalal University of Science and Technology, Sylhet
Duration: One year (2015-2016)
Expenditure of the project: Tk. 500000.00

Introduction

The coastal belt of Bangladesh is frequently lashed by tropical storms and associated surges which cause a tremendous loss of lives and properties every year. It is found that the major damage is caused by associated surge rather than by a storm itself. A proper warning system can mitigate the sufferings of the people along the coastal belt. At present Bangladesh Meteorological Department (BMD) can forecast an approaching storm as they receive information of the storm from various sources including satellites. But accurate forecast of the associated tidal surges at different locations is utmost important and that can best be done through a numerical model only and various numerical models have already been proved to be useful towards the same. So, to improve the warning system an operational tide and surge interaction model should be developed which can be used in real time prediction of the water levels at different locations along the coastal belt. In our study, we have attempted to develop a tide-surge forecasting model that is capable to incorporate the whole coastal belt and offshore islands of Bangladesh very accurately in the numerical schemes and the target has achieved through nestednumerical schemes. In our model, we have solved the vertically integrated shallow water equations using semi-implicit finite difference technique in Cartesian coordinate system. The effect of various factors that influence water levels, namely tide generating forces, river discharge, offshore islands have tested.

Objectives

To develop a operational forecasting model along the coast of Bangladesh so that the warning system may be improved during a storm period.

Methodologies

In this study, we have developed a surge forecasting model which is capable to incorporate the whole coastal belt and offshore islands of Bangladesh very accurately in the numerical schemes.We have used nestednumerical schemes and have solved the vertically integrated shallow water equations using semi-implicit finite difference technique inCartesian coordinate system. The effect of various factors that influence water levels, namely tide generating forces, river discharge, offshore islands have tested. Foranalysis and verification of our computed results, some locations along the coastal belt of Bangladesh have considered. We have collected the observed data of previous occurred storm from Bangladesh Meteorological Department (BMD) and BIWTA to compare our computed results. The above mentioned methods are well established methods that are used by different researchers in their works (see the References section).

Results

For the purpose of model verification, the model had been applied for some major storms that hit the coast of Bangladesh in the last ten years. Records show that SIDR 2007 and AILA 2009 storms are the most severe cyclonic storms having maximum wind speed 240 km/h and 120 km/h respectively. So we give more stress on the data of SIDR and AILA for computation of results. The tracks (paths) of the chosen storms are shown in Fig. 1.

Figure 2 depicts the computed water levels due to tide-surge interaction associated with SIDR 2007 storm at Hiron Point, Tiger Point, Patharghata, Kuakata and Chittagong. It may be observed that, the maximum water level is increasing with time as the storm approaches towards the coast and finally there is recession. At Hiron point a strong recession is occurred after 15 hrs of 15th November, earlier than in any other location and about 3 hrs before landfall of the storm. The recession takes place due to backwash of water from the shore towards the sea. In fact, Hiron point is situated far left (west) of the storm path and so the direction of the anti-clock wise circulatory wind becomes northerly (i.e. towards the sea) at Hiron Point long before the storm reaches the coast and thus driving the water towards the sea. It may be noticed that the beginning of recession delays as we proceed towards east as is expected. At every location, the peak surge is attaining before the land falls time of the storm. This is expected; as the circulatory wind intensity is highest along the coast when the storm reaches near the coast. The maximum elevation varies between 3.9m (at Hiron point) to 6m (at Chittagong). According to the Wikipedia website, the entire cities of Patuakhali (kuakata), Barguna (Patharghata) and Jhalokati district were hit hard by the storm surge of over 5 meters (16 ft). Thus, the results that come out through our model at these locations compare well with the results stated in Wikipedia website.

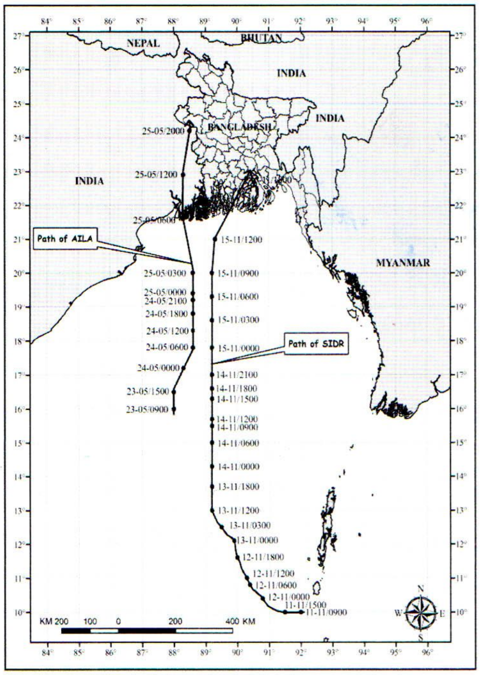


Fig.1: Observed track (path) of cyclonic storms SIDR and AILA.

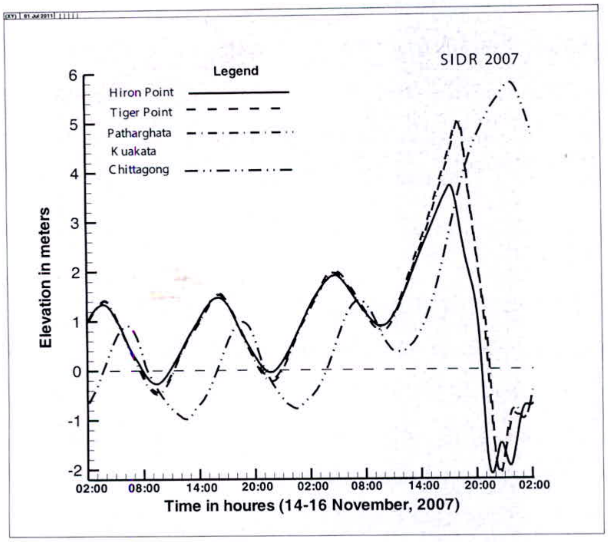


Fig. 2 Computed water levels due to the interaction of tide and surge at different locations associated with storm SIDR.

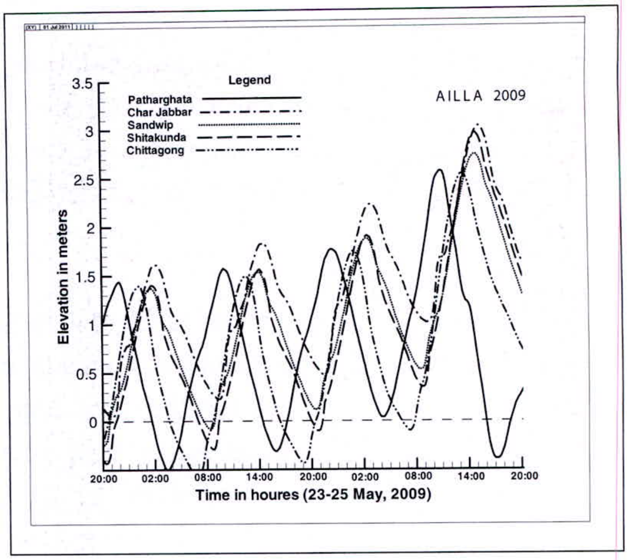


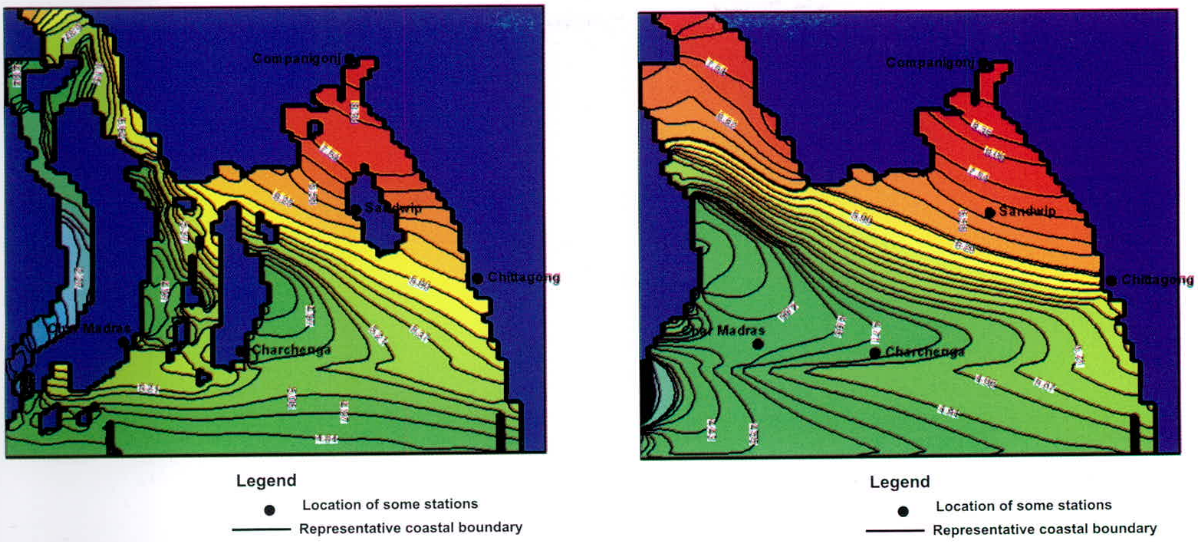
Fig. 3 Computed water levels due to the interaction of tide and surge at different locations associated with storm AILA.

Figure 3 depicts the computed water levels due to tide- surge interaction associated with AILA 2009 storm at Patharghata, Char Jabber, Sandwip, Shitakunda and Chittagong. The maximum overall water level due to the interaction of tide and surge at these locations are between 2.6 m and 3.4 m (Figs. 3). According to the Wikipedia website [http://en.wikipedia.org/wiki/Cyclone_Aila], there was 3 m (10 ft) surge height at the western regions of Bangladesh. According to the report of NASA's Goddard Space Flight, there was storm surge between 10-13 feet (3.048 m- 3.9624 m) high along the western Bangladesh coastline while land fall occurred. Thus the computed water levels for western coastal locations are found to be in good agreement with the observed ones.

Finally, a sensitivity test is done to examine the effect of offshore islands on the surge intensity along the coast. Figures 4 and 5 show our model simulated water levels along the Meghna estuarine region with and without inclusion of offshore islands for the storms SIDR and AILA respectively. It can be shown from these figures that when offshore islands are taken into account, water levels are found to be reduced. Thus it can be concluded here that offshore islands reduce surge intensity.

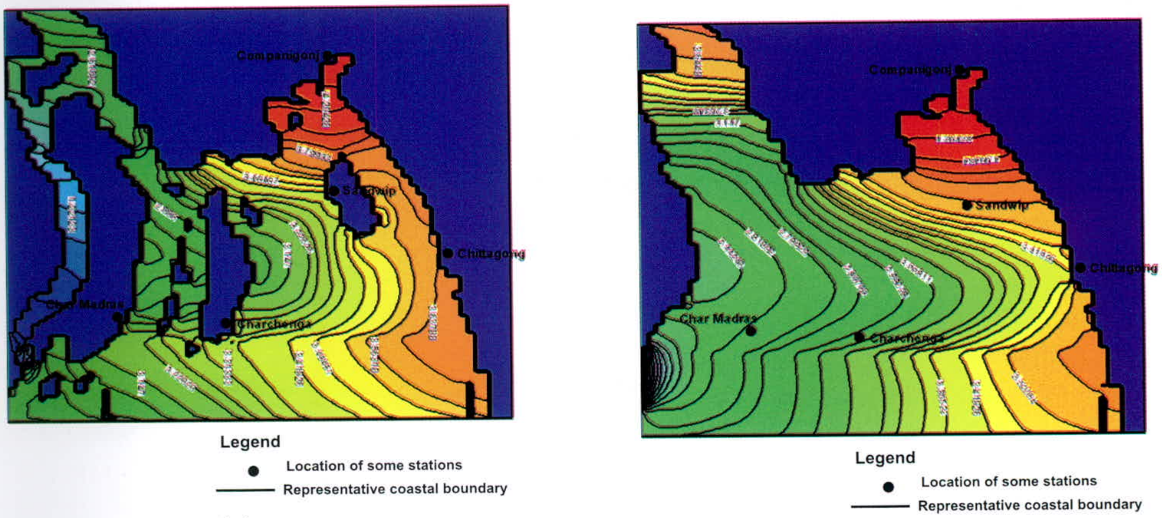
Conclusion

Results of our model shows that offshore islands reduce surge intensity. It is to be noted here that the study was conducted only including two major islands Sandwip and Bhola and the very fine resolution was not taken into account, where our present study is conducted including all the small and big offshore islands accurately in our model.



(a) (b)

Fig.4: Contours of our computed water levels (in the VFMS region of the computed domain) associated storm with SIDR 2007 (a) withislands and (b) without islands



(a) (b)

Fig.5 : Contours of our computed water levels (in the VFMS region of the computed domain) associated storm with AILA 2009 (a) with islands and (b) without islands

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Sustainable Management of Small Indigenous Fish Species (SIS) in the Padma River and Its Implications Policy in Bangladesh

Md Yeamin Hossain and Md Istiaque Hossaina

Location: Department of Fisheries, University of Rajshahi, Rajshahi , Bangladesh
Duration: One year (2015-2016)
Expenditure of the project: Tk. 1200000.00

Introduction

The conservation of aquatic biodiversity has gained great ecological importance over recent years and the climate change unswervingly affects fishery production along many pathways. There are approximately 260 species of freshwater fishes in Bangladesh. Of these, around 140 are classified as small indigenous species (SIS) that constitute a major part of inland capture fisheries. SIS are a food resource crucial in preventing malnutrition, and mineral deficiencies among the rural people (Thilsted *et al.,* 1997). The SIS represents a highly diverse fish assemblage that has been largely overlooked by fisheries scientist. It is, therefore, the proper time to take appropriate measures to conserve these small fish species improving their habitats, and thereby recommending suitable management policies. The River Padma, second longest river of Bangladesh, is believed to be an important spawning and feeding ground for riverine fish species (Hossain *et al.,* 2013). A large number of fishes including some commercially important species are fished by both small and large scale fishers throughout the year. However, the wild stocks have seriously declined in rivers and streams of Bangladesh by a number of natural and man-made factors that need to be considered within an ecosystem context. In this work we have studied some SIS species.

Objectives

The overall objective of this study is to deal with the sustainable management of SIS in the Padma River. But, the specific objectives of this proposed study were to:

- analyze the status (morphometric and conditions) of available SIS in the Padma River;
- estimate size at sexual maturity, spawning season, and fecundity of SIS;
- observe growth parameters, growth performance index and longevity of SIS;
- assess the stocks of SIS (total mortality and exploitation rate); and
- identify the main threats and provide recommendations for the conservation of SIS in the Padma River and other water-bodies in Bangladesh.

Methodologies

Samples were collected from the fisherman catch during July 2015 to June 2016 in Pabna and Rajshahi region. The samples were immediately placed in ice, then fixed in 10% formalin for laboratory work. The fixed specimens were sexed, weighed with 0.01 g accuracy, and measured to the nearest 0.01 cm. The morphometric relationships i.e., LWR were calculated by: $W= a*L^b$. Condition factors were calculated by the equations as i. $K_F =100\times (W/L^3)$ (Fulton, 1904); ii. $K_R = W/(a\times L^b)$ (Le Cren, 1951); iii. $K_A= W/L^b$ (Tesch, 1968). Relative weight (WR) was calculated as $W_R =(W / W_S) \times 100$ (Froese, 2006). The *Lm* was calculated by Binohlan and Froese (2009). Spawning season was estimated using GSI. Fecundity was calculated according to Hossain et al. (2010). The growth parameters were calculated by the equation of von Bertalanffy (1938). The growth performance indices (Ø') was calculated by Pauly and Munro (1984) and the longevity was estimated from the time series of LFDs. Stock of SIS were assessed using several index. Information for threats to SIS were collected through the survey to the fishers, fish farmers, traders, experienced persons, researchers, GO and NGO personnel and available literatures.

Results

A total of 78 species of fish under 9 orders and 25 families were recorded from the Padma River and Cyprinidae was the most dominant group (Figure 1), where 33 species were recorded as threatened. Among them, 11(33.3%) were vulnerable, 15 (45.5%) were endangered and 7 (21.2%) were critically endangered.

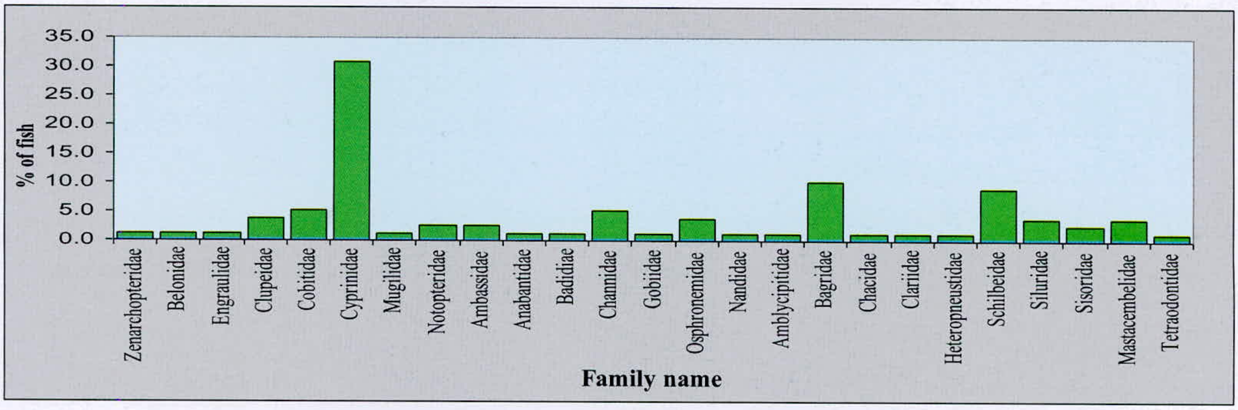


Fig. 1: Amount of fish (%) in different family in the Padma River, northwestern Bangladesh

The growth pattern was negative allometric ($b<3.0$) in *Botia dario, Botia lohachata, Clupisoma garua, Corica soborna, Pethia phutunio, Dermogenys pusilla, Ompok pabda, Mastacembelus armatus, Mystus bleekeri, Mysus tengra, Mystus vittatus, and Pethia ticto;* positive allometric ($b>3.0$) growth was found for *Labeo bata, Chanda nama, Mystus cavasius, Cabdio morar, Nandus nandus, Trichogaster fasciata,* but isometric ($b=3.0$) growth was estimated for *Esomus danricus* and *Trichogaster lalius.* The first sexual maturity was 7.32 cm for *B. dario,* 4.40 cm for *B. lohachata,* 15.18 cm for *C. garua,* 4.86 for *Chanda nama,* 9.40 cm for *D. pusilla,* 11.10 cm for *O. pabda,* 4.1 cm (male) and 4.7 cm for *P. ticto* (Fig 2), 4.0 cm for *E. danricus,* and 14.12 cm for *L. bata in* TL and Fulton's condition factor was best suited.

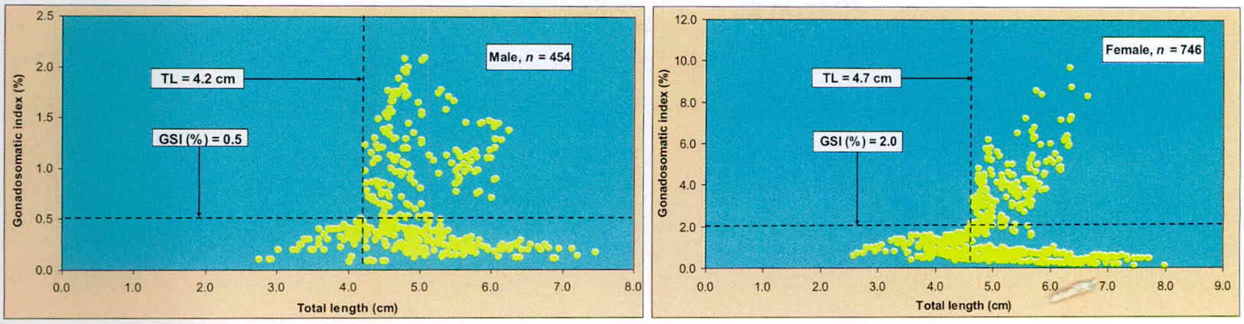


Fig.2: Showing the GSI based first sexual maturity of male and female *Pethia ticto* from the Padma River of Bangladesh of northwestern Bangladesh.

The spawning season was April to August for *Mastasembelus armatus* (Figure 3), May to September for *O. pabda* (Figure 3) and *Clupeisoma garua,* and April to September for *P. ticto* in the Padma River. The fecundity was ranged from 2230-8450 for *P. ticto* and 620-1544 for *D. pusilla.*

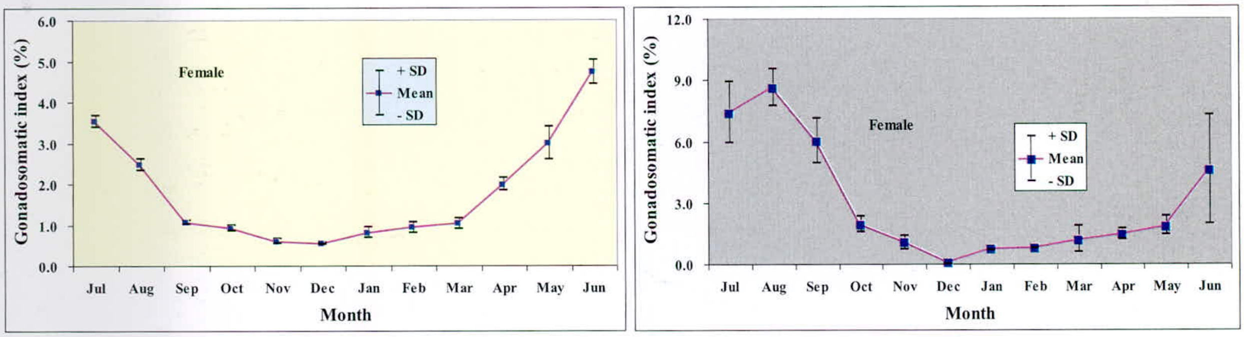
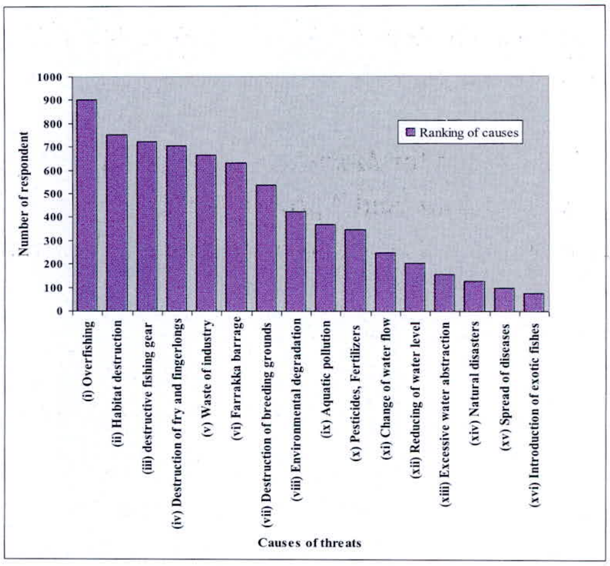


Fig. 3: Showing the spawning season of female *Mastacembelus armatus* (left) and *Ompok pabda* (right) on the basis of gonadosomatic index (%) in the Padma River of northwestern Bangladesh.

The K values were 0.53 year-1 for *O. pabda,* 0.38 year-1 for *M. armatus* and 1.16 year-1 for *P. ticto* in the Padma River. Also, growth performance index was 3.27 and 3.25 for male and female *M. armatus,* 2.35 and 2.39 for male and female of *O. pabda,* and 1.80, 2.16 for male and female *P. ticto* respectively, in the Padma River. The longevity of males and females of *M. armatus* were calculated as 3.0 and 3.33 years, and 5.66, 7.89 years for male and female *O. pabda* and for P. ticto was 2.6 year for male and 2.30 year for female. Total mortality was 2.27 year-1 for males and 2.19 year-1 for females of *M. armatus,* 1.72 year-1 for males and 1.92 year-1 for females of *O. pabda,* and 2.67 year-1 for males and 7.14 year-1 for females of *P. ticto.* Also, the exploitation rate was around 0.30 year-1 (30%) for males and females of *M. armatus,* 0.24 year-1 (24%) for males and 0.45 year-1 (45%) for females of O. pabda, and 0.58 year-1 or 58% for males and 0.62 year-1 or 62 % for females of *P. ticto* in the Padma River.

The results indicated that the fish biodiversity are declining due to a combination of several factors including over-exploitation, environmental degradation, natural disasters, pesticides and aquatic pollution, spread of diseases, uncontrolled introduction of exotic fishes, destruction of breeding grounds, excessive water abstraction, siltation, various ecological changes in its natural habit, and lack of proper management. In addition, the construction of Farrakka barrage by India in the Padma River and subsequent withdrawal of waters from the Padma River are the major causes for destroying its fish biodiversity (Figure 4). However, over exploitation and habitat degradation are the key factors for declining of fishes from the Padma River.



Conclusion

The main purpose of this study was sustainable management of small indigenous species (SIS) in the Padma River and its policy implications. Here, we have studied morphometric relationships, conditions and population biology including size at first sexual maturity, spawning season, and fecundity of some SIS. Also, growth parameters, stock assessment (total mortality, exploitation rate) and causes of declining which can support fishery managers in designing their management plans and managing the fishery. For sustainable management regular monitoring should be continued on first sexual maturity, spawning season, conditions of fish and water-body, growth performance and longevity, total, natural and fishing mortality, also the manmade and natural causes which may decline the fish diversity especially the SIS in the Padma River. The major causes for threats of fishes in the Padma River such as over exploitation and habitat destruction should take into concern.

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Development of Cryopreservation Technology for Freshwater Fish Embryos Using Ultrasound for Conservation

Sk Mustafizur Rahman and Md Nazmul Ahsan

Location: Fisheries and Marine Resource Technology (FMRT) Discipline, Khulna University

Duration: One year (2015-2016)

Expenditure of the project: Tk. 1250000.00

Introduction

Survival after cryopreservation has never been achieved with fish embryos. This failure has been attributed to the insufficient cryoprotectant (CPA) permeation prior to cooling (Rahman et al., 2008 and Cabrita *et al.,* 2006). The large size of fish embryos resulting in a low surface area to volume ratio also reduces the CPA permeation during cryopreservation (Hagedorn *et al.,* 1997). Vitrification, rapid cooling of liquid medium in the absence of ice crystal formation, has been suggested as a tool to overcome this problem. Although tremendous efforts have been made in the last decades, cryopreservation of eggs and embryos has made little progress.

Several innovative techniques (dechorionation, pressure, microinjection and electroporation) have been developed to accelerate the permeation of CPA into fish embryos. Some of these techniques showed promising results but none has led to successful cryopreservation.

Ultrasound is commonly used for drug delivery through mammalian skin, medical practices for diagnostic and therapeutic purposes (Mitragotri *et al.,* 1996). Ultrasound also enhanced the permeation of CPA into the embryos of zebrafish (Wang *et al.,* 2008; Silakes and Bart, 2010) and marine whiting (Rahman *et al.,* 2017). Thus, this study examined the suitability of ultrasound for the cryopreservation of Silver carp *(Hypophthalmichthys molitrix)* embryos.

Objectives

In this study, we have chosen the embryos of Silver carp, *H. molitrix* and Catfish, *Clarias batrachus* due to the fact that these species can spawn in captive condition, produce numerous eggs and finally short embryonic development. Here we present the data of Silver carp only. The broad objectives of this proposal are:

- to examine the toxicity of CPA single and mixture solutions to the fish embryos at different developmental stages.
- to observed the toxicity of ultrasound in the presence or absence of CPA mixture solution.
- to test the effectiveness of ultrasound in CPA mixture solution to prevent ice formation during rapid cooling (attempted vitrification).

Methodologies

Toxicity of single and mixture CPA solutions

The toxicity of six CPAs [dimethylformamide (DFA), propylene glycol (PG), methanol (MeOH), ethylene glycol (EG), glycerol (Gly) and dimethyl sulfoxide (DMSO)] to embryos were examined at three developmental stages (morula, somites and tail elongation). Embryos were exposed to CPAs at concentrations of 10-25% for 20 min. After exposure, embryos were incubated in Petri-dishes for hatching. Four to five CPAs were used to prepare twelve mixture solutions. Toxicity of the CPA mixture solutions were tested during stepwise impregnation (5-steps of 2 min) of somites embryos. The 5-steps were represented increases in concentration of 20%/step.

Effect of ultrasound

Embryos were exposed to ultrasound for 2 and 3 min in the presence/absence of CPA mixture solutions. Next, embryos were exposed to CPA mixture solution for 8 min (4-steps of 2 min) prior to ultrasound in CPA for 2 min.

Cryopreservation trial

Embryos treated with best conditions determined from previous experiments were exposed to liquid nitrogen (LN2). After 1-2 hr of storage, embryos were removed from LN2 and immersed in water bath at 40ºC for 5 sec for thawing. Thawed embryos were treated as described previously for survival and observed the degree of morphological damage after thawing.

Results

Toxicity of single and mixture CPA solutions

Hatching rates of embryos treated with the six CPAs varied markedly with the type and concentration of CPA (Figs. 1-3). Differences between developmental stages were not as marked as those between CPAs, but indicated that embryos at morula stage were slightly less tolerant to CPAs than other stages. Embryos exposed to PG were the least affected followed by those exposed to DMSO, MeOH, EG, DFA and Gly, in this order. Hatching dropped significantly when the embryos were exposed to increasing concentrations of CPAs. The survival rates of somites embryos impregnated with CPA mixture solutions (5-steps of 4 min) were sharply reduced to half for CS3 (48%), CS6 (40%), and CS12 (48%) than control (84%). CPA solutions CS2, CS5, CS8, CS9, CS10 and CS11 gave hatching between 7% and 26% while none of the embryos survived after exposure to CS1, CS4 and CS7 (Table 1).

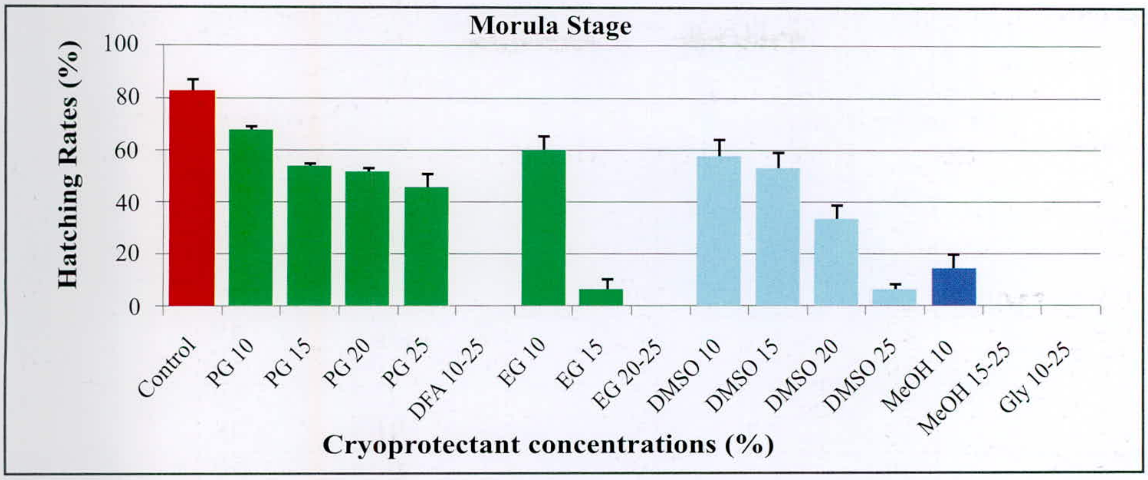

Fig.1: Hatching rates of silver carp embryos at morula stage exposed to different cryoprotectants and cryoprotectant concentrations for 20 min. DFA= dimethylformamide, PG= propylene glycol, MeOH= methanol, EG=ethylene glycol, Gly=glycerol, and DMSO= dimethyl sulfoxide.

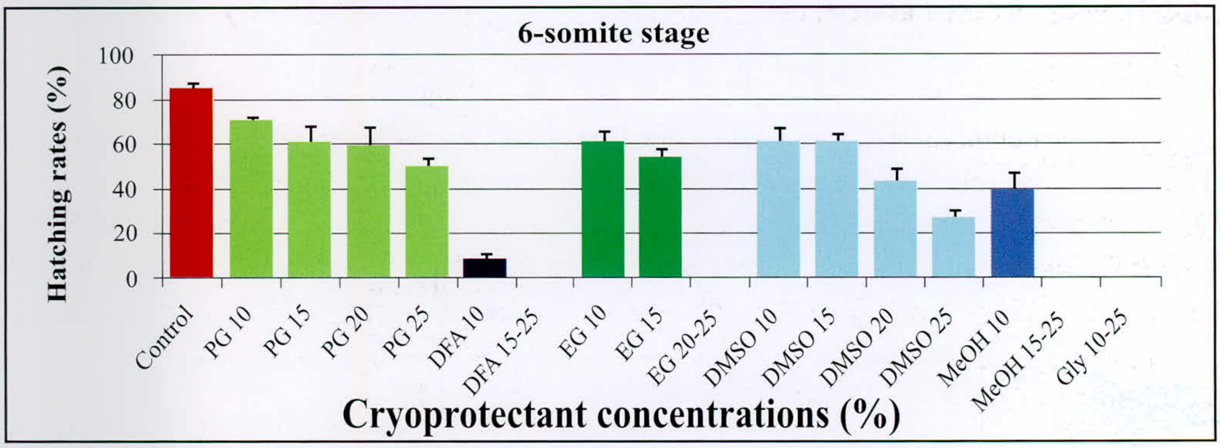

Fig.2: Hatching rates of silver carp embryos at 6-somite stage exposed to different cryoprotectants and cryoprotectant concentrations for 20 min.

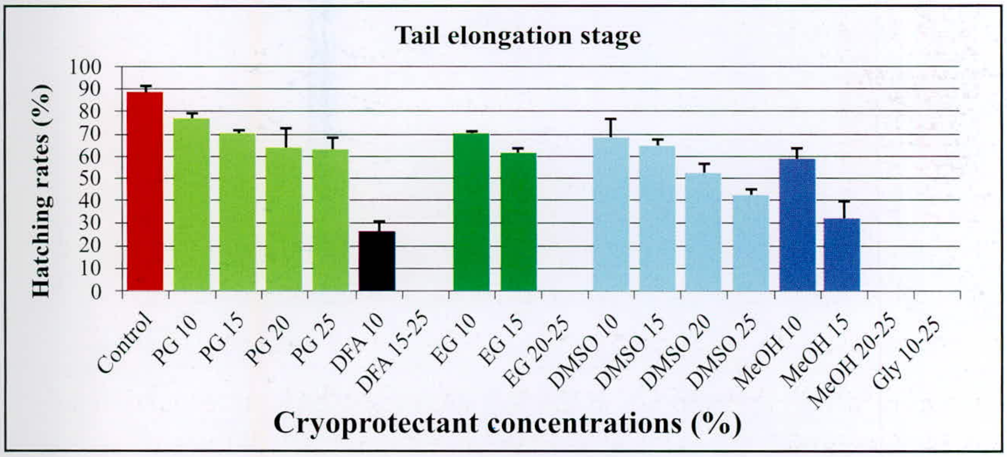

Fig. 3: Hatching rates of silver carp embryos at tail elongation stage exposed to different cryoprotectants and cryoprotectant concentrations for 20 min.

Table 1: Composition of the CPA mixture solutions used in this study and hatching rates embryos exposed to these mixture solutions.

Solutions	Composition (%)					Extender	Hatching rates (%)
	PG	DFA	DMSO	MeOH	EG	Water*	
CS 1	25		20	5	20	30	0
CS 2	25		15	5	15	40	12
CS 3	25		10	5	10	50	48
CS 4	20		20	5	20	35	0
CS 5	20		15	5	15	45	25
CS 6	20		10	5	10	55	40
CS 7	25	5	15	5	15	35	0
CS 8	20	5	15	5	10	45	12
CS 9	20	5	10	5	10	50	12
CS 10	15	5	15	5	15	45	7
CS 11	15	5	15	5	10	50	26
CS 12	15	5	10	5	10	55	48
Control						100	84

*** Hatchery water was used as extender.**

Effect of ultrasound

Hatching of somites embryos exposed to ultrasound (in water) were comparable for 2 min but reduced to 50% than that of controls. On the other hand, hatching rates declined sharply in the presence of CPA mixture. CPA mixture solutions, CS3, CS5, CS6, and CS12 had hatching rates between 5 to 15%. Remaining solutions did not give any viable embryos (Fig. 4) after ultrasound in CPA.

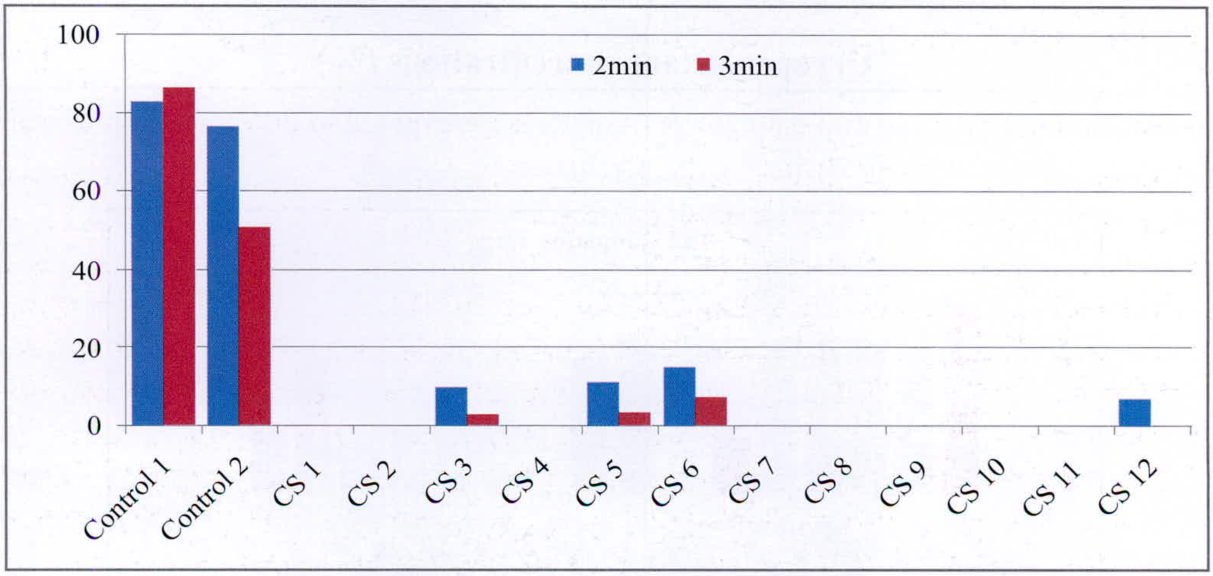

Fig. 4: Hatching rates of silver carp embryos at somites stage exposed to ultrasound in the presence/absence of CPA solutions for 0 (control), 2, and 3 min. Embryos of control 1 did not receive CPA solutions and ultrasound exposure while control 2 received only ultrasound.

Cryopreservation trial

Embryos plunged into LN2 after ultrasound-mediated CPA impregnation showed varied appearance after freeze-thawing ranging from apparently intact morphology to evident damage including breaking down of the chorion and leakage of contents. The majority, however, had clear damages such as mottling, loss or protuberances in the yolk mass, increased perivitelline space, and ruptured chorion (Fig. 5). Tail elongation stage is more tolerant to cooling than somites. However, even embryos with intact morphology did not show any developmental ability during incubation.

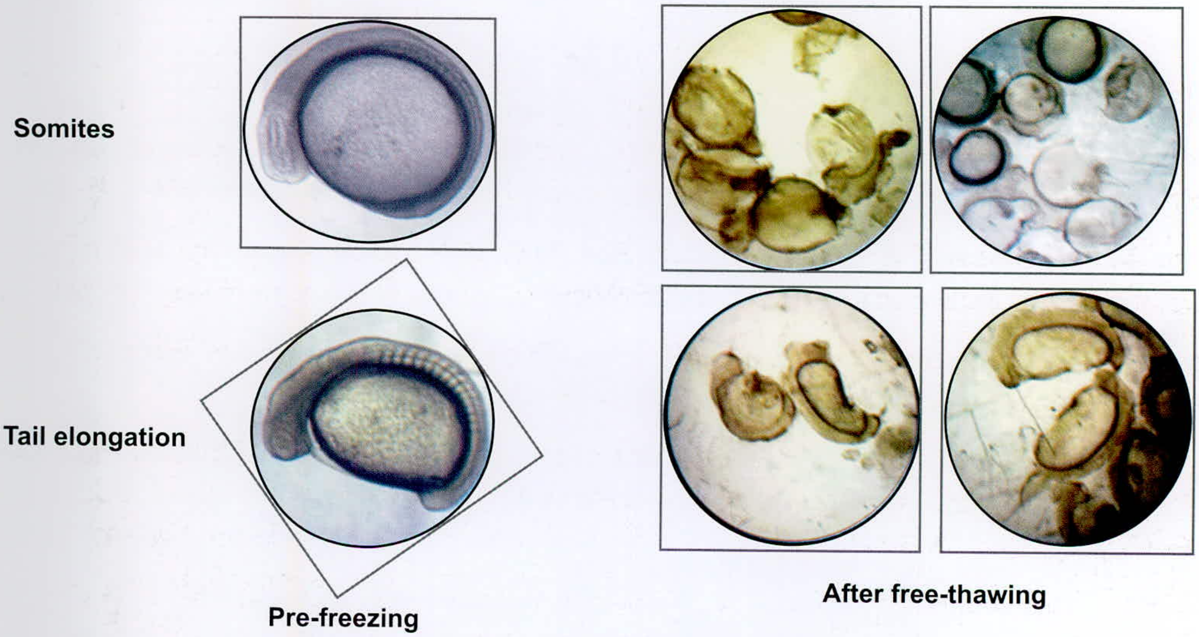

Fig. 5. Embryo morphology after freeze-thawing.

Conclusion

Cryopreservation of fish embryos requires the swift uptake of considerable amounts of cryoprotectant but this process is hampered by the low permeability of the egg chorion. Therefore, ultrasound is used to improve the permeability into the developed embryos of Silver carp *(Hypophthalmichthys molitrix)*. Embryos at three developmental stages (Morula, 6-somite and tail elongation) were immersed in six single cryoprotectants, DMSO, Gly, MeOH, PG, DFA and EG, in concentrations of 10, 15, 20 and 25% for 20 min. Hatching rates decreased with increasing cryoprotectant concentrations and toxicity to silver carp embryos varied in the order of PG < DMSO < EG < MeOH < DFA < Gly. Embryos at tail elongation stage were more tolerable to CPA solutions than morula and somites stages. Embryos tolerated well the mixture solutions of CS3, CS6 and CS12 (about 50%) than those of CS2, CS5, CS8, CS9, CS10, and CS11 (7%-26%). None of the embryos survived after exposure to CS1, CS4 and CS7. On the other hand, hatching rates of somites embryos sharply declined after exposure to ultrasound in the presence of CPA mixture solutions. Only CS3, CS5, CS6 and CS12 gave hatching rates between 5 to 15% while remaining solutions did not give any sign of survival embryo of silver carp.

A preliminary cryopreservation trial after ultrasound-mediated impregnation of somites and tail elongation embryos with CPA mixture solutions did not yield live embryos after freeze-thawing but show considerable proportion of morphologically intact embryos after freeze-thawing. Embryos at tail elongation stage were more tolerant to cooling temperature than somites stage. Embryos treated with CPA solution under the best conditions determined in this study did not resume development after attempted vitrification. These results suggest that sonication might be useful for fish embryo cryopreservation, if not alone, perhaps in combination with other techniques to enhance CPA permeation.

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Genotyping of Human Papiloma Viruses (HPV): Prevalence, Burden and Vaccine Approaches in Bangladesh

Mahmuda Yasmin and Ashrafun Nessa

Location: Department of Microbiology, University of Dhaka
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 3000000.00

Introduction

Globally, cervical cancer is the second most common cancer in women and the third most frequent cause of cancer death (Ferlay *et. al.,* 2002). It is now estimated that more than 80% cancers in cervix are associated with HPV. Over 100 HPV genotypes have been identified, a subset is associated with malignancy. HPV16 and 18 are the most prevalent oncogenic types (Smith *et al,* 2007). These associations indicate the importance of detecting, distinguishing, and quantitating both low risk oncogenic HPV infections for monitoring and treating disease development and progression. As a preventive measure, several candidate vaccine against HPV have been developed to prevent infections by HPV types 16 and 18, the most common oncogenic types, as well as against the common types HPV 6 and HPV 11 that cause genital warts (WHO 2010). In Bangladesh, there are some initiatives already been taken to aware people to take vaccine which would reduce the burden of cervical cancer and anogenital warts. But, to date not much study has been done to investigate the circulating genotype in this population. The proposed study therefore designed to take an initiative to find out the common genotypes in both rural and urban population in Bangladesh.

Objectives

Main objectives of this study are:

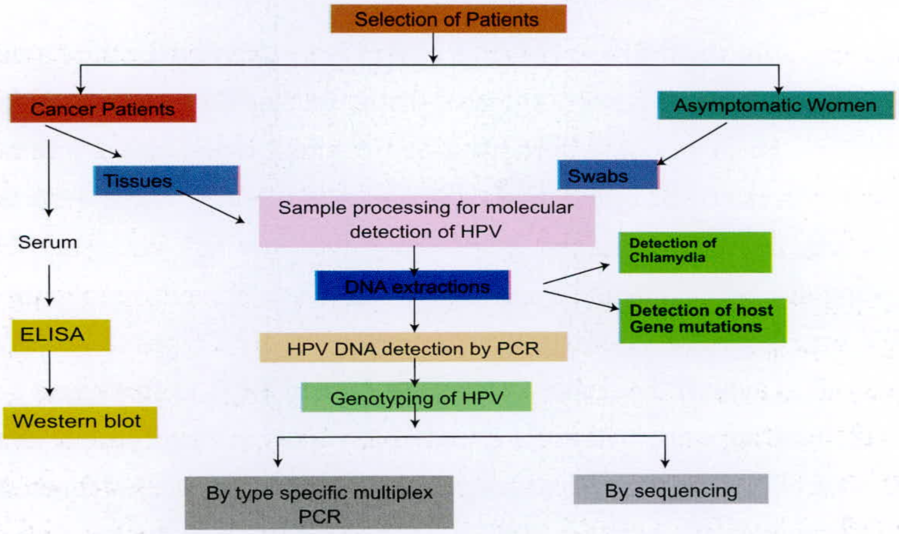
- To determine the prevalence of risk of cervical cancer in general female population of age group 25-50 yrs.
- To determine the Circulating genotype in the risk group (In both High risk and low risk groups).
- To determine the association of *Chlamydia* with HPV.
- To find out protein marker from serum of cervical cancer patient by western blot.
- To detect mutation in the genes with oncogenic potentials in cancer patients.

Methodologies

The study was conducted jointly at the department of gynae and obstetrics in Bangabandhu Sheikh Mujib Medical University (BSMMU) and the department of Microbiology. The cervical swab was collected by a trained nurse from 11 stations of BSMMU, namely tejgaon, uttarkhan and mugda of dhaka, kurigram, brahmanbaria, chadpur, khulna, lalmonirhat, nachol, and noakhali.

Please see below the work plan we have used. Samples were collected using a cytobrush and were kept in PreservCyt® solution (a commercially available methanol based preservative). The collected samples were kept in -20°C for further studies.

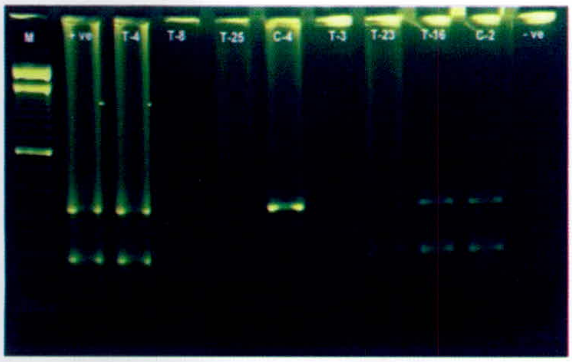
Work plan



Tissue samples from cervical cancer patient were collected from National Institute of Cancer Research & Hospital (NICRH), Mohakhali, Dhaka. All specimens were taken by experienced personnel, either by a trained nurse or physician and was kept in PBS solution. The collected samples were kept in -20oC for further studies.

Results

A total of 410 cervical swab specimens were collected between November, 2013 to August, 2016 from apparently healthy women. The mean age of the participants of this study was 30 years. By PCR amplification, 121 (29.5%) of them were found to be positive for HPV. There was also 115 cancer tissue samples collected from 115 cancer cases, 113 of them were HPV positive (98.26%). When genotyping was performed, 31 of 121 HPV-positive healthy women had HPV-16, and equal number of type 18 was also detected. On the other hand, 20 of them had both type 16 and 18 . When we analysed the genotypes of tissue samples collected from cancer cases; 44 of 113 had HPV-type 16 and 10 had type 18. This indicates that type-18 which is considered as more aggressive all HPV-types are not predominant in this country, although 43 of 113 cases had both type 16 and 18.



HPV types	Samples taken from asymptomatic individuals	Samples taken from cancer tissue
HPV-16	31	44
HPV-18	31	10
HPV-16 and 18 both	20	43
Other types	39	16
Total	121	113

The fig. shows the agarose gel picture of HPV genotyping and the Tables shows the overall

Analysis of demographic data: All the apparently healthy women were provided with a questionnaire evaluate their socioeconomic status, sexual behavior, age, husband's occupation etc and then correlated with their virologic status. From the analysed data, it has been found that most of the HPV positive cases belonged to the age group 41-50. Most of the women had experienced their first intercourse at the age of mid 20'. Again, husbands' occupation was found to have no impact on HPV stutus.

Polymorphism in marker genes: Among the high number of genes involved in different signal transduction and cell growth regulation pathways, five different genomic regions within the top three most frequently mutated genes in COSMIC database with a key role in the development of cervical cancers were selected to study mutation frequency in our patients. These genes are *EGFR* (Epidermal Growth Factor Receptor), *KRAS* (Kirsten rat sarcoma), and *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha). DNA from 46 cervical tissue samples were extracted and amplified by PCR, and sequenced through Sanger cycle sequencing method. *In silico* analysis shows that, in a total, 39 mutations were found in 28 patienst samples; 11 (23.91%) were found in *EGFR* gene fragments. On the other hand, 24 (52.17%) mutation were found in *PIK3CA* gene fragment, among which 2 were found in more than 1 patient. Four mutations (8.7%) were found in *KRAS* gene. Our study shows that except for *KRAS,* the frequency of observed mutations in our patients is higher than those reported earlier in other parts of the world.

***KRAS* Gene**	***EGFR* Gene**	***PIK3CA* Gene**
4 in 46 samples **8.7%**	11 in 46 samples **23.91%**	24 in 46 samples **52.17%**

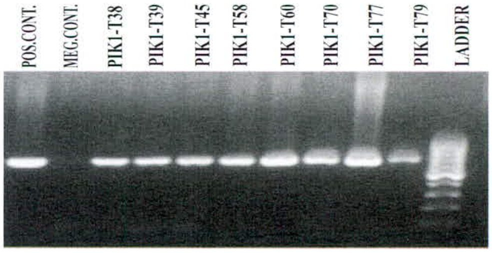


Table shows no. and percentage of patients having mutations in their genes. The fig. shows the agarose gel electrophoresis of PCR specific amplicon (~700bp) using PIK3CA gene.

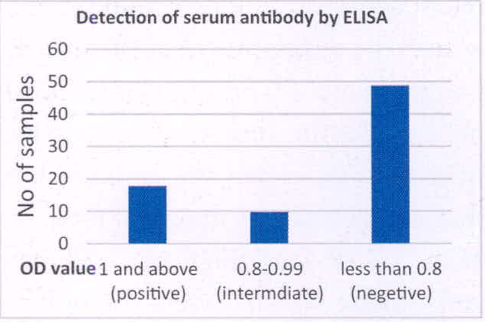
Individual gene mutation result

Association of Chlamydia with HPV: *Chlamydia trachomatis,* an obligate intracellular sexually transmitted pathogen, is suggested to be a cofactor of cervical cancer.

All 115 cancerous tissues and 170 cervical swab samples were tested for the presence of *C. trachomatis*. Among them, 48 of 115 tissue samples showed the evidence of co-infection with *Chalmydia*. On the other hand, 7 of 170 swab samples gave positive results, 4 of which from 125 HPV negative samples. This indicates that chlamydia co-infection prevail in cancer cases more prominently than that of asymptomatic individuals. And also it has an impact on cancer development.

Parameters	HPV (+ve) samples from tissue	HPV negative	Total number of tissue samples	HPV (+ve) samples from swab	HPV (-ve) samples from swab	Total number of samples
No of samples	**113**	**2**	**115**	**45**	**125**	**170**
Samples tested positive for *C. trachomatis*	**48**	**0**	**48**	**3**	**4**	**7**

Detection of serum antibody from cervical cancer patient by ELISA: Serum samples from 77 cervical cancer cases were obtained and tested for the presence of antibody to L1 antigen ((Human Anti-HPVL1 IgG) by ELISA. Only 18 of them were found to be positive and others are negative or their antibody level lies in between positive and negative.



OD value	No of samples
1 and above (positive)	18
0.8-0.99 (intermediate)	10
less than 0.8 (negative)	49

Detection of gene expression of cancerous patients was done by detection of some selected proteins using antibody to Serpin B3/SCCA, CEA, HMGB, Cytokeratin 19 fragment, which could be used as a biomarker for the HPV mediated cervical cancer. We have found most of the patients are positive for CEA and Cytokeratin 19 protein.

Conclusion

Human papillomaviruses (HPV) have emerged over the past 15 years as the most significant etiological factor for cervical cancer (Zur Hausen, 1994). HPV DNA can be detected in nearly all cervical carcinomas worldwide (Bosch *et al.,* 2002 ; Walboomers *et al.,* 1999), and increasing HPV prevalence rates are found with increasing severity of cervical dysplasia. Due to this strong association, it has been suggested that high-risk HPV detection might be used as a tool to identify women at risk for the development of cervical cancer (de Roda Husman *et al.,* 1994).

Molecular detection of HPV DNA in cervical samples was done by Polymerase Chain Reaction (PCR). A total of 410 cervical smear samples 115 cancerous tissue samples were analyzed for the detection of HPV. Among them, 29% of healthy asymptomatic cases and more than 98% of the cancer cases were found to be positive for HPV. This indicates that significant percentage of women in sexually active age harbor the virus and nearly all cancer cases are associated with the virus. This is the first detailed study of HPV-cervical cancer association.

Genotyping was also done using multiplex nested PCR amplification as well as by sequencing. In this study it has been established high-risk genotypes prevail in the cancerous tissues with significant higher rate than in the asymptomatic cases. In this study, we have also analyzed the other socioeconomic factors associated with HPV positive cases, detected the association of Chalamydia with HPV, detected antibody to HPV-L1 antigen, expression of host genes (SCCA, CYFRA21-1, CEA, and HMGB) by Western Blot, which could be used as cancer biomarker.

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Salmonellosis Control Program in Bangladesh: Development of Cost-Effective Pathogen Detection Method and Vaccine Production

Md Anwar Hossain

Location: Department of Microbiology, University of Dhaka, Dhaka
Duration: Three Years (2013-2016)
Expenditure of the project: Tk. 2800000.00

Introduction

Salmonella infection is one of the major constraints of livestock, specially in poultry farming that hindered its development in Bangladesh (Das *et.al.,* 2005; Kamaruddin *et.al.,* 2003). In recent days, the prevalence of salmonellosis in both breeder flock, commercial broiler and layer flocks is increasing day by day. Research works carried out in Bangladesh concerning salmonellosis in poultry had been limited so far to investigating the seroprevalence and antibiotic resistance of *Salmonella* and mortality and histopathology of poultry flocks (Islam *et. al.,* 2006, Ahmed *et.al.,* 2008, Hossain *et. al.,* 2010). Added to the literature includes geographical variation (Hossain *et.al.,* 2004) and poultry age variation (Sikder *et.al.,* 2005) of seroprevalence of *Salmonella*.

Although reports are available on the prevalence of salmonellosis in livestock and poultry, information regarding involvement of the non-host adapted serotypes in humans in Bangladesh is meagre and no effective local vaccine strains are reported. Our previous endeavour was to isolate multi-drug resistant *Salmonella* from poultry in Bangladesh along with detection of seroprevalence (Mahmud *et. al.,* 2011). Several zoonotic strains of *Salmonella enterica* was detected by our research team from five large poultry farms in Bangladesh. The present report will address a survey of zoonotic *Salmonella* types in Bangladesh covering the major districts including Naraangong, Dhaka and Gajipur. Furthermore, Silkworm larvae based method for characterization of virulent strain(s) and SYBR Real-time base Salmonella quantification method is being reported. The ultimate aim is mitigation of Salmonellosis from poultry to save the large economy of Bangladesh.

Objectives

- Countrywide survey on salmonellosis and *Salmonella* burden in poultry/livestock and assessment of economic loss
- Development of insect larvae based bioassay method for *Salmonella* virulent strain(s) characterizations and tracing the source of Salmonella transmission
- Development of mitigation method
- Development of skilled man power

Methodologies

Poultry samples was placed into each of two culture media: Tetrathionate broth (Tet) and GN Hajna broth (GN) and incubated overnight at 37°C for 24 h. Then approximately 100 µl from the GN culture was transferred into Rappaport R-IO medium (R-IO) (GN-R). At 48 h, 100 µl was transferred from the Tet culture into R-lO (T48-R). All GN-R and T48-R media were incubated overnight at 37°C, and then streaked onto brilliant green agar with sulfadiazine (BGS) plates. Additionally, the 48-h Tet culture (T48) was also streaked onto BGS for overnight at 37°C. Colonies having the typical appearance of *Salmonella* were to triple sugar iron and lysine iron agar slants and was incubated overnight at 37°C. Presumptive positive isolates were serogrouped using serogroup specific typing sera. DNA sequencing and microarray analysis were performed on seven gene fragments using the amplification and sequencing primers that are described on the MLST website. (Achtman *et al.,* 2012). Silk worm larvae based bioassay was done following the model previously established in Professor Sekimizu's laboratory, Japan (kato *et al.,* 2002) will be considered for Salmonella pathogenicity detection. Immunostaining was carried out and visualization of the Salmonella was done under fluorescence microscope.

Results

In 1st year investigations, we have report in details the establishment of methodologies – i. isolation and characterization of Salmonella spp. from field samples; ii. development of insect larvae based method for characterization of pathogenic *Salmonella* spp. and iii. molecular characterization of *Salmonella* spp..

In current 2nd year report, we have completed the development of Real-Time SYBR Green based PCR method for quantification of load in poultry samples and fomites; development of methods for molecular identification and route of transmission (ARDA, MLST and rDNA sequencing); and established a large pool of isolated and fully characterized Salmonella spp. isolates for further studies.

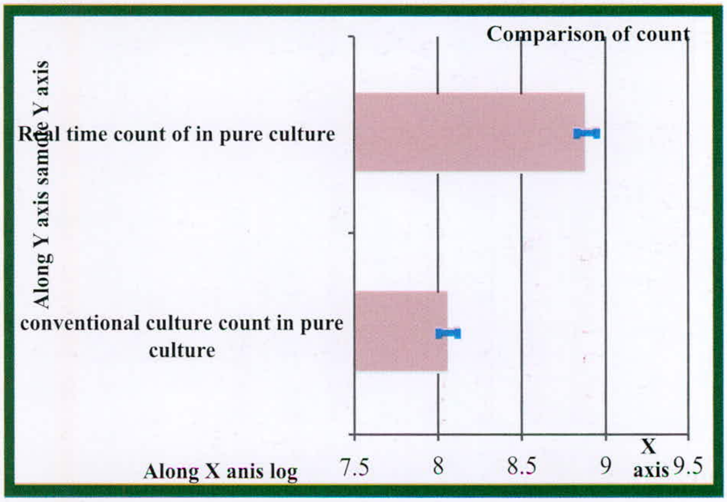

Fig. 1: Bar diagram representation of comparison between counts obtained from conventional culture and Real time PCR method from pure culture is shown. Count is converted to log scale and then the bar diagram is prepared by Microsoft office excel, 2007 (Count mean ± standard deviation).

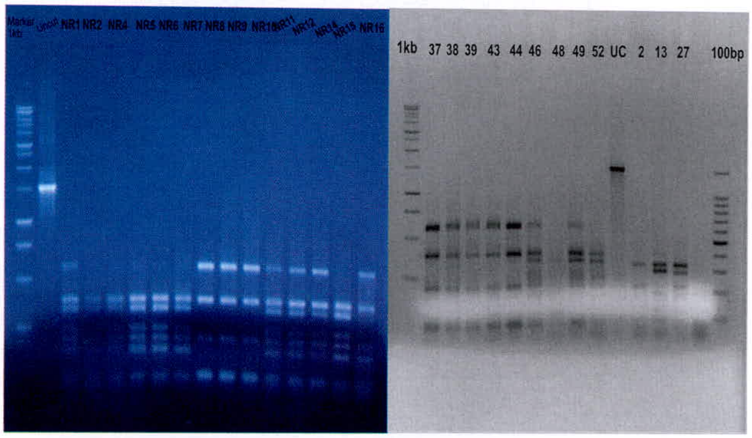

Fig.2: ARDRA profile (Alu I) of the isolated *Salmonella* spp.

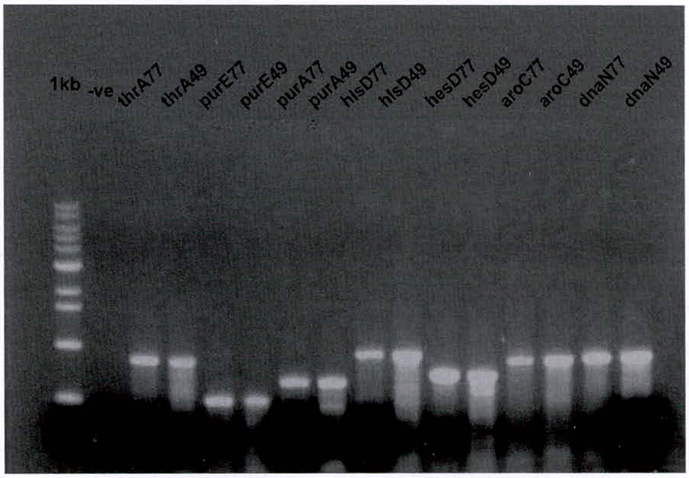

Fig. 3: Gel Electrophoresis of seven housekeeping genes of *Salmonella* spp for MLST analysis

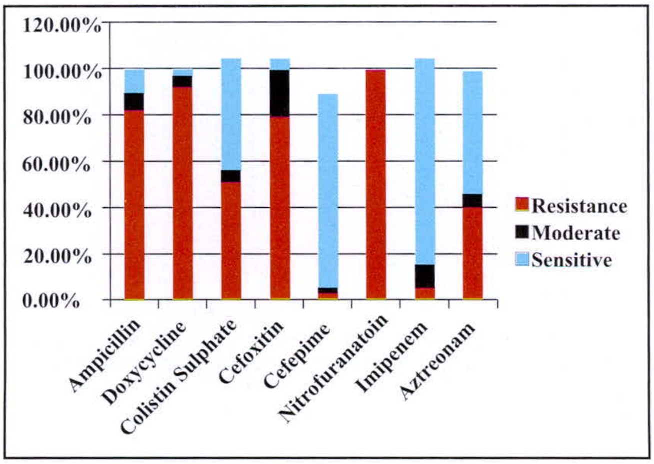

Fig. 4: Diagrammatic presentation of antibiotic resistance of *Salmonella* isolates

Isolation and Molecular Characterization of *Salmonella spp.*

Total 43 samples were collected from three different farms located at Rupganj, Narayanganj and Pandhoa, Jahangirnagar, Savar on 10th October 2015 and 1st March 2016 respectively. The samples were collected directly into Buffered Peptone Water and transported to the laboratory maintaining proper temperature. The samples were processed within 3-4 hours after collection. The sample types include cloacal swab, droppings, egg swab, handler's swab, feed and water. To develop a pool of *Salmonella* spp., we have isolated *Salmonella* spp. using culture techniques and the isolates were thoroughly characterized by biochemical and molecular techniques. Finally we isolated more than thousands of *Salmonella* spp., classified them according to their seroprevalence, host biasness and understanding of route of transmissions.

Conclusion

Salmonella spp. are zoonotic pathogens and now-a-days become alarming for public health due to its multidrug resistant properties. In present investigation, we are developing an effective surveillance methodology for its quantifications and route of transmissions. The methodology will be very effective for Salmonellosis containment and development of effective vaccine for poultry health.

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Population Genetic Structure and Demographic History of *Tenualosa ilisha* in Bangladesh and Indo-West Pacific: Implications for Conservation and Breeding

Kazi Ahsan Habib and Md Ekramul Hoque

Location: Dept. of Fisheries Biology and Genetics, Sher-e-Bangla Agricultural Universit, Dhaka
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 2500000.00

Introduction

The national fish of Bangladesh, Hilsa Shad, *Tenualosa ilisha* (Clupeidae, Clupeiformes) is an anadromous species distributed from Kuwait, Iran and Iraq coastal waters in the Persian Gulf to the west coast of India in the Arabian Sea and the Bay of Bengal (BoB). Hilsa is the largest and single most valuable fishery in Bangladesh and this fish is also commercially very important in India, Myanmar, Pakistan and Kuwait. In Bangladesh, Hilsa contributes 50-60% of the global hilsa catch and 12-13% of the national fish production (BOBLME, 2008). Different factors such as hydrological changes of spawning ground as well as overfishing, and habitat degradation are causing adverse effect on Hilsa production. Information on genetic variability and population structure of fish is very important for conservation, fishery management and artificial propagation. Though few researches on the population genetic structure of *T. ilisha* have been conducted on morphometric, allozyme and RFLP analyses, the results were contradictory (Mazumder and Alam, 2009). In the present study, an effective marker, mtDNA control region (d-loop) sequence has been used for assessing genetic diversity, and population structure of Hilsa. The analyses will also provide insights into historical demography and evolutionary process of the species that might have influenced by paleoceanographic condition of the Bay of Bengal.

Objectives

- To estimate genetic diversity in the species *T. ilisha*
- To assess the genetic structure of Hilsa among different populations throughout the distribution range in Indo-West Pacific region
- To draw phylogeographyof Hilsa Shad populations
- To illustrate demographic and evolutionary history of Hilsa

Methodologies

A total of 157 *Hilsa shad* individuals were collected from six locations (population) from its distribution range viz. Bangladesh (Chandpur-CP and Cox's Bazar-XP), West Bengal of India (IP), Myanmar (MP) Pakistan (PP) and Kuwait (KP). The individuals of all populations were collected when fish returned to their natal rivers for breeding except the population XP collected from off shore.

Genomic DNA was extracted from the fin or muscle tissue of the 95% alcohol fixed specimens using commercial DNA extraction kit. The first hypervariable portion of the mtDNA control region was amplified from the genomic DNA through the polymerase chain reaction (PCR) using the primer set (TilsDL-F and TilsDL-R) and temperature profile used by Habib *et al.* (2014). PCR products were examined by 1% agarose-gel electrophoresis with a standard size marker and purified by a PCR purification kit. The purified DNA was sequenced using Automated DNA sequencer 377 or 3100 (Applied Biosystems, USA).

The sequence data were edited and aligned with ClustalW (Thompson *et al.* 1994). Molecular diversity indices such as haplotype diversity (h), nucleotide diversity (π), average number of nucleotide differences (k), number of haplotypes (Nh), polymorphic sites (S), transitions (ti) and transversions (tv) for each population using the program ARLEQUIN (version 3.5, Schneider et al. 2000). Pairwise population genetic structure (i.e. fixation index, F_{ST}) and population panmixia (i.e. exact test) among populations; and hierarchical analysis of molecular variance (AMOVA) were also assessed using ARLEQUIN. The program MEGA 3.1 (Kumar *et al.* 2004) was used for reconstructing phylogenetic relationship (i.e. evolutionar relationship) among haplotypes of the d-loop sequences by neighbor-joining (NJ) method (Saitou and Ne 1987). Historical demography of the *T. ilisha* was examined by Tajima's *D* statistics, Fu's F_S test fo selective neutrality; and mismatch distribution (MMD) analyses implemented in ARLEQIN.

Results

Intra-specific genetic diversity

A total of 695 nucleotide long D-loop region were obtained from 157 individuals of six populations MP, XP, CP, IP, PP and KP after removing the ambiguous sequences near the primer ends. The sequences comprised 508 polymorphic sites. These polymorphic sites defined 107 haplotypes, of which 100 haplotypes were singletons, six haplotypes were shared between two populations and only a single haplotype was shared among three populations. No haplotype was found common for all of the six populations. Most of the nucleotide substitutions were transitional and 408indels were detected in the sequences of D-loop region(Table 1). Except KP (Kuwait), the nucleotide diversities (π) were very low for all of the five populations (MP, XP, CP, IP and PP) which ranged from 0.06 – 0.15 nucleotide differences per site while the haplotype diversities (h) were very high, 0.96–0.99 (Table 1).

Table 1. Genetic diversities of the D-loop region of six populations of *T. ilisha*

Populations (ID)	Natal River & Sea Area	N_i	Sb (ti+tv)	N_{id}	N_h	*h*	π	S
Myanmar (MP)	Irrawady&BoB	13	78 (74+4)	173	12	0.99	0.15	249
Cox's Bazar (XP)	BoB	35	56 (50+6)	322	29	0.99	0.11	361
Chandpur (CP)	Meghna&BoB	35	53 (46+7)	250	29	0.98	0.08	287
India (IP)	Hugli &BoB	35	34 (34+0)	249	19	0.96	0.09	276
Pakistan (PP)	Indus &BoB	14	50 (40+10)	39	12	0.98	0.06	86
Kuwait (KP)	Shatt al-Arab& Persian Gulf	25	15 (9+6)	115	14	0.84	0.62	130
Pooled		157	108 (91+17)	408	107	0.99	0.11	508

* Number of individuals (N_i), number of substitution (Sb), Transition (ti), transversion (tv), number of indels (N_{id}), number of haplotype (N_h), haplotype diversity (*h*), nucleotide diversity (π), number of polymorphic site (S).

Population genetic structure

Pairwise comparisons of F_{ST}values indicatesignificant ($p < 0.05$) genetic population structure between each of the population pair except populations of Bangladesh (XP, CP) vs. Myanmar (MP), and Pakistan (PP) vs. Myanmar (MP). Non-significant exact P values showed that XP, CP and MP are panmictic(random mating) populations (Table: 2).

Table 2. Pairwise F_{ST} (below diagonal) and significance of Exact *P* values (above diagonal) among populations of *T.ilisha* for mtDNA control region

Population	XP	CP	MP	IP	PP	KP
XP (Cox's Bazar, BD)	-	0.219	0.225	0.00	0.01	0.00
CP (Chandpur, BD)	0.002	-	0.173	0.00	0.01	0.00
MP (Myanmar)	0.005	0.006	-	0.00	0.01	0.00
IP (India)	**0.026	**0.026	*0.028	-	0.00	0.00
PP (Pakistan)	**0.016	*0.019	0.017	**0.032	-	0.00
KP (Kuwait)	**0.083	**0.086	**0.091	**0.994	**0.095	-

For F_{ST}: *$p < 0.05$ **$p < 0.01$

The genetic structure of the *T. ilisha* in the north-west Pacific was further investigated with hierarchical AMOVA that took into account geographical distribution (different natal river and adjacent seaenvironment). The best subdivision that would limit the intrapopulation variation and maximize the interpopulation differentiation of the samples was found to accord with the natal rivers and connected sea habitat. Significant grouping (i.e. significant Φ_{CT} values) was obtained when the populations were divided into four groups i.e. Myanmar-Bangladesh (Irawady and Meghna river), India (Huli river) of the Bay of Bengal, Pakistan (Indus river and Arabian sea) and Kuwait (Persian Gulf) (Table: 3).

Table 3. Hierarchical AMOVAs

No. of groups	Grouping pattern	Φ statistics(Φ_{CT})	*P*
02	Bay of Bengal(XP+CP+MP+IP) / Arabian Sea-Persian Gulf (PP+KP)	0.026	0.07
03	Bangladesh +Myanmar(XP+CP+MP) / India (IP) / Pakistan +Kuwait (PP+KP)	0.02	0.18
04	River Meghna and Off Cox's Bazar of Bangladesh + River Irrwaddy of Myanmar(XP+CP+MP) / River Hugli of India(IP) /River Indus of Pakistan (PP) / Persian Gulf- off Kuwait (KP)	0.047	0.05

Phylogeography and evolutionary history

In the phylogenetic tree, three distinct lineages were identified among the haplotypes supported by high (> 80) bootstrap values (Figure 1): two Bay of Bengal clades (Clade-1 and Clade-2) and an Arabian Sea-Persian Gulf Clade (Clade-3). In the Bay of Bengal, Clade-1 (termed as Hugli and Meghna river lineage) comprised of all the haplotypes of India and Bangladesh, six haplotypes of Myanmar and a single haplotype of Pakistan. Since some haplotypes of Myanmar are present in the Bangladesh-India lineage, geneflow (migration) occurs from Bangladesh and India (Hugli and Meghna estuary) to Myanmar (Irrawadi estuary). Similarly a little or almost no geneflow also occurs from Pakistan (Indus river estuary) to Hugli and Meghna estuary since a single haplotype of Pakistan is present in Bangladesh-India lineage. On the other hand, Clade-2 comprised only of six haplotypes of Myanmar (Irrawaddy lineage). Clade-3 harboured only the haplotypes of Pakistan and Kuwait (termed as Indus river-Pursian Gulf lineage). From the phylogenetic tree, it is clearly revealed that the neighboring Indus river-Persian Gulf lineage of *T. ilisha* was separated from the Bay of Bengal lineages.

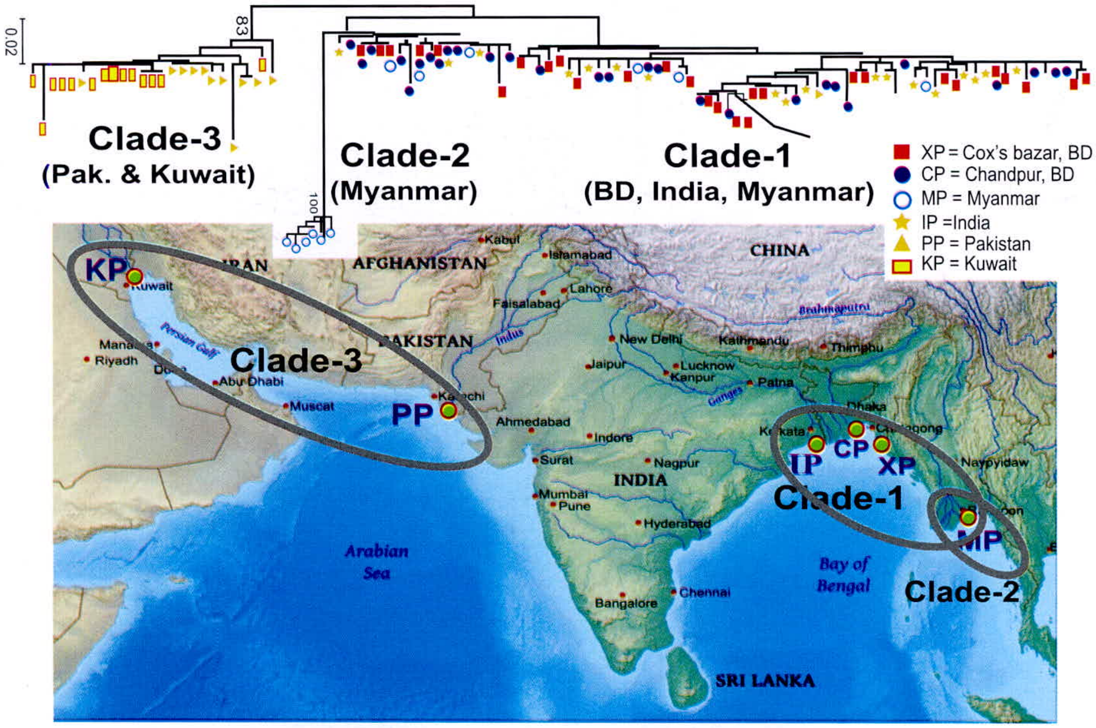

Figure 1: Phylogeography of *T. ilisha*

Demographic History:

In the neutrality tests, both of Tajima's *D* and Fu's F_S statistics for XP, CP, MP, IP and PP populations were non-significant (Table 4) indicating demographic equilibrium of these populations. For the KP population, significant negative *D* but non-significant negative FS value also suggests the history of demographic equilibrium.

Table 4. Tajimas'*sD* andFu's F_S statistics

Population	Tajima's *D*		Fu's *FS*	
	D	*P*	*FS*	*P*
XP	-0.81	0.22	0.23	0.56
CP	-0.67	0.27	-1.39	0.31
MP	1.92	0.99	1.49	0.69
IP	-0.08	0.53	8.49	0.99
PP	-0.84	0.19	0.46	0.55
KP	-2.32	0.00	2.40	0.68
Pooled	-0.91	0.18	-9.17	0.11

Mismatch distributions of the sequences were all multimodal for each of the populations (Figure: 2) further suggesting the populations have been at demographic equilibrium and subdivided into several units (Excoffier, 2004).

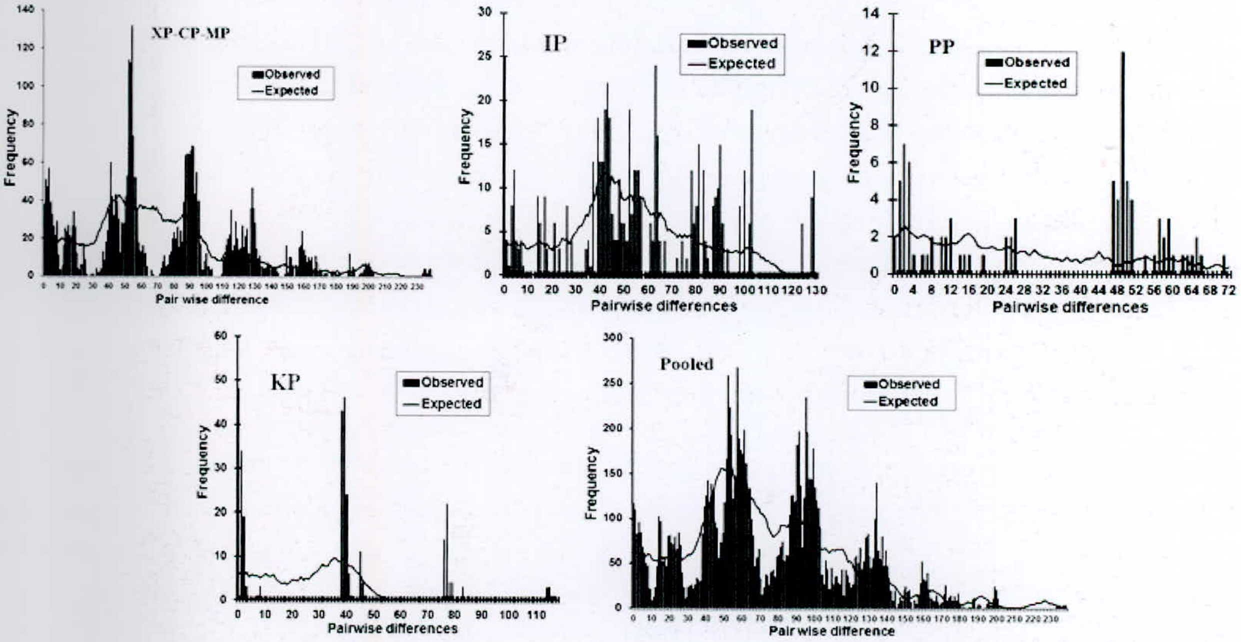

Fig. 2: The observed pairwise difference (*bar*) and the expected mismatch distribution under the sudden expansion model (*solid line*) for the populations of *T. ilisha*

Conclusion

The present studysuggests non-panmicticmtDNA gene pool for *T. ilisha* throughout its distribution range. There are clear genetic structure among Persian Gulf (KP), Arabian Sea (PP) and the Bay of Bengal (IP, CP, XP, KP) populations. However, inside Bay of Bengal, lack of genetic structure and population panmixia were found between Bangladesh (CP, XP) and Myanmar (MP) populations (i.e. frequent migration). Nevertheless, Hugli river (IP) showed geneticstructure with each of the CP, XP and MP populations within BoB. CP and IP populations were the spent or brood fish collected at breeding season from their natal rivers, Meghna and Hugli, respectively in this study.

The philopatry (tendency of organism to breed at or near their place of birth) possibly caused the significant genetic separation between CP and IP. On the other hand, the individuals of XP (Cox's Bazar) population were collected from the open sea which is very near to CP population. So, the XP population likely consists of the individuals of CP and did not show genetic differentiation between them. The population of Persian Gulf (KP) and Indus river estuary of Arabian Sea (PP) showed genetic structure comparing each of the populations studied.

Haplotype diversity of *T. ilisha* was found high for all of the populations. Several scenarios have been proposed to explain the maintenance of high haplotypic diversity within populations of an organism, including large population size, environmental heterogeneity, and life history traits that favor rapid population increase (Nei, 1987). In marine fishes, large population size is considered to be responsible for extraordinarily high levels of genetic diversity (Avise, 1998). Hilsa shadoccurs in the foreshore areas, estuaries, brackish water lakes and freshwater rivers of the western division of the Indo-Pacific faunistic region. Its marine distribution extends from Kuwait, Iran and Iraq coast in the Persian Gulf to the west coast of India in the Arabian Sea, the Bay of Bengal to northern Sumatra. Such large population size may account for the high levels of haplotypic diversity observed for *T. ilisha* in this study. High haplotypic diversity suggests large, stable, effective population size over time in the continental shelf fishes (Stepien, 1999) as showed byHilsa shad. In the study of phylogeography, three distinct lineages were detected among haplotypes formed during intra-specific evolution of *T. ilisha*. Those lineages are Meghna and Hugli river lineage (Clade-1), Irrawaddy river lineage (Clade-2) and Arabian Sea-Persian Gulf lineage (Clade-3) as showed in Figure 1. The neutrality test and mismatch distribution analysis suggest the history of demographic equilibrium for all of the populations of *T. ilisha.*

Assessment of genetic diversity and population genetic structure are essential for maintaining a productive fishery through effective management (Seeb *et al.* 1990). Different populations with unique genetic structure and/or geographically isolated stock should be managed as distinct units, and such units require separate monitoring and management (Salgueiro *et al.* 2003). Thus, the results of the present study will be helpful for making conservation strategy of Hilsa shad, one of the most important commercial species in the Indo-West-Pacific region. It is necessary to collect more hilsa samples in different seasons from different breeding sites of Bangladesh and study the genetic diversity and population structure for optimisingpresent hilsafishery management strategy of the country and for effective breeding program for aquaculture initiative taken by the Department of Fisheries of the Government of Bangladesh.

Publications from this Research

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Ionic Liquids and Their Binary Systems as Green Solvents for Sustainable Environment

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Location: Department of Chemistry, University of Dhaka, Dhaka, Bangladesh
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1700000.00

Introduction

Ionic liquids (IL) are a novel class of liquid salts with melting point below 100°C. ILs are very promising in the materials science field due to their negligible vapor pressure, non-flammability, high ionic conductivity, and high thermal, chemical and electrochemical stability (M. Freemantle, 2010). ILs are used as solvent media for synthesis, catalysis, polymerization, separation, and extraction processes (T. Welton, 1999; Carmichael *et al.* 1999, Armstrong *et al.* 1999; Huddleston *et al.,* 1998; Dai *et al.* 1999; Visser *et al.* 2000) and for the design of novel functional materials (Zhao and Bond, 2009; Zhao *et al.,* 2009). Adding conventional solvents to IL systems significantly influences their physico-chemical properties and may be exploited for manifold applications (Attri *et al.* 2011; Kavitha *et al.* 2012; Rilo *et al.* 2009). The fundamental properties of ILs provide a better perception about the structure property relationship (Tokuda *et al.,* 2004, 2005, 2006) and facilitate successful combination of IL and molecular solvents for targeted functionality (Plechkova and Seddon, 2008).

Three ILs – the hydrophobic 1-ethyl-3-methylimidazolium bis (trifluoromethyl) sulfonylimide, [EMIM][TFSI], and the hydrophilic 1-ethyl-3-methylimidazolium methanesulphonate, [EMIM][MS] and 1-ethyl-3-methylimidazolium tetrafluoroborate, [EMIM][BF_4] – and their binary mixtures with polar solvents were studied and compared by different thermoanalytical techniques.

Thermal properties and stability of binary mixture of [EMIM][MS] and water were investigated at different compositions using different thermochemical and analytical techniques. Thermal properties of binary mixture of [EMIM][TFSI] with ethanol and methanol were also studied at various compositions. Interactions in [EMIM][MS]-water, [EMIM][TFSI]-methanol, and [EMIM][TFSI]-ethanol systems were investigated using ATR-IR spectroscopy. Their densities, viscosities, and conductivities were analyzed using various thermodynamic models. Polarity of the binary mixtures was estimated by solvatochromic method using Reichardt's betaine dye (RBD) and its derivative as solvatochromic probes.

Objectives

The work aimed to accomplish the following objectives:

- To unveil novel route for designing green solvents with desirable chemistry for task-specific applications.
- To envisage the structural change and aggregation behavior of ILs induced by variation in compositions of polar solvents.
- To explore the possibility of utilizing binary IL-polar solvents at ambient condition.

Methodologies

The ILs [EMIM][TFSI], [EMIM][BF4], and [EMIM][MS] were obtained from Sigma-Aldrich and used without any further purification. All binary mixtures were prepared gravimetrically by using an analytical balance (Unilab UB-110) with a precision of ± 0.0001 g and sonicated (LU-2 Ultrasonic cleaner, Labnics Equipment) for 20 minutes.

Water content in pure ILs was determined by Karl-Fischer titration (Metrohm- Titrando 890) and was found to be less than 0.5% (w/w). Thermogravimetric measurements and differential thermal analysis of the neat ILs and their binary mixtures with water were conducted in N_2 atmosphere (50 mL.min^{-1}) with a Seiko Instruments thermo-gravimetry/differential thermal analyzer (TG-DTA 6200). Melting point of aluminum and heating rates of 5, 10 and 20°C were used to calibrate the equipment. The accuracy of temperature and mass, measurements was ± 0.1°C and 10-3 mg respectively. Density was measured with an Anton-Paar (Model DMA 4500) vibrating-tube density meter. Dynamic viscosities were measured using an Anton-Paar Lovis-2000 falling ball automated viscometer with an accuracy of $\pm 10^{-6}$ mPa.s. Specific conductance was measured with a conductivity meter, Jenway model 4510 (Camlab, UK) equipped with a dip-type pre-calibrated cell.

UV-visible spectra of RBD solutions in polar solvents, ILs, and binary mixtures of different composition were recorded by a double beam Shimadzu UV-visible spectrophotometer (model UV-1800 PC). Value of the λmax of the charge transfer band was used to compare the polarity of different media based on the empirical scale of solvent polarity, the ET(30) scale (T. Welton, 1999).

ATR-FTIR measurements with single reflectance were performed by using Shimadzu IR Prestige-21 spectrometer on a 0.5 cm2 zinc-selenide (Zn-Se) cell. The absorption spectra were accumulated for 50 times per sample with a resolution of 4.0 cm^{-1}.

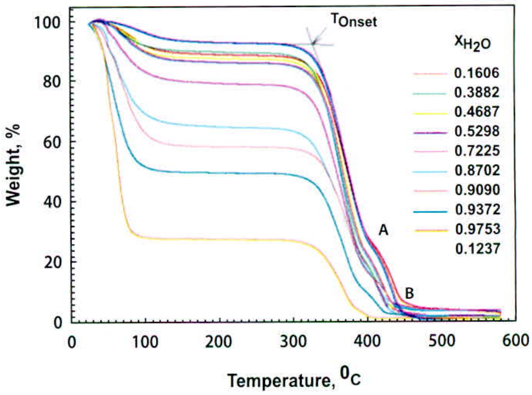

Fig. 1: Thermogravimetric curves of [EMIM][MS]-water system.

Three-step weight loss processes were observed for [EMIM][MS]-water, with the third step varying widely for different compositions, as shown in Fig. 1. The onset temperatures of [EMIM][TFSI]-ethanol and [EMIM][TFSI]-methanol mixtures did not vary, thus, polar solvents like ethanol and methanol does not alter the thermal stability of the [EMIM][TFSI].

Increase of density of IL-water mixtures with increasing amount of water shows a non-linear pattern, indicating interactions between these molecules (Fig. 2). Viscosity of [EMIM][TFSI]-ethanol and [EMIM][TFSI]-methanol systems decrease sharply with addition of ethanol and methanol.

The specific conductance of [EMIM][MS]-water increases with increase of IL until the maximum value is reached, after which formation of aggregates affects the change in specific conductance. [EMIM][TFSI]-ethanol and [EMIM][TFSI]-methanol mixtures also reach a maximum in specific conductance before declining with increasing amount of IL.

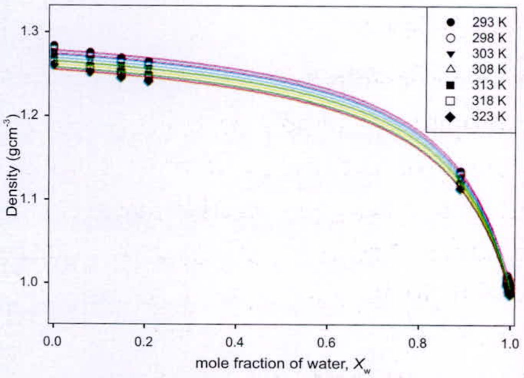

Fig. 2: mole fraction of water.

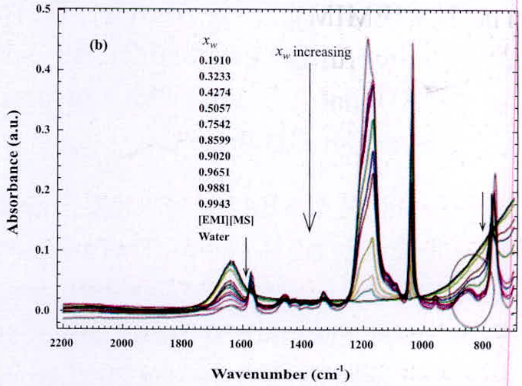

Fig. 3: ATR-FTIR spectra of [EMIM][MS]-water mixtures between 750-2200 cm-1.

ATR-FTIR spectroscopic analyses of [EMIM][MS]-water at very high concentrations of IL shows a new band at 1589 cm^{-1} with an isosbestic point located at 1583 cm^{-1}, which suggests the formation of a new complex from the direct interaction between [EMIM]+ cation and water (Fig. 3) (G. Socrates, 2008).

ATR-FTIR spectra of [EMIM][TFSI]-ethanol and [EMIM][TFSI]-methanol mixtures show shift in wavenumber of –CF3 band of the anion of the IL, signifying alteration of the structural orientation in the binary systems. This indicates interaction of the anion with the –OH of alcohols through hydrogen bonding (Miran $^{\text{et al.,}}$ 2011).

For [EMIM][MS]-water, λmax of the RBD and Cl-RBD dyes decreases with increasing water, indicating the increase in polarity of the system. For [EMIM][TFSI]-ethanol, polarity of binary mixture is higher at all compositions than the individual component. For [EMIM][TFSI]-methanol, polarity is higher than pure methanol only at high methanol content, and polarity decreases with increasing mole fraction of [EMIM][TFSI].

A slight increase in the potential window is monitored in [EMIM][MS]-water system, followed by a sharp fall with increasing water content. Relaxation of the ion-ion interaction by intrusion of water molecules into the inherent structure of [EMIM][MS] is mainly responsible downfall of potential window.

Conclusion

Physico-chemical properties such as density, viscosity and conductivity of both hydrophobic and hydrophilic ILs could be significantly influenced by the addition of polar solvents like ethanol, methanol, acetonitrile and water. Addition of these solvents perturbs the local structure of IL significantly and the extent and nature of interactions between IL and molecular solvents depend on the composition of binary mixtures. Activation energy and enthalpy change for viscous flow decrease gradually with addition of solvent to IL. Large positive values of excess free energy change of activation of binary mixtures of [EMIM][TFSI] indicate specific interaction leading to complex formation through intermolecular hydrogen bonding interaction between unlike molecules compared to like molecules. The electrochemical stability of [EMIM][MS]-water systems increases slightly with addition of water to pure IL and then finally decreases due to relaxation of the ion-ion interaction by intrusion of water molecules into the inherent structure of IL. Depending on the structure of IL and polar solvent, the variation of polarity of binary mixtures shows different trends. However, in all cases the polarity of binary systems has been found to be higher than those of pure ILs and solvents. Addition of neutral molecular solvent to the pure IL increases the conductivity to a maximum at a solvent reach region and then decreases near to zero. This is because the smaller solvent molecules separate cations and anions by means of incorporation into the aggregated structure of pure ILs. Thus changes in properties of the binary systems reflect modification of interaction of ILs in the microscopic level and structural rearrangement. Therefore, binary systems of IL with polar solvents may open up a new window to use and explore the task specificity of ILs under ambient condition.

Publications from this research

Marium M., Auni A., Rahman M.M., Mollah M.Y.A. and Susan M.A.B.H. 2017. Molecular level interactions between 1-ethyl-3-methylimidazolium methanesulphonate and water: Study of physicochemical properties with variation of temperature. *Journal of Molecular Liquids,* 225:621-630.

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Plasmonic Photovoltaics for Next Generation Solar Cells

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Location: Department of Electrical and Electronic Engineering, BUET, Dhaka
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1500000.00

Introduction

Today, one of the major challenges that our society faces is to provide low-cost and environment-friendly energy sources. The average usage of energy in today's world is ~2206 TW (https://www.cia.gov/), most of which is produced using fossil fuels.Although ~$1.5x10^5$ TW solar energy is available on earth (Lewis 2007), the use of solar cells is scarce due to their high cost and low efficiency.The solar cell cost can be significantly reduced by decreasing the material volume. However, a thinner solar cell faces with the adverse effect of decreased absorption of incident solar energy, and hence a decrease in the efficiency. Increased absorption of incident solar energy in thin- and ultra-thin-film solar cells over the entire solar spectrum is key to making them more efficient but less costly. Recently, differentnano-photonictechniques are being explored to increase the light absorption in thin-film and ultra-thin-film solar cells (Atwater and Polwan, 2010; Bermel *et al.* 2007; Ferry *et al.* 2010; Kelzenberg *et al.* 2010). Through this project, we addressed the challenge of light absorption in ~200-nm-thick ultra-thin photovoltaic layer for low-cost and highly-efficient next generation solar cells. We designed and characterized novel solar cell sincorporating the concepts of plasmonics. The designed solar cells show increased absorption and efficiency over the entire spectrum of solar energy.

Objectives

- developing simulation tools by solving Maxwell's equation to calculate the interactions of the incident light with the solar cell that can be used to design and characterize plasmonic solar cells;
- designing solar cells with ultra-thin layer thickness of only ~200 nm using plasmonic concepts;
- using plasmonic metal structures of different shape and size at different layersof solar cells for efficient light absorption;
- disseminating results through publications and workshops.

Methodologies

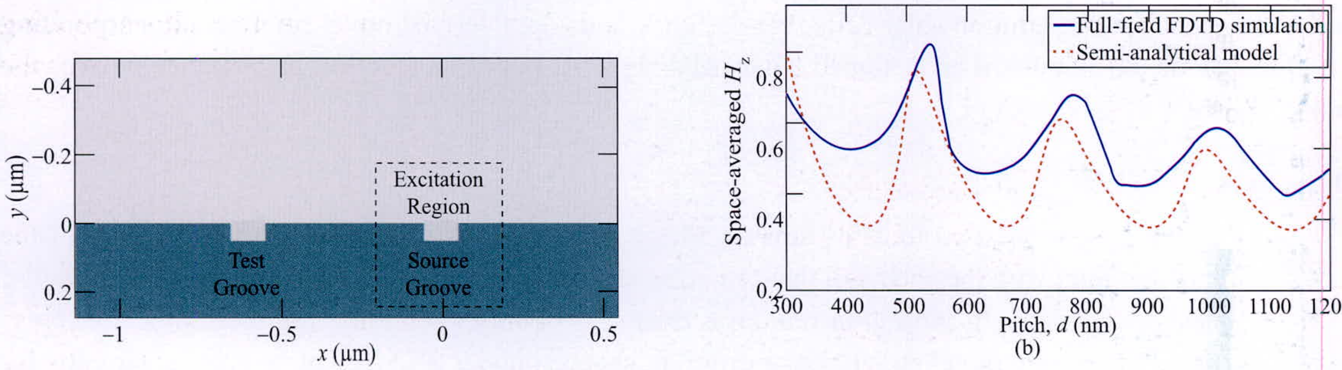
While ultra-thin-film solar cells with layer thicknesses ~200 nm significantly decrease material volume,light absorption is challenging as the layer thickness is much less than the wavelength of the incident light. Therefore, the design of ultra-thin-film solar cells in this project needed special methods that included:

- Using sophisticated simulationof time-resolved dynamics of the incident light to design and characterize the ultra-thin-film solar cells. The simulation tool solved full-field vectorial three-dimensional Maxwell equation. The optical properties of dielectric materials and metal were accurately calculated using a multi-coefficient model.
- Usingmetal structuresin a measured way to increase the excitation of plasmonic modes while keeping the light absorption inside the metal layer to a minimum. The coupled light to plasmonic modes is confined in a small volume of space and can be absorbed within the dielectric layer. The metal structures also scatter light and increase the pathway for increased absorption within an ultra-thinfilm.To increase light absorption, this project used following key techniques: (a) Corrugated metal layer at the bottom of the ultra-thin film, (b) dielectric strips on the top of the ultra-thin film, (c) an intermediate metal layer in a tandem solar cell between two ultra-thin films.

Results

i. Development of Design Software and Semi-Analytical Model

Sophisticated and robust design and characterization tools such as finite-difference time-domain (FDTD) and finite-difference frequency-domain (FDFD) simulators have been developed. These simulators implement mathematical models of interaction of light with materials to emulate response of practical solar cells. Since FDTD and FDFD simulators take considerable time to simulate the light interactions with solar cells, a semi-analytical model was developed that is much faster than FDTD and FDFD techniques but reproduces the interactions with sufficient accuracy (Figure 1).



(a)

(b)

Fig. 1: (a) Setup. (b) Space-averaged magnetic field versus pitch of the corrugated interface.

ii. Strip-Loaded Plasmonic Solar Cells

A single-junction solar cell using crystalline silicon with silicon strips on the top layer and metal grooves at the back contact was designed. This solar cell absorbs incident light over the entire spectrum of sunlight (400 nm to 1000 nm) andincreases light absorption by~46% compared to a reference planar structure (Figure 2).

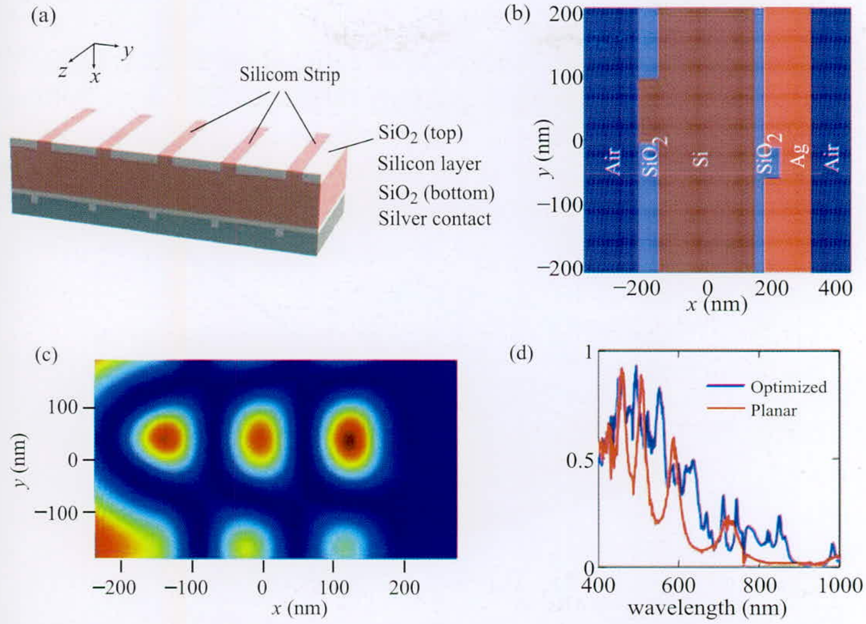


Fig. 2: (a) Schematic diagram. (b) 2D cross-sectional view. (c) Electric field profile at 820 nm, (d) Solar weighted absorption spectra.

iii. Plasmonic Solar Cells with Intermediate Metal Layer

a) Intermediate Metal Strips

A tandem solar cell with ultra-thin amorphous silicon (a-Si) and microcrystalline silicon (μ-Si) layers with intermediate silver (Ag) plasmonic strips has been designed. Intermediate Ag strips increases efficiency by supporting plasmonic resonances and collecting carriers from two ultra-thin films, thereby also resolves the issue of current mismatch. The design shows ~65% total solar absorption (Figure 3).

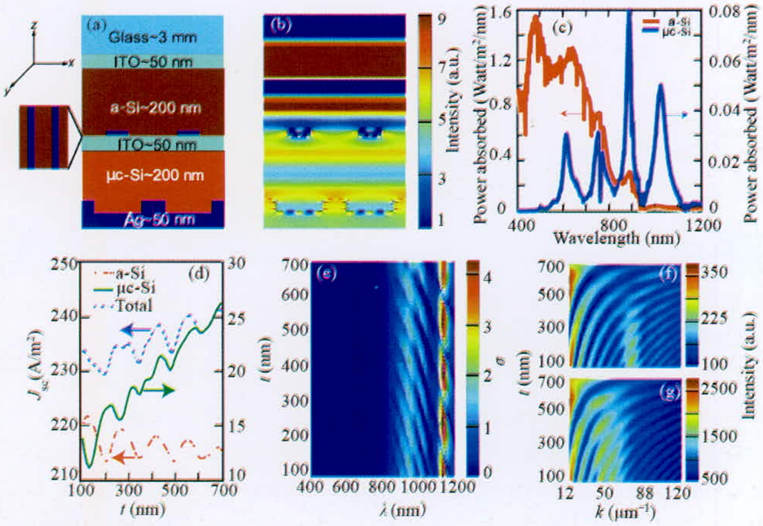


Fig. 3: (a) Cross section of the proposed structure, (b) Electric field intensity profile at 534 nm, (c) Absorbed power, (d) Short-circuit current, (e) Scattering cross-section, and Intensity for (f) 400–730 nm and (g) 730–1200 nm.

Intermediate Metal Nanohole Array

We designed and characterized ultra-thin-film tandem solar cellswith 6,6-phenyl C61-butyric acid methyl ester (PCBM) as top sub-cell and copper indium gallium selenide (CIGS) as bottom sub-cell with an intermediate metal nanohole array (Figure 4). The nanohole array supports extraordinary optical transmission, and therefore, light transmission from PCBM layer can be controlled and increased to CIGS layer (Figure 5). The intermediate layer increases plasmonic excitations and thus light absorption.

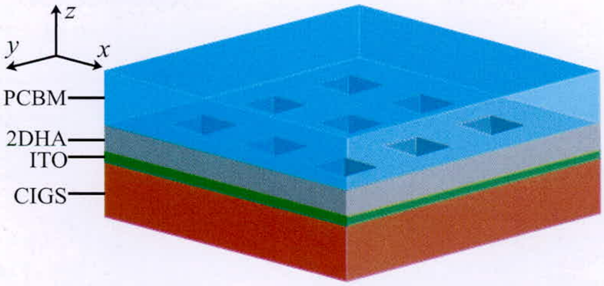


Fig. 4: Schematic illustration of ultra-thin-film tandem solar cell with intermediate nanohole array.

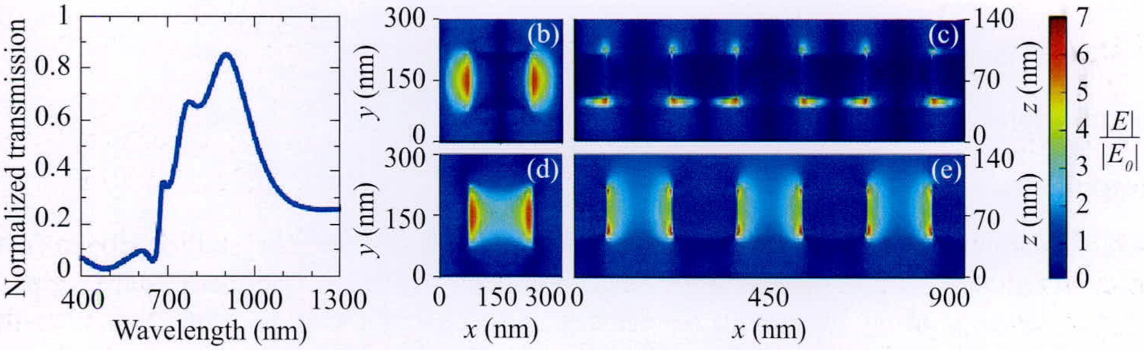


Fig. 5: (a) Transmission spectra into the bottom sub cell. Electric field profile at 764 nm in (b) *x-y* plane and (c) *x-z* plane, at 914 nm in (d) *x-y* plane and (e) *x-z* plane.

Conclusion

Solutions to the obstacles of using renewable energy are our paths forward to a sustainable future,which is the mostimportantobjective of today'sworld. Solar energy is certainly the ultimate choice for an energy source. This project contributed toward the solution of the challengeof making solar energy affordable and efficient. This project designed novel solar cell structures and explored in-depth physics that are immensely important to make the solar cell technology affordable and efficient. The designed solar cells show significant solar energy absorption in a ~200-nm-thick solar cell so that the efficiency normalized by the layer thickness is much greater than that of conventional bulk solar cells. Therefore, the price of generated power using the designed solar cells will be less than the conventional bulk solar cells. The designed single-junction silicon strip-loaded design offers ~46% absorption enhancement in an easy-to-fabricate structure. Although the designed structures with intermediate metal layer are relatively complex to fabricate, they also offer significant light absorption and solution to the current mismatch problem of a tandem structure. Additionally, due to the presence of the intermediate layer, the two tandem layers can be used in parallel. Also,the fabrication technology of nanoscale complex geometrical structures are seeing a lot of success recently and being introduced to the industry for many applications.

The designs and results of this project have been disseminated in the form of publications in journals and conferences, and in presentations in conferences and workshops/seminars. Two papers have been published in internationally reputed Journal of Physics that is published by American Institute of Physics and six papers have been published/presented in prestigious international conferences. The work on this project resulted in two Master of Science and three Bachelor of Science theses.

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EcoRadar: Devising an Ecological Radar System for Detecting Environmental Pollution in Bangladesh

A B M Alim Al Islam

Location: Department of Computer Science and Engineering, BUET, Dhaka
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 3000000.00

Introduction

Environmental pollution is one of the major threats to the development of human civilization. Different types ofenvironmental pollution such as air, water, and soil pollution cause various life-threatening diseases. Moreover, environmental pollution can also threaten economic progress of a nation.

As a developing country, Bangladesh is highly susceptible to environmental pollution. The first step oflimiting environmental pollution is to detect these phenomena. Until recently, modern technologies such aswireless sensor networks (WSNs) have started to play a crucial role in detecting environmental pollution.Consequently, we propose a WSN-based environmental pollution detection system in Bangladesh.

In our project, we have attempted to detect air pollution using WSN. Since we utilized networking in suchecological sensing, we have termed our project "EcoRadar". We have conducted it over a timeline spanning 3 (three) years utilizing a budget of 3000K BDT (Thirty lac Taka only) under funding of the Ministry ofEducation, Government of the People's Republic of Bangladesh. We have developed a new fully-functionaland independent sensor node for automated sensing of air pollution. We have performed real experiments by deploying the node in different settings. Experimental results confirm that this project exhibits a gleamingprospect towards enhancing the quality of living and enrich national economy of Bangladesh.

Objectives

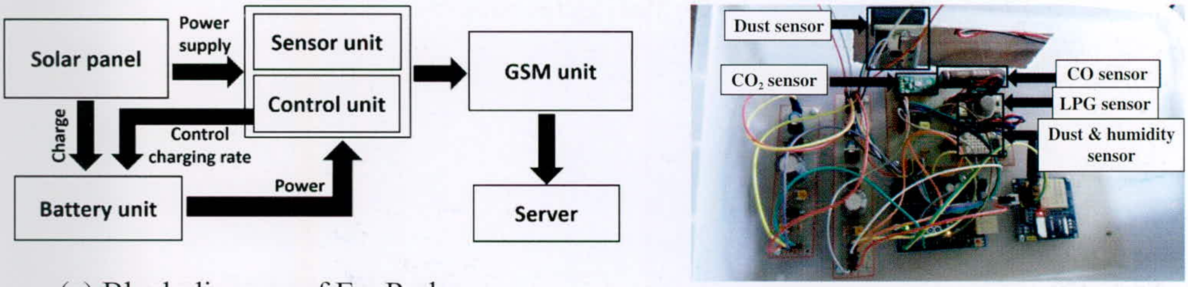
Bangladesh is widely known for experiencing a variety of environmental pollution such as air, water and soil pollution. We experience direct exposure to these pollutions. To escape from the severity of the consequences, several preventive measures have been taken in recent times. However, it is of utmost importance to meticulouslyidentify the presence of a pollutant and also determine the concentration the amount of pollutant.

In recent times, modern technology has come under investigation to identify and determine the extent ofpollution (Sohan *et al.,* 2016). The most prominent technology being exploited in this regard is wireless sensor network (WSN). AWSN is a wireless network of sensor nodes, which generally performs two different tasks: 1) Sense data fromthe surroundings, and 2) Transmit the sensed data towards a base station for further processing.

We intend to quantify different air pollutants in different locations in Dhaka Metropolitan City by usingsensitive sensors particularly constructed for this purpose (Aeroqual, Scientific). Moreover spatial and temporal analysis of thecollected data will facilitate identifying the pollution sources and their impact on the environment. Also, weplan the deployment of simple low-cost temperature and humidity sensors since such data may come in usefulto the Bangladesh Meteorological Department.

Methodologies

Here, we will discuss about the methodologies that we incorporated towards building up an independentsensing module.



(a) Block diagram of EcoRadar

(b) Prototype implementation of EcoRadar

Fig. 1: Block diagram and prototype of our sensing module

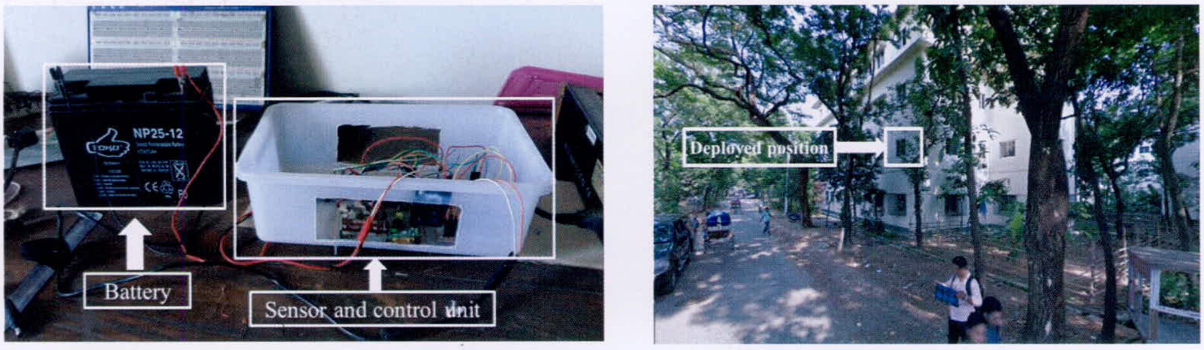
Choice of sensors: We incorporated the following sensors in our developed sensing module.

1. Figaro TGS-5042: This sensor has a long life, good long term stability, and high accuracy for COdetection.
2. Telaire T6713: It is ideal for CO$_2$ level detection for indoor air quality and energy saving applications.
3. DHT11: It is ideal for temperature and humidity sensing applications.
4. GP2Y1010AU0F Dust Sensor: This sensor is effective to detect fine dust particles such as PM$_{2.5.}$
5. MQ-2: It is used in gas leakage detecting equipment for detecting LPG, i-butane, propane, methane,alcohol, Hydrogen, smoke, etc.

Design and development: Figure 1a represents a simplified block diagram of EcoRadar. The sensor unit is designed to sense environmental data at fixed intervals. The control unit is responsible for charging up the battery during day time. Itis also responsible for calling the GSM module to upload sensed data to a server.Figure 1b shows our designed module based on the block diagram in Figure 1a.

Results

Here, we will discuss about the deployment settings and the results obtained from these settings. More data is available at http://taslim-arefin.net/works/ecoradar/.

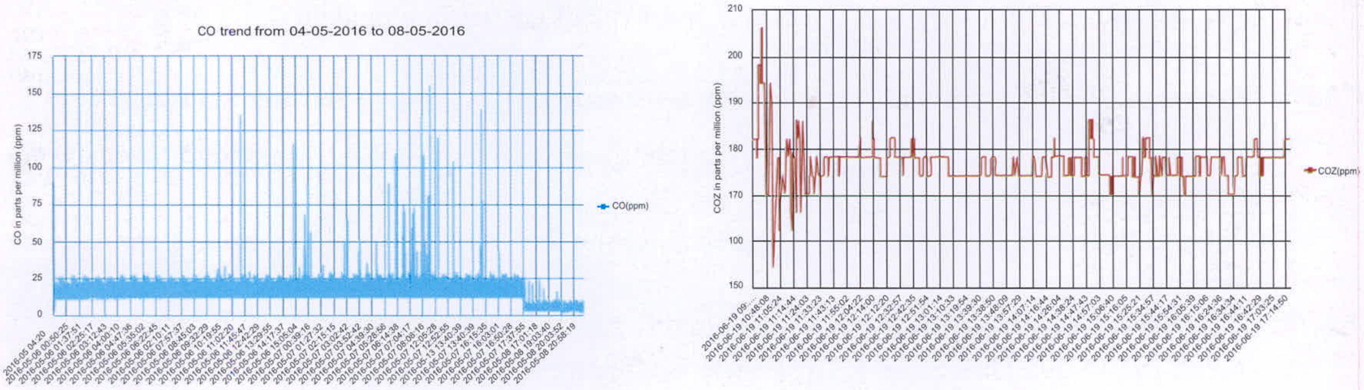


(a) Full setup with battery in indoor laboratory (b) Deployment at Azimpur colony

Fig. 2: Deployment of EcoRadar in two locations

Deployment: We deployed EcoRadar in the following locations:

1. Indoor Laboratory: As shown in Figure 2a, we deployed EcoRadar in an indoor laboratory to monitor indoor air quality.
2. ECE Plinth, BUET: We deployedEcoRadar at ECE Building plinth of BUET campus.
3. Deployment at Polashi: To accumulate outdoor data, we deployed EcoRadar at Polashi.
4. Deployment at Azimpur: Figure 2b shows a snapshot of deployment at Azimpur colony.
5. Deployment in different routes of Dhaka city: We deployed EcoRadar in different routes of Dhakacity (Chakraborty *et al.,* 2016).



(a) Trend of CO in 1st week of May, 2016 in indoor settings (b) Trend of CO_2 at Polashi in outdoor settings

Fig. 3: Trend of CO and CO_2 in indoor and outdoor settings

Gas Trends in Indoor Environment:

We monitored the gas trends in indoor environment extensively. Our sensor prototype module was stationedand running in full swing at our laboratory CSE-414, ECE Building, BUET. Figure 3a shows the data trend of CO during the month of May, 2016.

Gas Trends in Outdoor Environment:

We deployed our sensor module beside a busy road at Polashi, BUET for 7 hours. Using that data, we caninfer the 24 hours trend at the same place. Figure 3b shows the data trend of CO_2 after deploying atPolashi, BUET. Compared to indoor settings, CO, CO_2, temperature and humidity shows similar trends.LPG in outdoor environment is low, averaging 507 ppm for the day. However, this empirical data did not findany trace of particulate matter $PM_{2.5}$ during the day.The study in (Chakraborty *et al.,* 2016) shows empirical data in different routes of Dhaka city.

Conclusion

Detecting environmental pollution, specifically air is important. Air pollution is related with several air borne diseases. Moreover, the geographic importance of a location can vary based on its level of air pollution. In EcoRadar, we tried to demonstrate a portable air quality monitoring system. Here, we used CO, CO_2, LPG, $PM_{2.5}$, temperature, and humidity sensors to empirically measure air quality around BUET campus. The data gathered using EcoRadar is employed in statistical analysis.

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Pollution Assessment of Industrial Wastes and Management Strategies for Food Security

GKM Mustafizur Rahman, Md Giashuddin Miah, Md Mizanur Rahman and Mohammad Saiful Alam

Location: Department of Soil Science, BSMRAU, Gazipur
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 2500000.00

Introduction

There is rapid establishment of textile and dyeing industries in many areas of Bangladesh. Sadar and Sreepur upazilas of Gazipur, Savar of Dhaka and Valuka of Mymensingh areas are the commercially important areas where industrial clusters have developed as part of the rapid economic growth of the country. These industries have brought a range of problems one of which is serious soil and water pollution. Industrial effluents contain appreciable amount of heavy metals, which may accumulate in the soil, thus create crop damage (Fig.1) and make problem for safe and rational utilization of agricultural soils (Chen *et al.,* 2005).

Fig. 1: Damage of rice crop due to discharge industrial effluent

Long-term deposition of untreated industrial effluents into agricultural lands is known to have significant contribution to heavy metals in surface soils (Mapanda *et al.,* 2005). Such accumulation of trace metals in agricultural soils may affect food quality and safety (Sharma *et al.,* 2007). However, information on trace metal contamination in soil and their transfer to crops in the vicinity of industrial areas of Bangladesh is still lacking.

Objectives

The objectives of the present study were to

- Assess heavy metals build up and spatial distribution in agricultural soils in the vicinity of the industries.
- Find out the best mitigation options to reduce heavy metal toxicity

Methodologies

Socio-economic survey were conducted in industrial contaminated areas using structured questionnaire to assess the livelihood activities including farmers' perception on industrial contamination,production practices, income and expenditure, education and health care, and other related aspects of toxic pollutants of degradation of food quality and production, health hazards etc.Waste water and soil samples were collected from the selected different contaminated areas of Bangladesh (Fig. 2) and processed to analyze in the laboratory to determine toxic metals using standard methods. All sampling points were GEO-REFERENCED using GPS. Geo-statistical analysis was done for making GIS map. Moreover, a series of laboratory, pot and field experiments were conducted using different management options for identifying the best one to mitigate soil contamination; and also isolated and identified the microbes which could be degraded the toxic contaminants in the untreated industrial effluent.

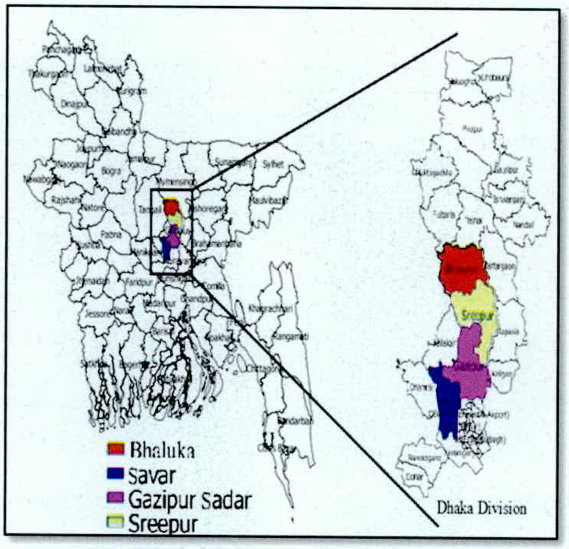


Fig. 2. Map showing the sampling sites of the study area

Results

Soil controls crops productivity and quality. It is assumed that heavy metals are severely added to the soil from the industrial waste water thus adversely affected the agricultural crop production. Findings showed in Table 1 illustrated that low lands are more vulnerable to the adverse situation followed by medium and highland. This is because discharged wastes ultimately deposited in the low land and remain there for a longer period of time unless washed away through flood water.

Table 1. Distribution of the respondents according to their responses on decrease in quality of soil

Types of land	Types of quality	Respondents					
		LD		MD		HD	
		No	Percent	No	Percent	No	Percent
High	Color	62	39	70	44	28	17
	Odor	105	66	55	34	-	-
Medium	Color	52	32	73	46	35	22
	Odor	58	36	82	52	20	12
Low	Color	-	-	38	24	122	76
	Odor	-	-	-	-	160	100

* LD= Low Decrease, MD= Moderately Decrease, HD= Highly Decrease

Mean concentrations of Cd, Pb, Ni, Fe, Zn and Cu was higher than their background values in the industrial contaminated paddy field soils of Dhaka division of Bangladesh indicating their increasing trend. The considerable spatial variation of heavy metal accumulation was observed in each command area of different industries. Heavy metals in the contaminated soils showed different spatial distribution patterns (Fig. 3).

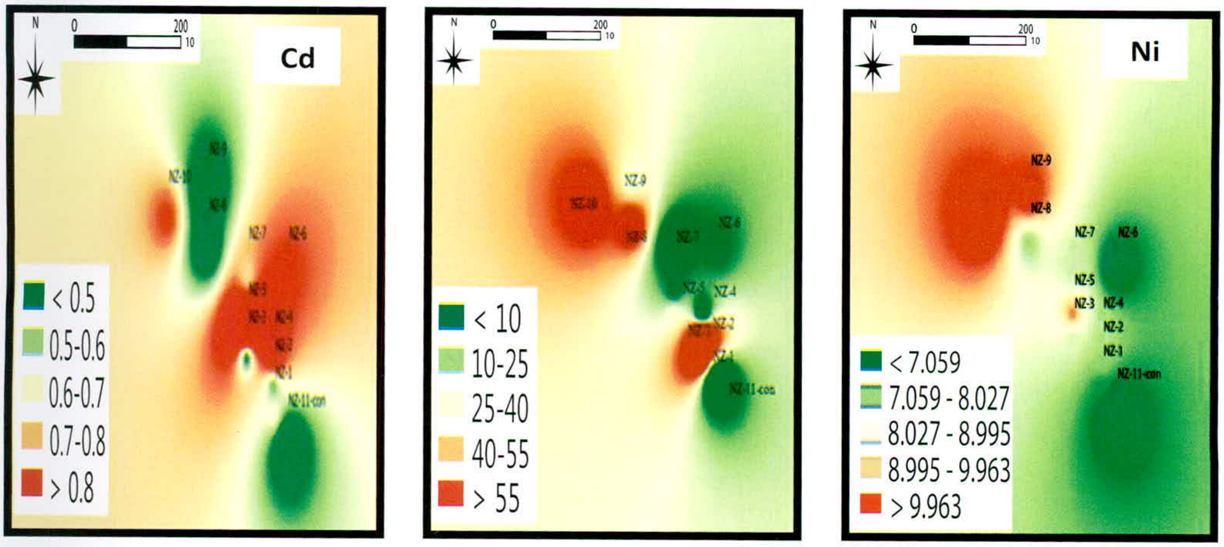
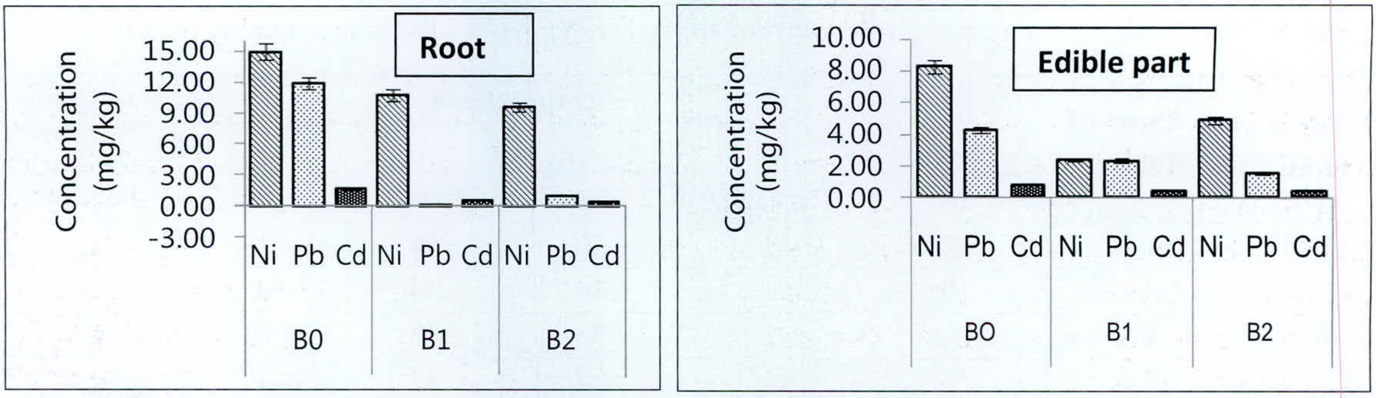


Fig. 3: Spatial variation and distribution of heavy metal in an industrial command area

The studied initial soils contained substantial amount heavy metals including Pb (305 mg/kg), Cd (1.84 mg/kg), Ni (53 mg/kg) as compared to reference value (control). The results of the pot experiment using different biochar revealed that rice husk biochar application reduced heavy metal contamination significantly in different leafy vegetables (Fig. 4). The observed findings might be due to formation of stable metal-organic complexes in soil.



Legends: B0=Without biochar, B1= Wood ash biochar, B2= Rice husk biochar

Fig. 4: Heavy metal concentration in root and edible part of Amaranth *(Amaranthus lividus)* under different biochar

Eleven (11) bacterial isolates were isolated according to the colony color and shape (Fig. 4). All isolated bacteria showed positive response to produce IAA. Among the 11 isolates, five of them were Gram positive and six of them were Gram negative. All the Gram negative 6 bacterial isolates were found as positive for KOH test and the entire Gram positive 5 bacterial isolates were found as negative for KOH test. Four of the bacterial isolates were positive for cellulase activity. Five of the bacterial isolates were found as positive for phosphate solubilization activity. Three of the bacterial isolates were found as positive in catalase activity. The results of the biochemical studies could be useful to know about characters of resistant bacteria population which could help to identify the species of bacterial isolates through further molecular characterization. Hence the tolerant isolates could be used for bioremediation of heavy metal toxicity in the industrial effluent contaminated soil and water.

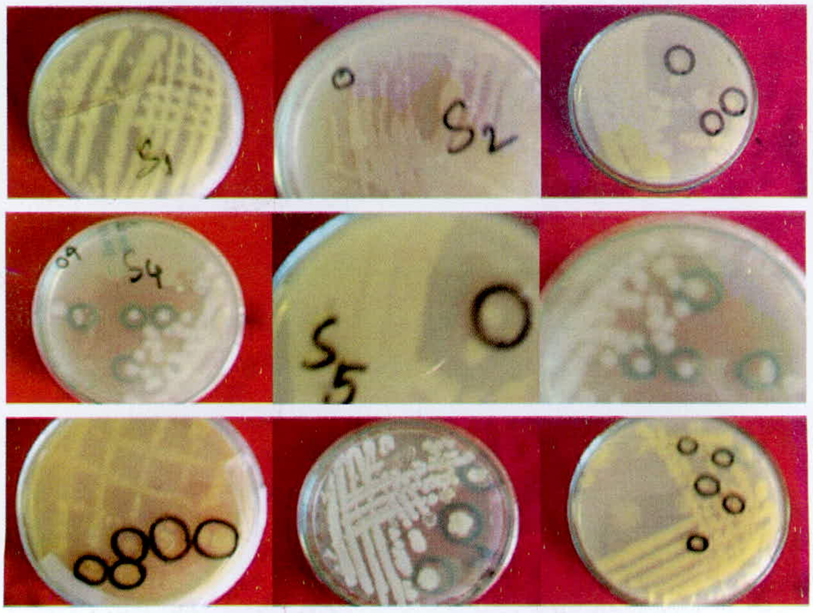


Fig. 5: Morphological characteristics of isolated colonies

Conclusion

The project entitled "Pollution Assessment of Industrial Wastes and Management Strategies for Food Security" was designed with the objective to assess the soil contamination through industrial wastes water and identify possible mitigation approaches. Socio-economic survey were conducted in industrial contaminated areas to find out the farmers perception on industrial contaminated irrigation water and its effects on land, crop yields, human health, domestic and aquatic animal health. Moreover a series of pot and laboratory experiments were carried out to generate information on heavy metal contamination in soil-water-plant system through untreated industrial waste water and possible mitigation approaches for reducing heavy metal contamination in soil and water system. Results from the study during this period showed that untreated and unplanned industrial discharges deteriorate not only the quality of soil, crop and environment but also directly affect the human, animal and aquatic animals. Thus, human being, domestic animals and aquatic animals are also suffering from adverse effects of industrial wastes dumped untreated. Elevated concentration of heavy metal accumulation in soil were detected in all studied command areas of different industries as compared to background values but still their concentration remain within the safe limit. Concentrations of different heavy metals like Cd, Pb, Ni, Fe, Zn and Cu was higher than their background values in the industrial contaminated paddy field soils of indicating their increasing trend. The considerable spatial variation of heavy metal accumulation was observed in each command area of different industries. Heavy metals in the contaminated soils also showed different spatial distribution patterns. Biochar was used as a potential tool for remediation of heavy metal in industrial contaminated soils through pot experiments and study results demonstrated significant reduction of heavy metals in leafy vegetables grown in industrial contaminated soil. Heavy metal tolerant bacteria could be potentially effective against heavy metal contamination in agricultural soil. Isolation and biochemical characterization of heavy metal tolerant bacteria was carried out in the laboratory to identify resistant bacteria against extreme unfavourable industrial contamination.

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Biochemical and Molecular Characterization of the Tea cultivars of Bangladesh to Validate the Authentic Quality of Tea in World Market

Md Abu Reza

Location: Department of Genetic Engineering and Biotechnology, Rajshahi University

Duration: Three years (2013-2016)

Expenditure of the project: Tk. 2650000.00

Introduction

Tea is one of the most important cash crops of Bangladesh which contributed of about 0.81% of the GDP in 2011 (Hossain *et al.* 2011, Ahammed, 2012). It is a dietary source of interesting compounds that helps preventing wide variety of diseases. Of them antioxidants are prevalently found in which promote cardiovascular functionality, prevent cancer and help in combating metabolic disorders. These antioxidants and other beneficial components of tea may vary substantially based on the difference of variety or cultivars as well as due to different geographical distribution. Bangladesh Tea Research Institute (BTRI) is involved in research and improvement of tea and has released a number of quality cultivars (Alam and Haque 2001, Aziz *et al;* 2011). However, we do not have any biochemical profile of our cultivated tea. Lack of biochemical data of our exported tea lowered our international market opportunity. Current project was undertaken to carry out the biochemical profiling of our tea cultivars to give authenticity to foreign importers. Moreover molecular/genotypic characterization through marker based techniques will give a tag to our tea varieties and help us to their phylogenetic analysis. Reports from the proposed study will contribute significantly to our agricultural improvements.

Objectives

The key objective of the proposed project is to perform a comprehensive biochemical profiling of the tea varieties of Bangladesh to make it as an authenticated product to the world tea market.

Specific Objectives of the Project:

- To perform biochemical characterization of the cultivars in order to select quality tea genotype.
- Determination of total polyphenol content
- Determination of total flavanoid content
- Determination of Total Antioxidant Capacity
- DPPH (1,1-diphenyl-2-picrylhydrazyl) assay to determine the scavenging capacity

General Objectives of the Project:

- To recommendthe best tea cultivars with superior biochemical properties
- To carry out genotypic characterization of the cultivars to assess the genetic diversity using random amplified polymorphic DNA (RAPD).
- To calculate genetic distance of the cultivars using RAPD data.
- To select the best clones from the genetic distance table for future tea breeding programme.

Methodologies

Present investigation was under undertaken to evaluate the genetic and biochemical variation present among 12 selected tea genotypes of Bangladesh. Morphological/agronomical data of 15 quantitative characters were recorded and statistically analyzed to calculate heritability and other quantitative traits. For biochemical characterization, total polyphenol, total flavonoid, antioxidant activities were determined using standard protocol of Velioglu and others (1998), Wang and Jiao (2000) and Prieto and others (1999) respectively. To assess the major alkaloids, RP-HPLC was carried out with 4 standards (viz. caffeine, Gallic Acid, Theobromine and Theophylline). Analytical Phenomenex C18 column was used as non-polar stationary phase and water-acetonitrile was used as mobile phase. Quantification of 4 alkaloids was done based on the retention time and peak area in comparison to standards. To evaluate the genetic diversity, molecular marker based technique RAPD was carried out. DNA was extracted from leave samples and subsequently PCR were carried out using RAPD decamerperimers. PCR products were analyzed on 1% agarose gel stained with EtBr. Results of the RAPD were analyzed by scoring the number of PCR bands and their size. Phylogenetic analysis and dendrogram was constructed from the scored result. Based on the results of the morphological, biochemical and molecular characterization data recommendation was made for superior tea genotypes.

Results

The 12 tea genotypes selected for this study were MZ/39, E/4, D/13, B2 × T1, Br/2/97, SDL/1, BT2, Ph/9/4, Ph/9/25, Ph/9/40, BS-67 and BT5 collected from the germplasm repository of Bangladesh Tea Research Institute (BTRI). Correlation coefficient analysis showed of 14 quantitative characters that the pluckable shoot yield/plant was positively and significantly correlated with height, length, breadth of the plucking surface and shoot density. Among 12 genotypes the highest antioxidant capacity was found in BT5. The DPPH assay of the methanolic extract of leaf also shows that the highest antioxidant activity (IC50 value of 39 μg/ml) in BT5 genotype. The biochemical and nutritional components viz., gallic acid, theobromine, theophylline, caffeine were analyzed through HPLC. The results reveal that among the 12 genotypes, Ph/9/25 showed the highest percentage of gallic acid, whereas no gallic acid was found in the genotypes D/13, B2×T1 and BS-67. The tea genotypes MZ/39, E/4, Ph/9/25 and Ph/9/40 showed the highest amount of total alkaloid content (theophylline, theobromine and caffeine). The highest amount caffeine content was recorded in Ph/9/40 followed by MZ/39 and Ph/9/25. The caffeine content was the lowest in BS-67. On the other hand, among all the genotypes, total catechin content was the highest in D/13 followed by E/4 and MZ/39. RAPD assay was performed to estimate the genetic diversity among the twelve tea genotypes. T decamer primers showed total 83 distinct score able bands of which 64 (77.18%) bands were polymorphic. The highest percentage polymorphic loci were found in OPC9 and the lowest loci (60%) in OPA9. The highest genetic distance was found between the Ph/9/25 and MZ/39 whereas the lowest genetic distance was found in SDL/1 and MZ/39. By The result indicates the genetic diversity among the tea genotypes and RAPD marker could be used for improvement of tea cultivars.

Conclusion

Present study reveals all the twelve tea genotypes used in this study could distinctly be identified by the presence and absence of unique RAPD bands.Therefore, RAPD markers provided a practical and effective method not only to evaluate the genetic diversity and relationships, but also for the identification of tea genetic resources in Bangladesh. In conclusion, the information generated from this study showed that there are significant genetic differences among the 12 tea cultivars studied and that they could be used to strengthen the future tea cultivar improvement programme effectively by incorporating potential progenitors from the available tea germplasm.

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Modification of Jute Fibre with Synthesized Fibre-Reactive Chitosan Derivatives for Improved Eco-friend Jute Products

Md Ibrahim H Mondal and Md Asadul Hoque

Location: Department of Applied Chemistry & Chemical Engineering, Rajshahi University
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 2500000.00

Introduction

Jute is the agricultural renewable natural raw materials, principally grown in Bangladesh and India. Cellulose occurs naturally almost in pure form only in cotton fibre, and in combination with lignin and hemicellulose in jute. Hence, jute is a composite fibre of cellulose, hemicellulose and lignin. Due to the presence of hemicellulose and lignin, jute has some limits in the use of textile fibres. However, with a view to minimize the undesirable properties and to enhance their effectiveness for intensified textiles and other uses, the present study on jute fibre is of prime importance.

Chitosan is a deacetylated derivative of chitin which is the major components in the outer shell of prawn (shrimp). Chitosan is biodegradable, biocompatible, bioactive and has antimicrobial properties. But, the solubility of chitosan is limited to moderately aqueous acid media (Zhang *et al.,* 2007).This represents a serious drawback to many of its potential applications. For this, the water soluble derivative of chitosan would be required to enhance the useability of said bio-polymer. To overcome the solubility problem, an attempt has been taken to prepare water soluble derivatives of chitosan which are safe and friendly substances for human usage. Many chemicals that are used as modifier from early for natural fibres are toxic to humans and do not easily degrade in the environment. The textile industry continues to look for eco-friendly processes that substitute for toxic textile chemicals.

Objectives

The present research work was undertaken to overcome the inherent limitation of jute fibre and tried to prepare value added products from prawn shell waste for textile sector and hence our environment would get safety from prawn shell waste pollution.

The specific objectives of the research work are as follows:

- To prepare chitosan from prawn shell waste.
- To prepare water soluble chitosan derivatives from chitosan.
- To modify jute fibre using chitosan and its ecofriend functional derivatives.
- To investigate the physico-chemical characteristics of the modified jute fibre.
- To save our environment from prawn shell waste pollution.

Methodologies

Jute fibre was scoured with soap and detergent, and bleached with aqueous sodiumchlorite (Farouqui and Mondal, 1989).

Prawn shell was processed to chitin and chitosan was obtained by deacetylation of chitin with NaOH (Islam *et al.,* 2015). N-octyl chitosan (NOCh) was prepared by suspending chitosan in methanol, and then octanal was added to it according to Bobu *et al.* (2011).

The procedure described for the carboxymethylation of chitosan was used for the preparation of carboxymethyl chitosan (CMCh) using monochloroacetic acid and isopropanol solution (Yeasmin and Mondal, 2015). Except these, N-(2-hydroxy) propyl-3-trimethyl ammonium chitosan chloride (HTAChC) and N-methylolacrylamide-N-(2-hydroxy) propyl-3-trimethyl ammonium chitosan chloride (NMA-HTAChC) were synthesized according to Park *et al.* (1996), respectively

Graft copolymerization of CMCh was carried out with a reaction of acrylic acid in presence of potassium persulphate (Sherbiny and Mahdy, 2010).

Elemental analysis, molecular weight, moisture content, ash content etc. of the synthesized samples were determined (Islam *et al.,* 2015).

The jute fibre (untreated or unmodified jute fibre) was grafted (modified) with chitosan and NOCh, CMCh, CMCh-g-AA, HTAChC and NMA-HTAChC derivatives solution in presence of initiator according to Bhuiyan *et al.* (2013). Their weight gain was determined (Islam *et al.,* 2015). The modified samples were characterized by FTIR, SEM and TGA. Moisture absorption, tensile strength and dyeing characteristics with reactive dyes were also investigated.

Results

The yield percent of chitin from dry prawn shell wastes, chitosan from chitin, NOCh, CMCh and HTAChC from chitosan, CMCh-g-AA from CMCh, and NMA-HTAChC from HTAChC treated with corresponding reactants are listed in Table 1. The yield percent increased with the increasing reaction steps. Except CMCh-g-AA, the chitosan derivatives are water soluble and their moisture absorption capacity are higher than that of chitosan. The weight gain (%) increased considerably with the increase of modifier (chitosan, NOCh, CMCh, CMCh-g-AA, HTAChC and NMA-HTAChC) concentration. This weight gain increased due to the absorption, adsorption and excess deposition of modifier on jute fibres at higher concentration. The modification was optimized on weight gain of each modifier. Experiment showed moisture absorption of modified jute fibres decreased, whereas tensile strength and molecular weight increased compared to that of unmodified fibres.

Table 1: Represents yield of chitosan derivatives, their solubility in water, moisture absorption and graft yield of jute fibres.

Name of chitosan derivatives	Yield, %	Solubility in water	Moisture absorption, %	Graft yield, %
Chitin	29.69%	-	7.95	-
Chitosan	15.56%	-	9.79	10.34
NOCh	95.60%	+	8.17	8.82
CMCh	285.65%	+	13.76	12.39
CMCh-g-AA	296.11%	-	6.83	13.28
HTAChC	113.68%	+	18.43	12.31
NMA-HTAChC	103.55%	+	17.71	16.01

The characteristic FTIR spectra of prepared chitosan, NOCh, CMCh, CMCh-g-AA, HTAChC and NMA-HTAChC, and their treated- jute fibresare shown in Figure 1 and Figure 2, respectively. Each peak of a sample spectra indicates the confirmation of the product sample and that of their modified sample indicates the incorporation of the modifier (chitosan derivatives) onto the fibre surface.

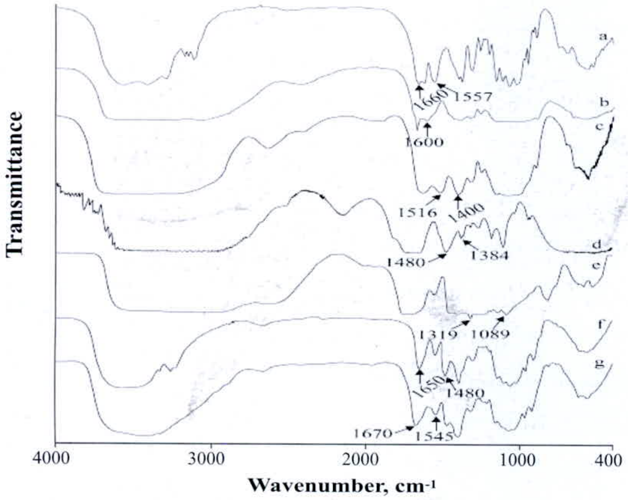

Fig. 1: FTIR spectra of prepared (a) Chitin, (b) Chitosan, (c) NOCh, (d) CMCh, (e) CMCh-g-AA, (f) HTAChC and (g) NMA-HTAChC

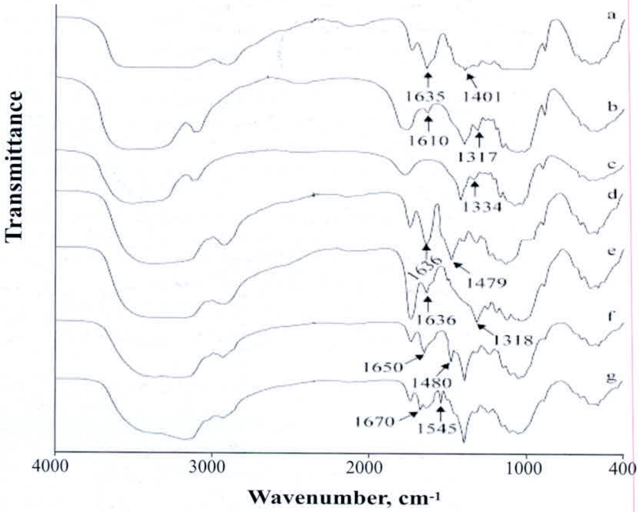

Fig. 2: FTIR spectra of (a) Jute fibre, (b) Chitosan modified, (c) NOCh Modified, (d) CMCh modified, (e) CMCh-g-AA modified, (f) HTAChC modified and (g) NMA-HTAChC modified jute fibres.

Figure 3 shows the SEM micrograph of unmodified and modified fibres respectively. The SEM micrograph represents the microporous surface of the untreated jute fibres. On the other hand, the modified fibre shows smoother surface due to the absorption of modifier on the fibre surface.

The initial decomposition temperature (T_i) of jute fibre, chitosan-, NOCh-, CMCh-, CMCh-g-AA-, HTAChC- and NMA- HTAChC-modified jute fibres are 205, 280, 250, 230, 272, 255 and 270^{O}C, respectively, and the corresponding char yields are 6, 28, 12, 28, 18, 22 and 24%, respectively. On the basis of initial decomposition temperature, thermal stability of the jute and modified fibres follows the order, unmodified jute <CMCh modified <NOCh modified <HTAChC modified < NMA-HTAChC modified <CMCh-g-AA modified< chitosan modified jute fibre.

Four reactive dyes, Reactive Orange 14, Reactive Brown 10, Direct Orange 31 and Direct Yellow 29 were applied to jute fibre. The dye exhaustion of chitosan, NOCh, CMCh, CMCh-g-AA, HTAChC and NMA-HTAChC modified fibres is higher than that of unmodifiedfibres. The modified fibres also showed better fastness properties.

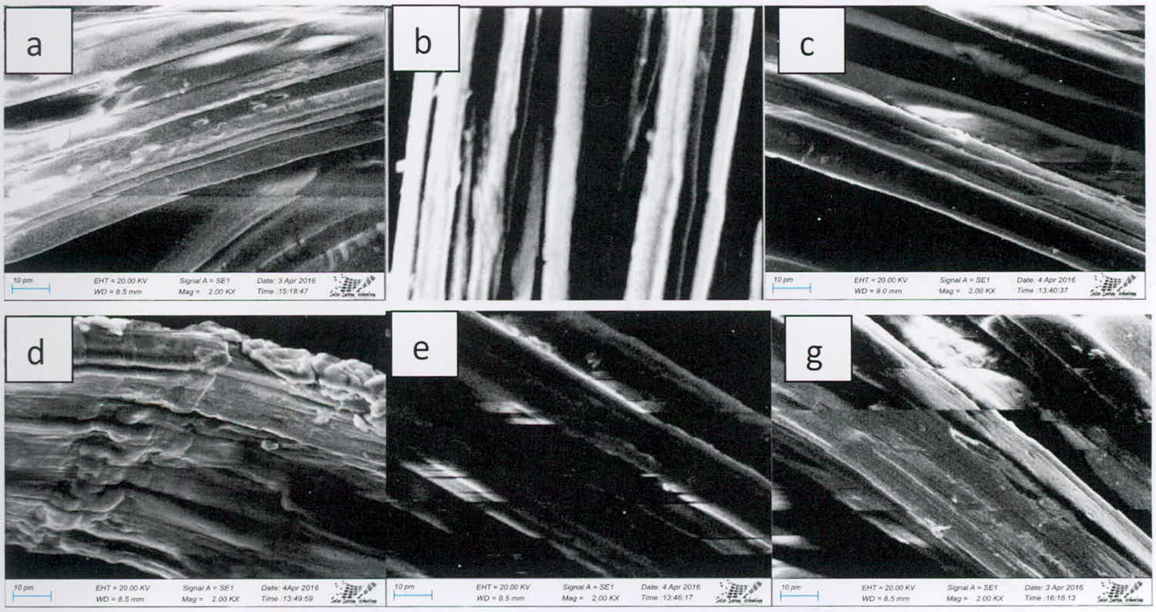

Fig. 3: SEM of (a) Jute fibre, (b) Chitosan modified, (c) NOCh modified, (d) CMCh modified, (e)CMCh-g-AA modified, (f) HTAChC modified and (g) NMA-HTAChC modified jute fibres.

Conclusion

Chitosan and its fibre reactive water soluble derivatives, were successfully prepared from abandoned prawn shell waste by chemical processes and examined by instrumental techniques. The prepared chitosans derivatives were used as biodegradable natural modifiers for jute fibres. Modifications were confirmed by FTIR spectra and SEM images of the untreated and treated fibres. For the modified fibres, chitosan and its derivatives acted as the bridges between the fibre surfaces and dye molecules, resulting in higher dye absorption for the reactive dyestuffs. On the other hand, modified fibres showed changed thermal stability, but increased colour depth and colour fastness properties. Thus chitosan and its derivatives from prawn shell waste would be a valuable in the functionalization of other materials. They can produce value-added products and they can easily be used as ecofriendly textile modifiers for improvement of jute, as well as other textile, fibre quality. The preparation of chitosan and its derivatives from prawn shell waste will help to save the environment from pollution in prawn processing areas.

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Optimization of the Conditions for Mass In Vitro Clonal Propagation of Gerbera (*Gerbera jamesonii* L.)

Md Monirul Islam and S M Abdullah Al Mamun

Location: Agrotechnology Discipline, Khulna University, Khulna
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 2000000.00

Introduction

Gerbera has a short history of cultivation as a cut flower in Bangladesh. However, this flower has gained popularity within short period and its cultivation area has been expanding every year. As this sector of floriculture is expanding, demand on gerbera plant propagules has been increased. In Bangladesh gerbera saplings is being propagated by division of clamps. But this method is somewhat slow and quality saplings and new genotype cannot be produced by this method. In most of the gerbera producing countries quality gerbera saplings are produced by the method of tissue culture (Cardoso and Teixeira da Silva, 2013). In Bangladesh few reports are available on gerbera propagation using *in vitro* techniques. We also need to boost breeding approach for generating new lucrative gerbera genotypes.

Objective

The project objective was to develop an easy and reliable protocol for mass in vitro propagation of quality gerbera saplings to meet the present demand.

Methodology

Plant material: Gerbera genotypes of various flower colour were collected from Godkhali, Jessore and established in a mother nursery bed at Khulna University campus and the nursery was named as 'Gerbera Research Center'.

Explants used: Capitulum, cotyledonary node, leaf blade, leaf midrib, Shoot tips, biological seeds.

Explantation: The explants were surface sterilized following conventional method using 70% ethanol and 0.2% mercuric chloride and rinsing them thrice in autoclaved double distilled water.

Inoculation: Sterilized explants were cultured onto MS (Murashige and Skoog, 1962) medium with various combinations of growth hormones

Incubation: The cultures were incubated in growth chamber, maintaining a 16 hours of photoperiod with a temperature of 25 ± 1^0C.

Phenomenon observed: *In vitro* Callus induction, morphogenesis from callus, direct organogenesis from explants, hardening of regenerated plantlets etc.

Data collection and analysis: At regular interval data were collected, processed and analyzed in computer with appropriate software.

Results

Experiment 1: Choice of Explant for Micropropagation of Gerbera

Immature and mature capitulum disk segments, leaf blade and mid rib from the genotypes *viz.,* Rubby Moon (Red), Risoluto (Yellow) and Arielle (White) were cultured on MS medium supplemented with NAA 0.5 mgL^{-1} + BAP 3.0 mgL^{-1} for callus induction and all explants responded to callus formation. The calli were transferred for regeneration on MS medium with BAP 3.0 mgL^{-1}. The calli proliferated in mass and turned into green but no regeneration was noticed. This experiment was repeated consecutive three years with other media combinations but no regeneration occurred from the capitulum. Son *et al.* (2011) worked on flower bud explants of three varieties of Gerbera and found MS medium supplemented with 3.0 mgL^{-1} BAP + 0.1 mgL^{-1} IAA as the best medium for culture establishment.

Fig. 1: Constructed 'Gerbera Research Center' and its inauguration

Fig. 2: Visitors of the project in the 'Gerbera Research Center'

Experimet 2: Plant Regeneration from Cotyledonary Nodes of *Gerbera*

Cotyledonary node explants were isolated from *in vitro* grown seedlings of five cultivars and were cultured on MS medium containing different combinations of cytokinins. Experiment revealed that both genotype and growth hormone had significant effect on multiple shoot formation. Yellow gerbera cultivar and BAP 1.0 mgL^{-1} performed better in multiple shooting. Micro shoots were subjected to auxin (NAA) treatment to induce roots and different concentration of NAA had been applied; NAA 5.0 mgL^{-1} produced maximum number of roots. Well developed plantlets were transferred *ex vitro.* For hardening a numbers of media was used, among them mixture of coarse sand garden soil (1:1) was most promising for all the genotypes studied.

Experimet 3: Multiple Shoot Regeneration from Mature Seed Explant of *Gerbera*

Matured seeds of nine gerbera genotypes were germinated on MS medium supplemented with BAP 3.0 mgL^{-1}. *In vitro* grown seedlings after 30 days of culture were transferred onto MS medium containing BAP 2.0 mgL^{-1} + NAA 1.0 mgL^{-1}. Multiple shoots were proliferated in

Fig. 3: Direct regeneration of gerbera from matured seed

Experimet 4: Indirect Plant Regeneration through Callus Culture Using Mature Seeds of *Gerbera*

Effect of gerbera genotypes and levels of IBA was investigated for callus formation and subsequent morphogenesis. Frequencies of callus were variable both with genotypes and IBA concentrations. 'Pale yellow' genotype and IBA 2.0 mgL^{-1} were identified most promising for callus differentiation. Multiple shoot regeneration was achieved on MS medium fortified with BAP 2.0 mgL^{-1} + IAA 1.0 mgL^{-1}. *In vitro* grown shoots were rooted on MS medium supplemented with 5.0 mgL^{-1} IBA. Maximum shoot regeneration (96%), shoot number callus-1 (9.86) and rooting (100%) was noticed for 'pale yellow' gerbera while 'white' genotype showed minimum plant regeneration (76%) and rooting (75%). Survival rate of regenerated plants ranged from 57% to 89%. Maximum survival rate was found for the genotype 'pale yellow' and lowest was recorded in 'white' gerbera genotype. Rooted plants were hardened before transplanting to soil. Rahman *et al.* (2014) conducted an experiment for propagation of a red Gerbera and viewed that lower concentration of BAP (1.0 and 2.0 mgL^{-1}) with NAA induced the explants to form callus. Akter *et al.* (2012) observed 95-100% root induction in the red, yellow and white genotypes. Ganesh *et al.* (2012) reported genotypic differences in plantlet survivability.

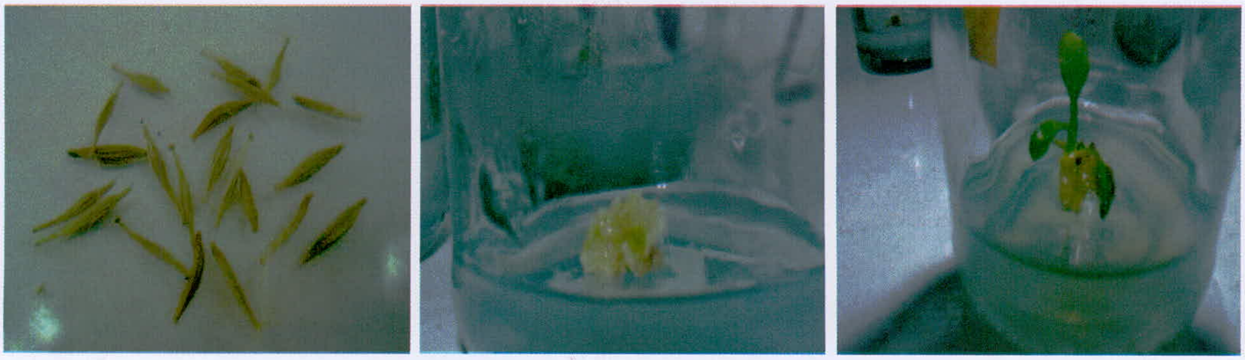

Fig. 4: Sequential steps of *in vitro* micropropagation of gerbera genotypes on growth regulators supplemented MS

Experimet 5: Indirect Plant Regeneration through Callus Culture Using Capitulum of *Gerbera*

Capitulum of gerbera was cultured on MS medium supplemented with different concentrations of growth regulators for *in vitro* regeneration and found better results from pale yellow genotypes for MS medium with 2, 4-D 4.0 mgL^{-1}. Kanwar and Kumar (2008) worked on plant regeneration from capitulum, shoot tip, leaf, petiole and other parts and concluded that gerberas are highly amenable to *in vitro* studies.

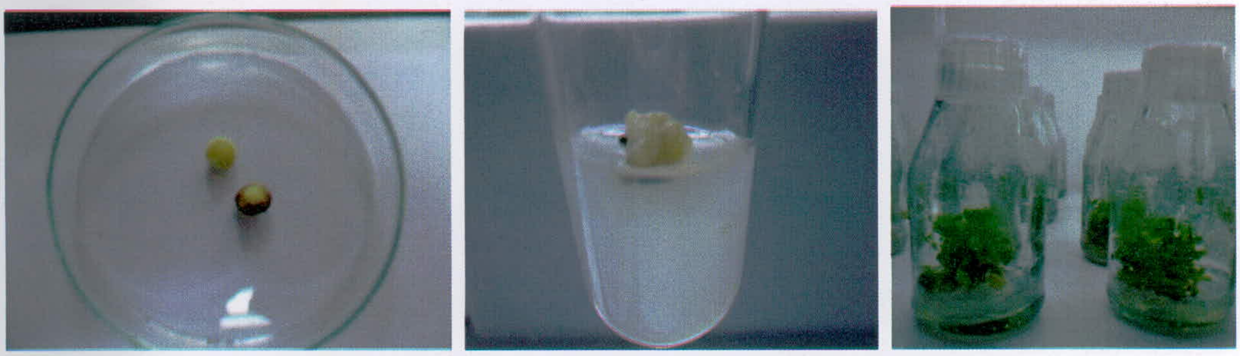

Fig. 5: Micropropagation of Gerbera from capitulum explant

Conclusion

On the basis of the studies conducted under this project it may be concluded that,

i) Mature seed, shoot tips and cotyledonary nodes from *in vitro* grown seedling are the most suitable explants for initiating micropropagation of gerbera;

ii) Performance of *in vitro* culture of gerbera is genotypic dependant;

iii) Growth regulators have significant effect on regeneration of plant;

iv) *Ex vitro* establishment of regenerated gerbera plants also depend on media and genotypes; and

v) Seed based micropropagation protocol may be a excellent tool for breeding new cultivars of gerbera.

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Salinity Tolerance Measurement of Indigenous Rice Germplasms of South West Saline Areas of Bangladesh

Md Yasin Ali, Debesh Das and Rahima Nusrat Remme

Location: Agrotechnology Discipline, Khulna University, Khulna
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1314000.00

Introduction

Bangladesh was a treasury of rice diversity. Nearly 10,000 land races of rice are considered to exist in Bangladesh (Haque and Miah 1990). With the expansive culture of modern varieties, the number of traditional rice cultivars reduced. Rice is a unique crop adapted to a wide range of climatic, edaphic and farming conditions; and its adaptability has resulted from a large genetic diversity which has been accumulated since the beginning of its cultivation by human. The landraces (local varieties of cultivated rice) vary widely in their morphological and physico-chemical characteristics. They are usually poor yielder but they are superior to modern varieties in terms of stability, resistance to biotic and abiotic stresses, quality characters etc. A number of reports showed that indigenous rice cultivars from Bangladesh possess a wide diversity in ecological, morphological and physiological characteristics (Islam 1990; Jahan *et al.*, 2003). Some of these varieties have high yield potentiality and higher consumer demand due to their grain quality compared to modern varieties. In saline areas of south west region of Bangladesh a number of indigenous rice cultivars exist in the farmers' field as these varieties possess some special characteristics. They have the capacity to tolerate various ranges of salinity, tidal surge and other stresses. However, in comparison to a large number of landraces, only a few have been evaluated. It is generally recognized that the evaluation of plant genetic resources is important and necessary for their utilization. However, it should be noted that a large number of landraces have been lost by the spread of elite modern varieties. This genetic erosion is the most serious problem facing rice breeding today. The landraces are characterized by their specific adaptability to various growing conditions, specially denoted by either resistance to diseases and insect pests or tolerance to environmental stresses. From these facts, the landraces are considered to be an important "cultural heritage". For preserving genetic diversity in order to use for variety improvement programs in future, the threatened rice varieties need to be collected, conserved and evaluated. Therefore, evaluation of indigenous rice germplasms of southwest saline region of Bangladesh will be done in respect of salinity tolerance.

Objectives

- To collect the indigenous rice germplasms from south west region of Bangladesh;
- To evaluate the collected rice germplasms for yield performance;
- To evaluate the collected rice germplasms for salinity tolerance; and
- To conserve the collected germplasms for future utilization in breeding purposes.

Methodologies

Study 1. Collection of indigenous rice germplasms

For collecting indigenous rice germplasms the research team visited different locations of southwestern part of the country time to time and collected the seed samples directly from the farmers during different growing seasons. Seeds of 60 varieties of aman rice, 11 varieties of aus rice and 13 varieties of boro rice were collected. The collected seeds were tagged and preserved for further research.

Study 2. Evaluation of germination, growth and yield performance of the collected rice germplasms under salt stress

A series of laboratory and field experiments were carried out in three distinct rice growing seasons viz. Aus, Aman and Boro seasons. The factorial experiment consisted of two factors such as - Factor A: Four different concentrations of salt solution (saline water) viz. S_0 = Control (distilled water), S1= 5 dS m-^{1}, S_2 = 10 dS m-^{1}, S_3= 15 dS m-^{1} and Factor B: Indigenous rice varieties (Aus, Aman and Boro). The experiments were laid out in a Completely Randomized Design (CRD) with three replications.

The laboratory experiments were conducted in the Agronomy Laboratory of Agrotechnology discipline, Khulna University to evaluate seed germination and seedling growth parameters of indigenous rice germplasms under different levels of salinity.

Germination parameters such as Germination Energy (%), Germination Capacity (%), Germination Percentage (%), Germination Speed (%) and seedling growth parameters such as Seedling growth rate (mg/day), Shoot length (cm), Root length (cm), Seedling dry weight (mg) were evaluated.

The field experiments were conducted in pot soil in three seasons viz. aus, aman and boro seasons to determine the growth and yield potentiality of the collected germplasms under salt stress. The following parameters were studied:

i) Plant height	ii) Days to flowering
iii) Total number of tillers plant^{-1}	iv) Number of effective tillers plant^{-1}
v) Length of panicle	vi) Total number of grains panicle^{-1}
vii) Number of filled grains panicle^{-1}	viii) Grain yield
ix) Biological yield	x) Harvest index

Results

The results revealed that all the germination and seedling growth parameters were gradually decreased with increasing salinity levels. Among the aman varieties germination capacity of Nonakochi, Enchi, Jataibalam, Chiniatap, Sahebkochi, Sadamota, Mondeswar, Kalomota, Talmugur, Mulagati, Boushohagi, Barjomuri, Motha, Sadagotal, Lalmota was found good whereas, varieties Gochi, Khainol, Sudha were poor. Seedling growth rate reduced drastically at 15dSm-1 salinity (46.76%). Varieties Nonakochi, Sahebkochi, Jingashail, Sadamota, Enchi, Jotaibalam exhibited higher seedling growth rate, while, lower seedling growth rate was observed in Rajashail, Khoinol, Sudha, Rupeswar and Gochi. Seedling vigour index was high in Talmugur, Khakshail, Harisanker, Murabajol, Barjomuri, Motha but that was low in Mulagathi, Kalomota, Swarna, Khejurchari, Hatibazor, Gopalvog.

Grain weight reduced significantly due to increase in salinity. Some varieties showed high yield potentiality such as Hoglapata (52.78 gpot^{-1}), Ranisalut (49.43 gpot^{-1}), Sudha (47.28 gpot^{-1}) , Ghunshi (45.69 gpot^{-1}), Rupeshwar (42.27 gpot-1). Some varieties showed moderate salinity tolerance while some were sensitive to high salinity. Varieties Ghunshi (45.69 gpot^{-1}), Ranisalut (49.43 gpot^{-1}), Hoglapata (52.78 gpot^{-1}), Sudha (47.28 gpot^{-1}) had high yield potentiality but showed susceptibility to high salinity (60 - 75% yield reduction in 15dSm^{-1}). Moderate yielder Moinamoti (29.49 gpot^{-1}), and low yielder Kumrogorh, Jamainaru, Horgoja, Kashfulbalam, Satin, Volanath, Kalomota, Talmugur showed moderate salinity tolerance (31- 48% yield reduction).

Aus rice varieties Nayantara, Kajollata, Chandromoni and Kaloshate were superior to other varieties considering their germination and seedling growth attributes whereas Kajollata, Nayanmoni and Kolmilota were performed better over others variety considering their growth and yield attributes under salt stress.

In case of boro varieties, Kusmus, IT and Ashan boro were found superior and Nayon tara, Bapoy and Nayon moni were inferior based on germination and seedling growth parameters under salinity stress.

Table 1. Effect of variety on seed germination parameters of fifteen local aman rice

Variety	Germination capacity (%)	Germination energy (%)	Seedling growth rate (mg day-1)
Rajashail	32.50 h	11.50 i	5.25
Benadol	70.50 f	37.33 g	12.5
Holdegotal	78.50 de	12.00 i	15.75
Lalgotal	80.50 d	17.33 h	13.63
Sadamota	98.67 ab	84.50 b	29.50
Khainol	16.00 j	5.50 j	3.75
Kutepatnai	76.50 e	52.50 e	15.00
Nonakochi	99.17 ab	79.00 cd	**33.00**
Sudha	19.83 i	7.50 ij	26.25
Enchi	99.17 ab	74.66 d	25.88
Jotaibalam	99.33 ab	**95.00 a**	29.00
Chiniatop	**99.50 a**	75.33 d	12.25
Kalojira	95.33 b	56.50 e	13.75
Sahebkochi	99.33 ab	83.00 bc	**33.00**
Dakshail	97.50 ab	79.00 cd	17.38
Jingashail	96.33 ab	83.33 bc	32.88
Rupeswar	40.83 g	8.67 ij	6.25
Katarivogh	86.00 c	44.33 f	16.13
Mondeswar	97.00 ab	43.66 f	25.63
Gochi	**15.67 j**	**4.17 j**	**2.75**
CV (%)	6.80	12.29	15.81
Level of Significance	0.01	0.01	0.01
LSD	3.45	4.73	-

The figures in a column having different letter(s) are significantly different at 1% level by DMRT.

Table 2. Effect variety on yield and yield contributing characters of fifteen local aman rice

Variety	Effective Tiller (no.)	Filled grain/panicle (no.)	Unfilled spikelet/ panicle(no.)	1000 grain weight (g)	Grain yield/pot (g)	Straw yield/pot (g)	Harvest Index (%)
V_1	10.67bc	98.83cd	14.72	35.18abc	36.92abc	55.58f	39.64ab
V_2	10.67def	100.33cd	15.88	37.26ab	32.73bc	87.42a	25.86d
V_3	10.42bc	103.42bcd	24.26	37.40ab	40.09ab	66.58b-f	37.48abc
V_4	8.08ef	122.83abc	28.11	38.48a	39.72abc	69.92bcd	33.13bcd
V_5	11.67ab	117.83abcd	29.57	34.60bc	47.28a	61.58def	43.04a
V_6	8.83cdef	101.67cd	14.72	33.72c	30.69bc	67.75b-e	30.96cd
V_7	10.00bcd	98.92cd	18.11	35.82abc	35.74abc	63.58c-f	35.45bc
V_8	10.33bcd	126.42ab	13.39	30.40d	39.19abc	57.25ef	39.83ab
V_9	10.58bc	130.00a	18.29	28.73d	41.27ab	72.42bcd	34.88bc
V_{10}	7.42f	97.92d	14.95	37.95ab	27.68c	74.67bc	26.89d
V_{11}	10.08bcd	101.75cd	20.37	36.49abc	37.52abc	57.17ef	38.81ab
V_{12}	9.58cde	96.50d	15.59	35.35abc	32.51bc	72.92bcd	32.23bcd
V13	12.42a	114.42a-d	20.57	30.48d	42.27ab	77.25b	34.91bc
V_{14}	10.08bcd	114.17a-d	23.66	28.71d	32.87bc	56.08f	36.77abc
V_{15}	10.08cde	107.33a-d	24.49	37.68ab	38.17abc	64.25c-f	35.38bc
CV (%)	20.82	23.42	69.53	10.28	34.41	18.30	23.17
Level of significance	0.01	0.01	NS	0.01	0.05	0.01	0.01
LSD value	1.66	20.61	-	2.87	10.29	9.91	6.56

The figures in a column having different letter(s) are significantly different at 1% level by DMRT. V1 = Rajashail, V2 = Sadamota, V3= Khainol, V4 = Kutepatnai, V5 = Sudha, V6 = Enchi V7 = Jotaibalam, V8 = Chiniatop, V9 = Kalojira, V10 = Sahebkochi, V11 = Dakshail, V12 = Jingashail, V13 = Rupeshwar, V14 = Katarivogh, V15= Gochi

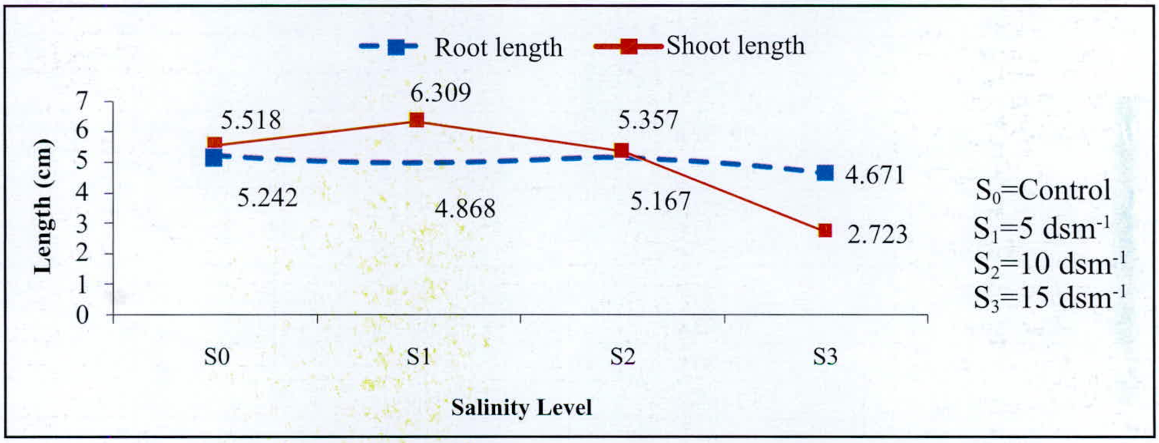

Fig. 1. Effect of salinity on root length and shoot length of indigenous aus rice varieties

Table 3. Interaction effect between salinity and variety on yield attributes of indigenous Aus rice

Variety	**Salinity level (dS m^{-1})**	**Grain yield/hill**	**Harvest index (%)**	**1000 grain weight (g)**
Noyon Tara	0	5.81 f-j	29.54 b-d	20.30
Ratul		10.92 c-h	33.14 a-c	23.47
Rani Ratul		10.26 c-h	19.29 g-j	20.77
Sri balen		9.86 c-h	18.74 g-k	24.89
Mala		16.07 bc	28.55 b-e	17.27
Kolmilata		19.00 b	32.85 a-c	22.07
Nayanmoni		13.30 b-e	26.33 b-f	22.07
Noncha		31.01 a	34.80 ab	23.46
Kopila ice		8.59 d-i	34.09 ab	17.44
Kajollata		15.30 b-d	40.67 a	20.81
Noyon Tara	5	4.13 g-j	24.20 d-g	18.86
Ratul		4.23 g-j	15.15 h-k	22.74
Rani Ratul		4.62 g-j	13.24 h-m	19.53
Sri balen		5.03 f-j	12.40 h-m	21.04
Mala		12.23 b-f	27.99 b-f	18.79
Kolmilata		10.87 c-h	26.35 b-e	19.25
Nayanmoni		10.94 c-h	25.17 c-g	23.65
Noncha		20.58 b-d	31.88 a-c	20.57
Kopila ice		2.53 ij	11.35 j-n	16.74
Kajollata		11.12 c-g	39.05 a	20.78
Noyon Tara	10	3.90 g-j	19.95 f-i	19.85
Ratul		2.41 ij	13.76 h-l	24.22
Rani Ratul		2.04 ij	10.62 k-o	16.93
Sri balen		3.11 ij	10.14 k-o	20.90
Mala		3.71 j	10.60 k-o	18.71
Kolmilata		8.58 d-i	20.56 e-h	17.75
Nayanmoni		5.04 f-j	20.33 e-h	20.34
Noncha		10.78 c-h	22.93 e-h	17.79
Kopila ice		1.77 ij	12.30 h-m	16.61
Kajollata		5.73 f-j	38.56 a	22.09
Noyon Tara	15	1.373 j	12.84 h-k	16.98
Ratul		1.67 ij	11.46 i-n	22.72
Rani Ratul		0.91 j	8.10 j-n	18.05
Sri balen		1.73 j	8.23 j-n	20.62
Mala		2.36 j	9.44 i-m	14.14
Kolmilata		5.24 ij	17.46 h-k	18.51
Nayanmoni		4.45 j	18.54 h-m	15.11
Noncha		5.58 h-j	17.47 h-k	17.92
Kopila ice		1.30 j	9.99 i-n	18.80
Kajollata		3.84 g-j	35.27 ab	24.41
CV		28.60%	18.50%	13.24%
LSD		6.169	7.38	-
LS		**	**	NS

The figures in a column having different letter(s) are significantly different at 1% level by DMRT.
** = Significant at 1% level, CV= Coefficient of Variation

Table 4. Effect of variety on seed germination parameters of local boro rice

Variety	Germination Energy (%)	Speed of germination (%)	Seedling dry weight (mg)	Seedling vigor index
Kusmus	65.49 a	62.49 bc	54.68 f	1092.63 ab
IT	67.17 a	78.84 a	90.03 ab	708.47 d-f
Sylhety boro	27.83 c	41.54 e	63.02 d-f	771.35 de
Tere balem	53.33 b	60.93 bc	62.92 d-f	962.35 bc
Shate boro	44.83 b	47.40 de	79.58 bc	1172.85 a
Abdul hai	24.00 c	25.40 f	91.70 ab	955.90 b-d
Bere ratna	63.50 a	67.11 b	70.06 c-e	947.04 b-d
Nayon tara	48.17 b	50.72 d	56.68 ef	827.70 c-e
Ashan boro	70.00 a	78.65 a	89.98 ab	1029.10 ab
Kali boro	51.17 b	56.34 cd	93.64 a	1091.11 ab
Bapoy	0.00 d	0.00 g	59.36 ef	944.89 b-d
Nayon moni	0.00 d	0.00 g	53.86 f	349.49 f
GS one	45.67 b	51.13 d	39.93 g	695.48 e
CV (%)	17.36	16.72	18.94	17.90
Level of Significance	**	**	**	**

The figures having same letter(s) in a column are not significantly different by DMRT.
** = Significant at 1% level, CV= Coefficient of Variation

Conclusion

A series of experiments were conducted at the Agronomy Laboratory and Dr. Purnendu Gain Field Laboratory of Agrotechnology Discipline, Khulna University, Khulna to evaluate the salinity tolerance of indigenous rice varieties of South western coastal area of Bangladesh. The results revealed that all of the germination and seedling growth parameters were gradually decreased with increasing salinity levels (0 - 15dS m-1). In Aman season, considering all the germination and seedling growth parameters, Moinamoti, Khejurchari, Swarna, Mulagathi, Hamai, Jotaibalam, Sadamota and Sahebkochi were found superior and Khainol and Gochi were found inferior. In case of growth and yield contributing characters, Moinamoti, Boushoghi, Hoglapata, Ranisalute, Khejurchari, Swarna, Mulagathi, Ghunsi Sudha, Kalojira and Rupeswar performed well. The Moinamoti can tolerate soil salinity up to 15 dS m-1 with better harvest index (21.53%) and Khejurchari, Swarna, Mulagathi, and Hamai can perform well up to 10 dS m-1 soil salinity compared to control.

Aus rice varieties Nayantara, Kajollata, Chandromoni and Kaloshate were superior to other varieties considering their germination and seedling growth attributes whereas Kajollata, Nayanmoni and Kolmilota were performed better considering their growth and yield attributes under salt stress.

In Boro season, rice varieties Kusmus, IT and Ashan boro were found superior and Nayon tara, Bapoy and Nayon moni were inferior based on germination and seedling growth parameters under salinity stress.

From the findings of the research it might be reasonably concluded that-

- A considerable number (84) of indigenous rice germplasms of Aus, Aman and Boro varieties were collected from south west region of Bangladesh.

- The indigenous rice varieties Nayantara, Kajollata, Chandromoni and Kaloshate, Nayanmoni, Moinamoti, Boushoghi, Jataibalam, Hoglapata, Ranisalute, Khejurchari, Swarna, Mulagathi, Ghunsi, Sudha, Kalojira and Rupeswar, Hamai Kusmus, IT and Ashan boro were might be considered as superior variety considering germination and seedling growth and yield attributes under salt stress which can be used as genetic resource for breeding purpose in future.
- These indigenous varieties will act as crop insurance under extreme salinity condition in future.

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Sarkar T. A., Ali M.A., Das D., Ali M.Y. and Remme R.N. 2016. Salt stress on germination and seedling growth of indigenous Aman rice*(Oryza sativa). Khulna University Studies,* 14(1&2): 49 – 57.

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Variability and Segregation Pattern in *Bipolaris sorokiniana* (Sacc.) Shoem

Sanjoy Kumar Adhikary and Md Monirul Islam

Location: Agrotechnology Discipline, Khulna University , Khulna
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 2500000.00

Introduction

Wheat production played significant role in ensuring food security in Bangladesh, India, Pakistan, and Nepal (Basheer and Atawnah, 2014). Wheat production become lessens with progressing time due to several biotic and abiotic factors. Global climate changes have a major impact on wheat production by increasing severity of diseases including leaf blotch (Hossain and Teixeira da Silva, 2013). Leaf blotch disease of wheat causes *Bipolaris sorokiniana* (Sacc.) shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph: *Cochliobolus sativus*) is an important and serious disease (Joshi and Chand, 2002). *Bipolaris sorokiniana* causes serious yield loss, it might be go up to 100% (Acharya *et al.,* 2011). Grain yield losses had been reported 15% to 16% in Nepal and Bangladesh (Bhatta *et al.,* 1997 and Alam *et al.,* 1998). *Bipolaris sorokiniana* is heterothallic fungi and exhibits variability (Aggarwal *et al.,* 2009). Detailed knowledge on pathological and genetic variability of *B. sorokiniana* is useful to observe epidemiological aspects of disease spread (Poloni *et al.,* 2009).

Objectives

Determination of pathogenic and genetic variability among isolates of *Bipolaris sorokiniana* isolated from different wheat growing regions of Bangladesh and to explore segregation pattern in the telomorph.

Methodologies

Sample collection area: Leaf blotch diseased samples were collected from dofferent Agroecological regions (AEZ) of Bangladesh.

Isolation, identification, purification and pathogenicity test of fungi: The fungus was isolated following tissue planting method (Mian, 1995). Morphological identification of fungi was done following Barnett (1972).

Experiment-1: Evaluation of pathogenic variability among the isolates of *Bipolaris sorokiniana*

For pathogenicity study susceptible wheat variety (Kanchan) was grown. According to Adhikary and Mian (2005) seven components of disease development were considered for pathogenicity assessment. Disease severity was measured on a standard scale of 0-5 as suggested by (Rahman *et al.,* 2011). The percent disease index (PDI) was calculated following a standard formula (McKinney, 1923).

$$\text{PDI} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum grade}} \times 100$$

Mean data of seven components of disease development were subjected to multivariate analysesby using SPSS statistics.

Experiment-2: Assessment of genetic diversity among the *Bipolaris sorokiniana* isolates using RAPD marker

PureLink fungal DNA purification kit was used for DNA extraction. Polymerase chain reaction (PCR) were carried out in a 20 µl volume containing 200 µm of each dNTP, 1× buffer, 1.2 mM MgCl2, 1 U of Taq DNA polymerase, 1µl primer and 50 ng of DNA. Amplifications were conducted in a thermo cycler using the following protocol: 94°C for 2 min followed by 40 cycles of 94°C for 30 sec, 30°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 5 min. Amplification products were separated by electrophoresis on 1% agarose gel. Study of genetic diversity, each of the DNA profile for each primer was recorded on the basis of binary data of the presence (1) versus absence (0) of the same length. The dendrogram was constructed following Unweighted Pair Group Method using Arithmetic Average (UPGMA).

Experiment-3: Explore genetic segregation patterns based on RAPD traits

Trait wise Segregation analysis Using Random Amplified Polymorphic DNA (RAPD) markers: Genomic DNA isolation, quantification andpolymerase chain reaction weresame as previous experiment 2.Trait wise segregation pattern was analyzed following Bucci *et al.* (1993). Subsequently, chi-square (χ2) calculation was done following Graph Pad Prism version 7.0 and goodness-of-fit ratio was calculated following Kar and Halder (2010).

Results

A total of 169 isolates were found after completion of standard isolation technique from leaf blotch diseased leaves of wheat, which collected from different wheat growing Agroecological regions (AEZ) of Bangladesh.

Morphological identification of Bangladeshi isolates of *Bipolaris sorokiniana*

Conidia of *B. sorokiniana* Germinated Conidia of *B. sorokiniana*

Fig. 1: Conidia of *Bipolaris sorokiniana* shows the identifying characters (bipolar germination)

Evaluation of pathogenic variability among the isolates of *Bipolaris sorokiniana*

Dendogram showing Hierarchical cluster groups (less virulent to more virulent) of *B. sorokiniana* isolates bases on seven component of disease development in wheat.

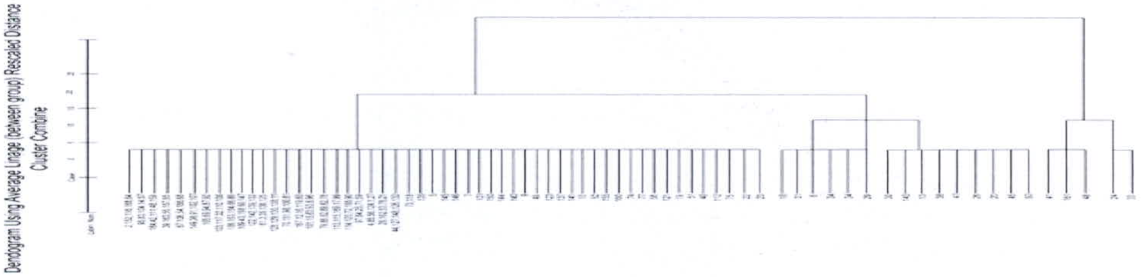


Fig. 2: Dendogram based on seven component of disease development in wheat

Assessment of genetic diversity among *Bipolaris sorokiniana* isolates using RAPD marker

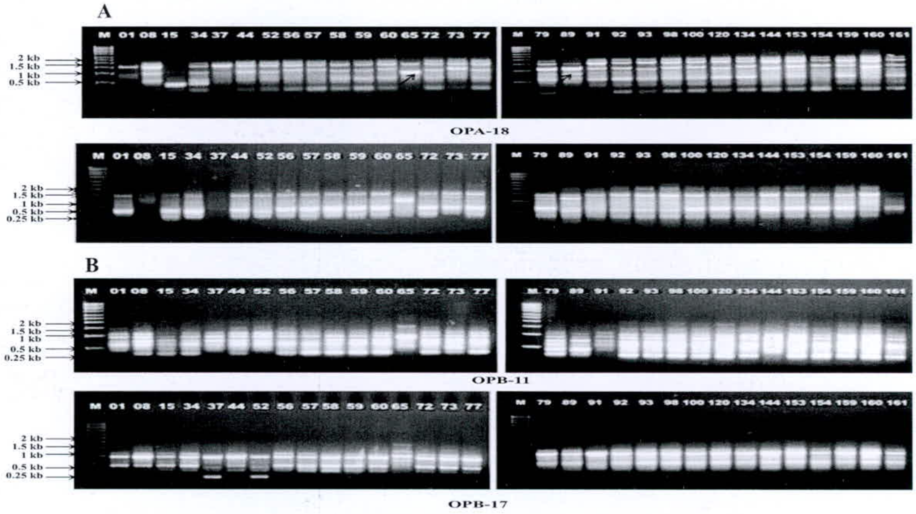


Fig. 3: A and B: RAPD profile of 31 isolates (01, 08 and so on) of *Bipolaris sorokiniana* generated by using OPB-18, OPA-18, OPB-11 and OPB-17 primers. M- Kilo base molecular weight ladder.

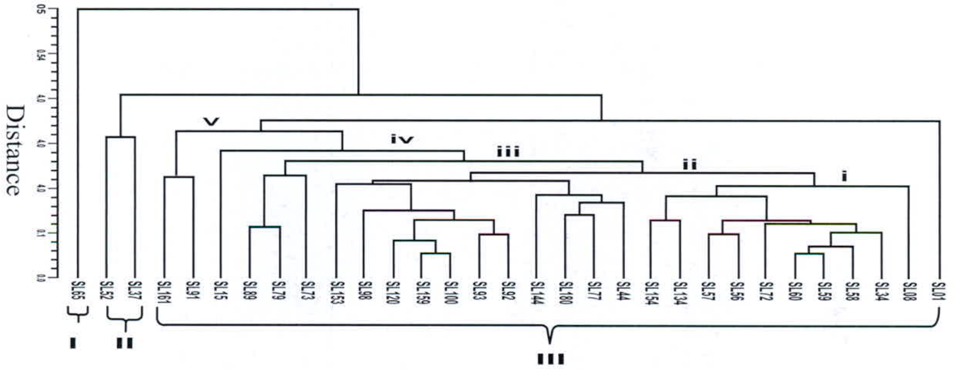


Fig. 4: Dendrogram constructed based on combined data obtained from using 9 primers in RAPD analysis of 31 isolates of *Bipolaris sorokiniana*

Exploration of genetic segregation patterns based on RAPD traits

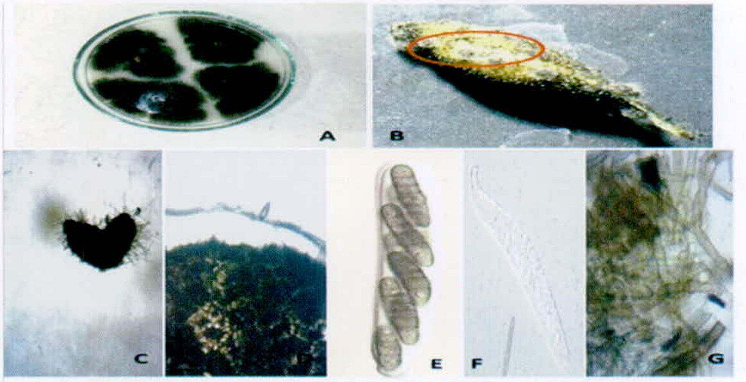


Fig. 5: Sexual structures (pseudothecia) over the barley seed after 21 days of incubation.

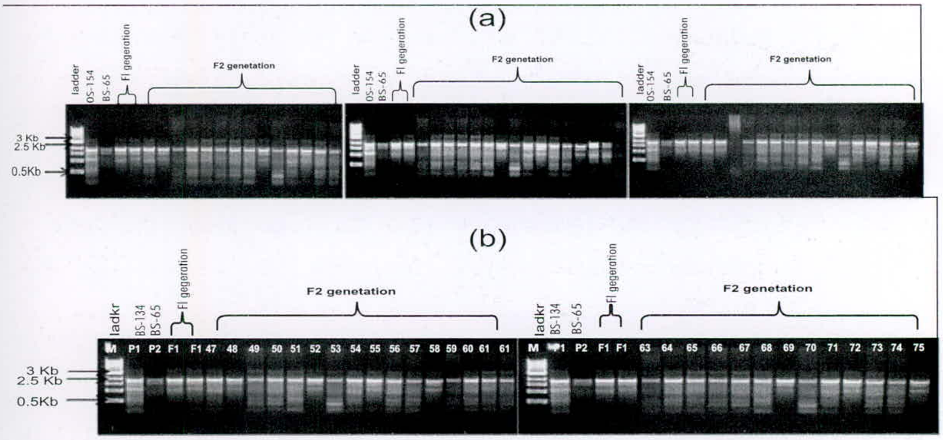
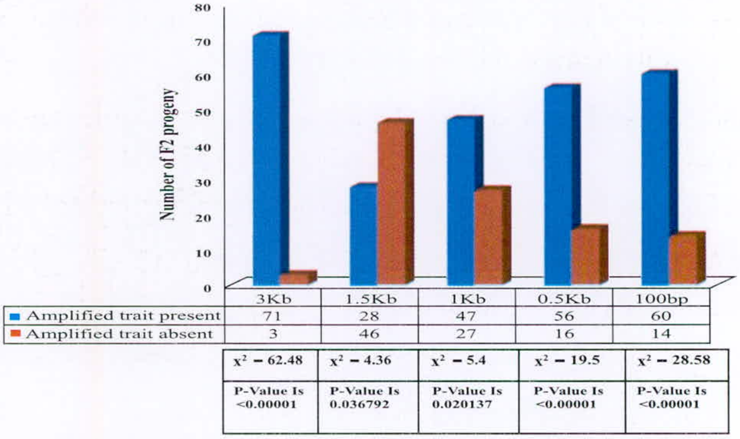


Fig.6: (a, b). DNA fingerprinting profiles of parent (BS-134 and BS-65), F1 generation and 75 F2 progeny of *Bipolaris sorokiniana* by using OPA-18 primer



	3Kb	1.5Kb	1Kb	0.5Kb	100bp
🟦 Amplified trait present	71	28	47	56	60
🟥 Amplified trait absent	3	46	27	16	14

x^2 – 62.48	x^2 – 4.36	x^2 – 5.4	x^2 – 19.5	x^2 – 28.58
P-Value Is <0.00001	P-Value Is 0.036792	P-Value Is 0.020137	P-Value Is <0.00001	P-Value Is <0.00001

Fig. 7: Trait wise segregation ratio based on DNA band size of 74 F2 progeny of *Bipolaris sorokiniana*

Conclusion

Pathogenic and genetic study revealed that *B. sorokiniana* is a highly diverse pathogen. Experimented 169 isolates of *B. sorokiniana* were grouped in five distinct clusters in pathogenic variability by multivariate analysis. In genetic variability study three clusters were found by performing RAPD analysis. Segregation pattern analysis of *B. sorokiniana* based on molecular approach by RAPD marker discovered that trait-wise distribution ratio was significant and each of the traits was fit to the 1:1 ratio, which follows Mendelian law of equal segregation.

Publications from this Research

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Mono-sex Culture of *Macrobrachium Rosenbergii* and Its Impact on the Economy of Bangladesh

Khandaker Anisul Huq and S M Bazlur Rahaman

Location: Fisheries & Marine Resource Technology Discipline, Khulna University, Khulna
Duration: Three years (2013-2016)
Expenditure of the project: : Tk. 2000000.00

Introduction

Freshwater prawn has huge potentiality in fisheries sector because of its universal appeal, good taste, high unit value and increasing demand in the world market. As a sub-tropical climatic condition and vast area of fresh water, Bangladesh has a unique opportunity to culture *M. rosenbergii* commercially (Akand & Hasan, 1992; Ahmed, 2001; Muir, 2003a). Aquaculture production has been affected by several biological factors, including gender, sexual maturity and age of the animals (Ahmed, 2004). A number of crustacean species exhibit bimodal growth patterns, in which males exhibit superior growth to females or vice versa (Ahmed, 2004). In *M. rosenbergii,* males have been recorded to have higher growth and Baghel *et al.* (2004) tried to alter the sex ratio using bio-encapsulated live *Artemia*. They also reported that in all male culture system the prawns reach to market size faster and make the ponds available for further culture to start with new crop. Considering the potentiality and opportunities of all male prawn culture, the present study was designed to test the growth and production performance of mono-sex male giant freshwater prawn at different stocking densities.

Objectives

- To compare growth, survival and production performance of mono-sex male with mono-sex female and mixed sex *Macrobrachium rosenbergii;*
- To identify the suitable stocking density of mono-sex prawn culture and to evaluate its economic profitability, and
- To increase farm production and to uplift the socio-economic condition of the marginal prawn farmers through disseminating the technology.

Methodologies

The experiment was conducted in experimental ponds (each 120 m^2) of FMRT Discipline, Khulna University. Well established methodologies for nursery pond preparation of prawn were followed with some modifications. Chemicals and fertilizers were applied at the following rate; lime at 1 kg/dec, rotenone at 200 g/dec, urea at 0.5 kg/dec and TSP at 0.5 kg/dec. Water depth of the ponds were always kept 1.0-1.5m.

The experiment was done by two distinct phases within the two years' time frame. In first year prawn PL was reared for 75 days (from July 01 to September 15, 2010) up to sex separation stage where stocking density was 25 PL/m2. The PL was nursed with 35 % protein. Feeding rate was 200 g/10000 PL and at the frequency of three times per day. After nursing, the prawns were stocked at 3 juveniles/m2 according to designed treatments with three replications (e.g. all male, all female and mixed- sex (50 % male and 50 % female)). Growth performance of prawns recorded throughout the 150 days culture period (September 16, 2010 to February 15, 2011). In grow-out pond SABINCO supplementary quality pellet feed containing 30 % protein was given at 10 % to 3 % of their body weight and two times a day. Sampling for length-weight data, water quality (pH, DO, transparency & salinity) were done monthly.

In second year prawn PL were reared for 75 days (July 01 to September 15, 2011) to separate all male juveniles. The all male prawn juvenile were stocked in three different densities 1, 2 and 3/m2 with 3 replications and reared for 165 days (September 16, 2011 to February 28, 2012). Same procedure was followed for pond preparation, feed and feeding, water quality, sampling and length-weight data collection and data analysis.

Results

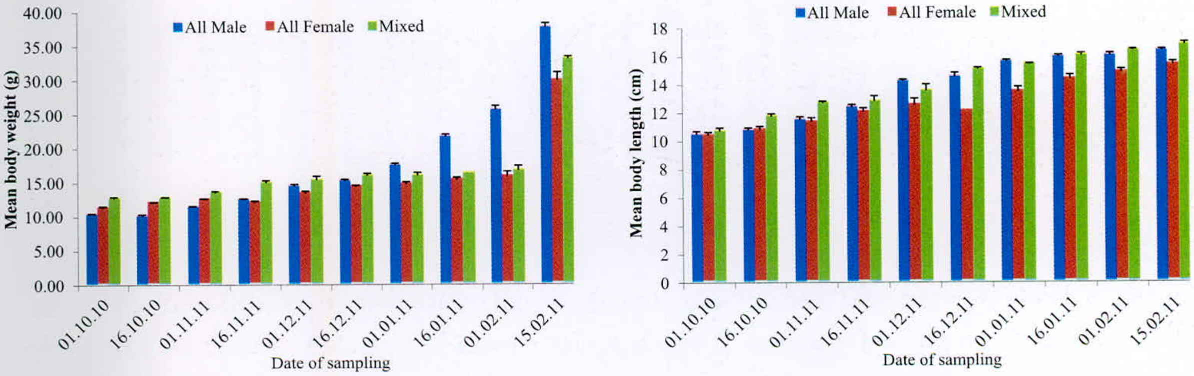

Fig. 1: Mean body weight and mean body length of 3 different groups of prawn revealed by fortnightly sampling. The error bars indicating standard error of fifteen different samples.

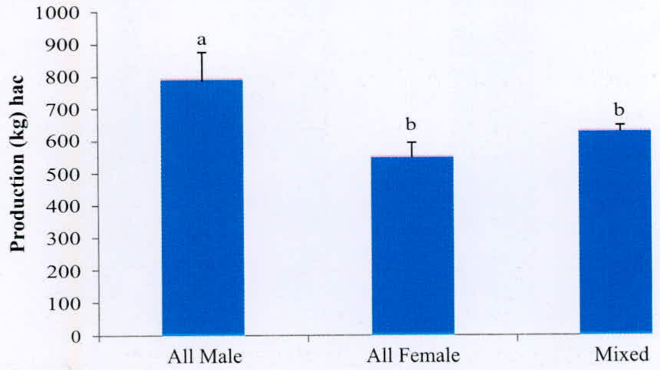

Fig.2: Production performace of all male, all female and mixed sex prawn after 150 days culture in earthen experimental ponds. The error bars indicating the standard deviations of three replicates. Significance test was done by one way anova using SPSS 16.0 version ($P < 0.05$).

Table 1. The survival rate, average individual weight and total production (mean±STDEV) of all male, all female and mix-sex prawn during the 1st year experiment for 5 months (1 September 2011 to 31 January 2012).

Culture type	Stocking density	Total Stocked	Total harvest (piece)	Survival rate	Average weight (g)	Mean harvest (g) in 120 m^2	Production/ha (Kg)
All Male	3	360	244	67.78±5.9	38.83±3.6	9.48±1.0	789.61±85.6^a
All Female	3	360	233	64.72±0.96	28.33±3.9	6.60±0.1	550.14±44.2^b
Mix-sex	3	360	227	63.05±2.17	33.07±5.8	7.51±0.3	625.51±22.8^b

The 1st year study revealed that after 5 months culture the growth, survival and production performance were higher in all male mono-sex prawn culture compare to all female and mix- sex (50%male and 50% female) culture of *Macrobrachium rosenbergii* practicing same stocking density in all 3 treatments. The highest production was obtained at 789.611 Kg/ha for all male and the lowest at 550.14 Kg/ha for all female and in mix-sex produced was found 625.511 Kg/ha.

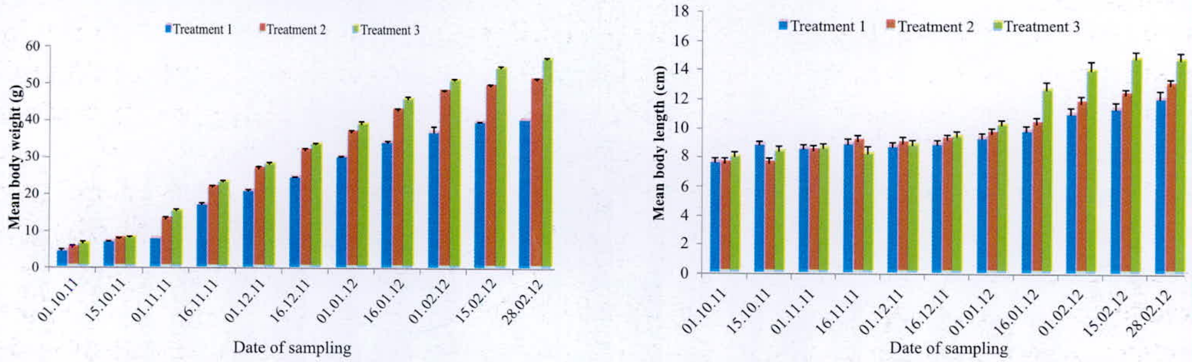

Fig. 3: Mean body weight and mean body length of all male prawn at different stocking densities (Treatment 1 = 3/m2, Treatment 2 = 2/m2, Treatment 3 = 1/m2). The error bars indicating the standard error of fifteen samples.

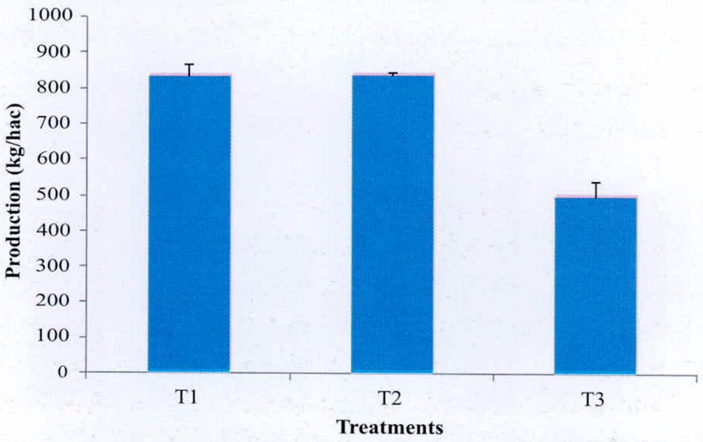

Fig. 4: Production performace of all male prawns at different stocking densities (T1 = 3/m2, T2 = 2/m2, T3 = 1/m2) after 165 days culture in earthen experimental ponds. The error bars indicating the standard deviations of three replicates. Significance test was done by one way anova using SPSS 16.0 version (P < 0.05).

Table 2. Total production (mean±STDEV) of mono-sex male prawn at different stocking densities for165 days culture (16 September 2011 to 28 February 2012).

Treatment	Stocking density/m^2	Total Stocked	Total harvest (piece)	Survival rate (%)	Average weight (g)	Total harvest weight (g)	Production/ha (Kg)
T1	3	360	242	67.22±3.0	41.42±1.8	10.02±0.4	835.30±34.9^{a}
T2	2	240	193	80.42±3.1	52.25±2.2	10.08±0.1	840.35±9.1^{a}
T3	1	120	103	85.83±3.0	58.27±1.5	6.00±0.5	500.15±42.0^{b}

In the second phase it was found that in case of all male mono-sex culture stocking density 2 juvenile/m^2 showed higher production (840kg/ha) than 3 juvenile/m^2 (835kg/ha) and 1 juvenile/m^2 (500kg/ha). Therefore, 2 juvenile/m^2 stocking density could be suggested to practice in the farmer's pond to get higher production and benefit.

Conclusion

The first year study revealed that the growth, survival and production performance were higher in all male mono-sex prawn culture compare to mono-sex female and mixed sex culture of *Macrobrachium rosenbergii*. In second year study, it was found that in case of all male mono-sex culture the stocking density 2 juvenile/m^2 showed best performance considering individual growth, survival and also the total production. Therefore, in case of all male prawn culture, 2 juvenile/m^2 stocking density could be suggested to practice in the farmers pond to get higher production and benefit. As the land and space have been becoming scarce day by day and today our understanding on productive ecology is still poor, various innovative technologies must be developed and applied to enhance productivity especially from the aquaculture sector in Bangladesh. Prawn has wide range of culture area from coastal to any freshwater reservoir over the country. As a developing country prawn has diverse contributions such as food production, employment opportunity for income-poor fisher-folks, and valuable foreign currency earnings. It is necessary to produce all male prawn PL through genetic study and using health safe sex hormone with PL feed.

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Morphological and Assemblage Patterns of Gastropod within Environmental and Habitat Variation of South-western Bangladesh

Salma Begum and Prosun Kumar Ghosh

Location: Environmental Science Discipline, Khulna University, Khulna
Duration: Three years (2013- 2016)
Expenditure of the project: Tk. 2200000.00

Introduction

South-western coastal region of Bangladesh (eg. Khulna, Jessor, Satkhira, Bagerhat, Gopalgang, Pirojpur districts and adjacent areas), is already vulnerable due to many development disasters, the expected impacts of climate change would manifold increased the current vulnerabilities. Since climate change is a dynamic phenomenon, in order to appreciate changing climate over the region or the region, efforts should be made to define one or more scenarios of a changing climate in relation to the area and its resources (Lewin 2006). The south western region of Bangladesh is blessed with world's largest mangrove Sundarbans, with its rich floral and faunal biodiversity. Gastropod mollusks are being popular for their socioecological significance in the Southwestern Bangladesh. Gastropods are one of the most diverse groups of mollusks which include (snails, whelks, slugs, limpets). The gastropods shells collected in the coastal areas are used in many different purposes, eg. making poultry and fish feed, lime production, ornamental usage, paint making etc. Gastropods meat is used in the coastal shrimp and prawn hatcheries as feed for mother shrimp in greater Khulna district and adjacent areas. The main harvest season for Gastropods is winter and early summer (November –March). There is a greater economic utility of Gastropod muscle and shell for its contribution in the growth, nutrition, and egg spawning in poultry & duck industry and in shrimp hatchery. It is also a nutritious food for human being and is randomly exploited by coastal communities. The exploitation of the Gastropod resources could have a direct effect to ecosystem and the socio-ecological environment of the southwestern region of Bangladesh.

Objectives

The main objective of the project is to facilitate ecological research related to climate change impacts on vulnerable coastal ecosystems of Bangladesh by calibrating an environmental indicator (e.g. *Pila globosa*) from local as well as regional level.

The specific objectives are-

- To investigate distribution pattern of the gastropods morphometry(as morphological assemblage) I relation to the physic chemical environmental conditions in the six localities (twelve sites) of south western region of Bangladesh;
- To compare shell and soft body morphometry of the gastropods in terms of dry shell, body dry mass of sampled areas;
- To evaluate whether morphometric differences can be related to differences in environmental (salinity and temperature) regime; and
- To recommend the socioecological activity and ecosystem management associated with gastropod mollusk.

Methodologies

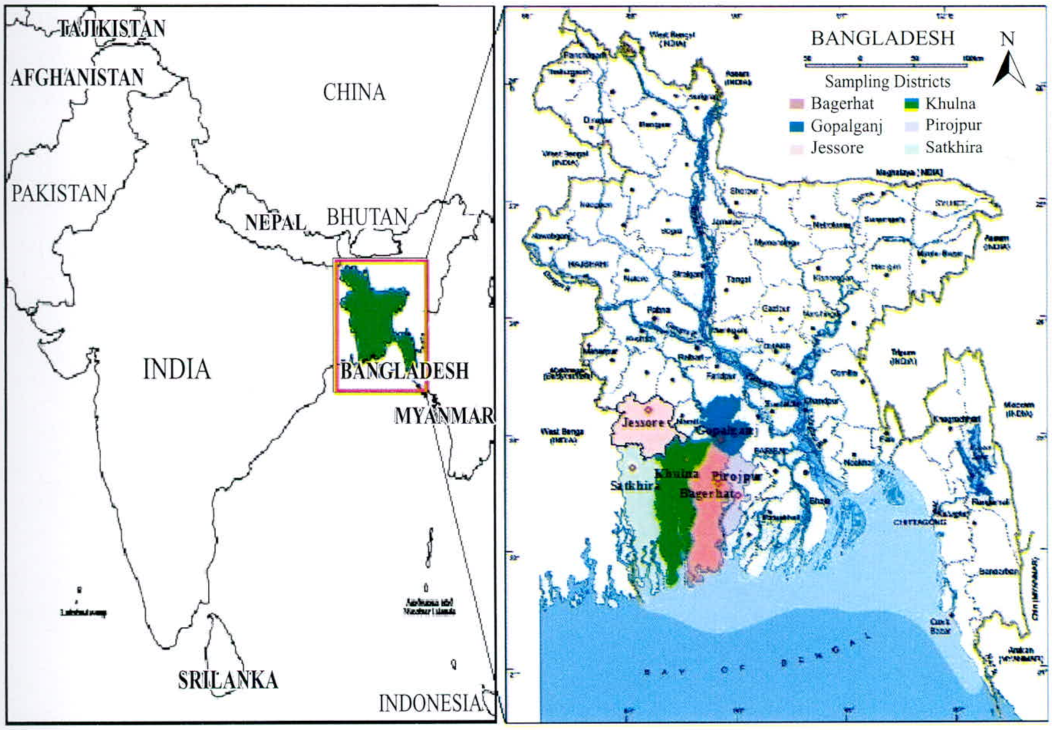

Fig 1: Location of sample collection areas for the gastropod

Sampling sites were as follows: Khulna (at three sites such as, Koyra, Paikgacha sadar and Chandkhali), Satkhira (Kaliganj, Shyamnagar and Munshigonj), Bagerhat (Bagerhat Sadar, Morrelganj and Sarankhola), Gopalganj, Jessore and Pirojpur. a total of 2160 samples were collected. Primary information and data were collected by focal group discussions (FGDs), sampled gastropods morphological (length, height, shell mass, softbody mass), habitat ecological (water and sediment quality such as pH, EC, TDS, Temperature, Ca, Mg, P, Na, salinity) parameters were analysed (APHA 1995; Begum et al.2009) in laboratory of environmental science discipline. Secondary data were collected from journals and books. The variables relationships were examined in correlation matrix, AVOVA test and descriptive analysis, factor analysis test to see the overall variability.

Results

Descriptive analysis showed Satkhira samples have the highest variability of body mass, this might be due to the close connection to sea water (Table 1, 2) which also resulting the monthly higher variation of shell thickness in Satkhira samples (Fig 2).

ANOVA found significant variation of water dissolved oxygen in Gopalgang-Pirojpur (p= 0.012), Khulna -Pirojpur(p= 0.023) ad Satkhira –Pirojpur (0.019). Sediment showed slightly alkaline. Result found that there are significant variations of heights (L-1), widths (L-2), aperture lengths (L-3), dry shell mass and thickness of the mollusks among different sites (Table 3) in the south-west coastal area of Bangladesh at 5% level of significance. R-mode factor analysis was done on the dataset.

Principal component analysis and Varimax rotation with Kaiser normalization were applied and found that five factors were extracted to statistically explain the variability of mollusks in the south-west-coastal region of Bangladesh such as above mentioned morphological factors, sediment salinity, water pH, Electrical conductivity and Total dissolved solids and Calcium. The workshop showed the conservative perspective and views of the stakeholders suggested the gastropod mollusk needs socio-ecological awareness involving the local people, controlled harvesting and need to conserve the useful animal.

Table 1: Descriptive statistics of the overall morphological parameters of the gastropod mollusks for different sites in the south-western coastal area of Bangladesh

South-west coastal area	**Morphological parameter**	**Statistics**					
		Maximum	**Minimum**	**Mean**	**Standard deviation**	**Median**	**Coefficient of variation**
Gopalganj (n=360)	L1 (cm)	6.25	3.10	4.49	0.65	4.50	14.56
	L2 (cm)	5.74	2.49	3.68	0.57	3.66	15.45
	L3 (cm)	4.92	2.00	3.49	0.47	3.46	13.58
	L4 (cm)	3.23	1.10	2.02	0.40	2.00	19.19
	Dry Shell mass (gm)	23.65	2.01	7.87	3.80	7.41	48.24
	Body dry mass (gm)	10.08	0.07	2.27	1.48	1.92	65.09
	Thickness (cm)	0.34	0.03	0.14	0.06	0.13	44.97
Khulna (n=360)	L1 (cm)	6.90	2.54	4.25	0.76	4.34	17.98
	L2 (cm)	5.23	1.83	3.52	0.76	3.58	21.69
	L3 (cm)	4.86	0.31	3.29	0.63	3.40	19.20
	L4 (cm)	3.23	0.80	2.02	0.47	2.10	23.05
	Dry Shell mass (gm)	22.70	1.38	7.39	3.61	7.25	48.85
	Body dry mass (gm)	6.93	0.24	2.21	1.35	1.87	61.02
	Thickness (cm)	0.45	0.01	0.13	0.06	0.13	42.28
Satkhira (n=360)	L1 (cm)	6.34	2.90	4.57	0.67	4.53	14.68
	L2 (cm)	5.20	2.10	3.68	0.61	3.62	16.45
	L3 (cm)	4.85	1.95	3.54	0.53	3.47	15.04
	L4 (cm)	3.00	1.10	2.09	0.37	2.13	17.78
	Dry Shell mass (gm)	22.94	1.09	8.33	4.08	7.73	48.95
	Body dry mass (gm)	8.30	0.39	2.81	1.74	2.29	62.11
	Thickness (cm)	0.35	0.01	0.14	0.06	0.14	45.67
Pirojpur (n=360)	L1 (cm)	6.69	2.10	4.29	0.75	4.34	17.57
	L2 (cm)	5.30	2.10	3.63	0.68	3.62	18.65
	L3 (cm)	4.74	1.80	3.32	0.60	3.38	17.95
	L4 (cm)	3.10	1.00	2.02	0.43	2.07	21.15
	Dry Shell mass (gm)	21.93	1.42	7.51	3.79	7.24	50.48
	Body dry mass (gm)	8.29	0.31	2.44	1.53	1.97	62.94
	Thickness (cm)	0.28	0.03	0.14	0.04	0.13	31.70

Table 2: Descriptive statistics of the overall morphological parameters of the gastropod mollusks for different sites in the south-western coastal area of Bangladesh

South-west coastal area	Morphological parameter	Statistics					
		Maximum	Minimum	Mean	Standard deviation	Median	Coefficient of variation
Jessore (n=360)	L1 (cm)	6.60	2.34	4.38	0.74	4.40	16.90
	L2 (cm)	5.60	2.07	3.60	0.63	3.57	17.43
	L3 (cm)	4.94	2.00	3.41	0.57	3.42	16.82
	L4 (cm)	3.10	1.09	2.05	0.37	2.10	17.37
	Dry Shell mass (gm)	25.62	1.23	7.38	3.75	6.62	50.80
	Body dry mass (gm)	11.56	0.37	2.36	1.64	1.81	69.63
	Thickness (cm)	0.25	0.04	0.13	0.04	0.13	32.66
Bagerhat (n=360)	L1 (cm)	6.27	2.85	4.41	0.68	4.38	15.42
	L2 (cm)	5.10	2.26	3.60	0.58	3.50	16.23
	L3 (cm)	4.64	2.20	3.39	0.51	3.40	15.12
	L4 (cm)	2.90	1.12	2.07	0.40	2.14	18.71
	Dry Shell mass (gm)	20.54	1.26	7.67	4.08	7.18	53.18
	Body dry mass (gm)	9.78	0.28	2.60	1.67	2.23	64.27
	Thickness (cm)	0.26	0.04	0.14	0.05	0.14	34.76

Table 3: Result of the one-way ANOVA test for the morphological parameters of the sampling sites in the south-west coastal area of Bangladesh

Parameter	F-value (df=2159)	p-value	Significant mean difference between groups	Remarks
L-1	10.29	0.000	Gopalganj-Khulna (p=0.000); Gopalganj-Pirojpur (p=0.000); Gopalganj-Jessore (p=0.040); Khulna-Satkhira (p=0.000); Khulna- Jessore (p=0.016); Khulna-Bagerhat (p=0.002); Satkhira-Pirojpur (p=0.002); Satkhira-Bagerhat (p=0.002); Bagerhat-Pirojpur (p=0.023)	Significantly vary in different sites of the south-west coastal area
L-2	3.33	0.005	Gopalganj-Khulna (p=0.000); Khulna-Satkhira (p=0.001); Khulna-Pirojpur (p=0.021)	Significantly vary in different sites of the south-west coastal area

Continued

Parameter	F-value (df=2159)	p-value	Significant mean difference between groups	Remarks
L-3	10.42	0.00	Gopalganj-Khulna (p=0.000); Gopalganj-Pirojpur (p=0.000); Gopalganj-Jessore (p=0.032); Gopalganj-Bagerhat (p=0.015); Khulna-Satkhira (p=0.000); Khulna-Jessore (p=0.006); Khulna-Bagerhat (p=0.014); Satkhira-Pirojpur (p=0.000); Satkhira-Jessore (p=0.002); Satkhira- Bagerhat (p=0.001)	Significantly vary in different sites of the south-west coastal area
Dry shell mass	3.16	0.008	Khulna-Satkhira (p=0.001); Satkhira-Pirojpur (p=0.005); Satkhira-Jessore (p=0.001); Satkhira-Bagerhat (p=0.023)	Significantly vary in different sites of the south-west coastal area
Thickness	3.97	0.001	Gopalganj-Jessore (p=0.000); Khulna-Jessore (p=0.042); Satkhira-Jessore (p=0.001); Pirojpur-Jessore (p=0.002); Jessore-Bagerhat (p=0.001)	Significantly vary in different sites of coastal area

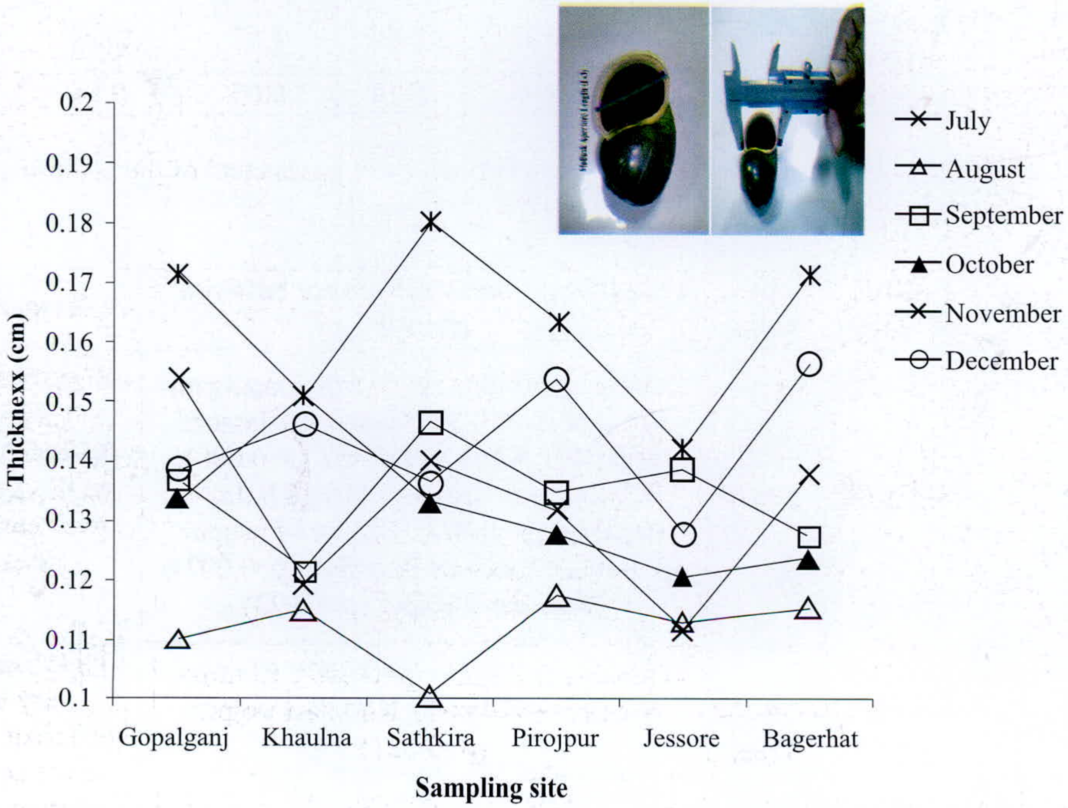

Fig 2: Monthly variation of thickness of the gastropods shell for different sites

Fig 4: Result dissemination workshop on Morphological assemblage of gastropod mollusk

Conclusion

The research project was the first attempt to understand the morphology of gastropod mollusk in the south western region in terms of the habitat and environment. The variation may need more localized data set and this result could act as a benchmark for the further detailed research. The mollusk research is necessary to know the past and in terms of the climate prediction in future. More local data is needed to calibrate this environmental recorder organism for the climate ecological research.

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Impact of Climate Change on Water Safety and Prevalence of Water-borne *Escherichia coli* and *Vibrio* spp. Associated Diseases in South-Western Coastal Communities of Bangladesh

Ms Selina Akter and Pravas Chandra Roy

Location: Department of Microbiology, Jashore University of Science and Technology, Jashore
Duration: Three years (2013 – 2016)
Expenditure of the project: Tk. 2000000.00

Introduction

Global Warming has already started to affect Bangladesh. The salinity intrusion and its disastrous effects in landscape, ecology and human health already created wide scale agony amongst the inhabitants in coastal belt. The remote communities at the South-Western coastal region are severely affecting by the scarcity of drinking water due to increased salinity of primary natural aquifers, which left secondary freshwater installations (rain water harvest etc.) the only choice. But still these sources can serve only seasonal supply. Scarcity induces contamination and imposes health risk. A considerable number of waterborne diseases like diarrhea and cholera cases with significant mortality are general features of these communities. The project assesses the seasonal fluctuation on surface-water quality, drinking water resources, vulnerability of the population to waterborne diseases as well as the impact of contamination in fish farming. The study addressed seasonal fluctuation in bacterial community structure specially that of Escherichia coli and Vibrio spp. in aquatic system. Identification of principle cleavage in water management and hygiene practices, tracking route of contamination and assessing vulnerable group in the community prone to devastating climate change are the major goals of the project.

Objectives

The aim of the project is to assess the effect of seasonal fluctuation on water quality, safety of drinking water, vulnerable age and sex group prone to waterborne diseases and their water management adaptation to climate change. The prevalence of two major waterborne bacterial pathogen, Escherichia coli and Vibrio spp. aimed to assess in natural aquatic systems and communities to establish its seasonal fluctuation, to identify public health risk and vulnerability.

Specific objectives

- Measurement of physicochemical parameters of both natural aquifers, natural fish farming lands and secondary drinking water installations for two consecutive years;
- Monitoring of water resources management and maintenance of personal hygiene;
- Advanced microbiological and molecular approaches to detect the major bacterial contamination in the both the aquatic system and drinking water sources; and
- Specimen collection and detection of etiological agent in clinical cases of waterborne diseases for prevalence study.

Methodologies

Measurement of physicochemical parameters such as Salinity, Conductivity, Total dissolved Solids, Dissolved oxygen, pH and Temperatures in the natural water system, like flowing river, canals and lakes as well as the small freshwater aquifers in the community to monitor the physical and chemical changes occurring between seasons.

Monitoring of the maintenance performances of the potable water management, evaluation of the possible route of drinking water contamination. Management of personal hygiene by using two indicators e.g., prevalence of UTI and assessing skin microbial flora.

Sampling (monthly) of water was done from drinking water sources, fish farming areas, rain water harvests from coastal communities of Bangladesh to detect pathogenic *Vibrio spp.* and *E. coli.*

Molecular detection of pathogenic bacterial population by polymerase chain reaction technique.

Monitoring of the waterborne disease prevalence in the community; specially including the survey on UTI chances in the community. Vulnerable age group and sex group determination, which are prone to water borne disease prevalence.

Results

A. Result on skin samples

The study has been carried out to isolate and identify bacterial isolates from human skin persisting with high level of arsenic from shallow tube-well and also with salinity from different saline contaminated water sources. The study also has been designed to investigate bacterial isolates from healthy human skin for a comparative analysis. To delineate the adaptive potential as well as tolerance to high arsenic concentrations, comparative growth rate and survival study of isolates has been done on media containg varrying concentrations of arsenic ($NaAsO_2$) and salt ($NaCl$).

Total Microbial Load is Comparatively Higher in Arsenic and Saline Contaminated Area

When compared to the two contaminated area, microbial count was found significantly lower in the skin of control populations. The total microbial count in arsenic area is much higher than the control area with a mean value of 338.13 x104 and 17.64 x104 respectively. The count is also comparatively higher in saline area (mean value 45.04 x104). The mean of total microbial load along with the mean total of Gram positive and also that of Gram negative bacteria from the three study are shown in the bar chart. In the chart a ratio has been used. The actual value is ten thousand times higher than the given value.

Gram Negative Bacteria Predominate Over Gram Positive in Arsenic Subjects Whereas the Scenario is Opposite in Saline Subjects

To verify whether there is any change of microbial composition of Gram positive or Gram negative microbes on the skin, a total 360 of which 117 isolates from unexposed subjects and 114 and 129 isolates were isolated from arsenic and salinity exposed subjects respectively. 66 out of 114 isolates were Gram negative in arsenic area which was found 1.4 times higher over the control group. On the other hand Gram positive bacteria predominate in the salinity exposed subjects. Among 129, 66 isolates were found to be Gram positive with another 15 unknown isolates.

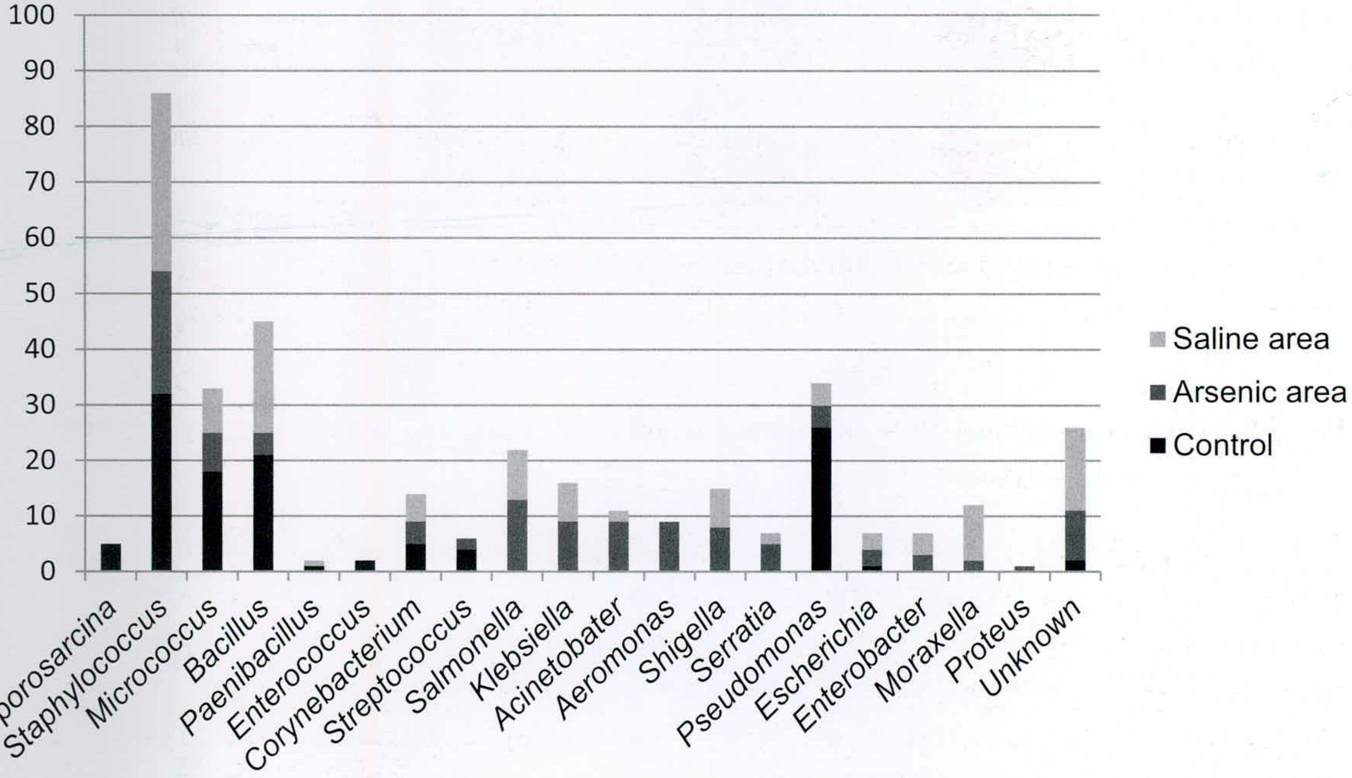


Fig. 3.1: Comparative analysis of the occurrence of various bacterial genera on skin of human population exposed to different water.

Results on samples of fish farming area

During January 2014 –January 2015, the present study was designed with the intention to detect shrimp pathogenic *Vibrio* spp. isolated from three regions in Bangladesh including Satkhira (Amian village), Sundarban (Kolagacia, Koromjol, Harbaria, Harbaria Ecotourism, Mongla Gath, Porkochia) and Southern Chittagong (Chera Dip, Naf River, Teknaf Beach, Laboni Point, Saint Martin). Extensive biochemical tests were performed in order to measure the variability of biochemical behavior among the strains. Detailed biochemical study revealed that all the strains had the biochemical characteristics typical of *Vibrio cholerae.* However variations were observed in some reactions.

Prevalence of different Vibrio spp. Isolated

Among different samples of the three regions of Bangladesh, a total of six Vibrio spp were identified. Those are V. parahaemolytus, V. cholera, V. alginolyticus, V. fischeri, V. harveyi and V. metschnikovii. A regional distribution pattern was observed (Figure 3.2).

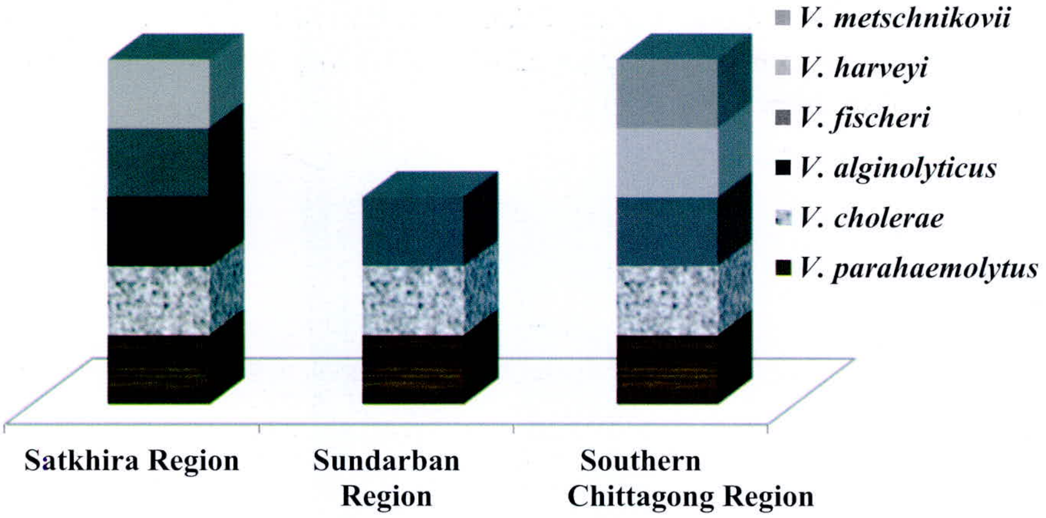


Fig. 3.2 Graphical representation of distribution of different Vibrio spp. among samples collected from three regions of Bangladesh.

Though a regional pattern of species wide distribution was observed in this study, prevalence pattern of different Vibrio spp were also affected by the type of samples taken. .

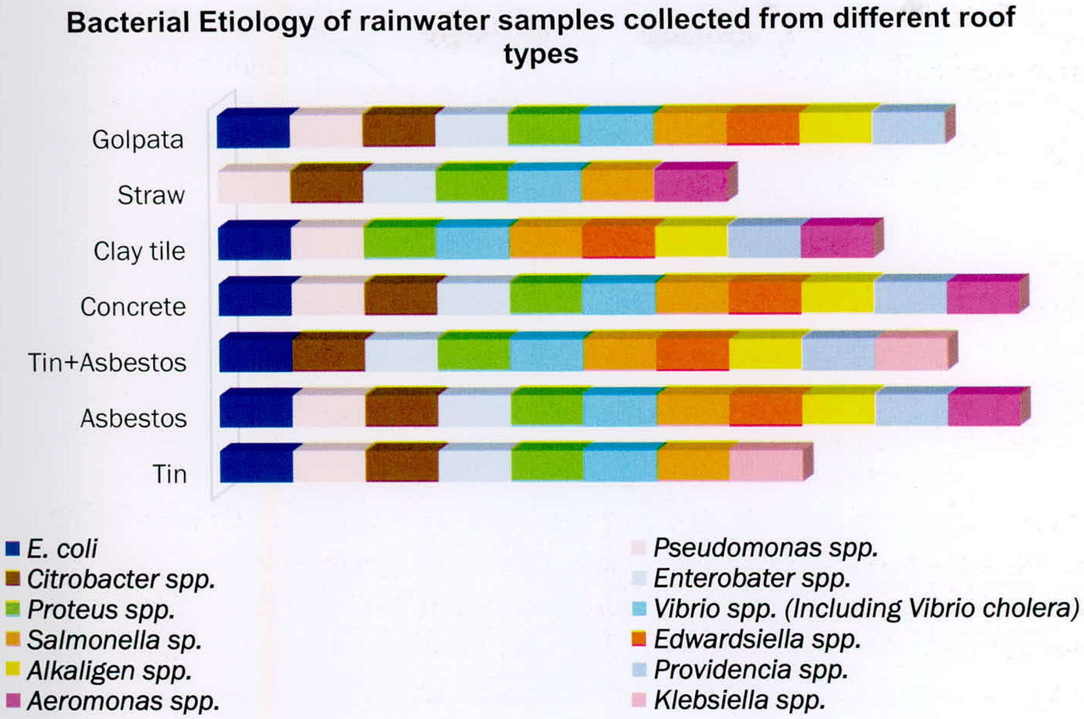
Results on rainwater harvest in coastal population

The aim of the study was to identify the bacteriological quality of rainwater harvest used by the local community of the salinity affected population.

Identification of principle cleavage in water management and hygiene practices.
To track the route of contamination and assessing vulnerability of the material used for roof making to the quality of rainwater harvest.

Total 40 rain water samples were collected from different houses located in the coastal area of Bangladesh within two districts i.e., Khulna (Goraikhali, Paikgacha) and Satkhira (Syamnagar).

Previous study confirmed that Shyamnagar and Paikgacha are severely vulnerable with water salinity problem (Hasan *et al.* 2013).



Conclusion

Aquaculture is an important commodity in the global fishery trade due to its increasing demand and competitive international price. According to FAO, shrimp alone contributes about 6.03% of the world's total seafood export. Shrimp has become a very important exportable commodity in Bangladesh, and is ranked second in earning foreign exchanges in national economy. Among the groups of microorganisms that cause serious losses in shrimp culture, the best known are bacteria because of the devastating economic effects they have on affected farms. *Vibrios* are the most common genera associated with crustaceans are common inhabitants of the aquatic environment including shrimp culture, which may also responsible for vibriosis, a well recognized cause for disease and mortality. *Vibrio* species have become a major source of concern for shrimp culture because of their close association with low survival rates in hatcheries or grow out ponds. New shrimp pathogens belonging to the *Vibrio* genus have been described although their virulence is not yet fully understood. Indeed, they may act as opportunistic agents in secondary infections or be true pathogens. The major objective of this study was to determine the occurrence of potentially pathogenic *Vibrio* spp. in different aquatic environmental samples, both from natural reservoir as well as potential area for shrimp and crab aquaculture. A total of 233 *Vibrio* spp. were isolated from three coastal districts of Bangladesh covering major aquaculture sites as well as natural pristine habitats of crustaceans. Forty nine samples (water, sediment, algal plankton, shrimp and crap) were processed so far, among which *V. parahaemolyticus* was found as the most prevalent (45% samples) vibrios. Significant numbers of samples were found to carry *V. cholera* (21%) and *V. fischeri* (18%). *V. alginolyticus, V. metshnikovii, V. fluvialis, V. vulnificus* and *V. cincinattiensis* were also isolated, though 3% vibrios hence remained unidentified.

V. alginolyticus was found only in aquaculture field (gher), and *V. metshnikovii* in coast line. *V. cholerae, V. parahaemolyticus, V. alginolyticus, V. fischeri,* and *V. harveyi* were found in both healthy shrimp and crab, which usually have potential to cause disease. The abundance of *Vibrio* spp. is expected because these are indigenous to brackish and marine environment, but potential pathogenic strains in the environment and shrimp pose risk to human health and economic loss.

Rain water can support the growth of many types of organisms when it comes in contact with substances which itself is contaminated. Contamination by bacteria like *Escherichia coli, Vibrio* spp., *Shigella* spp., *Salmonella* spp., is the main concern of water contamination, which could be life threatening to population depends on rainwater harvest. Total 40 rain water samples were collected from different location in the coastal area of Bangladesh, within 4 hrs of collection these water samples were pre-enriched in Nutrient Broth. 100 µL of pre-enriched sample were transferred to three different enrichment media. Isolated colonies on both MacConkey Agar as well as TCBs Agar were used for further biochemical tests for identification. Physico-chemical parameters were measured during sampling. Maximum microbial diversity obtained from rainwater harvested from Asbestos and Concrete roof. Maximum TDS obtained from rain water collected from roof made up of asbestos. Most prevalent organism were found as *E. coli* (24%) and *Enterobacter* spp. (22%). In a few cases *Klebsiella* spp. (2%) and *Alkaligen* spp. (2%) were also identified. The consumption of rain water collected from roof, especially from asbestos as well as concrete roof is risky. Many of the organisms that have been isolated from contaminated roof water have the potential for human pathogenicity, which under certain conditions can lead to infection and possibly disease outbreaks, notably gastrointestinal. Rain water harvesting roof should be clear enough to reduce the risk of such contamination.

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Addressing Low Cropping Intensity by Fitting Crops in Rice-Based Cropping Pattern in South-Western Bangladesh

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Location: Agrotechnology Discipline, Khulna University, Khulna
Duration: Three Years (2013-2016)
Expenditure of the project: Tk. 1800000.00

Introduction

Among the marginal lands worldwide, saline soil ranks top where the cropping intensity (CI) and productivity is low compared to the non-saline fertile lands. This holds true for south-western Bangladesh where salinity reduces crop productivity thus the CI is low. The arable land of this region remains fallow in the dry season (January – June) due to late transplanting and harvesting of aman rice (one crop in a year, CI 100%) because of, in addition to salinity; late and poor draining of soil and lack of good quality irrigation water (Karim *et al.* 1990; Mondal *et al.* 2006). According to Watt *et al.* (2013), to maintain crop production to keep pace with declining good land for agriculture (land using for cities, roads etc.), move to more marginal lands with new crops or management practices are the options for increasing CI. All options involve improved agronomic practices e.g., with less water, less fertilizer, and early crop establishment for avoiding salinity (Fischer and Edmeades 2010).

As no field crop is cultivated during the rabi and Kharif 1 season in this region, a crop that requires less water than that of rice (boro after aman) may be tested for its suitability to fit after t. aman for increasing CI. From this context, maize was selected for investigating its sowing window, nitrogen fertilizer requirement and response to saline water irrigation (which is abundant in the river and canal in this region).

Objectives

- To investigate the suitability of maize to grow after T. *aman* rice for increasing cropping intensity in the south-western Bangladesh (Khulna);
- To assess the nutrient (nitrogen) requirement of maize for grain yield under conventional and minimum tillage system in this region; and
- To investigate the response of maize to saline water irrigation.

Methodologies

We selected three locations with medium high land: two in Batiaghata Upazilla and one in Dakope (in polder 31) of Khulna district. In the three-year study, BRRIdhan 23 was in all sites as *T. aman* rice. Maize (BARI hybrid maize 7) was grown in all sites after *T. aman* in each year. Before *T. aman* season in the 1st year, a small pond was excavated in each site adjacent to the farmers' field to reserve rain water for irrigation.

In the first year, sowing date (six dates, 10 January to 2 March 2014, 10 days interval) trial of maize was done (sowing after full tillage and by hand dibbling) in three sites, to find out the suitable sowing window of this new crop in the south-western saline region.

In the second year, fertilizer (nitrogen, N) trial was done in all sites. The N treatment was 0, 50, 100 and 150% of the recommended rate of N for the selected maize variety (BARI 2014). One-third of N and other fertilizers were given during final land preparation, 1/3 after 28 days after emergence (DAE) and the rest 1/3 at 60 DAE.

In the third year, irrigation trial was done in two locations: one in Fultala, Batiaghata and another in Pankhali, Dacope, Khulna. The irrigation treatments were: pond water - control, canal water, 50% pond water + 50% river water, 50% pond water + 50% canal water. Recommended fertilizer dose was applied. All maize experiments were laid out following RCBD with four replications. The data of all maize experiments were analyzed statistically.

Results

All the maize experiment showed that this crop (a new crop in this region, introduced by the current project) can be fit after *T. aman* which increased CI from 100% to 200% in the project area.

In the sowing date trial in the first year, in general, maize grain yield was higher in the conventional full tillage than that of dibbling in all three sites. Among the sowing dates, sowing in February produced higher grain yield (~7.5 t/ha) that the early and late sowing dates (~6.5 t/ha). Plants in the last sowing were damaged at grain filling stage due to water logging created by early heavy rain.

In the second year, among the N treatments, the 100% and 150% recommended N produced statistically similar yield (8.5 and 8.7 t/ha, respectively). The 50% N (of recommended dose) produced grain 4.2 t/ha while the nil N produced much lower yield (1.5 t/ha).

Investigation in the first and second year showed that maize can be grown successfully in this region if the crop is irrigated with pond water. As the number of ponds and the water amount in them is not sufficient to cultivate maize in large areas, the third year experiment aimed at mixing saline water from canal and river with pond water to maximize the water volume for irrigation.

In the third year, the crop response at both sites (Fultala and Pankhali) were approximately similar, thus, the data of Pankhali is provided in Table 1. The maximum grain yield was found under pond water (control) irrigation (which is usual) followed by pond + canal water (78% of the control) and pond + river water (65% of the control) (Table 1). Lowest yield was observed under canal water irrigation which was 42% of that of control. Irrigation with saline water increased soil salinity that reduced the grain yield substantially (e.g., canal water irrigation).

The potential grain yield of this variety is maximum 11 t/ha in rabi season, grain yield is lower in the Kharif 1 season. Our studies were done in Kharif 1 season and in the marginal land (excessively wet at the sowing time and salinity during the reproductive stage of the crop), 5.8-7.5 t/ha with pond + canal water irrigation may give a handsome return.

Table1: Grain yield and electrical conductivity of irrigation water and topsoil (0-10 cm) of maize field in Pankhali site. Three irrigations were given. 'DAS' refers to days after sowing.

Irrigation	Water EC (dS m^{-1}) during irrigation			Soil ECe (dS m^{-1}) after irrigation (5 days after irrigation)			Grain yield (t/ha)
	1st (31 DAS)	2nd (56 DAS)	3rd (81 DAS)	1st	2nd	3rd	
Pond water	0.6	0.8	1.0	1.1 d	1.6 d	1.6 d	7.4 a
Canal water	7.0	10.0	14.0	3.1 a	4.2 a	6.0 a	3.1 d
Pond + river	5.3	7.2	9.0	2.5b	3.1 b	3.9 b	4.1 c
Pond + canal	3.8	5.4	7.0	1.8c	2.8 c	3.4 c	5.8 b
			CV (%)	6.2	3.4	5.3	5.5
			Level of significance	**	**	**	**

Conclusion

Main output of the project

1. Maize showed wide sowing window from January to February. For increasing cropping intensity, maize can be grown after *T. aman* in the project area where the land after aman rice harvest remains fallow.
2. The recommended nitrogen for BARI hybrid maize 7 may also be applied for maize in this region.
3. For irrigation, mixing of pond water with canal water can be used for maize cultivation.

The current study showed that in the south-western region, for increasing CI, maize can be grown successfully after *T. aman* if a small pond is excavated adjacent to farmers' field to harvest rain water for irrigation. Although the excavation of pond depends of farmers' willingness and economic ability, yield return from the second crop (maize in this study) will encourage them to cultivate dry season crops. In the fertilizer trial, the result showed that the recommended rate for the BARI hybrid maize 7 is also suitable for this region. In our study, maize crop required three irrigations and the harvested rain water in the small pond sometimes did not fulfill the irrigation requirement. To increase in the volume of pond water by mixing canal or river water may supplement the irrigation requirement. In irrigation trial in the third year, the mixture of pond plus canal water produced 5.8 t/ha grain of maize. This can give a substantial economic return to the farmers otherwise the land would have remained fallow – means no return.

Further study need to be done (i) on early establishment of dry season crops (*T. aman* should be HYV with short duration) that can avoid soil salinity: crop to be harvested before the salinity level becomes constraint to reduce yield; (ii) drain out of excess water after *T. aman* harvest to bring the soil moisture at field capacity for sowing seeds of dry season crops timely; (iii) in addition to surface drain, deep and/or sub-surface drain need to be tested.

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Screening the Antiviral Activity of Some Medicinal Plants Against White Spot Syndrome Virus (WSSV) – as Potential Way for the Sustainable Shrimp Production in Bangladesh

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Location: Fisheries and Marine Resource Technology, Khulna University, Khulna
Duration: Three Years (2013-2016)
Expenditure of the project: Tk.1000000.00

Introduction

White Spot Syndrome Virus (WSSV) can cause high mortality rates of 100% in black tiger shrimp, (*Penaeus monodon*) which may lead to the serious loss in the sustainable shrimp production in fisheries sector throughout the world including Bangladesh. It is reported that outbreaks of WSSV infection have wiped out within a few days the entire population of many shrimp farms and hatcheries (Sanchez-Paz 2010). Control of viral disease is quietly hard. Ironically till now no effective treatment could be developed to combat the virus. However, in India, Malaysia, Thailand there are some report to use some chemicals (formalin, sodium hypochlorite) and medicinal plants such as *Cynodon, Ceriops, Spirulina* sp have potency to protect shrimp from WSSV (Sudheer *et al.,* 2011; Balasubramanian *et al.,* 2007, 2006; Hemtanon *et al.,* 2005). To control the WSSV infections, Bangladesh also should develop preventing and curative method of this disease since shrimp production occupies an important position of national economy. Till date no research work has been conducted in this regard in Bangladesh. Realizing the situation an intensive investigation has been planned to find out a way to control the pathogen WSSV through using various medicinal plants, employing PCR technology (PCR and nested PCR) which is the most powerful technique to detect the virus.

Objectives

- Detection of White Spot Syndrome Virus (WSSV) infection using powerful technique of Polymerase Chain Reaction (PCR & nested PCR); and
- To investigate of the antiviral activity of the medicinal plants to control the WSSV infection.

Methodologies

Selection of study area and sampling:

To isolate and detect WSSV, shrimps were collected from different ghers of Satkhira Sadar upazila of Satkhira and Rampal upazila of Bagerhat for our study. Live shrimp *P. monodon* weighing from 19-31g and 25-38g respectively were randomly collected from the selected *ghers* during the period from August to September, 2014 and June to September, 15.

After collection, the samples were then immediately taken to the laboratory using oxygenated polythene bags containing the same *gher* water in a insulated styrofoam box. In each sampling, 5 shrimp were examined from each *gher*. To detect the pathogen, the organ samples of pleopods were removed aseptically, weighted and preserved at -200C for DNA extraction to detect the virus of WSSV.

DNA extraction and purification:

For DNA extraction from the infected shrimp, *AccuPrep®* Genomic DNA Extraction Kit from Bioneer was used. After extraction, DNA pellet was re-suspended in 80 µl of TE-RNAse (10mM Tris, 1mM EDTA). For each sample, DNA concentration was checked with 260 nm readings using a UV spectrophotometer (Micronal Mod. B382). The DNA purity was found by using following formula:

$$\text{Purity} = \text{Absorbance at } A_{260} / \text{Absorbance at } A_{280}$$

DNA amplification by a thermal cycler from the sample:

PCR reactions were carried out in 50ul of reaction mixture that consist of 10 mM dNTP, 1.5 mM $MgCl_2$, 1 mM each primer, 0.02 U Taq DNA polymerase (Invitrogen) in a PCR buffer (Tris-HCL, 10 mM; KCl,50 mM; pH 8.3). Diagnostic PCR for WSSV was carried out using the primers of 146 R2-F2 (941bp) and I.K 3-4 (298bp). The PCR was performred in a thermocycler for 35 cycles. Each cycle consists of three steps of denaturation, annealing and elongation, and final extention for 10 min.

To investigate of the antiviral activity of the medicinal plants to control the WSSV infection

A. Preparation of plant extract: *Freshly Cynodon* dactylon (locally named as 'Durba Grass'), *Momordica charantia* ('Korola') and *Aegle marmelos* ('Bel'), plant leaves were collected separately from field of Khulna University and washed with distilled water and dried in shadow. The shadow dried leaves were heated in oven at 50°C for 10 min and grinded by using mixer grinder (Capacitor start motor, WUHU motor factory, China). Approximately 200g of powdered plant material was macerated with 100% methanol at ratio of 1:10 and kept at room temperature for 72 hrs. Mixture was stirred every 24 h using sterile glass rod. This mixture was then filtered through Whatman® No.1 filter paper. Filtration procedure was done further twice for complete extraction of the bioactive compounds. The filtrate was then collected in a separate beaker and concentrated by evaporating the solvent. The extracts were then re-suspended in the methanol solvent before testing it for antiviral evaluation. The crude extract was kept in refrigerator (-20°C) until use (Khan *et al,* 1988; Jayanthi *et al,* 2013).

B. Required Dose Preparation: 0.30g and 0.45g of crude methanol extract of *C. dactylon, M. charantia* and *A. marmelos* were accurately measured by the electronic balance and taken in two different small vials. Then few (1ml) of methanol was added and triturated in unidirectional manner using a vortex mixer, after proper mixing the volume was adjusted by methanol for each and the concentration of these solutions became 100 mg/kg and 150 mg/kg of animal body weight.

C. Preparation of Viral Inoculum

The virus inoculum was prepared according to the method of Singh *et al.* 2005. In brief, a composite sample of pleopod and soft parts of the cephalothorax (500 mg) from collected *Penaeus monodon* was macerated in 10 ml cold PBS (in 100 ml solution, 1000 mM NaCl 1.37 ml; 1000 mM KCl 0.027ml; 1000 mM Na_2HPO_4 0.1 ml; 1000 mM KH_2PO_4 0.018 ml; double-distilled water 98.485 ml) with glass wool to a homogenous slurry using a mortar and pestle in an ice bath. The slurry was centrifuged at 12000 rpm in a refrigerated centrifuge at 4^0C, and the supernatant was filter-sterilized using a 0.45-µm-pore-size Millex®-GS MF Milipore™ MCE membrane (Milipore Corp., Ireland) (Fig.1). The preparation was stored at -20^0C until used.

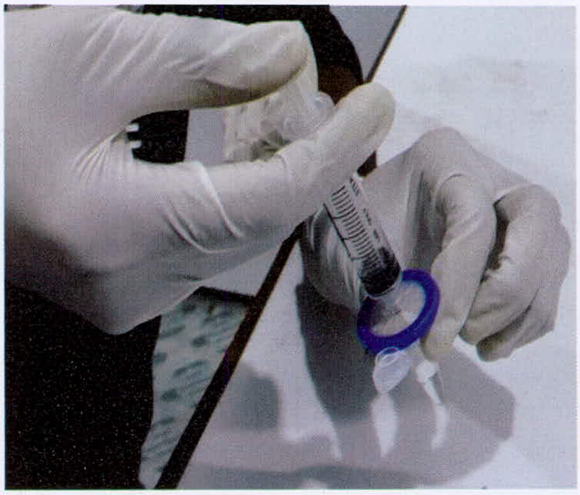

Fig. 1: Preparation of viral inoculum

Fig. 2: Intramuscular administration during the experiment

D. Experimental setup and Sampling

For *In-vivo* challenge test healthy looking 80 juvenile shrimps (Specific Pathogen of WSSV Free, SPF) were collected Batiaghata upazila. One day before the experiment all shrimps were collected and for acclimatization, transferred to the aquariums (4×1×1 feet) with having re-circulatory system in the physiology laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline. Water level, salinity, dissolved oxygen, water temperature of aquarium were respectively 10 inch, 5ppt, 4.5mg/L and 260C. The species, *Penaeus monodon* were fed with formulated shrimp feed and five shrimp were randomly collected in each time of sampling. In experiment, shrimp were injected between the 3rd and 4th segments of the pleopod (Fig. 2). Before and after injection, this surface was wiped with 70% ethanol. For positive control, a groups of 20 shrimp (maximum body weight = (14±2) g) were inoculated with 10µl of viral solution only whereas the other two groups (n=20) challenged with WSSV virus with the extract solution with the dose of 150mg/kg bw (Treatment-1) and lower dose of 100 mg/kgbw (Treatment-2) and kept in separate tanks.

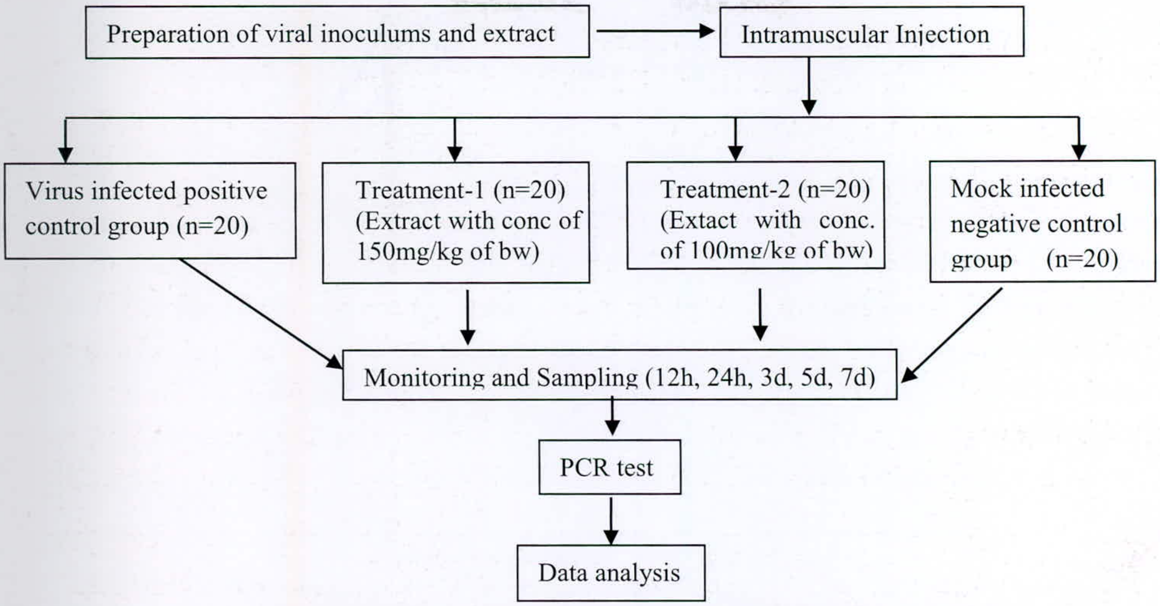

Fig. 3: Design (flow chart) of the experiment and data collection

Table 1: Mortality rate during the experiment in treatment-1 and treatment-2

In addition, a group of 20 shrimp were mock-inoculated with same dose of PBS and treated as negative control group (Fig.3). During the experiment mortalities were recorded and every time of sampling, 3 samples were randomly collected from each tank after 12h, 24h/day 1, day 3, day 5 and day 7 post inoculation (hpi). Then the samples were preserved in -80°C for pathogen confirmations. Further, representative sample of pleopods were subjected to PCR to indentify the antiviral activity of the extract solution.

Results

Results with respect to objective No. 1

From our result it can be seen that the virus was found to be present in the apparently healthy shrimp by powerful technique of PCR. The presence of WSSV in cultured shrimps was quite high in both Sathkhira and Bagerhat district which was 66% and 60% respectively by the one step PCR reaction using different primers of 146 R2-F2 (941bp) and I, K 3-4 (298bp).

A total of three *ghers* were sampled from each of Satkhira and Bagethat districts individually and a total of 30 samples from each *gher* were analyzed for WSSV and the result showed that the maximum intensity was observed with the band of 298 bp followed by the amplified product of 941bp. From our results, it was also seen that the presence of WSSV in cultured shrimps in Sathkhira district was quite high (66%) in the one step PCR reaction using different primers followed by Bagerhat which was 60%.

The variation in obtaining either or all of the three amplified products from different samples collected from different *ghers* indicates the virus load and severity of infection. When 298bp is +ve along, light infection is indicated. When both primers are +ve then moderate infection and if all the bands are produced then high infection occurred.

Results with respect objective No. II

In our experiment, two different concentrations (150mg/kg bw and 100mg/kg bw) of methanol extracts of leaves were tested experimentally against WSSV infected shrimps (*in vivo*). The results revealed that the methanol extracts of all three experimental plants leaf extracts showed potential antiviral activity against WSSD. The results also revealed that, all the shrimps survived at the whole study period and there were no visible signs of WSSV infection where the concentration was 150mg/kg of shrimp body weight (Treatment-1). Furthermore, 100% mortality was found in virus infected positive control group and no mortality, no band was found in mock infected negative control group and (Table 1 and Fig. 4)

Experimental condition of shrimp (n=160)	**Mortality during experiment (after administration viral solution and leaves extracts)**					**Mortality rate (%)**
	12h	**1d**	**3d**	**5d**	**7d**	
Positive control (n=20)	**0**	**0**	**13**	**7**	**0**	**100**
Negative control (n=20)	**0**	**0**	**0**	**0**	**0**	**0**
Mortality rate during the experiment using methanol extract of *A. marmelos*						
Treatment-1 (n=20)	**0**	**0**	**0**	**0**	**0**	**0**
Treatment-2 (n=20)	**0**	**0**	**0**	**2**	**5**	**35**
Mortality rate during the experiment using methanol extract of *M. charantia*						
Treatment-1 (n=20)	**0**	**0**	**0**	**0**	**0**	**0**
Treatment-2 (n=20)	**0**	**0**	**0**	**0**	**0**	**0**
Mortality rate durin g the experiment using ethanol extract of *C. dactylon*						
Treatment-1 (n=20)	**0**	**0**	**0**	**0**	**0**	**0**
Treatment-2 (n=20)	**0**	**0**	**0**	**0**	**0**	**0**

However, a mortality of 35% at the end of the experiment (Table 1) after post administration was found at the lower concentration of 100mg/kg of body weight (Treatment-2) and WSSV-specific DNA bands were visualized in the 5th and 7th day using I,K 3-4 (298 bp) primers in case of the extracts of *A. marmelos* (Fig.6). Simultaneously in case of *C. dactylon* and *M. charantia*, all the shrimps survived till the end of the experiment (Table 1) and no WSSV-specific DNA bands were found through PCR method using 146 F2-R2 (941 bp) primers even when nested PCR using I,K 3-4 (298 bp) was done (Fig.5). So from the above result, it might also be concluded that both of *C. dactylon* and *M. charantia*.were more effective than *A. marmelos*.

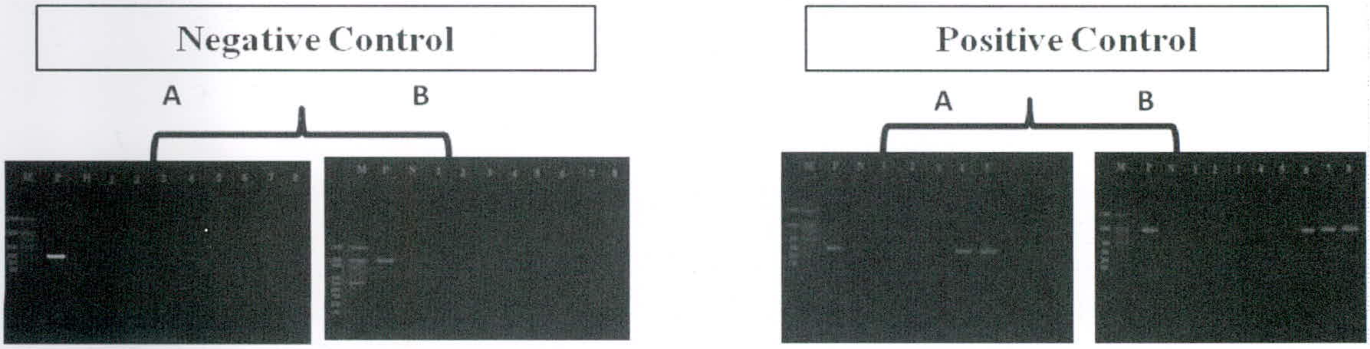

Fig.4: UV visualization of PCR product (Positive samples) electrophoresis in a 1.5% agarose gel. A) Where primer was I, K 3-4 (298 bp). B) Where primer was 146 F2 and 146 R2 (941 bp). Lane 'M' represents 100 bp DNA ladder or marker. The lanes indicate the time of sampling. 1=0h, 2=12h, 3=1day, 4=3day, 5=5day, 6=7day and P=Positive control, N=Negative control

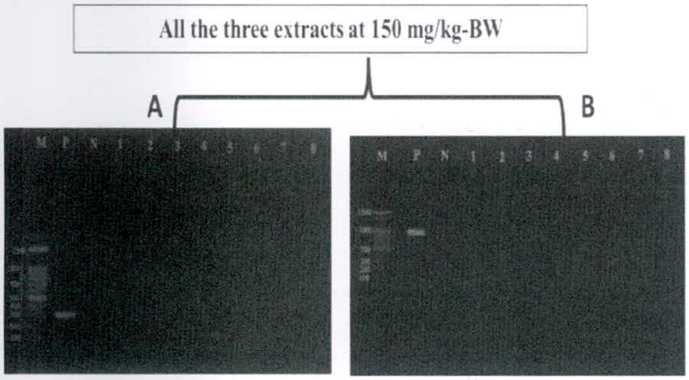

Fig.5: UV visualization of PCR product (Positive samples) electrophoresis in a 1.5% agarose gel. A) Where primer was I,K 3-4 (298 bp). B) Where primer was 146 F2 and 146 R2 (941 bp). Lane 'M' represents 100 bp DNA ladder or marker. The lanes indicate the time of sampling. 1=0h, 2=12h, 3=1day, 4=3day, 5=5day, 6=7day and P=Positive control, N=Negative control

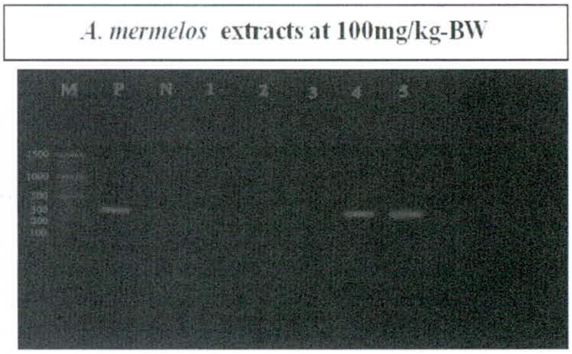

Fig.6: UV visualization of PCR product of *A. mermelos* extracts electrophoresis in a 1.5% agarose gel where primer was I,K 3-4 (298 bp). Lane 'M' represents 100 bp DNA ladder or marker. The lanes indicate the time of sampling. 1=0h, 2=12h, 3=1day, 4=3day, 5=5day, 6=7day and P=Positive control, N=Negative control

Overall the result was the methanol extracts of all the plants of *C. dactylon, M. charantia* and *A. mermelos* leaves extract are quite capable of protecting *P. monodon* from WSSV infection So the study could establish an eco-friendly herbal remedy against WSSV in shrimp aquaculture which will help to establish a sustainable shrimp production in our country as well as all over the world.

Conclusion

Precisely, the study has brought to light the presence of the anti WSSV property in *A. marmelos, M. charantia* and *C. dactylon* well known plant found growing in several countries of the world. The antiviral activity was confirmed through PCR analysis. The in vivo studies of the host (treated with *A. marmelos, M. charantia* and *C. dactylon*) revealed the potency of the drug to combat the deadly virus. So the study will help to establish an eco-friendly herbal remedy against WSSV in shrimp aquaculture. Also because of the low production cost and easy to adapt system any industry can produce the remedy commercially to establish a sustainable shrimp industry in our country as well as all over the world. However, more studies are required to evaluate the possible mode of action and optimizing the dose for each plant by which no mortality and no manifestation of WSSV symptoms would occur needs to be find out using different tools of biochemistry and molecular biology.

Publications from this research

Sayeed M.A., Mohim Al M, Md. and Banu G. R. 2016. Antiviral potential of *Momordica charantia* leaves extract against White Spot Syndrome Virus (WSSV) of black tiger shrimp *Penaeus monodon*. Seventh Biennial Fisheries Conference & Research Fair. Bangladesh Fisheries Research Forum (BFRF). P. 70.

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Blackleg and Hemorrhagic Septicemia in Cattle of Climatic Change Delta Region (Satkhira) with that in Cattle of Semi-changeable Endemic Region (Sirajganj) with their Control Measures

Md Mokbul Hossain and Md Ershaduzzaman

Location: Bangladesh Agricultural University, Mymensingh, Bangladesh

Duration: Three Years (2013-2016)

Expenditure of the project: Tk. 1500000.00

Introduction

Cattle all over in the world including Bangladesh die from different bacterial infectious diseases mainly by blackleg and hemorrhagic septicemia (Jones *et al.,* 1996; Radostits *et al.,* 2007). Disease pattern of humans and livestock vary with the interaction of causal agents, host response and environmental factors. The southwest region of Bangladesh is very much prone to climatic change. The disease pattern in humans and livestock in this area may be changed by introducing some emerging or reemerging or appearance of a new form of disease by changing the virulence of causal agents (Saini *el al.,* 1991, OIE 2012). The causes of calf morbidity-mortality, epidemiology and nature of etiology of bacterial infectious diseases of cattle especially blackleg and hemorrhagic septicemia needs to be verified through systemic investigation. The nature of causal agent of blackleg and hemorrhagic septicemia will be determined by culture, staining, biochemical tests (conventional methods), PCR for molecular diagnosis, gene sequencing for phylogenetic analysis, antibiogram against commonly used antibiotics and sequencing of causal agents and comparing with vaccine bacteria and bacteria isolated from semi-changeable climatic zone and that of climatic change delta region. These isolated bacteria may be preserved and used for seed bacteria for vaccine production of blackleg and hemorrhagic septicemia in Bangladesh in future.

Objectives

- Isolation and identification of the causal bacteria of blackleg (BQ) - *Clostridium chauvoei* and hemorrhagic septicemia (HS)- *Pasteurella multocida;*
- Gross and histopathological study of affected organs in natural cases and experimental pathology in laboratory animal;
- To determine the antibiotic sensitivity of causal bacteria of these diseases; and
- Molecular characterization of isolated bacteria by PCR and sequencing.

Methodologies

Sample collection from sick or dead cattle

In sick cattle appropriate samples were collected for bacteriological investigation. In case of diseased animals swab, fluid or blood were collected for isolation and identification of bacteria and in dead cattle, the internal organs such as liver, lung, kidney, spleen, etc. were collected for bacteriological and histopathological diagnosis of diseases.

BQ- From dead cattle the affected muscle had been collected for bacteriological and histopathological diagnosis of diseases.

HS- Fluid and affected tissue, lung, liver and spleen had been collected.

Direct Staining:

- BQ-affected muscle had been collected to demonstrate *Clostridium chauvoei* by staining.
- HS- smear of lungs. Liver, spleen from dead animal to demonstrate *Pasteurella multiocida* by staining.

1. Isolation and identification

Culture: Collected sample had been processed, cultured and subcultured in different broth and agar media (Nutrient broth, Cooked Meat Media, Nutrient agar media, Blood agar media, Eosin Methylene Blue (EMB) agar media, MacConkey agar media, *Salmonella-Shigella* agar media etc.).

Staining: Cultured bacteria had been stained- Gram's stain, Leishman's stain etc.

Biochemical test:

Five basic sugars (dextrose, sucrose, lactose, maltose and mannitol) fermentation test, MR-VP test, indole test, catalase and oxidase test had been carried out.

2. Pathological study:

The collected samples were examined grossly and then fixed in 10% buffered formalin and then processed for histopathology using routine method (Luna 1968). The slides were examined under microscope for pathological changes.

3. Antibiotic sensitivity Test:

Antibiogram was carried out to know the sensitivity using commercial discs with routine procedure.

4. PCR and Sequencing:

PCR of the isolated bacteria were carried out by using commercial kit.

Primers used for the detection of *Cl. chauvoei*

Specificity	Primers	Sequence (5'-3')	Amplicon size (bp)
Flgelin gene (*fli*C) *Clostridium chauvoei*	FlaF	AGAATAAACAGAAGCTGGAGATG	535
	FlachR	TACTAGCAGCATCAAATGTACC	

Miyashiro *et al.* (2007), Brazilian Journal of Microbiology (2007) 38:491-493

Primers used for the detection of *Pasteurella spp*.

Specificity	Primers	Sequence (5'-3')	Amplicon size (bp)
P. multocida	KMT1T7	ATC-CGC-TAT-TTA-CCC-AGT-GG	460
	KMT1SP6	GCT-GTA-AAC-GAA-CTC-GCC-AC	
P. multocida type B	KTT72	AGG-CTC-GTT-TGG-ATT-ATG-AAG	620
	KTSP61	ATC-CGC-TAA-CAC-ACT-CTC	

Lichtensteiger *et al.* (1996). Journal of clinical microbiology, Dec. 1996, p. 3035–3039

Results

Isolation and identification:

Blackleg

For the isolation and identification of the causal organism 2 muscle samples of blackleg (1 from satkhira and 1 from Sirajgonj) had been collected.

Clostridium chauvoei could not be isolated from the suspected blackleg cases and histopathology of affected muscle revealed Zenker's necrosis of muscle tissues.

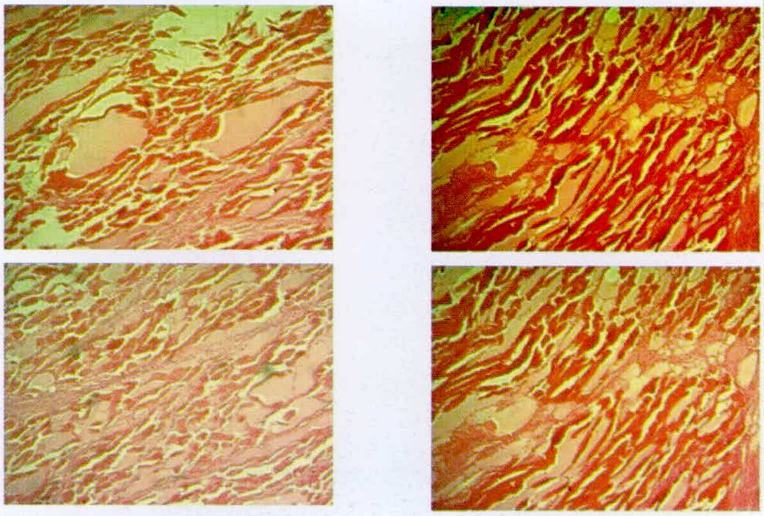


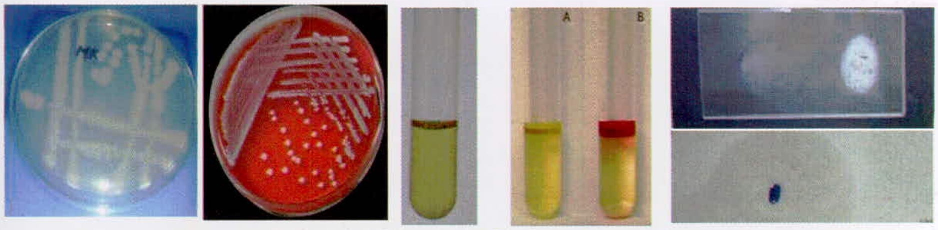
Fig: Zenker's necrosis of muscle tissues from four section of two cases

Hemorhagic septicmia

20 samples of clinically suspected HS (8 from Satkhira, 10 alive and 2 dead case from Sirajgonj) were collected from clinically infected cattle. A total of 18 edematous fluid samples from brisket region and histopathological samples (2 dead cattle from Sirajgonj) were collected to study pathological lesions.

Five samples out of 20 suspected HS cases (18 clinical and 2 dead cases) were found positive for *Pasteurella multocida* by routine methods.

Collection of samples



Culture in nutrient and blood agar

MR-VP -ve

Indole +ve

Catalase and Oxidase +ve

Fig: Culture and Biochemical tests

Antibiotic sensitivity

Antibiogram was carried out to know the sensitivity of the *Pasteurella multocida.*

- Highly sensitive to gentamaicin and ciprofloxacin
- Medium sensitive to tetracycline and amoxicillin
- Less sensitive to erythromycin and ampicillin

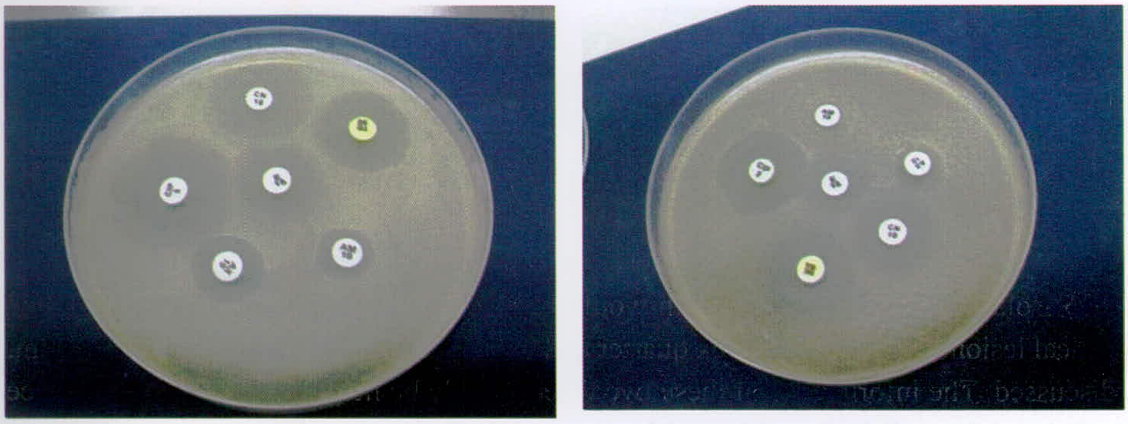


Fig: Antibiogram of *P. multocida*

Gross and histopathological study

- Grossly, hemorrhage and congested lungs and liver, frothy secretions from lungs
- Edema, hemorrhage and inflammation in brisket tissue
- Capsular thickening, inflammatory infiltration, edema of alveoli were visible in lungs
- In liver, there was hemorrhage and congestion with inflammatory infiltrations
- The experimental pathological of the isolated bacteria will be determined by experimental inoculation in mice

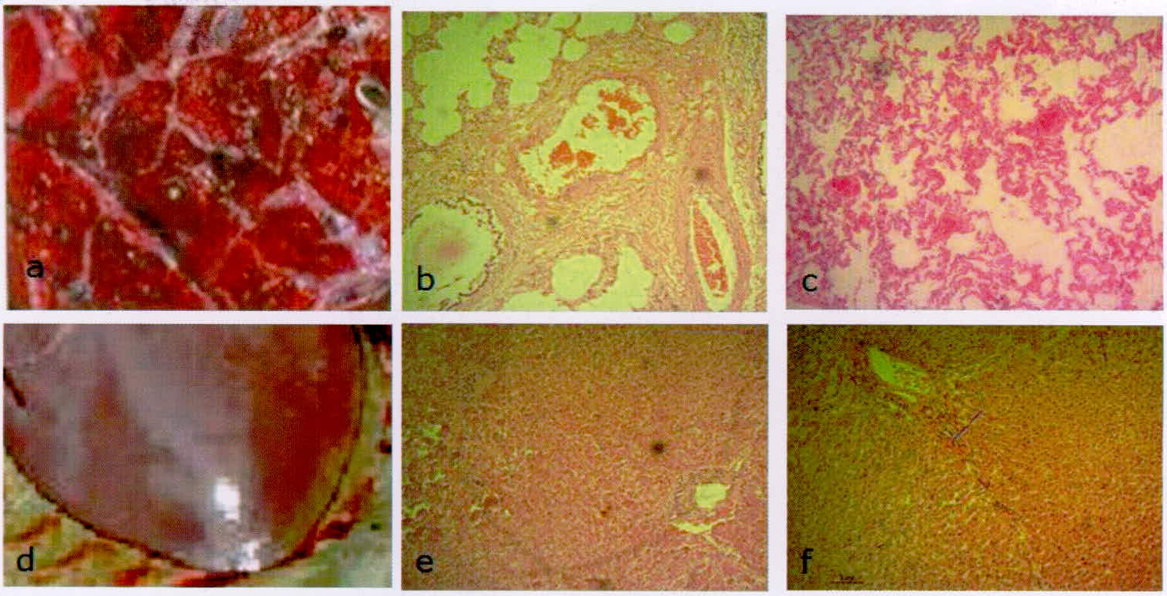


Fig. (a,b,c): Gross and histo-pathological lesions in lungs: Grossly, hemorrhage, congestion and frothy secretions; histo-pathologically, hemorrhage, congestion, edematous alveoli, inflammatory infiltration.

Fig. (d,e,f): Gross and histo-pathological lesions in liver: Grossly, hemorrhage, congestion; histo-pathologically, hemorrhage, congestion and inflammatory infiltration

PCR

In case of black leg, the two suspected BQ infected muscles didn't show any amplicon in PCR using the used primer. None of them (n=5) were found positive in PCR in case of *Pasteurella* using the mentioned primers. So no sequencing was carried out in both the cases.

Conclusion

We have got 5 isolates of *P. multocida* which would be used for further studies in future. The gross and histopathological lesions of suspected black quarter and hemorrhagic septicemia, the antibiogram, PCR test have been discussed. The information of these two diseases may be helpful to control them successfully in Bangladesh.

Publications from this research

Hossain M.M. and Rashid M. 2015. Blackleg and hemorrhagic septicemia in cattle of climatic change delta region (Satkhira) with that in cattle of semi-changable endemic region (Sirajganj) with their control measures. Bangladesh Agricultural University Research Progress Vol. 26, p 11.

Rashid M., Khan M. A. H. N. A. and Hossain M. M. 2016. Blackleg and hemorrhagic septicemia in cattle of climatic change delta region (Satkhira) with that in cattle of semi-changable endemic region (Sirajganj) with their control measures. Bangladesh Agricultural University Research Progress Vol. 27, p 22.

Rashid M., Badhy S. C., Khan M. A. H. N. A. and Hossain M. M. 2017. Pasteurellosis in cattle and buffalo: A microbiological and pathological study. Proceedings of Bangladesh Society for Veterinary Education and Research Annual Scientific Conference XXIII, p 58.

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PCR-based Method to Detect Pathogenic Microorganisms in Exportable Shrimp and Fish Products of Bangladesh

Md Shaheed Reza and Md Naim Uddin

Location: : Department of Fisheries Technology, Bangladesh Agricultural University
Duration: Three year (2013-2016)
Expenditure of the project: Tk. 1600000.00

Introduction

In Bangladesh, shrimp and fish industry is the most important among the agro-based industries. It contributes 3.69% of the GDP with an export earning amounting US$ 58.25 million in 2014-15 financial year (DoF, 2016). Bangladesh is already among the top 10 exporters of shrimp in the world. However, the export of shrimp and fish products have less market demand in the developed countries because of the emerging sets of food safety and agricultural health standards along with buyers' changing requirements (Islam *et al.,* 2009). In 1997, Bangladesh suffered a major setback in its export market due to the presence of pathogenic microorganisms in shrimp products and non-compliance with HACCP regulations. The common pathogenic bacteria that were included in the list were *Salmonella* and *E. coli*. The conventional microbiological methods for detection of these bacteria, however, usually include multiple subcultures and biotype or serotype identification steps and, thus are laborious and time-consuming (Swaminathan and Feng, 1994). Rapid and easy detection of pathogenic organisms is, therefore, needed to maintain healthy food (Feng, 1992). Polymerase chain reaction (PCR)-based is regarded as the method of choice that can rapidly identify pathogenic bacteria in fishery products if present even in low concentrations.

Objectives

The specific objectives of the research project were;

- Development of protocol for detection of pathogenic microorganisms in exportable shrimp and fish products using PCR-based techniques; and
- Improvement of quality assurance program of exportable shrimp and fish products.

Methodologies

During Year-1, a total of 10 frozen shrimp products were collected from processing industries of Khulna and Cox's Bazar regions and transported to the Dept. of Fisheries Technology, BAU, Mymensingh in an ice box packed with sufficient amount of ice. Isolation and identification of pathogenic *Salmonella* and *E. coli* were done based on morphology, staining, cultural and biochemical characteristics. For PCR, DNA were extracted from pure culture by boiling method. In brief, 100 µl of deionized water was taken and a pure bacterial colony from overnight culture was gently mixed with deionized water.

The tube was transferred into boiling water and boiled for 10 min then immediately to ice for cold shock about 10 min and centrifuged at 10,000 rpm for 10 min. Supernatant from the tube was collected and used as DNA template for PCR. PCR for amplification of *E. coli* afa and ECO genes was performed using the specific primers reported by Naravaneni and Jamil (2005). The amplified DNA fragments were then separated on 2% agarose gel and visualized with ethidium bromide staining.

Results

Since the capacity of all assays to monitor bacteria in any food product depends on the efficacy of PCR, one of the key factors in obtaining the PCR fingerprinting patterns from those bacteria is the efficiancy of the DNA extraction procedure. In the present study, genomic DNA was successfully isolated from bacteria using rapid boil method (Fig. 1) during Year-1.

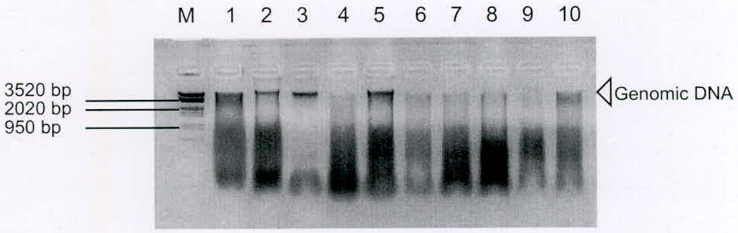

Fig. 1: Ethidium bromide stained bacterial DNA on 1.8% agarose gel isolated from *E. coli* (Lane 1-5, bacteria obtained from Khulna shrimp products and Lane 6-10, bacteria obtained from Cox's Bazar shrimp products). Lane M is marker (NipponGene).

From Fig. 1, it can be visualized that majority of the genomic DNA bands are located around 2-3 kb regions. And it is expected that the DNA templates are of good quality both qualitatively and quantitatively.

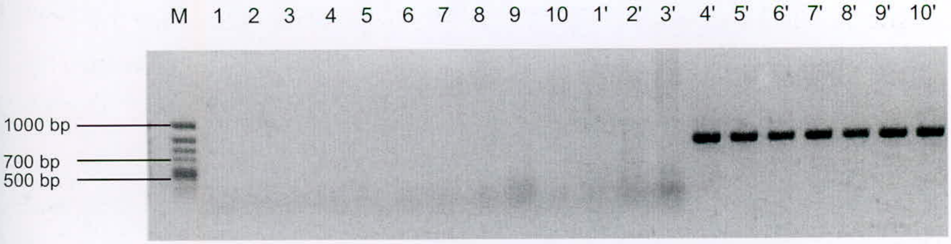

Fig. 2: PCR amplification of E. coli afa gene and 16S rRNA gene (positive control). Lane M, DNA marker; lane 1-10, afa PCR products of representative *E.coli* isolates obtained from frozen shrimp products; lane 1'-10', positive control (16S rRNA gene) for corresponding 1-10 samples.

In Fig. 2 the presence of *E. coli* in the frozen shrimp samples were confirmed by *E. coli* 16S rRNA gene specific primers (positive control). The overall prevalence of pathogenic *E. coli* was however 70% (7 out of 10) among the frozen shrimp products analyzed.

In the present study, afa gene was selected to identify pathogenicity of *E. coli* which is specific to *E. coli*. It was reported that afa gene clusters share a highly conserved region, including afaA, afaB, afaC, afaD, and afaF genes, genes which encode regulatory, chaperone, and usher functions (Garcia *et al.*, 1996). For identification of pathogenic strains of *E. coli* primers based on the afa gene, our study showed that none of the isolated strains of *E. coli* were pathogenic and hence amplification did not occur. Similar incident was also reported by Naravaneni and Jamil (2005).

Conclusion

Although the research project was approved for 3 (three) years, due to some technical difficulties, the work could not be continued after 1 (one) year. During this 1 year, we have isolated and tested pathogenicity of one bacterial genera out of the two that was originally approved in the research proposal. Our studies have shown that the prevalence of *E. coli* was comparatively higher (70%) among the 10 frozen shrimp samples analyzed. Since the acceptable upper limit for total and fecal coliforms in frozen fish and shrimp is 100 and <3 MPN/g, respectively (ICMSF, 1996), the prevalence of 70% *E. coli* in our sample is acceptable. The good side of the research work was that the *E. coli* isolates obtained from frozen shrimp products from Cox's Bazar and Khulna were not pathogenic to human. Further studies is needed for testing more genomic islands of *E. coli* responsible for pathogenicity.

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Studies on Leaf Blight (*Bipolaris Sorokiniana*) of Wheat and Development of Sustainable Environment Friendly Technology for Production of Wheat

Ismail Hossain and Mohammad Shahjahan Monjil

Location: Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1000000.00

Introduction

Wheat (*Triticum aestivum* L.) is third most produced valuable cereal on earth. It is also one of the most important staple food crop next to rice in Bangladesh. It has more protein than different nourishments. Grains are rich in proteins, carbohydrates, vitamins and minerals. The cultivation of wheat increases in Bangladesh in relation to previous years. Comparatively the less production cost with relatively more profit of the contemporary crops are considered as main reasons for increase of wheat cultivation in the country. Seed is one of the most important technologies for crop production. There are many causes of low yield of wheat. Among them poor germination of seeds affects the yield of wheat. For successful crop production there is no other alternative but to use good seed. Wheat is attacked by about 20 different diseases at various stages of its growth in Bangladesh among them, leaf spot/ leaf blight/black point (*Bipolaris sorokiniana*) is an important seed-borne as well as airborne disease of wheat in many wheat growing countries of the world including Bangladesh. This disease impaired seed germination and significant reduction in seedling vigour and grain yield. This disease resulted upto 25% yield reduction at farmer's field and it could 100% yield reduction in case of severe infection. Many researchers have tried to find safe and economical methods to control plant diseases by using extracts of different plant parts as plant extracts have antifungal effect and can be used as fungicidal seed treatments and spray for the control of wheat disease. Biological control 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, significance of this method is that incorporation of this control method will allow reduction in the use of chemical fungicides.

Objectives

Develop a sustainable, environment friendly management model of leaf blight (*Bipolaris sorokiniana*) for successful production of wheat in Bangladesh.

Methodologies

Diseased leaf and seed samples of wheat were collected from different wheat growing areas of Bangladesh and taken to the laboratory immediately and Isolation, purification and identification of disease causal pathogen, *Bipolaris sorokiniana* was done following the method of Hossain and Azad (1992). The Physiologic race of *B. sorokiniana pathogen* was studied following the method of Chauhan and Pandy (1995) and Pathogenic variation was studied following the method of Hossain and Azad (1992).

In in-vitro extracts of selected botanicals viz. Black cumin, Margosa, datura, Nayantara, Henna, Tulsi, Dodder, Allamanda, Lemon grass, garlic, Onion, Basok and Zinger as well as BAU-Biofungicide as biocontrol means were evaluated by following cup method and excised leaf method of Hossain and Schlosser (1993), where Bavistin was used as a chemical check.

21 Seeds samples of 8 varieties of wheat (*Triticum aestivum*) were collected from 11 different Upazillas of Bogra and Naogaon districts for Dry inspection, determination of moisture following CIMMYT method (Gilchist,1985) and Seed health test by Blotter method (ISTA, 1996). Then management of seed borne fungi using treatments T_0 = Untreated Control, T_1 = Mehedi leaf extract (1:10), T_2 = Basok leaf extract (1:10), T_3 = Chirota plant extract (1:10), T_4 = Merigold leaf extract (1:10), T_5 = BAU Bio-fungicide (3%), T_6 = Provax (0.4%), T7 = Tilt (0.3%) and T_8 = Bavistin (0.3%) were tested following seed treatment method. Germination test (ISTA, 1996), Vigor test (ISTA 2001) and seedling vigour was determined following the formula of Baki and Anderson (1972).

Plot trials were laid out in RCBD having three replications for each treatment. Seed samples of wheat (*Triticum aestivum* L.) var. kanchan which is highly susceptible to leaf blight (*Bipolaris sorokiniana*) was selected for plot experiment. The treatments were same as of treatments used in case of seed treatment study. Wheat plants were assessed in the field. Disease severity of wheat of each plot was assessed following the double digit scale prescribed by CIMMYT after Saari and Prescott (1975). Data on different parameters viz. No. of seedling/m2, height of seedlings, disease incidence (%), height of plants (cm), length of ear (cm) ,1000-grain weight, grain yield and % healthy and diseased grain were recorded.

Finally experiment was carried out in the farmer's field of the Charabari, Tangail sadar, Tangail. The field experiment was conducted by using wheat seeds (*Triticum aestivum*) of two varieties, the highly susceptible variety Kanchan and moderately susceptible variety Prodip were used for experimental purpose. Selected extracts of botanicals viz., Neem (1:10) and Garlic(1:10); BAU-Biofungicide (3%) as biocontrol means and chemical fungicide viz. Tilt 250 EC (0.2%) were used in the experiment, where RCBD design was used. Spray schedule and seed treatments were done as per treatments. Selected plant extracts, BAU-Biofungicide, and Tilt 250EC were tested for controlling leaf spot of wheat. The spray schedule was started just after appearance of leaf spot symptom and three sprays were maintained at 15 days interval. Wheat plants were assessed in the farmer's field. Disease severity of wheat in each plot of both Kanchan and Prodip were assessed following the double digit scale prescribed by CIMMYT after Sarri and Prescott (1975). Data on different parameters viz. No. of seedling/m^2, height of seedlings, disease incidence (%), height of plants (cm), length of panicle (cm) ,1000 - grain wt (g), grain yield and % healthy and diseased grains were collected.

Results

Effect of different plant extracts, BAU-Biofungicide and chemical fungicides were evaluated for controlling mycelial growth of *Bipolaris sorokiniana*. All the plant extracts and BAU-Biofungicide reduced mycelial growth significantly over control under different growing periods. Mycelial growth under different treatments varied significantly, where maximum mycelial growths were recorded under control and minimum in Bavistin followed by BAU-Biofungicide. BAU-Biofungicide showed maximum growth inhibition by 67.36 % over the control. Among the plant extracts, garlic was found superior in controlling mycelial growth of *B. sorokiniana.* Fungicides, Bavistin and Tilt showed excellent effect in controlling mycelia growth of *B. sorokiniana*.

A total number of 8 fungal species belonging to 6 genera were associated with wheat seeds. The fungi encouterd were *Bipolaris sorokiniana* (0.5 to 30.5%), *Alternaria tenuis* (0.5 to 25%), *Fusarium moniliforme* (0.0 to 33.5%), *Fusarium oxysporum* (0.0 to 10.5%), *Aspergillus flavus* (0.0 to 6.5%), *Aspergillus niger* (0.0 to 8.5%), *Curvularia lunata* (0.0 -7.5%) and *Penicillium* spp. (0.0 – 2.5%). Percent of germination of seeds ranged from 73.5 to 98.0% that varied from variety to variety as well as one location to another (Fig.1). The highest germination was recorded in seed sample of wheat variety BARI gom 28 collected from Rampur under Manda upazilla of Naogan and the lowest in wheat variety Kanchan collected from Dupchanchia of Bogra.

Fig.1: Incidence of seed borne fungi (Blotter method)

Effect of seed treatment with selected plant extracts (Mehedi, Basok, Marigold and Chirota), BAU-Biofungicide and chemical fungicides (Bavistin, Tilt and Provex) on germination and seed borne fungi infection of wheat seeds (Blotter Method) was tested. Germination of seeds ranged from 56.0 to 98.0% that varied from treatment to treatment. The highest germination was recorded in case of seed treatment with BAU-Biofungicide and the lowest germination was found in seed treatment with Tilt. Superior effect of controlling seed borne fungi was recorded by seed treating seeds with BAU-Biofungicide and Provax. Vigor index ranged from 955.50 to 3186.24%, while the highest vigor index was recorded in seed treatment with BAU-Biofungicide and the lowest in seed treatment with Tilt (Fig.2). Maximum disease severity (99) was recorded in untreated control at all counts, but minimum disease severity (56) was recorded in case of using seed treatment plus foliar spray with Tilt followed by BAU-Biofungicide (Fig.3).

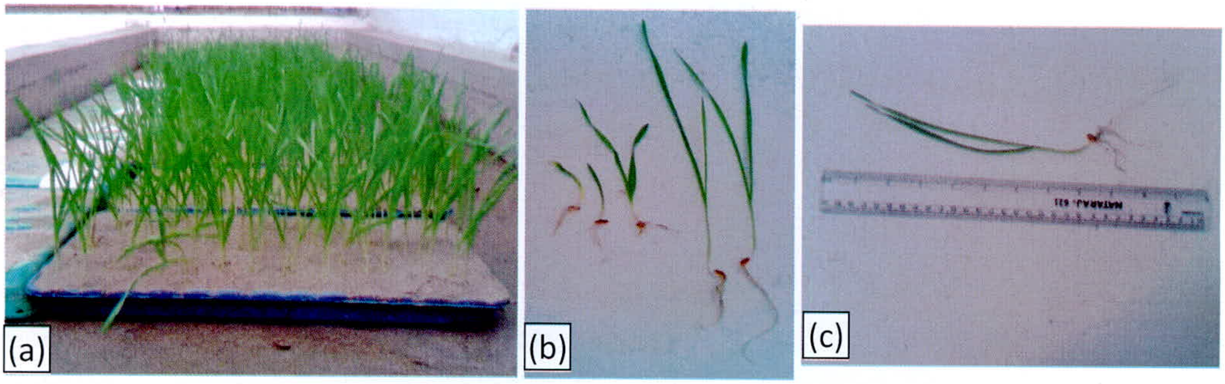
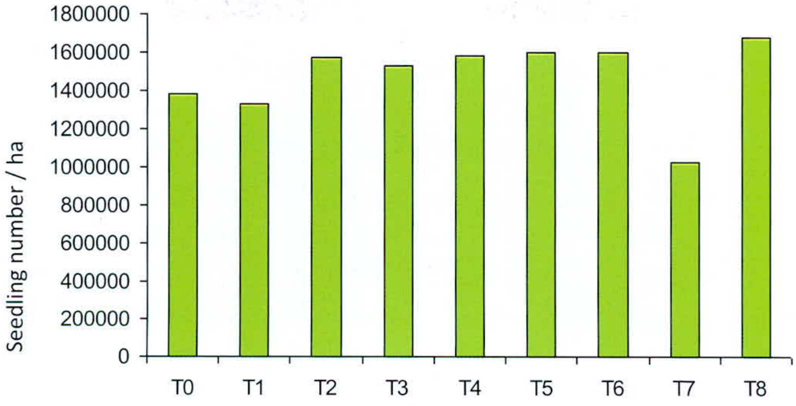


Fig. 2. Effect of seed treatment with selected plant extracts, BAU-Biofungicide and fungicides on % seed germination and vigor index of wheat seeds cv. Kanchan (Tray method): (a) Seedlings on tray, (b) different type of seedlings and (c) measuring of seedlings

Fig. 3: Showing leaf spot / leaf blight symptoms caused by *Bipolaris sorokiniana*

Seeding stand in the field under different treatments varied widely (Fig.4). Healthy field showing bright colored ears and dull brown ears were found in infected field (Fig.5). Variable effects of treatments on grain yield were recorded, where 35% and 37% higher grain yields were obtained by using BAU-Biofungicide and Tilt, respectively (Table-1).



T_0 = Untreated Control, T_1 = Mehedi leaf extract (1:10), T_2 = Basok leaf extract (1:10), T_3 = Chirota plant extract (1:10), T_4 = Merigold leaf extract (1:10), T_5 = BAU Bio-fungicide (3%), T_6 = Provax (0.4%), T_7 = Tilt (0.3%), and T_8 = Bavistin (0.3%).

Fig. 4: Effect of seed treatment and foliar spary with selected plant extracts, BAU-Biofungicide and chemical fungicides on seedling number/ha at 30 DAS of wheat cv. Kanchan (Field Plot experiment)

Fig. 5: Effect of seed treatment and foliar spray with selected plant extracts, BAU-Biofungicide and fungicides on disease severity of wheat cv. Kanchan (a) healthy field and (b) field having infection.

Table 1. Effect of different treatments on % seedling mortality, healthy Grains /ear and grain yield/ ha

Treatment	**% seedling mortality**	**Healthy Grains /ear**	**Grain yield (t/ha)**
Control	55.5	25.5	2.14
Neem extract (seed treatment)	46.5(-16.22)	29.5(+15.69)	2.53 (+18.22)
Neem extract (seed treatment +spray)	45.0(- 18.02)	31.0(+22.00)	2.41 (+12.62)
Garlic extract (seed treatment)	37.0(-33.30)	31.5(+23.53)	2.50 (+16.82)
Garlic extract (seed treatment +spray)	45.5(-18.02)	32.0(+25.49)	2.24 (+4.67)
BAU- Biofungicide (Seed Treatment)	41.5(-25.23)	36.5(+43.14)	2.81 (+13.31)
BAU- Biofungicide (Seed Treatment + spray)	42.5(-23.42)	37.0(+45.10)	2.89 (+ 35.05)
Tilt as spray	42.5(-5.41)	39.5(+54.90)	2.95 (+37.85)

Data in parentheses in column-wise indicate increase (+) or decrease (-) over untreated control

Conclusion

The experiments were carried out in *in-vitro* condition, in field Lab of Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and in the farmer's field of the Charabari, Tangail sadar, Tangail. In case of experiment in Farmer's plot, eight treatments comprising T_1 = Control (untreated), T_2 = Neem extract @1:10 (seed treatment), T_3 = Neem extract (seed treatment@ 1:10 + foliar spray @1:10),

T_4 = Garlic extract @1:10 (seed treatment), T_5 = Garlic extract (seed treatment@1:10 + foliar spray @1:10), T_6 = BAU-Biofungicide @ 3% (seed treatment), T_7 = BAU-Biofungicide (seed treatment @ 3% + foliar spray @ 3%) and T_8 = Tilt, a chemical fungicide @ 0.2% as foliar spray. Minimum seedling mortality of both the varieties of wheat, Kanchan and Prodip was recorded when BAU-Biofungicide was used as seed treatment in combination with foliar spray that resulted up to 24.48% reduction of seedling mortality over untreated control. BAU-Biofungicide as seed treatment in combination with foliar spray also showed superior effect in increasing plant height, no. of effective tillers formation and length of panicle followed by Tilt as foliar spray in case of both the wheat varieties. BAU-Biofungicide and Tilt showed similar effect in case of grain formation. BAU-Biofungicide and Tilt resulted 35.05% and 37.85% higher grain yield over control From the present study it may be concluded that BAU-Biofungicide as a biocontrol means as well as environment friendly ways of plant disease control in a unique means can successfully be used for controlling leaf blight of wheat in Bangladesh. This innovation need to be extended and forwarded to farmers of Bangladesh for successful and bumper wheat cultivation in the country.

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Najnine F., Akter, M. A. and Hossain I. 2016. Effect of plant extracts, BAU-Biofungicide and chemicals on Bipolaris leaf blight of wheat cv. Kanchan. Asian Australians. *Journal of Bioscience and Biotechnolgy,* 1 (2): 206-212.

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Conservation of Endangered Fishes in the Brahmaputra Basin: in-situ and Ex-situ Approach

Mostafa A R Hossain and Mohd Golam Quader Khan

Location: Department of Fisheries Biology & Genetics, BAU, Mymensingh
Duration: Three year (2013-2016)
Expenditure of the project: TK. 1000000.00

Introduction

A number of fishes are considered as endangered due to their gradual disappearance from the waterbodies of Bangladesh due to a number of reasons. While little can be done to bring back lost species, currently a number of fish are near-extinction, needing immediate measures to conserve. Where perennial tributary bacame seasonal due to several factors, establishing fish sanctuaries (refuges where fish are protected during dry season and grow and attain maturity for spawning in next monsoon and release millions of eggs) can help to restore fish biodiversity.

Under proposed project a fish sanctuary will be established with the direct participation of the fisher in the Old Brahmaputra using a bottom-up approach. There is a need to prioritize important threatened fish and develop propagation and restocking program. Considering biodiversity status and consumer demand, an endangered fish selected for proposed project is Arwari. Induced breeding of some of the fish species has been ongoing for many years in BAU and BFRI. Unfortunately, these individual trials are taking place in isolation and included only a few aquacultured species. There is a need to prioritize important indigenous and threatened species of Bangladesh and develop a long-term propagation and restocking program. Such a program would then complement the gene bank.

Objectives

The main objective of the proposed project is to conserve biodiversity and enhance production of important indigenous fish species in the Brahmaputra Basin. The objectives of this study are: to establish a permanent fish sanctuary in the Brahmaputra basin with the direct participation of the local fishers using community based approach (*in-situ*) and to develop breeding and mass propagation protocols for one important indigenous fish species and release their spawn in the selected waterbodies for stock enhancement (*ex-situ*)

Methodologies

Study area

The study area covers a fishing community of Mymensingh. The poor and disadvantaged fishing communities are selected on the basis of set criteria. Fifty households (HH) are selected from the fishing community. Focal points (male and female) are selected as representatives. Initiative goals and purposes are introduced to the focal points so that they can explain the detail to others during initial activities.

The sanctuary site selection

Based on the data of the water quality parameters, month-wise fluctuation of water depth, current, velocity and navigation, a proper location for the sanctuary is selected. The sanctuary is named Matshyarani. The core area of the sanctuary is 2000 m^2. The sanctuary area was clearly marked and all people followed the rule. Any decision regarding the sanctuary was taken on a participatory basis. With the help of two ocha, Matshyarani sanctuary is sampled twice a week to study the fish abundance.

Fish collection

Collection of live fish was not possible due to the scarcity of fish. Dead fish was collected and their morphometric and meristic study was carried out. The freshly dead fish numbering 30 were collected to study the morphometric and meristic characters, gonado somatic index (GSI) and other features.

Results

Table 1: Morphometric Characters

Characters	Sample 1	Sample 2	Sample 3
Total length (cm)	37	34	44
Standard length (cm)	30	26.5	33.5
Fork length (cm)	32	28.5	37
Pre-Dorsal length (cm)	13.5	11.7	14.5
Head length (cm)	9	7.5	10.1
Pre-Orbital length (cm)	3	2.3	3.5
Eye length (cm)	1.5	1	1.2
Post-Orbital length (cm)	4.5	4.2	5.4
1st dorsal fin base (cm)	4.3	4	5.5
Inter-Dorsal space (cm)	5.5	4.7	6.4
2nd dorsal fin base/Adipose fin base (cm)	3.9	3.3	3.9
Length of caudal peduncle (cm)	4.8	3.4	4.9
Caudal peduncle depth (cm)	3.7	3.2	4.1
Anal fin base (cm)	3.5	3.5	4.2
Pectoral fin base (cm)	6	5	6.7
Weight (g)	501.7	336.1	710

Table 2: Merestic Characters

Characters	**Sample 1**	**Sample 2**	**Sample 3**
Dorsal fin ray	I/8	I/8	I/8
Anal fin ray	10	10	10
Pelvic fin ray	6	6	6
Pectoral fin ray	I/7	I/7	I/7
Caudal fin ray	17-18	17-18	17-18
Dorsal spine length (cm)	5	4.4	5.6
Pectoral spine length (cm)	6	5	6.7
Number of barbells	8 (Two are large)	8 (Two are large)	8 (Two are large)
Length of barbells (Large one)	20	17.5	20.5
Adipose fin	Present (Continuous)	Present (Continuous)	Present (Continuous)

Morphometric and meristic characters of the studied *H. menoda*

Table 3: Sampling in Matshyarani

Sl	Name of species	No. of species	Length (cm)	Weight (g)
01	Puti	112	11	15.6
02	Chhep chela	12	8	7
03	Titputi	68	5	4
04	Kakila	27	16.5	8.8
05	Baila	44	7	21.0
06	Chirka	2	6.5	2.3
07	Kanpona	23	3	2.1
08	Gulsha	24	19	60
09	Katari chela	35	13	16
10	Gutum	56	6.2	11.24
11	Chela	34	7	9.1
12	Gang gutum	3	5	11
13	Golda	45	20	100
14	Chaka	3	20	89
15	Baim	12	29	102
16	*M. malcomsuni*	38	7	4.4
17	*M. rudae*	123	2.2	2
18	*M. rosenbergii*	22	19	45
19	Vagna	22	23	106
20	Chanchan puti	4	9	11
21	Carpio	2	45	1450
22	Kholisha	10	4	3.2
23	Mola	45	5.7	8
24	Dhela	10	4	4.1
25	Tengra	23	13	28

Table 4: Animal other than fish

Sl	Name of the species	No.of sp.	Length (cm)	Weight (g)
01	Small long snail	534		
02	Apple snail	95		
03	Mussel	123		
05	Frog	34		
06	Crab	23		
07	Eagle	4		
08	Kingfisher	4		
09	Back swimmer	66		
10	Ranatra	11		
11	Snake	2		

Some of indigenous fish and other animals observed in Matshyarani Sanctuary

Conclusion

Due to Matshyarani, fish production in this section of the river Brahmaputra has increased. A number of threatened fish and invertebrates are restored and overall aquatic biodiversity improved. Income of fishers increased and socioeconomic status of fisher families uplifted. More fish are available in the local market. As it is expected, sanctuary as a mean of biodiversity conservation already taken up as part of national fisheries policy in Bangladesh.

At the policy level, to restore fisheries in each flood protected area where migration of fish has been restricted, a fish sanctuary should be established through government support for community participation. This should have an emphasis on including fishers in sanctuary planning and management as they may not be involved in practice in any committees that have been established for water management.

Where there is no suitable water body for establishing a sanctuary but the area retains sufficient water for a reasonable period for growth and breeding of fish, then silted up water bodies (khas fishery) should be excavated within the area to be set aside as fish sanctuaries. Once suitable habitat is restored and protected, beel-floodplain resident fishes that may have been lost from that flood protected area may be re-introduced there.

While details of management and planning involve local government and communities, sanctuary establishment needs a national strategy and guidelines with supporting rules and amendments to legislation. As part of the coordination of inland capture fisheries and wetland management and protection, an apex national committee is needed which would include sanctuaries as an important part of its remit, with the secretary, MOFL as the chairperson, Joint Secretary of MOL as Vice Chairman and DG, DOF as Member Secretary of the committee and with membership from MOFE, MOWR, MO LGRD, etc. The breeding biology of all the indigenous threatened fishes of Bangladesh like Hemibagrus menoda should be properly studied before approaching the mass seed production technology to enhance their stock in the open water

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Investigation on Health Issues of Some Endangered Freshwater Fishes of Bangladesh

Gias Uddin Ahmed and Md Ali Reza Faruk

Location: Department of Aquaculture, Faculty of Fisheries, BAU, Mymensingh
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1500000.00

Introduction

There are 260 fish species in Bangladesh (DoF, 2015) while 143 small indigenous species play an important role in the national diet of Bangladesh. Small indigenous species have a high nutritional value in terms of both protein content and micro-nutrients. Provide food and supplementary income to the majority of the people. But, these small fishes are in great threat, endangered and some are extinct from the inland water bodies of Bangladesh. Diseases in freshwater fishes of Bangladesh are great threat to achieve optimum production and become a limiting factor to achieve desired success of aquaculture Ahmad (1998). Fish parasites can cause mortalities of fishes in culture operations. They attack fishes and destroy them or make wounds or disease on their flash, thus making inedible (Cheng, 1964). Bacterial diseases are the most common infectious problem of commercial fish farms and ornamental fishes. Clinical and histological methods are important diagnostic tools by which various types of diseases can be identified. Thus, the aim of the study is to identify the seasonal variation of diseases and pathology of the small indigenous fish species through above mentioned methods and techniques.

Objectives

- To investigate aqua-ecological parameters of the habitats;
- To investigate parasites from fish form the investigating areas;
- To investigate microbial load in fish and water; and
- To investigate the seasonal variation of diseases in selection to different habitats.

Methodologies

To investigate health condition of some endangered freshwater species present investigation was carried out to save the fishes from further reduction. The study areas were Kailla beel, Mymensingh, Kangsa river near Jaria, Netrakena, Noli beel, Kapasia, Gazipur and Surma river, near Gobindaganj, Chatak, Sunamganj. PRA tools such as focus group discussion (FDG) were conducted with fisherman, people living surrounding water bodies, and fish retailers. Twenty to twenty five people were selected (on the basis of their knowledge, skill) for that discussion (Fig. A).

Results

Sampling for fish and water were carried out at monthly intervals. The experimental fishes were Baila, Colisa. Gutum, Rani, Kuchia and Tara baim. The sampled fishes were examined clinically and histologically by following standard process (Fig. B). Bacterial load in liver and kidney fish samples and water and parasitological investigation were also carried out monthly from every sampling stations by following standard procedures. Water qualities of four sampling stations were determined on water temperature, DO, pH, alkalinity, ammonia, hardness and nitrite by respective test kids during sampling at monthly intervals.

A. FGD in Noli Beel.
B. Sample collection
Result: in Fish Disease
Results: Lab for Histology
Results: and Bacteriology.

Clinically the fishes had almost bright normal appearances in rainy and autumn seasons, whereas, in winter season, the fishes had reddish lesions, dark or faded color and deep ulcer with reduced mucus externally (Figs. D and E). Again from summer season, injury of fishes recovered to give almost normal appearances. Pathologically all the organs of most of the fishes were near to normal in structure during rainy and autumn seasons. Whereas, in late amtumn, the organs had minor pathology like necrosis and vacuums. However, during winter structure of most of the organs were deteriorated to have pathologies like necrosis, pyknosis, hemorrhage, vacuums, melanocytes and missing of the part of organs. Water quality parameters were almost normal in all the seasons except in winter season, when temperature, DO, alkalinity and hardness were decreased and ammonia was increased in most of the sampling sites. The highest bacterial loads was found in liver of tara baim 8.14 x 10^{13} CFU/g followed by kidney of gutum 5.66 x 10^{13} CFU/g and water was 1.64 x 10^{7} CFU/ml from Kailla beel during winter season. The lowest bacterial loads were recorded in liver of gutum 13.4 x10^{12} CFU/g from Surma river followed by kidney of gutum 6.2 x 10^{12} CFU/g from Noli beel water sample was recorded 3.8 x 10^{6} CFU/ml from Noli beel in summer season (Fig. C). Bacterial loads in fishes and water were more or less similar in rainy and autumn seasons. Sampling stationwise, the highest parasitic infestation was found in fishes of Kailla beel followed by fishes of Kongsa river, fishes of Surma river and fishes of Noli beel during winter season and minimum was found summer season. Parasitewise, Fishes were highly infested by digeneatic trematode followed by monogeneatic trematode, protozoan, cestode, acenthocephala and nematode. Fishwise, highest parasitic infestation was found in baila followed by tara baim, gutum, rani, kochia and colisa.

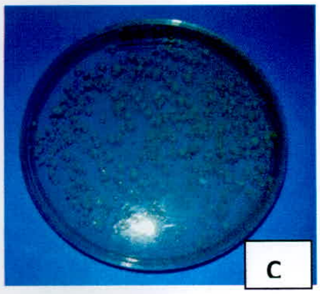

Fig. C: Bacteria Colonies from water of Noli Beel in Winter.

Fig. D and E: Clinical appearance of baim and baila from Kailla Beel in winter season.

Conclusion

Water quality parameters like DO and P^H did not show much variation among seasons and sampling stations. Temperature and alkalinity were extremely lower and ammonia and nitrite values were higher in winter compared with other seasons. This fluctuation could be the cause of clinical and pathological variations of the organs of fishes in winter season. In the present study, water bodies wise, the highest bacterial loads was found in fishes and water of Kailla beel followed by fishes of Kongsa river, fishes of Surma river and fishes of Noli beel. Season wise, maximum bacterial loads in fishes and water was found in winter and minimum in summer. Bacterial loads in fishes and water were more or less similar in rainy and autumn seasons. The highest parasitic infestation were recorded in fishes of Kailla beel followed by fishes of Kongsa river, fishes of Surma river and fishes of Noli beel and highest parasitic infested fish was baila followed by tara baim, gutum, rani, kochia and colisa. On the other hand, maximum parasitic infestation in fishes was observed in winter season followed by rainy, late autumn, autumn and summer seasons. Clinically winter was the most affected season with huge clinical signs and comparatively less injuries and abnormalities were observed in rainy, late autumn, autumn and summer seasons. Histologically also most of the pathologies were observed in fish organs of winter season and comparatively less pathologies were found during rainy, late autumn, autumn and summer seasons. However, incidence of disease outbreak of fresh water fishes of Bangladesh might be related to seasonal variations and environmental factors. So it could be mentioned that, proper management techniques should be taken for open water fishery especially during winter season in order to save these important fishes from extinction.

Publications from this research

Ahmed G. U., Rahman M. M., Faruk M. A. R. and Chandra K. J. 2016. Seasonal Variation of Parasitic Infestation in Perches from Beel Fisheries of Bangladesh. *Asian Australas. J. Biosci. Biotechnol.*, 1(2): 371-379.

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Ahmed G. U. and Rahman M. M. 2017. Investigation on Health Issues of Some Endangered Freshwater Fishes of Bangladesh, Presented Annual Research Reports at the Bangladesh Agricultural University Research System (BAURES) Annual Workshops held on 2013, 2014, 2015, 2016 and 2017 at Bangladesh Agricultural University, Mymensingh.

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Milk Urea Nitrogen as a Tool to Determine Optimum Feeding Levels for Maximum Milk Production in Dairy Cows

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Location: Department of Livestock Production and Management, Sylhet Agricultural University, Sylhet

Duration: Three years (2013-2016)

Expenditure of the project: Tk.2650000.00

Introduction

Milk Urea Nitrogen (MUN), a fraction of milk protein that is derived from Blood Urea Nitrogen (BUN), may be a useful tool that may help monitoring of any change required in the feeding and management of a herd. Average MUN values may range from 100 to 140 mg/L (Carlsson and Pehrson, 1994; Moore and Verga, 1996). The liver convert ammonia to urea to be excreted or recycled and it diffuses freely across the cell membranes, and, therefore, MUN concentrations represent BUN. If, BUN values are elevated the MUN will be elevated. If MUN values are high, a herd may experience wasting of feed protein along with excess excretion of nitrogen into the environment causing pollution. If MUN values are too low, the rumen microbial protein yield may be reduced thereby limiting milk production and milk protein yield (Brodrick and Clayton, 1997). Availability of rumen degradable carbohydrate, a key factor that provides energy to the rumen microbes to convert ammonia into microbial protein often affects MUN values. Feeding too much dietary protein, and diets containing higher level of degradable and/or soluble protein, such as urea, may enhance MUN, even a diet contains a normal level of total crude protein (CP). Variation in lactation yield and genetic quality of cows fed diets containing similar level of nutrition, especially of protein, may also affect MUN contents. Evaluation of MUN content of milk during the collection time may give a good indication on protein availability to cows from the diets fed to them.

Thus, determination of MUN values of cows available in different regions considering variations in plane of nutrition, feed availability, seasons and genotypes is important, this would provide database for developing relations between MUN content and dietary nutrition, and further, it would facilitate undertaking research works for MUN indicator development to monitor dairy cow feeding and nutrition. These MUN indicators may guide farmers for better feeding of their cows to increase productivity, and to bring more economic benefits and nutrition for their family.

Objectives

- Conduct a bench mark survey on the feed systems, feed composition, milk composition prevailing in different regions and seasons;
- Evaluate MUN of cows considering nutritional and non-nutritional factors; and
- Determine optimum feeding levels of crude protein (CP) and readily degradable carbohydrate (RDC) to optimize MUN content for maximize milk production.

Methodologies

Three experiments were conducted to investigate milk urea nitrogen as a tool to determine optimum feeding levels for maximum milk production in dairy cows. In 1st experiment, a total of 100 dairy farmers, 50 dairy farmers in each region and season were selected to know the feeding systems of cows, chemical composition of feed stuffs and milk composition. In 2nd experiment, a total of 160 cows, 10 cows in each of native (local cow) and crossbred (local × Holstein Friesian) origins (genotype) differing in lactation yield were used in $2\times2\times2\times2$ factorial experiment using CRD to evaluate milk urea nitrogen of cows considering regions and seasons. In 3rd experiment, twenty (20) multiparous crossbred cows with 323.63 ± 44.08 (mean ± SD) kg body weight in the early stage of lactation (3-10 d after parturition) of 2nd to 4th parity were randomly allocated to two levels of dietary crude protein (8.0% and 12.0%) without (0.0%) or with 15.0% sugarcane molasses on dry matter basis. Four isoenergetic diets differed in CP were formulated according to their requirements (ARC, 1994). All data were subjected to ANOVA following the principles of CRD using computer package GENSTAT (Lawes Agricultural Trust, 1997) and SEM differentiated treatment means.

Results

Feeding practices followed by farmers in different feed bases and seasons were based on rice straw, small quantity of green grasses from various sources with small supplementation of concentrates.

MUN concentration positively correlated with intake of dietary CP.MUN concentration was higher under good base (38.86 mg/dl) than poor feed base condition (28.55 mg/dl). Season did not affect MUN concentration. MUN of the local and crossbred cow was found 31.97 and 35.44 mg/dl, respectively. The stage of lactation did affect the level of MUN. MUN was lower below 100 days in milk (31.48 mg/dl) than above 100 days in milk (35.93 mg/dl).

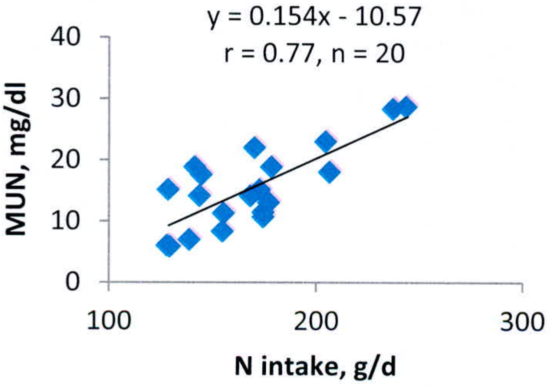

Fig. 1: Relation between N intake and MUN

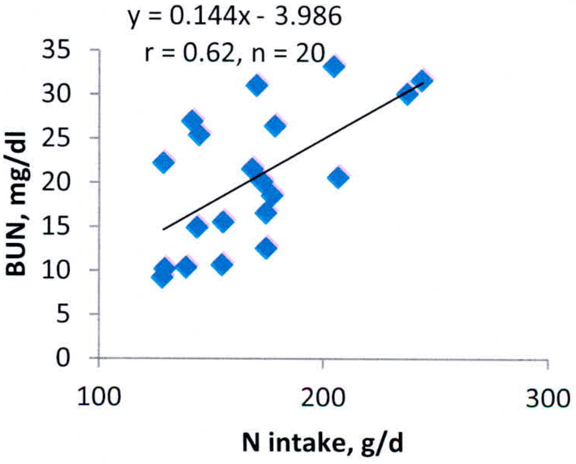

Fig. 2: Relation between N intake and BUN

DMI positively associated ($p<0.01$) with MUN ($r = 0.80$) and BUN ($r = 0.66$). Intake of N significantly correlated with MUN ($r = 0.77$, $p<0.01$) and BUN ($r = 0.62$, $p<0.01$) concentration (Figure 1 & 2).

Table 1. Effect of dietary crude protein (CP) on milk production,MUN and BUN of crossbred cows

Parameter	CP, % of DM		SEM	F-value
	8.0% CP	12.0% CP		
Intake and yield				
DMI, kg/d	10.05	10.44	0.295	0.360
N Intake, g/d	145.8	192.5	7.210	<0.001
4%FCM, kg/d	7.13	7.65	0.411	0.384
Milk components, g/kg				
Fat	46.10	42.70	1.530	0.133
Protein	36.36	37.65	0.442	0.055
Efficiency, yield/intake				
Milk/DMI	0.653	0.703	0.0289	0.238
Milk N/N intake	0.003	0.003	0.0002	**0.002**
MUN, mg/dl	13.45	17.61	1.400	0.052
BUN (mg/dl)	18.70	22.30	1.550	0.121

Dietary CP had a significant effect on N efficiency in milk N. The MUN and BUN concentration were significantly (P<0.01) increased by dietary CP treatments. In this result, 1% changed in the dietary CP did significant change in the MUN content (1.04 mg/dl). This observation was consistent with other studies (Davidson *et al.,* 2003; Groff and Wu, 2005; Promkot and Wanapat, 2005).

Table 2. Effect of water soluble carbohydrate (molasses) on milk production, MUN and BUN of crossbred cows

Parameter	Molasses, % of DM		SEM	F-value
	0.0% Molasses	15.0% Molasses		
Intake and yield				
DMI, kg/d	9.86	10.64	0.295	0.082
N Intake, g/d	161.7	176.4	7.210	0.163
4% FCM, kg/d	7.62	7.16	0.411	0.442
Milk components, g/kg				
Fat	46.70	42.00	1.530	0.044
Protein	37.39	36.62	0.442	0.239
Efficiency, yield/intake				
Milk/DMI	0.669	0.688	0.0289	0.641
Milk N/N intake	0.00371	0.00338	0.00016	0.163
MUN, mg/dl	10.83	20.22	1.400	<0.001
BUN (mg/dl)	14.60	26.50	1.550	<0.001

Soluble carbohydrate (molasses) did not produce any difference in the live weight change, but dietary crude protein with the presence of 15.0% molasses produced significant ($p<0.01$) difference in live weight change. The association between BW and MUN was positive ($p<0.05$).

The milk yield increased and 4% FCM decreased with the presence of 15% molasses, but these were not significant ($p>0.05$). The content of fat significantly ($p<0.01$) decreased 7.82% with the presence of 15% molasses than without molasses. Efficiency of milk per kg intake of DM and milk N per g of N intake did not produce any difference with or without molasses. Milk protein concentration and yield increased from 31.6 g/kg and 0.49 kg/day at 156 g/kg DM molasses inclusion up to 33.6 g/kg DM and 0.59 kg/day at 468 g/kg DM molasses inclusion.

Conclusion

Feeding practices followed by farmers in different feed bases and seasons were based on rice straw, small quantity of green grasses from various sources with small supplementation of concentrates.

MUN concentration positively correlated with intake of dietary CP. MUN concentration was higher under good base (38.86 mg/dl) than poor feed base condition (28.55 mg/dl). Season did not affect MUN concentration. MUN of the local and crossbred cow was found 31.97 and 35.44 mg/dl, respectively. The stage of lactation did affect the level of MUN. MUN was lower below 100 days in milk (31.48 mg/dl) than above 100 days in milk (35.93 mg/dl).DMI positively associated ($p<0.01$) with MUN ($r = 0.80$) and BUN ($r = 0.66$). Intake of N significantly correlated with MUN ($r = 0.77$, $p<0.01$) and BUN ($r = 0.62$, $p<0.01$) concentration.

Dietary CP had a significant effect on N efficiency in milk N. The MUN and BUN concentration were significantly ($P<0.01$) increased by dietary CP treatments. In this result, 1% changed in the dietary CP did significant change in the MUN content (1.04 mg/dl). Soluble carbohydrate (molasses) did not produce any difference in the live weight change, but dietary crude protein with the presence of 15.0% molasses produced significant ($p<0.01$) difference in live weight change. The association between BW and MUN was positive ($p<0.05$).

The milk yield increased and 4%FCM decreased with the presence of 15% molasses, but these were not significant ($p>0.05$). The content of fat significantly ($p<0.01$) decreased 7.82% with the presence of 15% molasses than without molasses. Efficiency of milk per kg intake of DM and milk N per g of N intake did not produce any difference with or without molasses. Milk protein concentration and yield increased from 31.6 g/kg and 0.49 kg/day at 156 g/kg DM molasses inclusion up to 33.6 g/kg DM and 0.59 kg/day at 468 g/kg DM molasses inclusion.

It may be concluded from experiment 1 that the plane of nutrition as a result of variation in feed availability may affect MUN concentration of dairy cows. From experiment 2 it was observed that on-farm diets having variations in CP affect MUN content of milk and experiment 3 shows that crude protein had positive impact on milk yield and milk composition. MUN content of milk positively correlated with intake of CP and rumen degradable protein. Thus, when increase rumen degradable protein intake of cows then increase MUN level in milk.

Publications from this research

Islam M. R., Uddin M. N., Akanda M. R., Khan M. M. H., Baset M. A. and Belal S. A.2015. Effect of milk urea nitrogen of dairy cows in relation to breed. *Iranian Journal of Applied Animal Science.* 5(2), 279-283.

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Synthesis and Application of Nanomaterials

Muhammad Younus and Mohammad Mizanur Rahman Khan

Location: Department of Chemistry, Shahjalal University of Science and Technology, Sylhet
Duration: Three years (2013-2016)
Expenditure of the project: Tk. 1000000.00

Introduction

Introduction Nanocomposite is a multiphase solid material where one of the phases has one, two or three dimensions of less than 100 nanometers (nm), or structures having nano-scale repeat distances between the different phases that make up the material. Polymer based nanocomposites are very promising due their various promising properties such as morphology, electrical conductivity, thermal properties, and so on. Among polymer based composites like polyanilne (PANI) based composites have various potential applications in nanochemistry and polymer science.

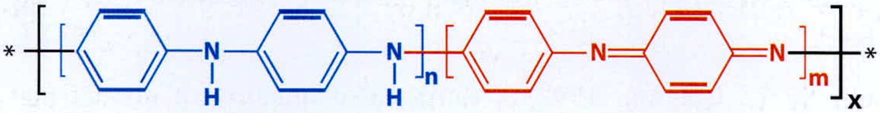


Fig. 1: Molecular structure of polyaniline, n+m = 1, x = half degree of polymerization

The mechanical, electrical, thermal, optical, catalytic properties of such nanocomposite will differ markedly from that of the component materials.As a conducting polymer, PANI (Figure 1) has been attracted considerable attention due to its chemical stability, unique doping bahaviour, abundant morphologies and relatively easy sysnthesis technique (MacDiarmid, 2001, Ma *et al.* 2004, Gospodinova and Terlemezyan, 1998.) The incorporation of different nanomaterials such as metallic, metal ions or semiconducting nanomaterials (mostly metal oxides) are using for the synthesis of PANI nanocomposites and thus are very promising to alter or improve the properties of PANI nanofibers. In the present project work, synthesis, properties and application (as a catalyst) of PANI based metal oxide nanocomposites were studied.

Objectives

The research objectives are mainly emphasizing on the following subjects-

- To synthesize PANI based composites in a simple chemical oxidative polymerization method;
- To understand the interaction between polymer matrix and metal oxides of the prepared composite materials;
- To examine the thermal stability of the synthesized composites;
- To analyze the photoluminescence property of the synthesized composites; and
- To apply the prepared composites as a catalyst in Sonogashira coupling reaction to form new C-C bond.

Methodologies

Synthesis of PANI/ZrO_2 and PANI/Pd nanocomposites

The PANI/ZrO_2 composites were synthesized by 'in situ' polymerization in the presence of ZrO_2 nanoparticles and HCl as the dopant (Khan *et al.* 2012). The preparation process for PANI/ZrO_2 composites was as follows: ZrO_2 Nano powder and 3.149g of Ammonium persulfate was taken in 100 ml of 1M HCl solution under vigorous stirring for 15-20 min at room temperature. 1mL of aniline was added drop wise and immediately after 2 mL of sodium hypochloride was added similar way. After the completion of mixing, the solution was kept steady for 30 mins to settle down the composite in fine form. Afterwards, the residue was collected through centrifugation at 3000 rpm for half an hour each time and finally collected in a sintered crucible. The collected dark green composite was kept 24-36 hour for drying purpose. Repeating same procedure PANI/ZrO_2 composites of varying amount of ZrO_2 (from 0.005g to 1.00g) were synthesized.

Similarly, PANI/Pd nanocomposites were synthesized according to the procedure reported in reference (Khan *et al.* 2012).The synthesized PANI/Pdcomposites were examined as a catalyst in Sonogashira Coupling Reaction (Norio *et al.* 2004, Huq *et al.* 2016).

The synthesized composites were were characterized by means of FTIR, UV-Vis spectroscopy, FESEM, energy dispersive X-ray spectroscopy (EDS), Thermogravimetric analysis (TGA), Photoluminescence (PL) spectroscopy, and 1H NMR.

Results

The possible incorporation of ZrO_2 and Pd in PANI was revealed by both Fourier transform infrared spectroscopy (FT-IR)and ultraviolet-visible spectroscopy (UV–vis). For instance, as presented in Fig. 2, Fig. 3 and Fig.4, respectively. Table 1 shows the total information on the vibrational frequencies of the Zr-O-Zr bond system in different composites.

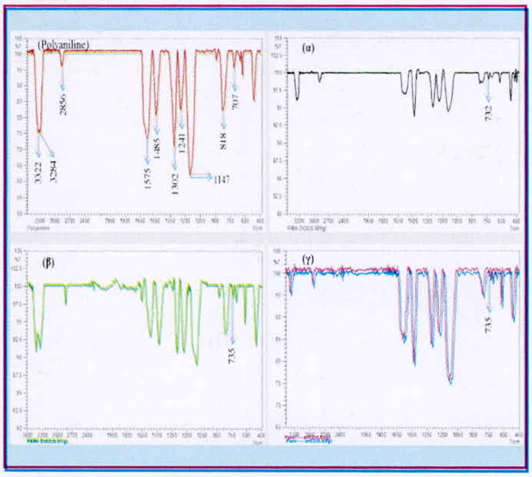


Fig. 2: FTIR spectrum of Polyaniline (PANI) and PANI-ZrO_2 (α, β, γ) composite nanofibers (Where α, β and γ represent PANI-ZrO_2 composites obtained from 0.005g, 0.01g, 0.05g loading of ZrO_2 respectively).

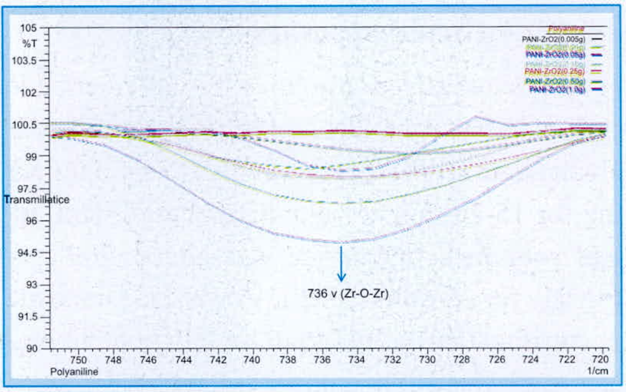


Fig. 3: Increasing of intensity of Zr-O-Zr asymmetric vibrational peak with the increasing of ZrO_2 in PANI matrix.

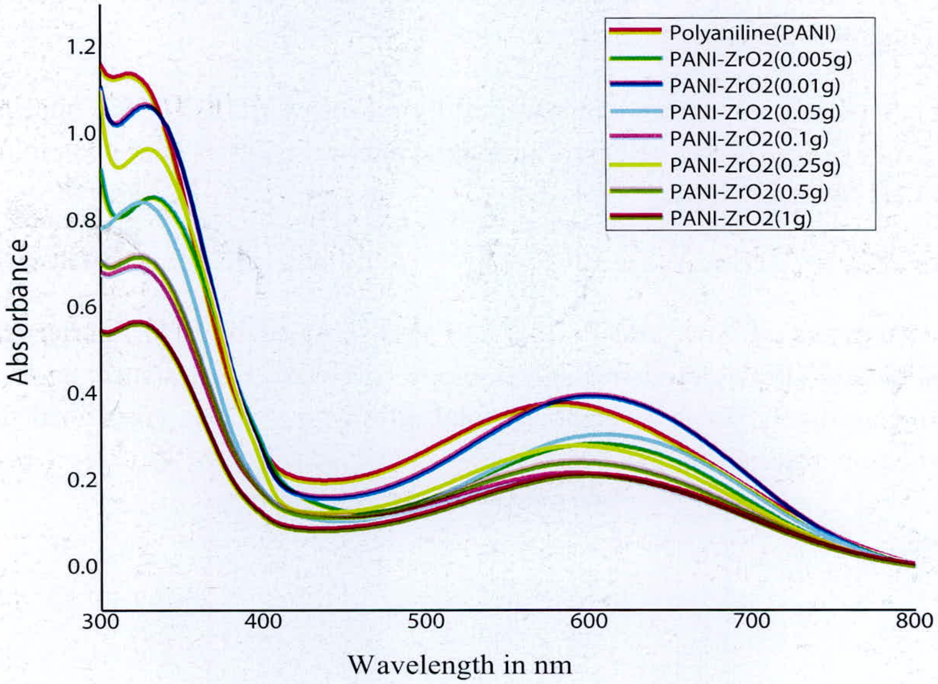


Fig. 4: Absorption peaks of PANI and PANI/ZrO_2 composites.

Morphology of PANI/ ZrO_2 composite nanofibers were examined by FESEM analysis. Typical FESEM micrographs of the PANI/ZrO_2 composite nanofibers are shown in Figure 5(a,b) and Figure 6, respectively. These micrographs confirm the formation of an abundance of nanofiber for ZrO_2 loadings.

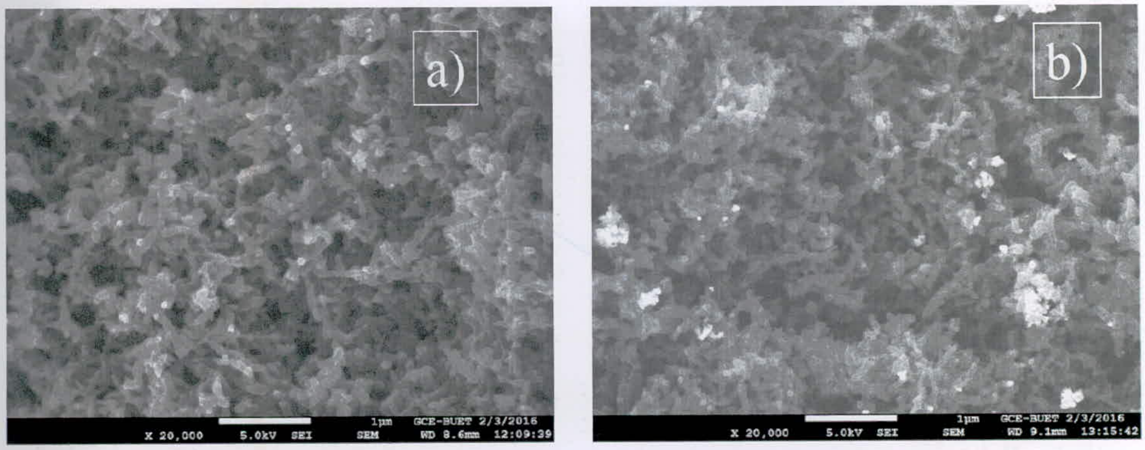


Fig. 5: FESEM image of the PANI/ZrO_2 composite nanofibers obtained at (a) 0.01 g and (b) 1.00 g loading of ZrO_2.

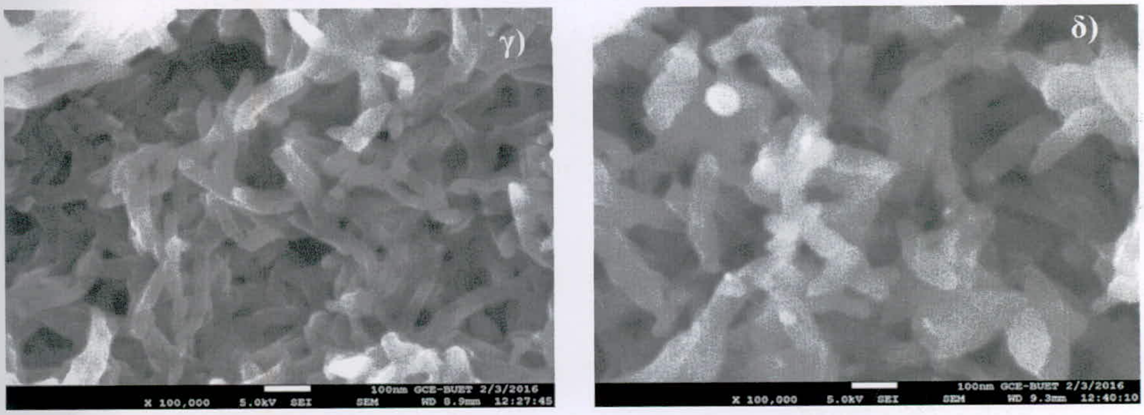
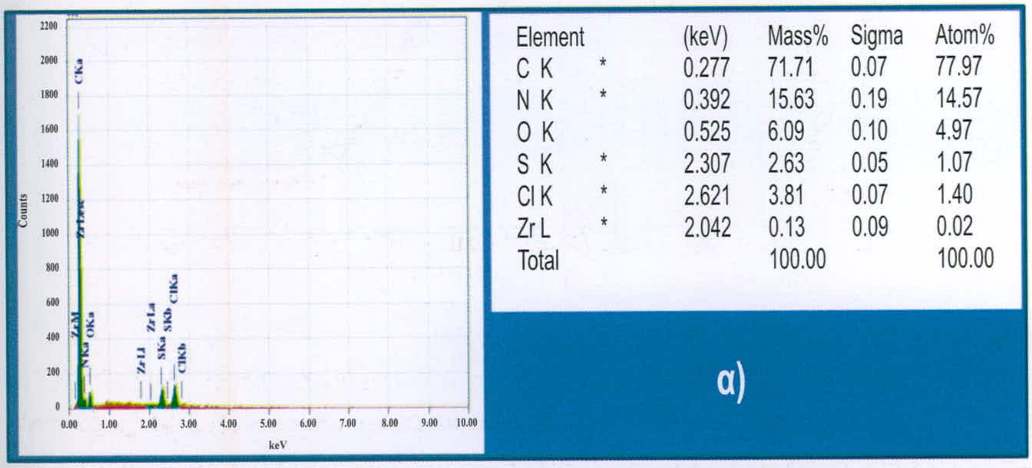


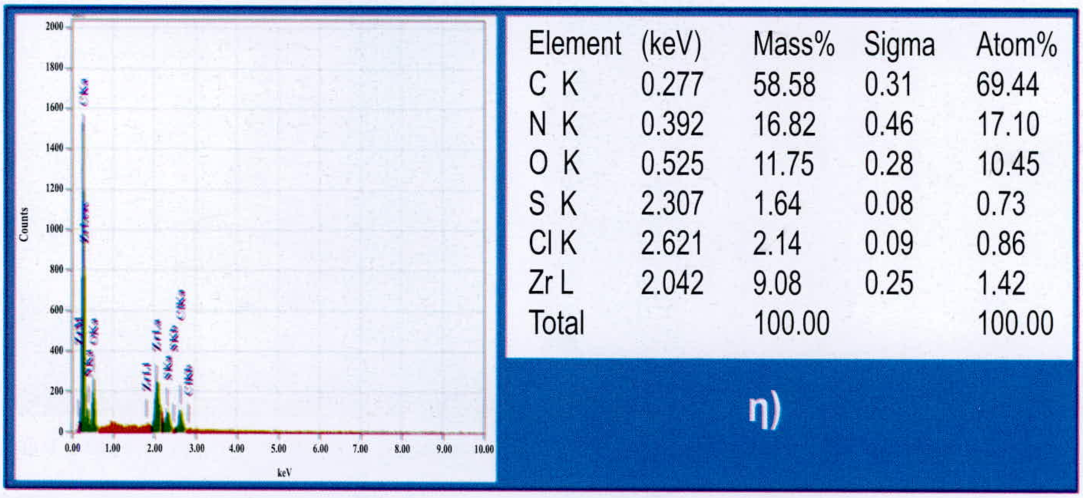
Fig. 6: FESEM images of sample γ and δ (Where γ and δ represents PANI/ZrO_2 fiber composites of 0.05g and 0.1g ZrO_2 loading respectively).

Elemental composition was analyzed byEnergy dispersive X-ray (EDS/EDX) spectroscopy (Figure 7 and Figure 8).



Element		(keV)	Mass%	Sigma	Atom%
C K	*	0.277	71.71	0.07	77.97
N K	*	0.392	15.63	0.19	14.57
O K		0.525	6.09	0.10	4.97
S K	*	2.307	2.63	0.05	1.07
Cl K	*	2.621	3.81	0.07	1.40
Zr L	*	2.042	0.13	0.09	0.02
Total			100.00		100.00

Fig. 7: Elemental composition of sample α (Where α represents PANI/ZrO_2 fiber composite of 0.005g ZrO_2 loading).



Element	(keV)	Mass%	Sigma	Atom%
C K	0.277	58.58	0.31	69.44
N K	0.392	16.82	0.46	17.10
O K	0.525	11.75	0.28	10.45
S K	2.307	1.64	0.08	0.73
Cl K	2.621	2.14	0.09	0.86
Zr L	2.042	9.08	0.25	1.42
Total		100.00		100.00

Fig. 8: Elemental composition of sample η (where η represents PANI/ZrO_2 fiber composites of 1.00g ZrO_2 loading).

Thermal stability of the prepared composites was investigated by TGA. TGA (Figure 9) data showed that with the increasing incorporation of ZrO_2 in the matrix of polyaniline (PANI), thermal stability of the composites rises.

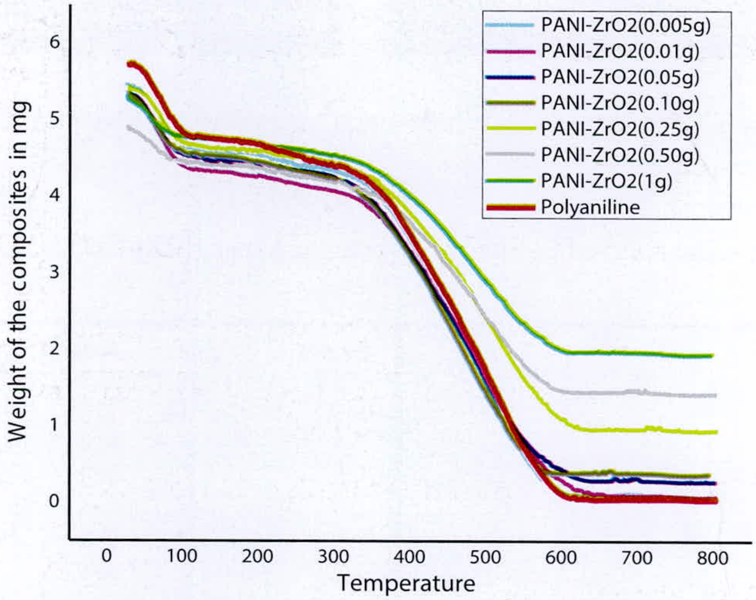


Fig. 9: TGA thermogram PANI and PANI/ZrO_2 composites.

Photoluminescence property of all the composites were investigated as shown in Figure 10and Figure 11, where the continuous emission spectra of PANI/ZrO_2 composites can be appreciated for 0.005 and 1.00 g loading of metal oxide.

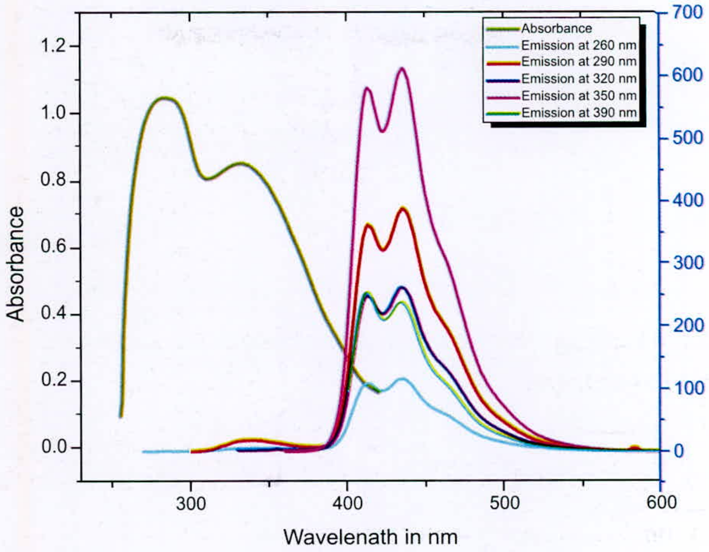


Fig. 10: PL spectrum of PANI/ZrO_2 (0.005g) composite.

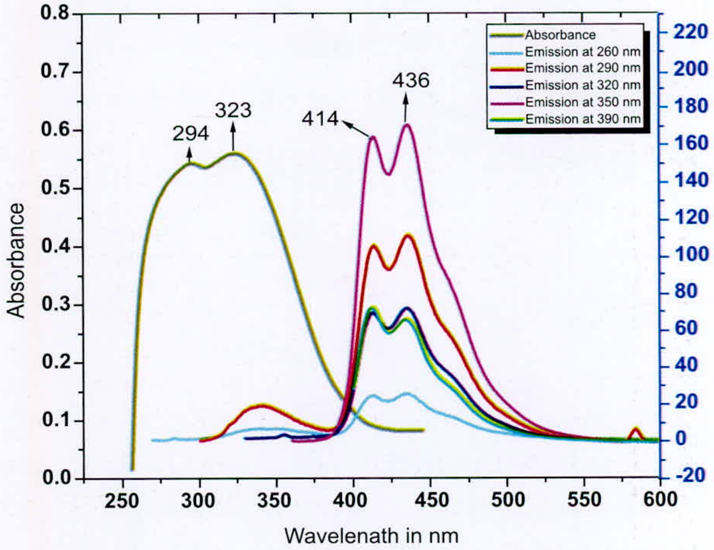
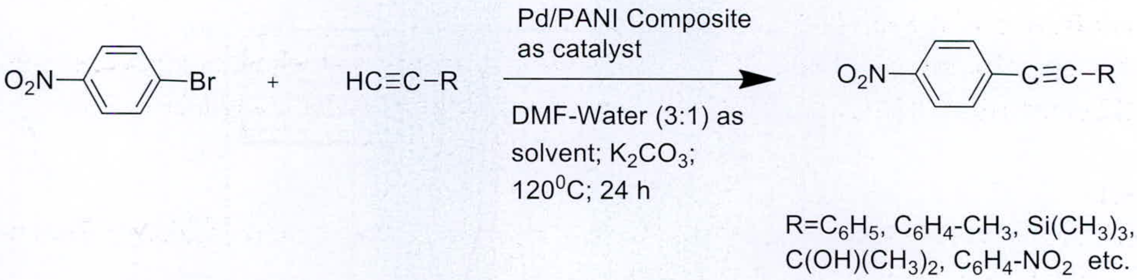


Fig. 11: PL spectrum of PANI/ZrO_2 (1.00 g) composite.

Catalytic Activityof PANI/Pd composites

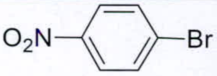
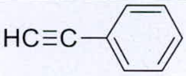
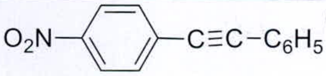
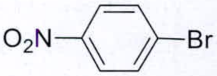
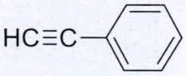
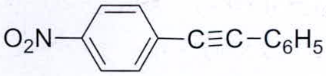
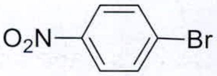
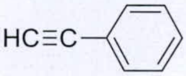
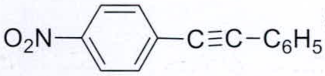
Sonogashira Coupling reaction of 4-nitrobromobenzene with different terminal Acetylenes

The synthesized Pd-PANI composite was employed as the catalyst for the Sonogashira coupling reactions (Scheme 1). The effects of catalysts were investigated using 4-nitrobromobenzene with different terminal acetylenes as shown in Table 1.



Scheme 1: Sonogashira coupling reactions of 4-nitrobromobenzene with different terminal acetylenes using Pd-PANI composite as catalyst.

Table 1: Optimization of the Sonogashira cross coupling reaction

Entry	4-nitrobromobenzene	Acetylene	Solvent	Product	Yield (%)
1			Diisopropylamine		0
2			DMF		7.5
3			DMF-Water (3:1)		84.1

Conclusion

A series of polymer matrix nanocomposites α, β, γ, δ, ε, ζ and η have been synthesized where α, β, γ, δ, ε, ζ and η denote the PANI/ZrO_2 composites of different amount of ZrO_2 loading 0.005g, 0.01g, 0.05g, 0.10g, 0.25g, 0.50g and 1.00g respectively.The freshly prepared PANI-ZrO_2 composites was investigated by means of FTIR, UV-Visible, FESEM/EDX, TGA and Photoluminescence Spectroscopy (PL). FTIR measurements revealed the possible incorporation of metal oxide in PANI. This is also confirmed by the UV-visible measurements, indicating the shifting of the absorption peaks with the addition of metal oxide. FESEM analysis showed that the addition of metal oxide between the range of 0.005 and 1.00 g, always produces nanofibers with regular and uniform surface morphology and without secondary growth and agglomeration of primary nanoparticles. TGA data showed better thermal stability for PANI-ZrO_2 composites material as compared to PANI nanofibers. In PL study β and γ composites have the highest and the lowest emission intensity respectively where the other composites have the emission intensity in between these two composites. Thus the photoluminescence property of the polyaniline compound is controllable with the inclusion of ZrO_2. The prepared PANI/Pd composites are effective as acatalystinsonogashira coupling reactions.

Publication from this Research

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