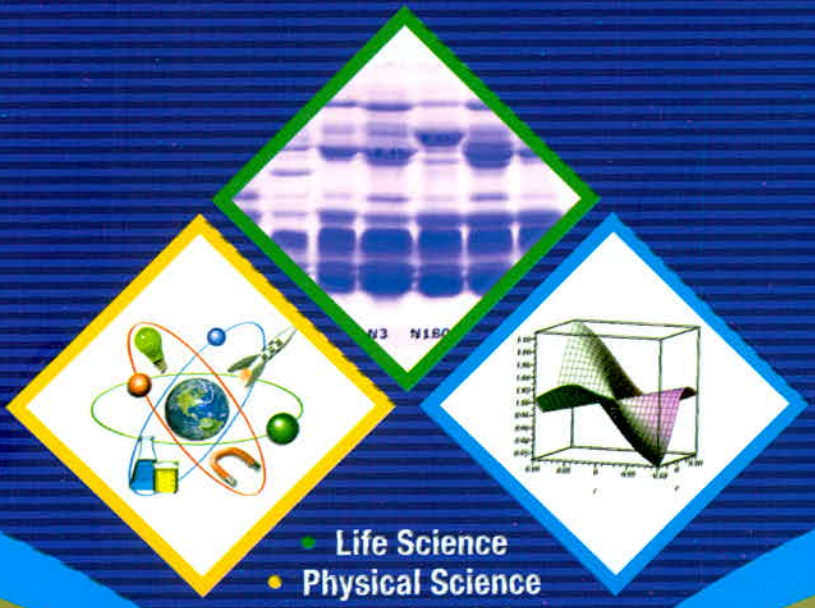
Report on Advanced Research
2010-2013



- Life Science
- Physical Science
- Mathematical Science

Grants for Advanced Research in Education

Secondary and Higher Education Division
Ministry of Education

Report on Advanced Research
2010 - 2013

Grants for Advanced Research in Education (GARE)

Ministry of Education
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بسم الله الرحمن الرحيم

PRIME MINISTER
GOVERNMENT OF THE PEOPLE'S REPUBLIC OF BANGLADESH

Message

I am happy to know that Ministry of Education has already financed a good number of projects under the program Grants for Advanced Research in Higher Education (GARE). The Ministry is now going to publish the first progress report on these completed research projects.

The Awami League government has been marching ahead with a vision to turn Bangladesh into a middle income country by 2021 and a developed one by 2041. In this regard, we need extensive research in all branches of knowledge especially in the field of science to generate knowledge and master the state of the art technology. Our government has been providing funds to the university teachers for strengthening their research and research management capabilities. It will upgrade the knowledge of understanding, generating answers of the unanswered questions and utilizing the usable outcomes for the wellbeing of the people. Priority of research depending on the demand of the nation should be set through the process of discussion among the academics and researchers.

I believe that the publication of the research report would bring the outcomes of the projects before other academics, researchers, post graduate students and other people.

I hope that the Ministry of Education will continue funding the research activities of the talented teachers of the universities and will publish subsequent issues of the research report for greater interest of the country.

I wish all out success of the project report.

Joi Bangla, Joi Bangabandhu
May Bangladesh Live Forever.

Sheikh Hasina

Nurul Islam Nahid 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 funded under Grants for Advanced Research in Higher Education (GARE) during the period 2009-2012. Quality research is imperative in higher education which may generate results that are examinable by peers, methodologies that can be replicated and knowledge that can be applied to enhance our capacity for solving national problems. Every pensive academician in higher education having distinguished academic background has the capacity of doing research. The fundamental requirement for research is an enquiring mind in order to recognize that there are questions that need answers.

The acquisition of knowledge is a continuous process which results through exploring the environment and asking questions. As an individual's knowledge increases, questions become more complex and answers are sought from professional experts, reference books and specialized journals. Research is the verification and strengthening of these limits of knowledge; assessing what is known up to the point, defining unanswered questions and devising ways of answering them in an organized and meaningful way. So, it may be noted that research is the systematic activity directed towards objectively investigating specific problems in order to establish relationships between and among variables. It seeks to answer specific questions without ambiguity. However, our research in higher education should be future oriented and designed to benefit learners rather than the researchers themselves. To meet the challenges of future successfully, higher education and research should progress simultaneously in Bangladesh.

In spite of our limited resources, Bangladesh Government is fully committed to provide required fund for research in higher education, because research methodologies give teachers the tools to analyze and make informed decisions about their practice. Research helps the teachers to share knowledge with colleagues and students to update and upgrade them. Too often researcher looks backward where there are lessons to learn to make it more participatory. I trust the achievement of our researchers will be demonstrated through patenting innovations and publication of their research findings in reputed national and international journals.

I hope, this progress report will be an asset specially for those who are interested in conducting research in higher education in future to contribute satisfactorily in our national development.

Nurul Islam Nahid M.P

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

Message

I am happy to learn that Secondary and Higher Eduation Division, MoE is going to publish the first progress report on the completed research projects under the program "Grants for Advanced Research in Higher Education" (GARE) funded by Bangladesh Government. Bangladesh is committed to improve the standard and ensure quality in higher education as the higher education plays a key role in making the use of the contribution of all citizens. The paramount significance of research is that it leads to generate knowledge and improvement in teaching and learning situation. Research requires a high level of alertness in planning, executing, observing, recording and reporting. Therefore, it develops in the researcher's scientific attitude of objectivity, curiosity and critical outlook. Research provides the teachers professional growth through a deeper understanding of pedagogical practices and psychology of learning and equips them with problem solving and leadership skills. So the Bangladesh Government is allocating sufficient fund for research in higher education through GARE for upgrading the knowledge and skills of teachers engaged in higher education. However, research must always be of high quality in order to generate knowledge that is applicable outside to the research setting or laboratory. The outcomes of the study undertaken should have implication whatever small it may be, for making policy, implementing project and adopting innovation.

A nation's prosperity can be chartered to a large extent by the contributions of its most gifted and talented citizens. When a nation fails to recognize and develop the talents of a large percentage of its population, it limits its ability to compete in the future. Through the research we are conducting, we may identify program components that will successfully stimulate advanced academic knowledge and skills in higher education with and operative commitment to reaching traditionally underserved students for their productive development.

Publishing the research achievement is an appropriate initiative to make our research known to national and international community. It may reflect the progress achieved so far in research in higher education with GARE funding and benefit the users interested in future research.

I express my appreciation to researchers, reviewers, experts and different committee members for their contribution and supports in GARE program. I thank the officials of Secondary and Higher Education Division and BANBEIS for providing their efforts in preparing this progress report.

সোহরাব

Md. Sohorab Hossain

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

Preface

One of the greatest assets of Bangladesh is its people and since witnessing exceptional national growth, the time has come to focus the investments in its human resources. Thus, for all intents and purposes, there is no alternative for development without a percentage of highly skilled experts. It is widely known that economic growth in this modern era largely depends on the welfare of its people and largely on development of science and technology in the country. The country has been riding on an influx of economic growth on the back of the manufacturing industries. Recently, however, the industries have taken a turn towards a more service oriented one, creating demand for a more skilled labour force. Research and development of science and technology are the integral parts of higher education in any society and the government of Bangladesh recognizing its importance, has allocated funds for advanced research in all branches of sciences and social science.

A selection committee was later formed for reviewing and choosing research funding with the director of BANBEIS as its member secretary. The members of this selection committee are chosen from different universities. Eminent scientists from a diverse background including physical sciences, biological and agricultural sciences and also from social sciences were selected to work in the committee. The committee started its work with the right enthusiasm from day one and as of today, three distinct groups of scientists have received research funds from this scheme. The researchers in the first phase completed their works and submitted the project completion report (PCR), while the researchers at the second and third phases are at various stages of completion.

Here, in this compilation we have presented the summarised versions of the completed projects. This publication highlights the achievements of our young, talented scientists. We hope that this booklet serves as a source of knowledge, encouragement and aspiration specifically for the budding scientific community as well as the general public. The accomplishment from this project leads me to believe that we have tapped in to an area with high potential and more importantly it has opened door to create further research opportunities for the young scientists in future.

Mesbahuddin Ahmed

Md. Fashiullah
Member Secretary
Editorial Committee &
Director
BANBEIS

Foreward

This is a great pleasure for me that we are going to publish the first publication on completed Advanced Research Project, supported by Ministry of Education under Grants for Advanced Research in Higher Education (GARE). Now Bangladesh Government is giving much emphasis on advanced research because, for the proper development of a country there is no alternative but 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 the country as a middle income country by 2021 and developed country by 2041. For that the Government has taken a massive program, advanced research is one of them. Recently the Government of Bangladesh is providing its remarkable budget in education sector. So we may hope that in research sector now 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 regards. 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

Introduction

The Government of Bangladesh, appreciating the role of science and technology in the national development of this very highly populated country, have decided to promote research and are offering funds through various agencies including through the Ministry of Education. Heavily publicized jute genome sequencing is one of the projects the Government have generously funded to put the country in the map of the state of the art technology, and for extracting benefit of the technology for the development of the national economy. The Ministry of Education is keen to facilitate creation of intellectual capital that can address today's issues, improve the quality of life, and provide momentum for economic growth by facilitating research and development across the country. Researchers and faculty members of universities and institutions of higher learning are invited to submit focused project proposals leading to solution of important national and regional problems. The proposals are evaluated by experts in the fields, and befitting funding made available based upon the potential of the proposal and the credential of researchers/investigators submitting the proposal.

Objectives

The Grants for Advanced Research in Higher Education (GARE) started funding from 2009 for research in public and private universities, Post-Graduate colleges of National and Open universities, Public Medical and Engineering universities. The objectives of this research program are:

- Funding higher research for technology innovation, adaptation and extension, specially identifying comparative advantage in the country and its successful application;
- Development of necessary number of critical manpower for latest technology exercise and application
- To reduce the total production cost and enhance 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 development actions; and
- To provide priority in basic and applied research.

Area of research

- Mathematical sciences
- Life sciences
- Physical sciences

Eligibility for research grant

- The research team/individual researcher should have experience in presenting papers in International/National Seminar/Symposium/Workshop.
- On-going number of research programs, adequate physical facilities and international relationship of receiving organization and published papers of the researcher must be satisfactory.
- The topic of the research should reflect the national demand and technical suitability of its application in the country.
- Priority is given to the researchers who have already considerable achievement in research in his/her field of specialization.

Projects included in this publication

This publication includes a total number of 36 Project Completion Reports (PCR) submitted by Principal Investigators of the projects and covers all the areas of research under the purview of the Grants for Advanced Research in Higher Education, MoE. There are 29 PCR from life science, 6 from physical science and 1 from mathematical science in this publication. The reports are of high standard based on original work of the researchers. The learned and reputed Principal Investigators described their study in brief indicating the outcomes of their research.

Conclusion

All professionals need to be able to trust the source of information and strict research ethics provide that assurance. Teaching does involve creative thinking and experimentation. Individuals and professional groups need to know what works and why does a teacher's action lead to improved student performance, increased motivation, commitment, better behavior and so on. Practitioners have to comply to policy, but that does not mean following a prescribed formula. Teachers can adapt it to fit the individual needs of their own students. But teachers are accountable. The public must have faith in the profession and attitudes to education vary across many social groups so the performance of teachers need to be demonstrated through the publication of research findings.

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Chapter 1

Funding Year 2010-2011

Antioxidants from Low Priced Fish: Screening and Evaluation

Md Golam Sarower and Wasim Sabbir

Institution: Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna
Duration: Two years (2010 - 2012)
Expenditure of the project: Tk. 1000000.00

Introduction

Antioxidants are one of the most important dietary functional components because they inhibit pathogenesis of various diseases such as cardiovascular disorders, diabetes, cancer, and inflammation. Reportedly, reactive oxygen species (ROS) have been linked to over 100 disorders (Halliwell and Gutteridge, 1999). Therefore, for maintaining a healthy biological system, it is critical to have the balance between oxidation and antioxidation. A variety of polyphenols, flavonoids, anthocyanins, vitamins have been reported as showing antioxidant (Kähkönen *et al.*, 1999). Widely used synthetic antioxidants are now under question due to their side effects like carcinogenicity (Gülçin *et al.*, 2007). Therefore, in response to the growing consumer concern about importance of dietary intake of antioxidants, and search for antioxidants and/or antioxidant from natural source have gained interest. Knowing the nutritional status of fish species usually eaten by the people could contribute to remedying problems of malnourishment and obesity. Although the potential antioxidant activity of some plants and fruits (Adib *et al.*, 2010; Mannan *et al.*, 2013) have been reported recently, it is not well addressed in fish, one of the major sources of dietary functional components. Small indigenous fish species (SIS) play a great role in protein supplement. Indigenous knowledge about these species and about their health benefits is high among rural population. This study was principally sought to assess antioxidant activity of the species concerned, and proximate composition, which indicate the desirability of the species from nutritional perspectives. Foods with rich protein and antioxidants are an essential component to a healthy well balanced diet.

Objectives

The specific objectives of this research are:

- to screen several low priced fish species including SIS, herbivore, carnivore, omnivore as the potential source of natural dietary antioxidants ;
- to evaluate the antioxidant activity of fish flesh extracts using reactive oxygen species (ROS) scavenging assay(s); and
- to know the biochemical composition of selected fish.

Methodology

Sample collection and preparation

Forty six fish species including eight marine SIS, eight freshwater SIS, ten herbivorous, ten carnivorousand ten omnivorous fish species were collected from different fish markets in Khulna, Bangladesh. After washing with distilled water, the muscle from collected fishes was separated from fish body except *A. mola.* Because of smaller size whole *A. mola* was used in sample preparation. Then 100 g fish sample was homogenized in ethanol and filtered it. The filtrate was kept in the shaking water bath at 40°C for drying. The fish extract and ascorbic were taken in a small vial and serial dilutions were prepared in ethanol and 0.004% DPPH were added to measure free radical scavenging activity.

Figure 1: Homogenizing fish samples

Estimation of antioxidant activity

Initially antioxidant activity was determined by TLC method. After applying DPPH on the TLC plates, yellow or whitish color on purple background was observed in the ethanol extracts of fishes. Then, radical scavenging activity of fish extracts against stable DPPH was determined by the slightly modified method explained by Brand-Williams et al. (1995). Freshly prepared DPPH solution (0.004% w/v) was taken in the test tubes, extracts were added to the tubes and shaken vigorously so that the final volume would be 3 ml. In the dark condition the tubes were allowed to stand for 30 min for the reaction to occur. The absorbance was determined at 517 nm using a spectrophotometer. First, the % inhibitions of DPPH free radical was measured, then % inhibitions were plotted against concentration and the inhibitory conc. 50% (IC50) was measured.

Analysis of proximate composition

Standard methods (AOAC, 2005) were followed for the analysis of proximate composition.

Statistical methods

The results were expressed as Mean ± SD. T-test was used to examine the difference between antioxidant level of freshwater and marine water species.

Results

Antioxidant Potential and Nutrient Content of Selected Small Indigenous Species (SIS) of Fish

The antioxidant level and nutrients in SIS of Bangladesh; eight marine and eight freshwater were measured by Thin Layer Chromatography (TLC) and 2, 2-Diphenyl 1-picrylhydrazil, 95% (DPPH) free radical scavenging method. The IC50 determined by DPPH varied from 327.04±0.06 µg/ml to 1888.21±0.10 µg/ml of wet weight. The highest antioxidant activity was observed in *Heteropneustes fossilis* followed by *Mystus gulio, Hemirhamphus gaimardi, Mystus vittatus, Megalaspis cordyla, Silonia silondia, Colisa fasciatus, Amblypharyngodon mola, Oxygaster phulo, Gobioides anguillaris, Chela laubuca, Plotosus canius, Channa orientalis, Mugil cephalus, Coilia dussumieri and Tetraodon cutcutia* (Table 1). The protein and lipid contents of the selected SIS ranged between 21.43 to 8.59%, and 7.22 to 1.75%, respectively. The study suggests the presence of potent antioxidant and appreciable amount of nutrients in selected fish samples. The top four potential SIS species consist of appreciable amount of protein and antioxidant are *Mystus gulio, Silonia silondia, Heteropneustes fossilis and Colisa fasciatus.*

Antioxidant Potential and Nutrient Content of Selected Herbivorous, Carnivorous and Omnivorous Fish Species

This study reports the antioxidant potential and chemical composition of muscle from 30 fish species of different feeding habits namely carnivore, herbivore and omnivore. Different in vitro assays used for determining antioxidant potential of extracts of fish species were: thin layer chromatography (TLC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. No significant difference of antioxidant activity or nutrient content was observed among carnivorous, herbivorous and omnivorous fishes. The IC50 measured by DPPH method varied from 154 to 2139 µg/ml of wet weight. The lowest IC50 value was observed in *C. idella* followed by *P. ticto, C. striatus, C. punctatus, L. rohita, A. testudineus, M. cordyla, O. mossambicus and G. giuris (*Table 1). The moisture content of samples ranged from 62 - 85%. The protein content was high in *C. idella* (24.3%) and low in *L. calbasu* (10.57 %). The lipid and ash content analyzed in the selected fish species ranged from 1.17-7.94% and 1.31-4.80%, respectively. Overall, the results suggest that fish of different feeding habit can be exploited for their antioxidant and nutrients components and used for consumption as well as value addition in food formulations.

Table 1: Antioxidant activity and proximate composition of freshwater and marine SIS, herbivorous, carnivorous and omnivorous species.

Scientific name	Local name	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	IC_{50} (µg/ml)
Freshwater species						
Amblypharyngodon mola	Mola	77.51±0.59	12.77±0.46	4.40±0.49	3.08±0.11	609.40±0.01
Channa orientalis	Cheng	72.09±0.57	16.86±0.58	3.91±0.58	5.09±0.57	1106.50±0.07
Colisa fasciatus	Khalisa	68.56±0.59	18.91±0.68	4.03±0.33	6.67±0.48	579.23±0.23
Heteropneustes fossilis	Shing	72.53±0.66	16.33±0.66	5.03±0.12	4.18±0.30	327.04±0.06
Mystus vittatus	Ayre	75.61±0.54	13.59±0.54	3.88±0.38	4.51±0.57	405.02±0.09
Oxygaster phulo	Chela	82.28±0.59	8.59±0.55	2.30±0.59	3.64±0.22	750.19±0.17
Plotosus canius	Kain magur	76.35±0.53	18.18±0.58	1.75±0.59	2.91±0.43	1029.17±0.21
Tetraodon cutcutia	Potka	73.78±0.58	17.21±0.22	4.07±0.17	3.51±0.46	1888.21±0.10
Marine species						
Chela laubuca	Chela	74.65±0.48	16.43±0.92	3.01±0.03	3.04±0.13	930.47±0.02
Coilia dussumieri	Amadi	75.13±0.25	11.97±0.09	3.06±0.25	6.09±0.19	1161.00.±0.17
Gobioides anguillaris	Shagor bele	74.26±0.25	15.10±0.98	1.87±0.02	3.86±0.05	786.20±0.15
Hemirhamphus gaimardi	Ekthuta	75.09±0.24	17.16±0.27	2.14±0.05	2.14±0.17	398.41±0.08
Megalaspis cordyla	Kawa	68.96±0.81	17.91±0.96	7.22±0.26	3.18±0.44	481.04±0.07
Mugil cephalus	Parshe	69.38±0.74	18.38±0.78	4.20±0.42	6.31±0.51	1152.15±0.07
Mystus gulio	Nuna tengra	64.52±0.98	21.43±0.64	5.62±0.68	4.42±0.39	364.18±0.05
Silonia silondia	Shilong	68.06±0.37	19.20±0.50	6.18±0.43	4.32±0.56	489.20±0.11
Herbivore						
Ctenopharyhngodon idella	Grass carp	78.07±0.70	12.65±0.41	4.93±0.18	3.19±0.03	154±0.02
Hypophthalmichthys molitrix	Silver carp	78.01±0.11	16.01±0.30	2.83±0.61	2.56±0.11	963±0.24
Labeo bata	Bata	74.51±0.39	21.24±0.62	2.06±0.03	2.31±0.06	966±0.34
Puntius gonionotus	Thai Shorputi	73.05±0.37	20.21±0.46	4.25±0.37	2.73±0.14	1679±0.18
Puntius ticto	Titputi	79.2±0.20	17.41±0.01	1.49±0.07	1.37±0.13	268±0.28
Labeo calbasu	Kalbasu	80.05±0.19	10.57±0.06	3.59±0.06	4.80±0.49	982±0.07
Labeo rohita	Rui	73.5±0.07	17.85±0.02	3.56±0.08	2.80±0.26	397±0.02
Tenualosa ilisha	Ilish	81.26±0.23	12.42±0.27	1.17±0.06	3.78±0.82	1011±0.05
Pomadasys hasta	Sada datina	76.09±0.49	18.45±0.31	3.54±0.17	1.87±0.47	588±0.06
Amblypharyngodon mola	Mola	68.45±0.21	18.90±0.31	7.50±0.10	3.69±0.09	520±0.09
Carnivore						
Channa punctatus	Taki	74.78±0.09	18.43±0.51	4.57±0.41	1.59±0.61	346±0.04
Channa striatus	Shol	69.71±0.32	24.30±0.24	3.47±0.13	2.20±0.17	328±0.01
Mystus aor	Ayre	72.45±0.25	20.12±0.08	5.21±0.05	1.98±0.58	1170±0.12
Notopterus chitala	Chital	74.69±0.41	15.42±0.23	5.68±0.07	3.27±0.39	2139±0.21
Notopterus notopterus	Foli	78.07±0.01	18.32±0.70	2.66±0.18	1.31±0.04	1757±0.15
Harpadon nehereus	Loyta	79.76±0.22	13.76±0.42	4.56±0.13	2.03±0.08	829±0.09
Lates calcarifer	Vetki	85.43±0.04	11.64±0.03	1.32±0.10	1.52±0.14	1482±0.05
Megalaspis cordyla	Kawa	74.87±0.50	17.54±0.13	5.39±0.08	2.19±0.51	408±0.03
Polynemus paradiseus	Taposhi	73.97±0.40	15.54±0.32	5.43±0.19	3.23±0.52	1111±0.07
Trichiurus haumela	Churi	68.21±0.12	23.26±0.33	5.87±0.19	2.39±0.44	949±0.02
Omnivore						
Anabas testudineus	Koi	76.59±0.10	18.25±0.19	1.30±0.06	2.32±0.07	398±0.11
Mackerel sp	Konkon	77.24±0.41	13.70±0.52	2.69±0.43	4.01±0.05	531±0.06
Pampus chinensis	Rupchanda	77.15±0.45	14.30±0.04	3.45±0.28	3.10±0.02	600±0.08
Rhinomugil corsula	Khorsula	75.98±0.31	13.67±0.25	6.87±0.43	3.12±0.12	1266±0.09
Scatophagus urgus	Bishtara	78.34±0.51	17.98±0.42	2.17±0.27	1.90±0.47	872±0.02
Catla catla	Catla	76.8±0.42	12.87±0.25	6.87±0.03	2.79±0.43	538±0.03
Cyprinus carpio	Common carp	68.6±0.17	22.34±0.18	4.06±0.51	4.23±0.43	824±0.17
Glossogobius giuris	Balia	70.2±0.23	21.37±0.05	5.91±0.17	1.30±0.57	492±0.19
Oreochromis mossambicus	Tilapia	71.95±0.16	15.85±0.05	7.94±0.23	4.26±0.7	411±0.07
Oreochromis niloticus	Nilotica	79.12±0.14	11.45±0.37	5.32±0.17	2.52±0.28	1028±0.05

Conclusion

The top four potential SIS species consist of appreciable amount of protein and antioxidant are *Mystus gulio, Silonia silondia, Heteropneustes fossilis and Colisa fasciatus.* Therefore, this low cost available SIS of Bangladesh could be an excellent source of nutrient and antioxidant thus provides us medicinal values. Among the herbivorous, carnivorous and omnivorous fish *Ctenopharyhngodon idella, Channa striatus* and *Anabas testudineus* species showed highest antioxidant activities. Studies on the specific type of antioxidants in the fish species concerned can be done in future.

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Online Learning and Evaluation System for Database Design, Tuning and Programming in SQL in a Client-Server Environment

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Institution: Department of Computer Science and Engineering, BUET, Dhaka 1000

Duration: One Year (2011 to 2012)

Expenditure of the project: Tk. 600000.00

Introduction

The project titled "Online Learning and Evaluation System for Database Design, Tuning and Programming in SQL in a Client-Server Environment" is an e-Learning project. Database Management System (DBMS) is a core course of Computer Science and Engineering and the database design and programming are essential parts of any software development during study period and system development after graduation. So the students must gain sufficient knowledge in these areas. The main activities of the project were to analyze the course contents of Database Management System and identifying the areas to implement the e-Learning system, design the different components of the system, and implement the system. The eLearning system has been developed for effective learning of database design and programming in SQL.

Objectives:

The main objective of this project is to develop an eLearning system for effective learning of database design and programming.

Methodology

Analysis: Firstly, the contents of DBMS, the programming in SQL, the database design and related topics were analyzed.

Design: Secondly, the eLearning system modules including user interfaces, the problem bank creation, the user management, student learning and evaluation management, performance monitoring and data set management modules were designed.

Implementation: Thirdly, the eLearning system was implemented in a client-server environment.

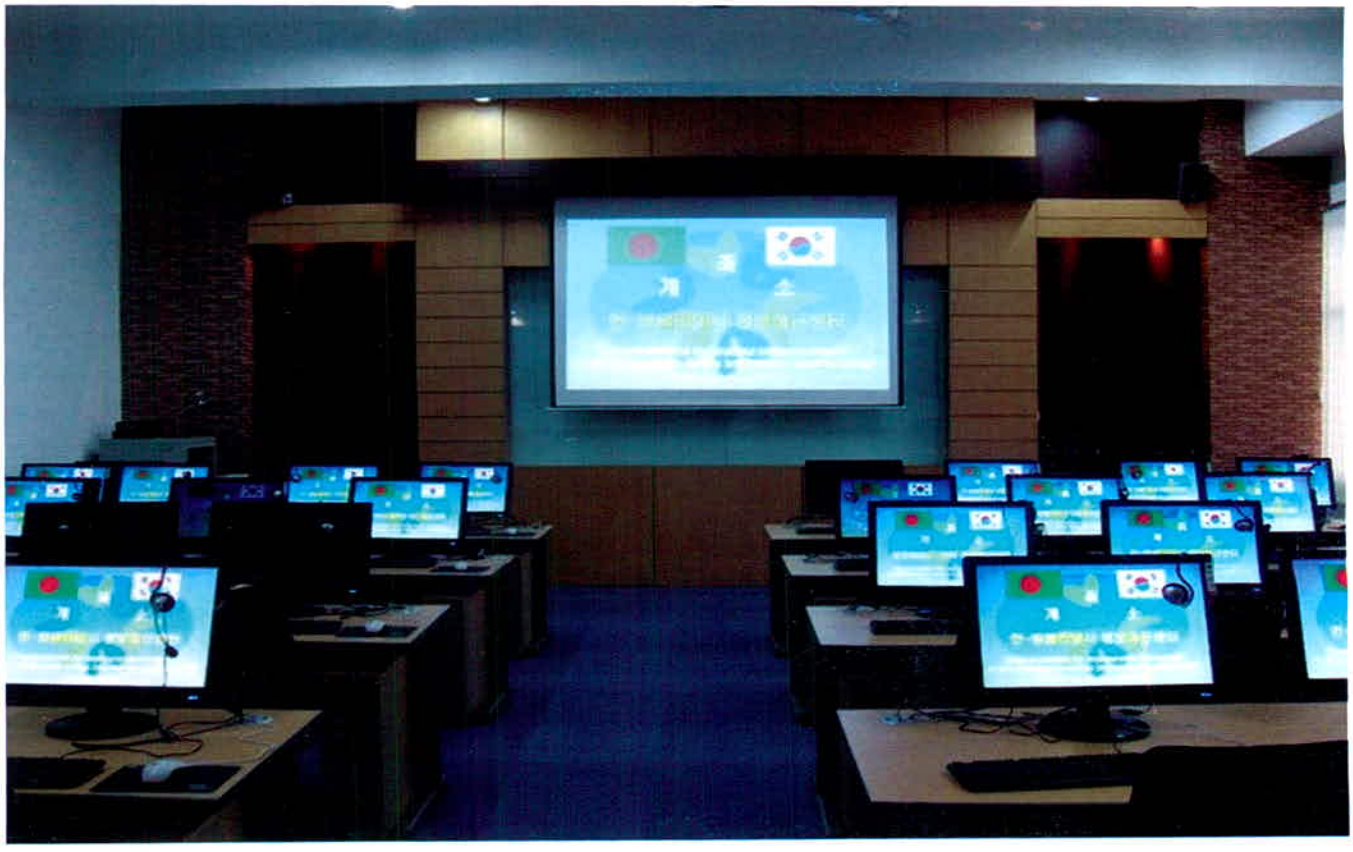

Figure 1: Database Laboratory (IAC Lab) of the Department of CSE, BUET where the eLearning of Database System has been implemented in a server and students use it.

Results

The result of the research is an eLearning system that has been described below in short. The eLearning system consists of five interrelated modules: the problem bank creation, student learning, progress monitoring, user management and data set management modules. The interface for problem-bank creation and management module is given in Fig. 1. Each SQL query problem has to be defined based on some schema. In the interface, the top part defines the schema (title of the schema, ERD design, list of tables, details of data types). After schema definition, SQL problems are defined as per the schema. The problem is defined in a structured form so that the solution is unique. Each problem is associated with a complexity value. For simplicity, we have considered the complexity values from levels 1 to 5 as given in part 3 of Fig.1. Part 4 shows the standard answer of the SQL problem as prepared by database teachers. Different buttons to test the standard SQL expression, prepared by the teacher, are shown in part 5.

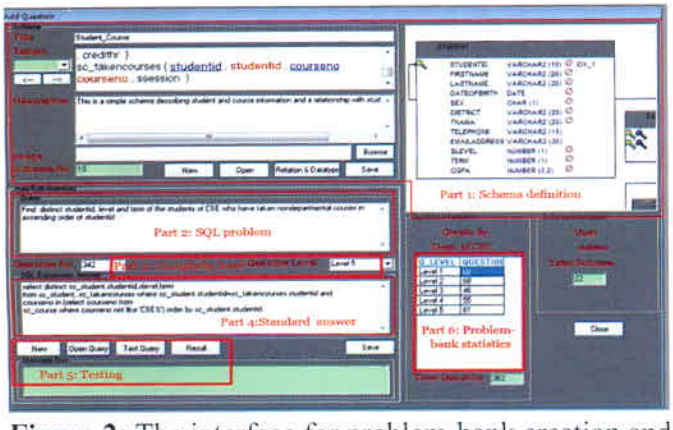

Figure 2: The interface for problem-bank creation and management module

Editing the existing answers of any SQL problems from the problem-bank, verifying the result of any SQL expression, and saving any solution of the problems are also given in part 5. Part 6 shows the statistics of the problem-bank.

Reuse of problem-bank for learning/teaching and evaluation

Figure 2 shows the interface for reusing the problem-bank for learning/teaching as a blended approach by generating test sets with different options. In the interface, part 1 is the statistics of problem bank showing how many problems of different complexity levels are there, how many problems of different levels are available to create the test set, etc. Part 2 is used to set different parameters of the test set, e.g., duration of the test set type of the test set, number of sets to allocate alternatively in a round-robin fashion to students of same class, and at the valid date and time of the session. Part 3 is used to reset the problem-bank to reuse the problems. In traditional learning system, students are not encouraged on life-long learning. But in this eLearning system, when students will find that even after regular course work, there are still many problems in the bank remaining unsolved and these are related to real-life problem-domain, they will be interested in future learning.

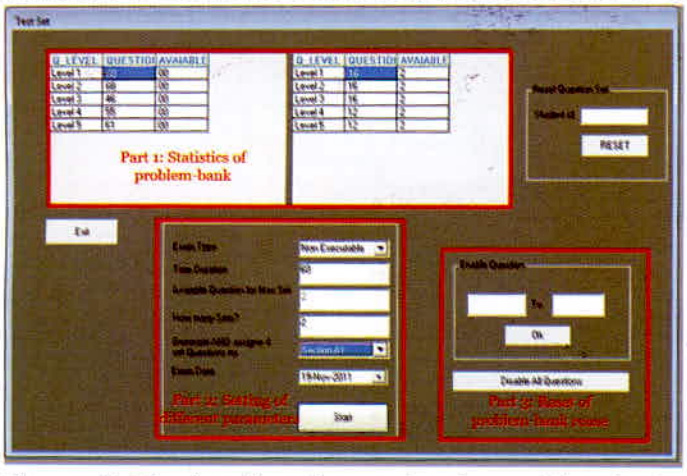

Figure 3: The interface for reusing the problem-bank

Use of test set for learning and evaluation

A test set can be allocated to both teacher and the students and they can log in to the system in learner-mode. Teacher let the students to solve the problems by themselves and the teachers can support them to solve the problem and learn. If the students totally fail to solve the problem, then the teacher can do it partially to support the students learning. If some students can solve the first problem they can proceed by their own. Those are not able to solve at all, the teacher can solve the problem and show the students about the solution and explain the solution. Learning about the solution, student will do it again by himself and develop the confidence about the problem-solving. During students' performance evaluation phase, the test set are allocated to the students and students solve the allocated problems individually and submit the solution in predetermined time

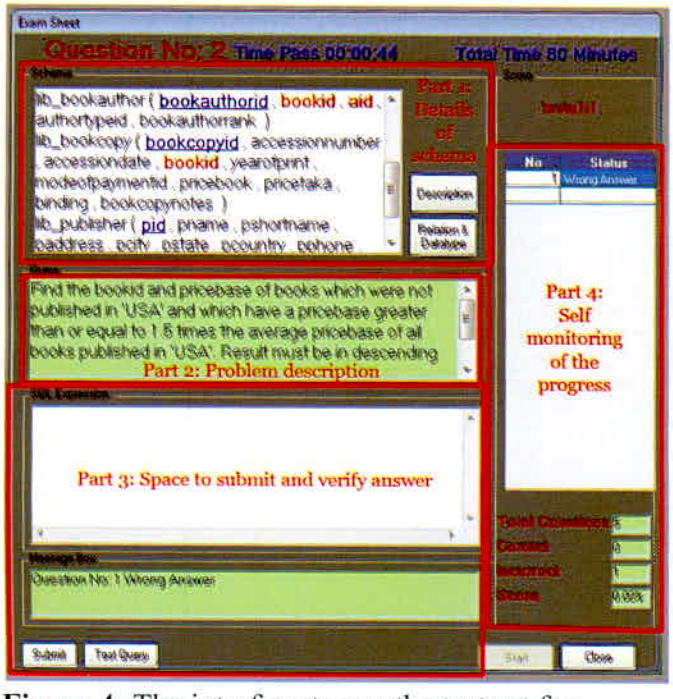

Figure 4: The interface to use the test set for learning and evaluation

Figure 3 shows the interface to use the test set as self-learning or self-evaluation or instructor-led-blended teaching/learning by the students. Part 1 shows the details of schema for the corresponding problem of the test set. Problems and schema are always linked up. Part 2 is the description of the problem. Part 3 is the user space to submit the solution of the problem, test the solutions, and submit the solution. Part 4 is self-monitoring of the progress. For every problem, student will be able to know instantly whether his submission is correct or not and the total score obtained.

Progress monitoring by the teacher

Teacher can monitor the progress of all students by the interface given in Figure 4. In this interface, part 1 shows the students' test history. Part 2 shows the performance details of a particular test that has already been completed or is under progress. Part 3 shows the details of schema for a problem that is selected in part 2 for checking the details of the student's submissions. Part 4 shows the standard solution of the problem and the students' submissions. Both submissions can be seen here to see the results and verify the correctness of students' submissions.

Conclusion

The eLearning system has been implemented in a server and client has been installed to all of the workstations of the database laboratory in the department of Computer Science and Engineering, BUET. The course teachers of database course can create assignments using the existing question bank or can create new assignments that are again stored in the problem-bank after the class. The eLearning system has been using in the database laboratory course and an evaluation was done on the effectiveness of learning outcome and it was found very much satisfactory.

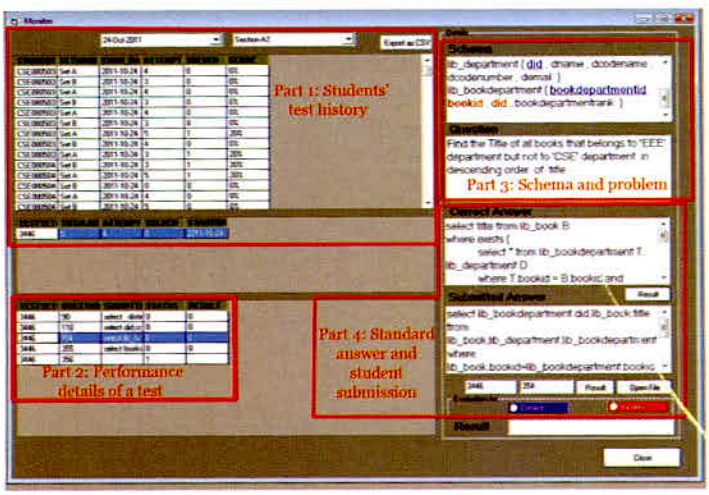

Figure 5: Interface for progress monitoring of students

Publication

Hoque ASML, Islam MM, Hossain MI, and Ahmed MF 2012. Problem-Based e-Learning and Evaluation System for Database Design and Programming in SQL. *International Journal of e-Education e-Business, e-Management and e-Learning,* IACIST PRESS, 2(6):537-541.

Use of Anesthetics in the Live Land Transport of Rohu, Silver Carp and *Catla* Fingerlings

Mahmud Hasan and Salim Ahmed

Institution: Department of Fisheries, University of Dhaka, Dhaka-1000

Duration: One Year (2010 - 2011)

Expenditure: Tk. 1500000.00

Introduction:

Anesthetics have been used in fish transport to minimize stress and mortality (Barton and Peter, 1982; Carmichael et al., 1984; Olsen *et al.*, 1995). The use of anesthetics generally depends on their efficacy, availability, cost-effectiveness and safety. Of the commonly used anesthetics, MS 222 (3 aminobenzoic acid ethyl ester methane sulfonate) is the most widely recommended anesthetic for fish handing and transport (Gilderhus and Marking, 1987; Ross & Ross 1999). Durve (1975) recommended seven of thirteen anesthetics including quinaldine and benzocaine suitable for use in the live transport of mullet seed, *Mugilcephalus, Lizadussumeiri* and *Lizatade.* Tertiary amyl alcohol and butyl alcohol have been used for transport mullet, *Mugiltrichodon* post larvae (Alverez-Lajonchere and Moreno, 1982). Gilderhus and Marking (1987) screened the efficacy of 16 anesthetics and recommended four, MS 222, quinaldine sulfate, benzocaine and 2-phenoxyethanol. Matson and Riple (1989) demonstrated that only metomidate is effective in rapid anesthesia in cod, *Gadusmorhua* compared to MS 222, chlorobutanol, benzocaine and phenoxyethanol, and MS 222 and benzocaine are chemically similar. Malmstrom *et al.* (1993) showed that metomidate is safe, and effective at a low-dose compared to MS 222 in inducing anesthesia in Atlantic halibut, *Hippoglossushippoglossus*. Massee *et al.* (1995) demonstrated that MS 222, quinaldine sulfate and metomidate are equally important anesthetics for larval red drum. Quinaldine was the most effective sedative in anesthetization in coral reef fish (Munday and Wilson, 1997). Quinaldine at 9 µL L-1 produced the quickest induction and recovery in sea bream (Hseu *et al.*, 1998). Clove oil was also used as an alternative to MS in reducing handling stress in rainbow trout (Wagner et al., 2003); in juvenile chinook salmon (Chow and Heath, 2000) and in rainbow trout (Wagner *et al.*, 2003). Metomidate and clove oil have been shown to be less stressful than MS 222 and quinaldine (Small, 2003). No difference was found on feed intake using MS 222, clove oil and CO2 in steelhead trout, *O. mykiss* (Pirhonen and Schreck, 2003). While clove oil and metomidate prevented the rise of cortisol in Atlantic salmon, benzocaine did not (Iversen *et al.*, 2003). Coyle *et al.* (2004) recommended MS 222, benzocaine, quinaldine, 2-phenoxyethanol, metomidate, clove oil for warm water fishes. Hasan and Bart (2007) reported on the use of quinaldine and benzocaine with aerators for fingerling transport of rohu *Labeorohita* and silver carp *Hypophthalmichthysmolitrix.* However, use of MS 222, tertiary amyl alcohol, butyl alcohol and 2-phenoxyethanol with compressed oxygen on the live transport of rohu, silver carp and catla, *Catla catla* fingerlings has never been reported.

Objectives

The overall objective of this study was to improve the livelihoods of the poor carp seed traders by improved and sustained income and reduced vulnerability through improved fish seed transport techniques. The specific objectives were:

i. To determine the effects of MS 222, Tertiary amyl alcohol, butyl alcohol, and 2-phenoxyethanol on the survival and RNA/DNA ratio in silver carp, rohu and catla fingerlings and

ii. To determine the doses of anesthetics for long distance high density safe transport of carp fingerlings with and without compressed oxygen.

Methodology

Experimental fish and transport simulation procedures:

Silver carp, catla and rohu fingerlings (8 to 12 cm) were obtained from carp seed market, Jessore and sued as experimental animals. On farm experiments were conducted. The fingerlings were conditioned. Fingerlings were placed into 200 L plastic drums at 400 g/L. The transport vessels were filled with well water mixed with anesthetics (Tertiary amyl alcohol, butyl alcohol, and 2-phenoxyethanol) with continuous oxygenation. Each experimental vessel was given oxygen injection.

Experimental design:

Factorial design was used with three replications. The experimental variables were the transport methods and trip durations. The control of the current methods are being practiced by the fish seed traders (size of the fingerlings/fry, loading density, handling and transport method that is the hand agitation). Fish mortality rate and RNA/DNA ratio were measured as the levels of stress experienced by the fingerlings.

Silver carp fingerlings with 2-PE at 115 µL/L

Silver carp fingerlings with Quinaldine at 200 µL/L

Silver carp fingerlings with Benzocaine at 20 mg/Land

Silver carp fingerlings in the drum with TAA oxygen injection

Figure 1: Preliminary trials and final transport simulation of silver carp fingerlings

Sedatives and oxygen

MS 222, tertiary amyl alcohol, butyl alcohol and 2-phenoxyethanol were used as anesthetics. The proper dose for final experiments was determined in a series of preliminary trials. Compressed oxygen cylinders from Bangladesh Oxygen Limited (BOL) were hired from local market.

Fish samples from each drum (n=5) were collected before loading ("0" time) and at different time periods after loadings of transport. Dead fingerlings were removed and water quality variables were also monitored in the transport vessels before loading and after loading at different time interval.

Data analysis

Mortality rate data will be square root transformed before statistical analysis. The RNA/DNA ratio data were compared by using ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data were analyzed using statistical software SPSS version 10.0 with the level of significance at $p<0.05$.

Results

Rohu fingerlings at a rate of 400 g/L can be safely transported for 9 hours by using pure oxygen injection with or without sedatives with little or no immediate and delayed mortality by using 200 L plastic drums. Catla fingerlings at a loading density of 400 g/L can also be safely transported for 6 hours with pure oxygen injection in plastic drums by using 2 PE and benzocaine. Injection of pure oxygen resulted in nearly 20% immediate and 7% delayed mortality. Similarly, quinaldine with oxygen resulted in 7% immediate mortality and 2% delayed mortality. Silver carp fingerlings can be transported without any mortality by using pure oxygen and tertiary amyl alcohol. Loading density was 400 g/L and the transport system was 200 L plastic drums. Silver carp may also be transported at the same loading density by using only oxygen and 2 PE with little immediate and delayed mortality.

Conclusion

Carp fingerlings are being transported using pure oxygen injection into the plastic drum water in Jessore area.

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Synthesis and Characterization of Low Cost Carboxy Methyl Cellulose (CMC) Derivatives of Different Grades from Knitted Rag for Textiles and other Uses

Md Ibrahim H. Mondal and Faisul Islam Farouqui

Institution: Dept. of Applied Chemistry & Chemical Engineering, Rajshahi University, Rajshahi 6205
Duration: One year (2010 - 2011)
Expenditure of the project: Tk. 310000.00

Introduction

Carboxy Methyl Cellulose (CMC) is one of the most versatile cellulosic derivatives of the present day world and tremendously used in textiles and many other industrial fields including food products, paper, cosmetics, pharmaceuticals and adhesives . Hence it is a vast important in the textile manufacturing and in every day life. Bangladesh is a textile industry based country and large amount of CMC is being imported to meet its demand for textile sector in every year and the importance of CMC is increasing day by day.

It has been reported that CMC was prepared from different cellulosic sources by different researchers following many different methods. But no worth mentioned work has been performed on the synthesis of CMC from knitte drag. Knitted rag contains high amount of good quality cellulose and huge amount of knitte drag are deposited as textile was tesin different garment industries that have virtually no use. The wastes contain both colored and non-colored knitte drags. These are normally dumped in nearby open space, and degraded naturally, hence these created environmental pollution. As the knitte drag contains high amount of good qualityα-cellulose, it can easily be collected with almost free of cost, will b ea great source for the manufacture of CMC and other cellulose derivatives. In this report, a multiple-step carboxy methylation technique will be adopted for producing lucrative CMC, with good solubility and fair degree of substitution (DS).

Objectives

The objective of the present work is to explore the use of this knitte drag by synthesizing different grades CMC by applying multiple steps carboxy methylation techniques. The novelty of this technique is there-use of the used ethanol in carboxy methylation.

Methodology

The knitted rag, a cellulosic material, was collected from Mozart Knit Ltd, Ashulia, Saver, Dhaka. Chemicals were purchased as analytical grade and used without further purification. The collected non-colored knitte drag was washed with 1% Na_2CO_3 solutionat 60^0C and the colored knitted rag was bleached with H_2O_2.

The CMC was synthesized by the conversion of cellulose (knitted rag) to alkali celluloses wollen in aqueous NaOH and a surplus of 95% ethanol as solvent with mono- chloro acetic acid. The synthesis of CMC was carried out by basification and etherification techniques. The synthesized crude Na-CMC was purified with ethanol and then dried in a desiccators over P_2O_5.

Degree of substitution (DS), molecular weight by "Mark-Houwink–Sakurada" equation, CMC content, Na Clin Na-CMC, moisture content, water absorption and gel content were determined. Grafting of CMC was carried out with methyl methacrylate (MMA), tensile strength was measured by using at ensile strength tester. IR-spectra of CMC and graft were recorded using KB rpellet technique with a FTIR spectrometer (FTIR-8900, Shimadju, Japan). The surface morphology of washed and CMC modified cotton fabric was examined by SEM.

Results

The synthesis of Na-CMC from knitted rag was carried out according to the Scheme 1. The potentiality of the process is the re-use of ethanol after washing of crude Na-CMC. The yield of Na-CMC obtained from carboxy methylation process at one to seven steps and the corresponding solubility in water, DS, molecular weight, CMC content, NaCl and others materials of the prepared CMC was determined and are listed in Table 1. It can be seen from Table 1 that the yield of CMC increased with the increase of the number of reaction steps in the optimized condition of carboxy methylation. The Na-CMC obtained from multiple steps carboxy- methylation showed a highly solubility in water than the product of single step. DS increased very fast at the initial step, but after the 4th step the increasing rate is slow. This is happened due to the substitution reaction which decreased the number of OH groups very fast.

The molecular weight of Na-CMC obtained was determined by viscosity method and calculated by using "Mark-Houwink-Sakurada"equation, $[\eta]=KMa$. The molecular weight of the prepared Na-CMC increased gradually with the increase of reaction steps [Table 1]. There as on is that as the DS increased with successive steps, number of OH groups attached to cellulose molecule was replaced by carboxymethyl groups.

Table1: Preparation of Na-CMC, and determination of DS, solubility, molecular weight, CMC, NaCl and others in synthesized Na-CMC.

Reaction steps	Yield of Na-CMCg	Solubility in water	DS	Molecular weight	CMC %	NaCl %	Others %
1	3.60	Partially soluble	0.91	153,886	72.60	3.07	24.33
2	7.51	Soluble	1.64	187,888	76.80	3.29	19.91
3	10.52	Highly soluble	2.46	213,796	79.00	3.44	17.56
4	12.31	Highly soluble	2.72	224,543	81.40	3.51	15.09
5	13.04	Highly soluble	2.80	241,046	82.80	3.58	13.62
6	14.04	Highly soluble	2.82	246,603	83.80	3.65	12.55
7	14.94	Highly soluble	2.84	252,231	85.00	3.65	11.35

The percentage of CMC and NaCl increasd gradually with the increase of DS as well as number of reaction step sand at the same time percentage of other materials decreased. This result confirms the increase of CMC with the increase of reaction steps (Table 1).Maximum graft yield was formed when CMC film was grafted with 80% MMA (Table 2).

Table 2. Effect of monomer concentration on modification of CMC film with MMA.

No. of experiments	Monomer concentration, %	Grafting yield, %	Grafting Efficiency, %
1	40	10.29	25.74
2	60	12.76	19.60
3	80	20.58	19.40
4	90	13.51	15.15
5	100	12.35	12.35

Table 3. Determination of grafting properties of 2% CMC film (DS=2.71).

Samples name	Moisture content, %	Water absorption, %	Gel content %	Tensile strength, kg	Elongation At break, %
CMC film	2.86	7.69	99.47	1.5	2.6
Grafted CMC film	1.08	1.78	97.03	2.8	1.7

The results of physical properties, such as moisture content, water absorption, gel content, tensile strengt hand elongation at break, of CMC and grafted CMC are listed in Table 3. The decrease of moisture content, water absorption, gel content and increase of tensile strength proves that MMA had been successfully grafted or incorporated with the prepared CMC film.

The FTIR spectra of standard CMC film (DS=0.8), prepared CMC film (DS=2.71) and MMA grafted CMC film are shown in Fig.1. From Fig.1, it can be seen that the peaks assigned at 163cm^{-1} and 142cm^{-1} indicate the presence of carboxy methyl substituent in both standard CMC and prepared CMC films. An extra absorption peak at 1741 cm^{-1} in Fig. I suggests that MMA had been successfully grafted onto prepared CMC film. This result is supported by SEM.

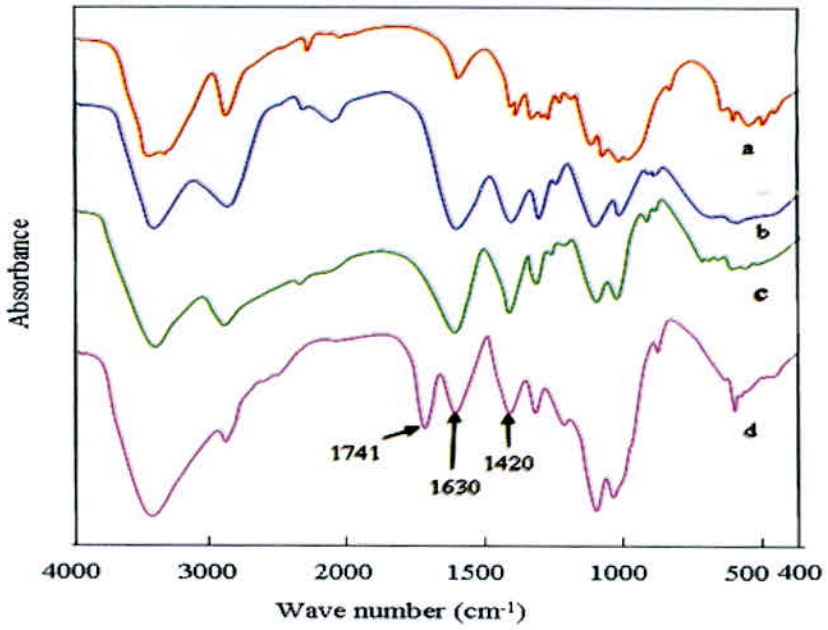

Figure 1: FTIR Spectra of Knitte drag (cellulose), standard CMC film (DS=0.8) prepared CMC film (DS=2.71) and grafted CMC film.

Conclusions

High performance CMC (high DS, purity and MW) was successfully produced from the high content cellulosic knitted rag, a cellulosic wastes of garment industries. Production of low cost and different grades CMC from knitted rag by the novel technique can be considered a feasible alternative way for generating value-added product and contributing to solving environmental problems resulting from damped knitted rag.

Publication

Alam ABMF and Mondal IH 2013. Utilization of Cellulosic Wastes in Textile and Garment Industries: 1.Synthesis and Grafting Characterization of Carboxy methyl Cellulose from Knitted Rag, *Journal of Applied Polymer Science,* 128(2), 1206-1212, (USA).

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Chapter 2

Funding Year 2011-2012

Generation of Doubled Haploid (Dh) Boro Rice Genotypes from Intervarietal Crosses Through Androgenesis to Select Materials for Better Adaptation to Climate Change

Md Shahidur Rashid Bhuiyan and Md Ekramul Haque

Institution: Sher-e-Bangla Agricultural University, Dhaka.

Duration: Three years (2011 - 2014)

Expenditure: Tk. 900000.00

Introduction

Boro rice has become a leading rice crop of our country through the last ten years. Its record production was 17.6 lakh metric tons during 2007-2008. Our high yielding boro rice varieties require a long period of 150-165 days to mature. The long duration of boro rice crops allow them to face more adverse conditions as a result of climate change, particularly at the early period of summer. Another culture using anther of F1 plants can regenerate haploid plantlets, the chromosome number of which can be doubled by treating with colchicine solution producing homozygous lines quicker than usual selfing method. These homozygous doubled haploid plants will allow selection of short durated boro rice with higher yield potential. Moreover, the reduction of maturity period of boro rice without much reduction in yield would allow early harvest that could avoid early summer adverse conditions.

Objectives

1. To develop boro rice materials quickly through androgenesis and chromosome doubling;
2. To select short duration materials of high quality boro rice to utilize them directly or indirectly in varietal improvement programme; and
3. To allow quicker harvest of boro rice crops with minimum damage to adverse situation.

Methodology

The seeds of the six varieties were sown in the seed beds of the experimental farm of Sher-e-Bangla Agricultural University, Dhaka on 12 and 22 November and 02 December of 2011. The 30 days old seedlings of the three Aus varieties were transplanted along with three varieties of Boro rice thrice at 10 days interval in crossing blocks for better synchronization of flowering. The 3 aus varieties were BR21, BR24 and BR26 having average life cycle of 105-115 days with yield of 3.0-4.0 ton/ha. The 3 boro varieties were BR28, BR29 and BRRI Dhan 36 with the life cycle of 140-160 days with average yield of 5.0-7.5 ton/ha.

The plants of the six varieties used for crossing program were transferred to the tubs for crossing in a half diallel fashion. Emasculation of panicles of selected female plants was done and pollens were dusted on

the emasculated panicles of female plants and after pollination the panicles were covered and tagged. Matured seeds were collected from each cross, sun dried and stored separately in paper bags with proper labeling. Three to four hundred crosses were made for each of the cross combination. The F_1 seeds of the 15 cross combinations were sown in seed beds in 22 November 2012 and the seedlings of 25 days were transplanted in the field on 17 December 2012. The plants of the 9 Aus x Boro cross combinations were transferred to tubs before one month ahead of anthers for using them in anther culture.

For anther culture, closed flower buds were collected from the hybrid plants of the 9 cross combinations at the early to mid uninucleate stage. Panicles thus collected were subjected to cold shock at 4^0C and 7 days in dark. The spikelets were then sterilized with 70% ethanol for few seconds and then transferred to 0.1% mercuric chloride solution for three-five minutes. The spikelets were rinsed five-six times by sterile distilled water and then placed horizontally on petridishes containing callus induction media.

For callus induction, N_6 media supplemented with 2, 4-D (2mg/l), kinetin (0.5 mg/l) and maltose (3%) showed the best results. The pH of the medium was adjusted to 5.8 using 1N NaOH/1N HCl and solidified with agar-agar (0.8%) and the media were autoclaved at 121^0C for 20 minute. Cultures were incubated at 28^0C ± 2^0C in dark for three-six weeks. After 3–4 weeks of incubation the pollens of the responsive anthers of the hybrids started to produce callus. Modified MS medium supplemented with NAA (0.25 mg/l) and maltose/sugar (3%) was selected for callus regeneration. Calli that grew and showed compact textures with 1–2 mm size were transferred to culture bottles containing 25 ml regeneration medium and the cultures were incubated under artificial light (2000 lux) at 25 ±1^0C for callus regeneration. Green plants that have reached 3–5 cm height were transferred to culture tubes containing 15 ml of rooting medium. MS medium supplemented with NAA (1.0 mg/l), Kinetin (0.1 mg/l), maltose (5%) and agar-agar (1%) was used for solidification.

The 0.5% colchicine solution gave the best result for doubling the chromosome number of the haploid plantlets. Before placing them in the net house they were hardened in the laboratory windows by increasing temperature gradually. The plants with well-formed roots were transferred to pots in the net house. Finally they were reared in net house with extra care and management. Most of the H_1 plants produced a few mature H_2 seeds only. The seedlings obtained from the H_2 seeds were produced in tubs under good management in the net house. The seedlings were transplanted to separate tubs for producing H_2 plants. A few H_2 plants with short duration with better yield per plant were selected. The H_3 seeds of the selected H_2 plants were collected for preliminary yield trial.

Data were recorded on number of anthers inoculated, number of anthers formed, no. of callus plated, number of plantlets regenerated and treated, number of doubled haploid plants obtained, number of H_2 seeds collected, number of H_2 plants obtained and finally number of H_2 plants selected.

Results

The F_1 seeds of nine combinations viz. BR 21 × BRRI Dhan 28, BR 21 × BRRI Dhan 29, BR 21 × BRRI Dhan 36, BR 24 × BRRI Dhan 28, BR 24 × BRRI Dhan 29, BR 24 × BRRI Dhan 36, BR 26 × BRRI Dhan 28, BR 26 × BRRI Dhan 29 and BR 26 × BRRI Dhan 36 were used for anther culture because of the facts that only these crosses involved Aus × Boro crosses. Anther culture was initiated to induce plantlets through callus formation. All the 9 F_1 combinations responded to callus formation. The frequency of callus formation was within the range of 3.73% to 12.38% (Table 1). The highest frequency was observed in the cross combinations where BRRI Dhan 29 was used as one of the parents. The crosses BR 21 × BRRI Dhan 29, BR 24 × BRRI Dhan 29 and BR 26 × BRRI Dhan 29 showed more than 10% callus formation while the lowest callus formation was observed in the combination BR 21 × BRRI Dhan 36 followed by the combination BR 26 × BRRI Dhan 36 showing that the lowest frequency of callus formation was being mostly conferred by the parent BRRI Dhan 36. Days to initiation of callus were more or less similar in all the cross combinations ranging from 18-23 days.

Table 1: Induction of callus from the anther of 9 F_1s of rice

Hybrids	No. of anthers inoculated	No. of anther formed callus	Frequency of callus (%)	Days to initiation
BR 21 × BRRI Dhan 28	425	29	6.82	20 – 22
BR 21 × BRRI Dhan 29	415	45	10.84	22 – 24
BR 21 × BRRI Dhan 36	375	14	3.73	18 – 21
BR 24 × BRRI Dhan 28	426	35	8.32	20 – 22
BR 24 × BRRI Dhan 29	428	53	12.38	21 – 13
BR 24 × BRRI Dhan 36	378	33	8.73	18 – 21
BR 26 × BRRI Dhan 28	400	22	5.50	21 – 23
BR 26 × BRRI Dhan 29	372	41	11.02	21 – 23
BR 26 × BRRI Dhan 36	426	20	4.69	19 – 21

More or less 100 calli were planted for each of the cross combinations (Table 2). Haploid plantlets were obtained in all the cross combinations showing variation. However, both green and albino types of plantlets were obtained in all the nine cross combinations. Highest number of 22 green plants were obtained from the anther culture of the hybrid of BRR 21 × BRRI Dhan 29 followed by BR 26 × BRRI Dhan 29.

The green haploid plantlets were treated with 0.5% colchicine solution in order to double the number of the chromosome. The treated plants that became doubled haploid were confirmed by counting the chromosome number of the cells. The highest percentage of doubled haploid plants was obtained in the hybrid combination BR 24 × BRRI Dhan 29 followed by BR 21 x BRRI Dhan 29 (Table 2). The H_1 plants in the pots did not show good growth probably because of tissue culture shock. However, many of them produced seeds, though number of seeds per plant was very low.

Table 2: Regeneration of haploid rice plantlets and doubled haploid plants from the anther derived calli of 9 F_1s of rice

Hybrid combinations	No. of callus plated	No. of haploid plantlets treated	No. of doubled haploid plants	% doubled haploid
BR 21 × BRRI Dhan 28	100	6	2	33.33
BR 21 × BRRI Dhan 29	110	22	13	59.09
BR 21 × BRRI Dhan 36	110	17	7	41.18
BR 24 × BRRI Dhan 28	100	16	5	31.25
BR 24 × BRRI Dhan 29	100	9	6	66.66
BR 24 × BRRI Dhan 36	105	6	2	33.33
BR 26 × BRRI Dhan 28	100	12	3	25.00
BR 26 × BRRI Dhan 29	108	18	10	55.55
BR 26 × BRRI Dhan 36	105	16	3	18.75

The highest number of H_2 (seeds from the H_1 plants) seeds were obtained from the cross combination BR 21 × BRRI Dhan 29 that produced 101 seeds. The three combinations BR 21 × BRRI Dhan 36, BR 24 × BRRI Dhan 28 and BR 26 × BRRI Dhan 29 produced 79, 65 and 58 seeds respectively (Table 3).

Table 3: Number of H_1 and H_2 plants obtained and number of plants selected from H_2 populations

Cross combinations	No. of H_1 plants	No. of H_2 seeds	No. of H_2 plants	No. of H_2 plants selected
BR 21 × BRRI Dhan 28	2	30	26	2
BR 21 × BRRI Dhan 29	13	101	81	5
BR 21 × BRRI Dhan 36	7	79	35	0
BR 24 × BRRI Dhan 28	5	65	37	2
BR 24 × BRRI Dhan 29	6	35	21	3
BR 24 × BRRI Dhan 36	2	39	32	1
BR 26 × BRRI Dhan 28	3	41	27	3
BR 26 × BRRI Dhan 29	10	58	38	6
BR 26 × BRRI Dhan 36	3	21	11	0

Maximum number of H_2 seeds from each cross combinations were sown in the tubs and ultimately seedlings were transplanted separately to different tubs. A good number of H_2 plants were obtained. The highest 81 H_2 plants were obtained from the cross combination BR 21 × BRRI Dhan 29 following by the combination BR 26 × BRRI Dhan 29 and the cross combination BR 24 × BRRI Dhan 28 and by the cross combination BR 21 × BRRI Dhan 36 which produced 38, 37 and 35 H_2 plants respectively (Table 3).

Photograph 1: Haploid Plantlets and doubled haploid plants (H_1) obtained through anther culture and H_2 plants obtained from H_1 Plants.

A total of 22 H_2 plants were selected from the six cross combinations based on their yield contributing traits. The plants that matured within 130 - 140 days with good yield per plant were selected. The highest 6 H_2 plants were selected from the combination BR 26 × BRRI Dhan 29 followed by 5 H_2 plants from BR 21 × BRRI Dhan 29. No H_2 plant was selected from the two combinations- BR 21 × BRRI Dhan 36 and BR 26 × BRRI Dhan 36. The selected H_2 plants produced enough H_3 seeds that would be grown for trial along with the standard checks.

Conclusion

Inter-varietal crosses were carried out among 3 aus and 3 boro varieties and anthers of the F1 hybrids were used for culture followed by doubling the chromosome number of the hybrid through colchicine treatment. Out of 122 haploid plantlets treated 51 doubled haploids (H_1) was obtained which produced 469 H_2 seeds. Ultimately 308 H_2 plants were obtained of which 22 plants were selected based on shorter duration, quality of grain and grain yield. The H_3 seeds collected from H_2 plants would be grown in preliminary trial along with standard check varieties for evaluation to select lines that could avoid early summer adverse conditions.

Publication

A student Mst. Shaila Begum (Reg. No. 06-02117) of the Dept. of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka has been awarded MS degree through the submission of the thesis entitled "Haploid Plant Regeneration through Pollen Culture in Rice *(Oryza sativa* L.)", the research of which was conducted with the support of the project.

Studies on the Limnology, Hydrology, Fish and Fisheries of the River Halda and Its Four Linked Rivers (Sangu, Chandkhali, Sikalbaha Channel and Karnaphuli)

Mohammad Ali Azadi, Munira Nasiruddin and M Atiqur Rahman
Institution: Department of Zoology, University of Chittagong, Chittagong - 4331

Duration: Three years (2011-2014)
Expenditure : Tk. 1000000.00

Introduction

Tidal River Halda, one of the tributaries of the River Karnaphuli is well known for the collection of fertilized eggs of major carps *(Catlacatla, Labeorohita, Cirrhinusmrigala and Labeocalbasu)* during monsoon period (April-June), from which commercial carp fry and brood fish are produced since time immemorial. So, in situ conservation of this natural renewable energy resource will bring huge economic value in the future. Major carps inhabit in the River Sangu, migrate to the River Halda for breeding during spawning period through the Chandkhali, Shikalbaha and Karnaphuli rivers. Thus to conserve and protect the fisheries resources of the River Halda, the conservation of fisheries resources of other four Halda linked rivers (Sangu, Chandkhali, Shikalbaha and Karnaphuli) are essential. A few short works have been done on the River Halda (Azadi 1979, Patra and Azadi 1984, 1985a, 1985b, Azadi 1985, Azadi 2004), but no work on limnology, hydrology and fish and fisheries of those linking rivers has yet been done. So, this investigation was undertaken for three years period (May 2010-April 2013) under the sponsorship of Ministry of Education.

Objectives

To determine the limnology, hydrology, fish and fisheries of the River Halda and its four linked rivers i.e. Karnaphuli, Shikalbaha, Chandkhali and Sangu with special reference to i) Water quality ii) Fish diversity and fishery status iii) Phyto and zooplankton abundance and diversity iv) Major Carps spawn fishery v) Habitat degradation and vi) To formulate recommendation for restoration of habitat degradation and enhance the fishery.

Methodology

For performing the above mentioned objectives, this research was planned for three years (May 2010-April 2013). Samplings were made twice monthly in eight stations namely Station-1 (Satter-Ghat), Station-2 (Ramdash-Hat) and Station 3 (Maduna-Ghat) in the HaldaRiver, Station 4 (Kalur-Ghat Bridge) and Station-5 (Firinghi Bazar Ferryghat) in Karnaphuli River, Staion-6 (Bellapara-Bridge) in Sikalbaha River, Station-7 (Toilardip-Bridge) in lower Sangu, and Station-8 (Barkal-Bridge) in Chandkhali river. Due to long distance (65.9 km from Station 1 to Stations 7 & 8) and otherconstraints, eight stations were selected at reasonable distance in five rivers for samples and data collection. Monthlyfor two years (May, 2010-April, 2012) Limnological, Hydrological (Physical: air and water temperature, turbidity, conductivity, total dissolved solids; Chemical: dissolved oxygen, free CO_2, pH, alkalinity, total hardness.

calcium, chloride, salinity) and Biological (phytoplankton, zooplankton) samples were collected and analysed. Some limnological parameters (Temperature, turbidity, conductivity, TDS, pH, Salinity by Hanna Instruments, USA; Refractometer, Japan; EC 4DGIT, China) were tested during sampling time and some (DO, free CO_2, alkalinity, total hardness, calcium, chloride) were tested in the laboratory within 4-5 hours of collection using Hach Test-Kit-Model-FF2, USA and APHA (2005). Plankton (Phyto and Zoo) were collected by Plankton net (Hydrobios-55, Germany) and identified following APHA (2005) and Ward and Whipple (1959). Fish samples and Fisheries related data were collected from the fishing spots and interviews with the fishermen. CPUE (Catch per unit effort) Kg/gear/day is the average catch rate and calculated as: CPUE = W/N (where W= total weight of fish recorded from gear sampled; N= Number of gears sampled).Historical carpspawn/fryproduction data were compared with the present rate of production to see the effect of climate change and man-made interventions on the spawn/fry production. Fisheries species were identified following standard books. SPSS and Microsoft Excel were utilized for data processing.

Figure 1: Collecting procedure of eggs

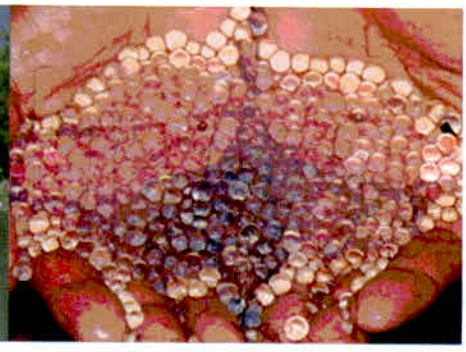

Figure 2: Eggs of carp

Results

Monthly limnological and hydrological minimum, maximum and yearly mean±SD data (n= 192) for two years (April 2010-May 2012) from eight stations of five rivers (Halda, Karnaphuli, Shikalbaha, Sangu and Chandkhali) varied as follows: Air Temperature:18^{O}C-36^{O}C, mean 27.21±3.76^{O}C; Water temperature: 19^{O}C-33^{O}C, mean 27.55±3.31^{O}C; Transparency (Turbidity):8.0 cm to 44 cm, mean 18.87±5.99 cm, maximum turbidity prevailed in Karnaphuli and Sangu rivers; Conductivity: 53 mS/cm to 7819 mS/cm, mean 389.76 ±856.33 mS/cm, always low conductivity was in Halda River and highest in Karnaphuli; TDS (Total Dissolved Solids):0.0 ppt- 5.68 ppt, mean 0.30±0.84ppt; Salinity: In the entire Halda and in the study areas of Shikalbaha, Sangu and Chandkhali rivers no salinity was observed round the year, except in November 2011 (3.0 ppt, at Firinghi Bazar point, station 5) in one of the study areas of the Karnaphuli River, near to estuary; pH: 7.40-8.80, mean 7.94±0.24; DO (Dissolved Oxygen): 1.63 mg/l-9.36 mg/l, mean 5.57±1.50 mg/l; lowest DO was recorded at station 5 of Karnaphuli river, a pollution prone zone and highest DO at Halda; Calcium: 4.01-833.67 mg/l, mean 18.00±59.88 mg/l; highest calcium was prevailed in Karnaphuli River; Total Hardness as $CaCO_3$: 17.00-1080.00 mg/l, mean 92.13±145.12

mg/l; total Hardness was highest at station 5 of Karnaphuli River, near to estuary; Alkalinity: 25.0-575.00 mg/l, mean 65.86±40.06 mg/l; Chloride: 2.20 mg/l to 2980 mg/l, mean 40.08±223.28 mg/l, highest chloride was at Station 5 of Karnaphuli River.

Biological:Phytoplankton-31 genera of phytoplankton belonging to 5 groups (Blue green algae-7, Green algae-6, Non motile green algae-5, Desmids-4 and Diatoms-9) were identified from the five studied rivers. The lowest and highest number of phytoplankton encountered as 1547.48/m^3 (Sangu) and 3597890/m^3 (Halda), mean 178307.08±421058.91/m^3 (n=192). Zooplankton-18 genera of Zooplankton belonging to 5 groups [(Cladocera (4)- *Daphnia, Diaphanosoma, Alona, Moina,* Copepoda (3)-Cyclops, *Nauplius, Metanauplius,* Rotifera (7)- *Brachionus, Monostyla, Kertella, Conochilus, Filinia, Tricchocerca, Euchlanis,* Protozoan (3)-*Paramecium, Volvox, Ceratium* and Mollusc (1)- *Helisoma*)] were identified from the five rivers. The lowest and highest number of zooplankton encountered as 0-5803.05/m^3 in Halda and Karnaphuli and 297889.88/m^3 in Karnaphuli with a mean 56,047.35±43930.38/m^3 (n=192).

Fish and Fisheries: 25 types of gears under 10 categories (Surrounding, Brush-shelter, Cast net, Set bag Net (SBN), Gill Net, Scoop Net, Drag Net, Hook line, Trap, and Wounding) were used to catch fish in the five rivers. A total of 130 species (112 finfish and 18 shellfish) under 14 orders, 48 families and 94 genera were recorded from the five rivers. Out of 130 species, nine species were vulnerable, 12 endangered and 5 were critically endangered as per IUCN (2000). Three were exotic. Four species (*Moringua macrocephalus, Oryzias dancena*, *Ophieleo trisaporos, Opistognathus nigromarginatus*) were newly recorded from the Halda as well from inland waters of Bangladesh and two species (*Barilius bendelisis* and *Neolissochilus hexagonolepis*) are reported here for the first time from the River Sangu.

CPUE (Catch per unit effort): Yearly mean highest and lowest CPUE was 8.839kg/gear/day (by set bag net-SBN) and 0.693 kg/gear/day (by cast net) in Karnaphuli, 6.580 kg/gear/day (SBN) and 0.560 kg/gear/day (cast net) in Chandkhali, 5.674 kg/gear/day (SBN) and 0.519 kg/gear/day (cast net) in Shikalbaha, 5.674 kg/gear/day (SBN) and 0.519 kg/gear/day (cast net) in Halda and 8.116 kg/gear/day (SBN) and 0.608 kg/gear/day (cast net) in Sangu River respectively.

Major Carps Spawn Fishery: Negative ($P<0.05$) trend of major carps fry production was observed in Halda River since 1945 to1955 (fry 2470 kg to 1096 kg, r=-0.5879), 1974 to 2000 (fry 1904 kg to 1117 kg, r=-0.834), 2000-2008 (fry 1117-169 kg, r=-0.720) and 2008 to 2015 (169 kg to 50 kg, r=-0.095) due to climate change and manmade causes (carp overfishing, blocking of water flow of 12 tributaries by 12 sluice gates at five spawning zones, loss of five spawning grounds due to loop cutting in lower Halda, and two rubber dams at upstram). 200-500 boats and about 2000 egg collectors were engaged in carps spawn fishery. Price of four days old fries varied from Taka 20,000 to 60,000/kg.

Conclusion

Limnological and Hydrological conditions in the River Halda were found to be congenial for major carps and other fishes. Lower Karnaphuli was detected as highly polluted, other three rivers (Sangu, Chandkhali and Sikalbaha) habitat was good. Climate change and some anthropogenic causes declined carp fry production in Halda River. For sustainable fish, fishery and biodiversity, Halda and its four linked rivers (Sangu, Karnaphuli, Chandkhali and Shikalbaha) should be managed, protected and conserved.

Publication

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Pollution of Antibiotics and Resistant Bacteria: Prospective Studies on Spreading of Antibiotics Resistance, Food Hygiene and Aquaculture in Bangladesh

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Institution: Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh

Duration: One Year (2013-2014)

Expenditure of the project: Tk. 2000000.00

Introduction

The persistent environmental pollution with antibiotics and resistant bacteria and its entry into food chain is potentially devastating, threatening the availability of safe food. There are growing concern on environmental pollution with antibiotics and resistant bacteria and bioaccumulation of antibiotics or their metabolites in food chain. Knowledge on 'antibiotics and resistance bacterial pollution of environments and its spreading through food chain' as well as integrated approach to address the problems is very limited. Particularly, my group has taken an integrated approach to address the problem and the present data consists of an elaboration of the overall approaches.

Objectives

The objectives of proposed study

1. Are there any correlations between pattern of antibiotics pollution and development of environmental resistant bacteria;
2. What are the antibiotics resistance pattern in bacteria in wastewater of hospitals, poultry and veterinary? How these resistant bacteria spread and transfer resistance markers vertically and horizontally; and
3. Do antibiotics and resistant bacteria enter into food-chain? The correlation between antibiotics bioaccu mulations and resistant bacteria load in food predicts the pollution distribution pathways in Bangladesh.

Methodology

Collection of Samples: Standard laboratory practice, sanitary and clinical safety measures were maintained during all types of sample collection.

Isolation of resistant bacteria: Plate count Agar method established in our laboratory was used for total and resistant bacteria count in wastewater/poultry of hospitals, dairy and poultry farms samples. The isolates were identified according to phenotypes and genotypes.

Determination of multi-drug resistant pattern: Bacterial susceptibility to different antimicrobial agents was measured in vitro by employing the modified Kirby-Bauer method by measuring zone sizes (in millimeters). Commercially available antibiotic discs (Oxoid, Basingstoke, UK) were used for the test. The zone diameters were interpreted into susceptible, intermediate or resistant categories according to the

which is susceptible to all the tested antimicrobial agents, were used as a control isolate for susceptibility studies.

Identification of genetic diversity of the resistant bacteria: Standard molecular biology techniques were used for identification of bacterial genetic diversity as well as types of antibiotics resistant genes. All these methods are established in our laboratory such as- 16S rDNA sequencing and analysis, ARDRA (arbitrary ribosomal DNA restriction analysis) and specific resistant gene amplification and sequencing.

Quantitative analysis of Antibiotics: High performance liquid chromatography (HPLC) method for quantitative analysis of chloramphenicol, ciprofloxacin, levofloxacin and tetracycline were established in our laboratory. The samples were extracted by solid phase extraction of antibiotics by SPE cartage-Biotagelsolute ENV+ followed by HPLC separation and quantifications.

Plasmid Analysis and Sequencing: Qiagen plasmid isolation and gel extraction kits (QIAGEN Sciences, Maryland, USA) were used for plasmid isolation and purification. Big-Dye (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) procedure combined with Applied BioSystem DNA sequencer ABI-prism 310 genetic analuzer (ABI Prism, USA) was used for DNA-sequencing.

Storage of Bacteria and Plasmids: Isolated bacteria were stored at -20/-80^{O}C in 15% glycerol NB or after freeze drying. Purified plasmids were stored at -80^{O}C.

Development of resistant genes/plasmid databank: A resistant genes/plasmid bank and dataBank (web base) using the findings of the proposed research project is currently under development.

Results

Clinical Waste Water (CWW) pollute environment with antibiotics and multidrug resistant bacteria: Healthcare facilities and agro-farms like hospitals, clinics, dairy and poultry have no /or limited waste management systems in Bangladesh. We have studied 3 medical college hospitals, 1 dairy farm and 5 poultry farms CWW and clearly demonstrated that- (i) CWW of Dhaka, Chittagong, Enam Medical College, Savar Dairy farm, and five poultry farms of Savar area pollutes the environment with multi-resistant bacteria (MRB); (ii) CWW spread resistant gene(s) pool; (iii) Some of the isolates have zoonotic potentials; and (iv) CWW pollutes ecological water bodies with antibiotics and resistant pathogens with zoonotic potentials. These results further suggest that unacceptable level occurrence of MRB in CWW may transfer resistant genes vertically and horizontally in diverse microbial community to pollute our environment.

Bacterial Isolates are Multi-resistant: During this study, 18 different antimicrobial agents were used to observe drug resistant pattern. The overall result showed that most of the isolates were resistant to more than 5-10 antibiotics, but poultry isolates were susceptible to Amikasin and Polymyxin B.

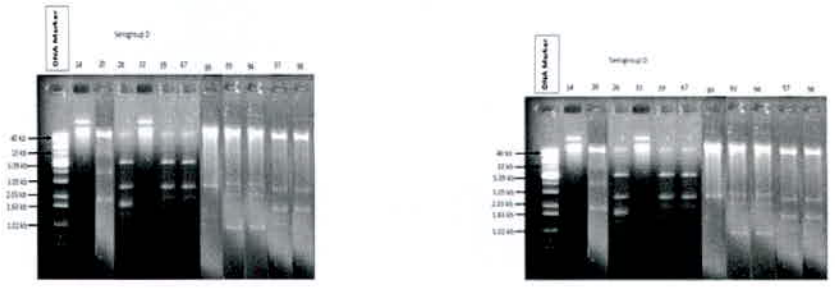

Figure 1: Plasmid profile showing several plasmid free antibiotic resistant bacteria

Plasmid profile, ARDRA, DNA sequencing, phylogeny and detection of zoonotic isolates: Among isolates, few isolates showed the presence of multiple numbers of plasmids and the restriction of the amplified fragment of the 16s rDNA gene with AluI generated five different profiles (A, B, C, D, E). Representative isolates from different ARDRA groups were selected for detail sequencing of 16 rDNA and phylogenetic analyses. Salmonella spp. and Enterobacter spp. were obtained as predominant group. Most of those correlated to that of the gene sequences of corresponding zoonotic strains –S. enterica serover Typhimurium and *Enterobacter cloacae.*

CWW pollutes environment with Antibiotics: CWW of hospitals (Dhaka Medical College and others) discharged ciprofloxacin to ecological water bodies at the range of 2.4-100 µg/L during our analysis period 2011 and 2012.

Conclusion

We have clearly demonstrated that CWW originated in hospitals and agri-farms pollutes environment with antibiotics and zoonotic MRB at unacceptable levels. Our findings suggest the need of a national survey on antibiotics and antibiotics resistance pollutions occur due to CWW in Bangladesh and its impact on public health.

Publication

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Application of Molecular Techniques for the Rapid Detection of Animal Diseases Communicable to Human being Targeting Visceral Leishmaniasis (VI), Malaria Human and Bovine Tuberculosis, Johne's Disease and Brucellosis

Md Abu Hadi Noor Ali Khan and Emdadul Haque Chowdhury

Institution: Department of Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Duration: Three years (2011-2014)

Expenditure of the project: Tk.1150000.00

Introduction

The predominantly agrarian nature of animal husbandry practices in Bangladesh provides ample opportunities for intermixing of animal species on the grazing lands and composite small holdings of livestock maintained by nearly 70% of the rural population. There are about 23.4 million cattle, 1.86 million buffalo, 33.5 million goats, 1.1 million sheep and considerable number of (actual figure not known) pigs in Bangladesh. Diseases are the major constraints of livestock development in Bangladesh (Khan *et al.*, 2015). Now a days, Visceral leishmaniasis, Malaria, Tuberculosis, and Brucellosis, becoming serious problems of public and animals health and affecting around 500-700 million people in the world (Maudlin *et al.*, 2009). Dogs, cat, cattle, sheep, goat, monkeys, swine, domestic and wild birds are often said to be involved with the dissemination of the diseases among themselves and as well as to human being. In addition, these diseases are on the World Organization for Animal Health list of significant diseases.

Bangladesh is having a highly and densely populated ecosystem and very often human beings are catching a number of transboundary diseases through direct or indirect contact with the infected/carrier animals. Until recently although we do have few laboratory techniques to detect such diseases, the technology is not well understood/ remains in the literature. A more intense, productive, cheaper and efficient research tools and techniques need to develop to frame those diseases at the early onset and therefore, strategies can be engineered in order to control/ prevent mass devastation caused by the outbreak of those diseases. This research study was, therefore, designed to adapt/design/apply techniques to diagnose those diseases in animals and developed strategies to prevent/control important zoonotic diseases like Visceral Leishmaniasis, Tuberculosis (human, bovine and John's diseases), Brucellosis, and Malaria and help the nation to keep surviving with less pain.

Objectives

1. To develop a more intense, productive, cheaper and efficient laboratory tools for the detection of Visceral Leishmaniasis, Tuberculosis (human, bovine and Johne's diseases), Brucellosis and malaria;
2. Identify carrier animals of the important zoonotic diseases; and
3. Develop strategies towards prevention and control of the disorder at early onset

Methodology

- Clinicopathological investigation of suspected or sick animals (Luna 1968)
- Collection of samples from suspected human and animals (Al-Mamun *et al.*, 2016)
- Active surveillance of important zoonotic diseases (Hossain *et al.*, 2016)
- Collected suspected tissue from the slaughtered/ dead animals (Kasfi *et al.*, 2015)
- PCR detection of zoonotic diseases (Labony *et al.*, 2015; Hossain *et al.*, 2016)
- Detection of diseases using impression smears (Khan *et al.*, 2012; Dey *et al.*, 2013)
- Formulate preventive and or control strategy (Khan *et al.*, 2012; Khan *et al.*, 2015)

Results

Tuberculosis:

- Out of 696 cattle tested, 23 (3.30%) were positive to Tuberculin tests (Table 1)
- Holstein Frisian cattle (N=21), appeared highly susceptible to tuberculosis (Figure 1)
- A dairy cow at Mymensingh district and a cow at Shavar, Dhaka were co-infected with both Bovine TB and Avian TB
- Dairy cattle were infected with Bovine TB, human TB and avian TB, these are widely zoonotic. Out of 11 cattle sample tested in PCR, two were infected with *M. tuberculosis*, nine with *M. bovis* and two with para TB
- Out of 20 human samples tested, 11 appear to be infected with tuberculosis. Seven of which were infected with *M. tuberculosis* and four with *M. bovis*.

Leishmaniasis:

- Out of 16 jackals, 50 cattle, 60 goats and 50 dogs investigated, 10 jackals, three cattle, five goats and 11 dogs found to carry kinitoplast minicicrcle DNA of L. *donovani* a causal agent of VL (Figure2)
- While testing the animal and human samples using PCR targeting 18S rRNA gene, only known positive human sample and three dogs samples produced amplicons selective for *L. donovani* (VL).

Brucellosis:

- Out of 190 sera sample tested using RBPT and iELISA from the cattle of Dairy farm, Bangladesh Agricultural University and meat market, Mymensingh, 2.63% and 1.05% cattle appeared positive to brucellosis respectively (Figure 3)
- Histopathological of aborted fetus due to brucellosis showed diffuse fibrosis and reactive cellular infiltration around the placental epithelia and in placental tissues.

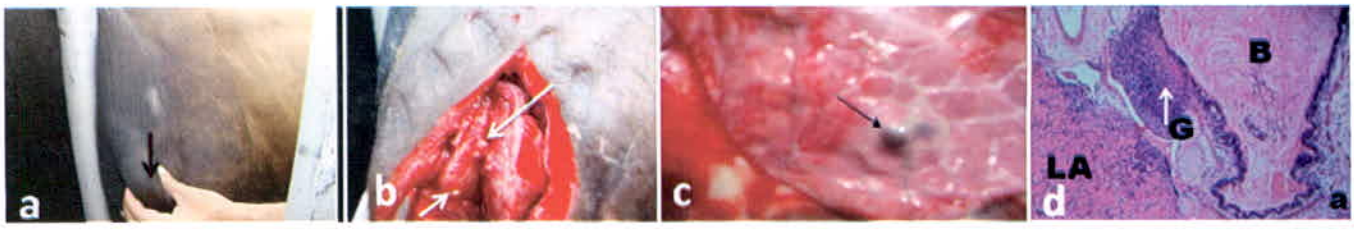

Figure 1: Investigation of tuberculosis (TB) in dairy cattle (a). During biopsy (b, arrow) cream color nodular masses were seen in lymphnodes. Following necropsy calcified nodular mass (c, arrow) was seen in lungs. The histopathological examination revealed granulomas (d, white arrow) in lungs (H&E staining, 10x) parenchyma.

Table 1: Results of intradermal tuberculin tests onto the male and female cattle in selected dairy cattle of Bangladesh. Out of 696 cattle tested, 23 were positive to Tuberculin tests reaction.

Location of farms	No. of cattle tested	Intradermal Bovine PPD	Intradermal Avian PPD	Overall + Ve reactivity
Mymensingh	86	4 (4.65%)	1 (1.16%)	5.81%
Mymensingh	70	1*	Nil	1.43%
Sirajong	19	1	Nil	5.26%
Bogra	56	nil	Nil	0.00%
Sylhet	47	2	Nil	4.26%
Chittagong	141	3	Nil	2.12 %
Savar (Dhaka)	234	9	Nil	3.85 %
Tangail	03	1	Nil	33.33%
Gazipur	20	1	Nil	5.00%
Sirajong	20	1	Nil	5.00%
Total	**696**	**23**	**01**	**3.30%**

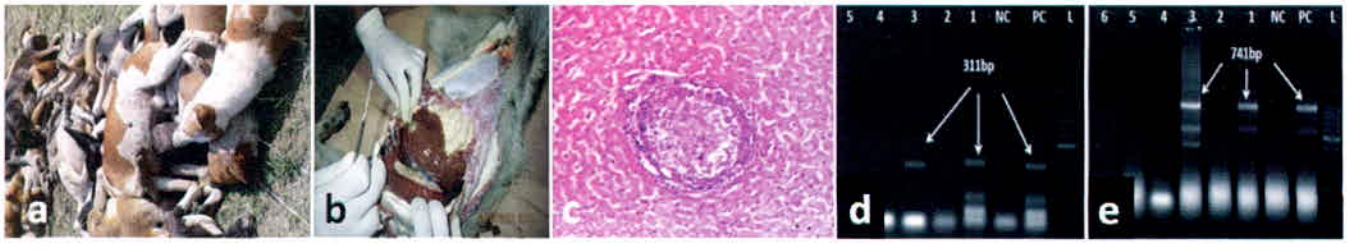

Figure 2: Investigation of Leishmaniasis (Kala-azar) in dogs (a and b). Following histopathology, granulomas was seen in liver. Out of 50 dogs investigated using PCR, only two of them found to infect with *Leishmania donovani,* causal agent of Kala-azar.

Figure 3: Investigation of brucellosis in dairy cow. The infected cow aborted her calf (b) at the age of eight month pregnancy and showed retained placenta (a, circle). Following Rose Bengal plate test, the serum samples (c, white arrow) agglutinate test antigens.

Conclusion

- This research project enabled the Principal Investigator and his team to design and adapt protocols to diagnose accurately TB, Kala-azar and Brucellosis within shortest period of time
- Cattle and human in Bangladesh were infected with tuberculosis (TB). Out of 11 cattle and 11 human samples investigated, 02 cattle and 07 human found to infect with *M. tuberculosis*. Infectivity due to *M. bovis* was seen in nine cattle and four humans. Co-infectivity in cattle due to *M. bovis* and *M. avium subsp var Paratuberculosis* were seen in two cases
- Street dogs found to carry *L. donovani* (Kala-azar) in their visceral organs
- Seroprevalance of brucellosis were seen in 2.63% and 1.05% dairy cattle as detected by RBPT and iELISA respectively. More specific and easily detection tool is required to diagnose brucellosis and reducing load of zoonotic spread
- It needs to screen all of the elderly dairy cattle by tuberculin test and PCR and require slaughtering all of the reactor cattle to minimize zoonotic spread of TB
- Street dogs have been carrying *L. donovai,* an etiological agent of Kala-azar and may play role towards transmitting infection to human
- Tuberculosis, Leishmaniasis and Brucellsois are endemic in a nimals and human in Bangladesh, they are extremely zoonotic; require extensive investigation to detect the actual scenario of field infectivity and design preventive strategies accordingly.

Publication

A PhD Thesis entitled "The incidence, pathology, diagnosis and molecular characterization of bovine tuberculosis in Bangladesh" was successfully completed in 2015.

A full length paper entitled "Designing polymerase chain reaction (PCR) technique for the detection of specific causes of tuberculosis (TB) in dairy cattle and human" published in USA in the *Journal of Veterinary Science & Medical Diagnosis* 2016, 5(4).

Adapted and designed PCR protocols for the rapid and accurate detection of TB, Leishmaniasis, Brucellosis in man and animals.

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Development of Indigenous (Desi) Chicken of Bangladesh as a Meat Type Bird Through Improved Nutrition and Management

Sachchidananda Das Chowdhury

Institution: Department of Poultry Science, Bangladesh Agricultural University Mymensingh-2202

Duration: Three Years (2011 - 2014)

Expenditure : Tk. 800000.00

Introduction

It is estimated that there are about 245 million chickens and 46.5 million ducks in Bangladesh. The per capita consumption of poultry meat in 2006 was 3.63kg and at present chicken contributes 35.25% of total meat production of the country. The indigenous *(desi)* chicken constitutes the major part of family poultry in Bangladesh. Currently, national share of commercial to family poultry with respect to meat production is 60:40. The fact is that meat from indigenous poultry are preferred widely by consumers because of good taste, lean meat, better pigmentation and also suitability for preparing different dishes although they are costlier than commercial broilers marketed today. Native chicken production has been potentiated by improved nutrition, management and health care in Bangladesh.

Desi chicken may be more productive with improved diets when reared in confinement but growth target or weight at marketing is yet to be determined as per demand of the consumers. Nutritional manipulation to develop *desi* chicken as a meat type bird is to be carried out with diets of adequate nutrient density by rearing them both in confinement and under traditional scavenging system. This research project is aimed at developing the indigenous *(desi)* chicken of Bangladesh as a meat type bird by growing them up to different target weights by providing diets of different nutrient density in order to find out the most economic way of their production.

Objectives

- To use locally available feed resources for the formulation of improved diets of indigenous *(desi)* chicken;
- To develop indigenous *(desi)* chicken of Bangladesh as a potential resource by increasing their body weight and meat yield characteristics by means of improved feeding; and
- To identify management systems for indigenous *(desi)* chicken for exploring their growth potential.

Methodology

The research project was implemented at the Poultry Farm of Bangladesh Agricultural University, Mymensingh and Vobkhali village at Mymensingh Sadar Upzilla. Feed ingredients were procured from local market of Mymensingh town. Feed samples were chemically analyzed for the determination of proximate components according to the methods of Association of Official Analytical Chemists (AOAC, 2005). Diets were formulated after reviewing available feeding standards making variations in nutrient contents so that one or more nutrient packages appropriate for feeding indigenous chickens to achieve target weight could be identified.

Table 1: Feeding trials of the project

Feeding trials	Title of the experiment	Management
1	Growth performance of *desi* chicken reared in confinement with diets of varying protein concentrations up to target weight of 750g	Floor management in confinement
2	Growth performance of *desi* chicken reared in confinement with diets of varying nutrient concentrations up to target weight of 850g	Floor management in confinement
3	Growth performance of d*esi* chicken reared in confinement with diets of varying protein concentrations from 12 weeks of age to the point of lay	Floor management in confinement
4	Growth performance of *desi* chicken reared in confinement with diets of varying nutrient (energy and protein) concentrations from12 weeks of age to the point of lay	Floor management in confinement
5	Growth performance of *desi* chicken reared in confinement with diets of varying energy concentrations from 3 weeks of age to target weight of 950g	Floor management in confinement
6	Growth performance and meat yield of *desi* chicken reared under village condition with diets of two different energy concentrations up to target weight of 950g under scavenging system	Scavenging system in rural households.
7	Growth performance and meat yield of *desi* chicken reared in confinement with diets of two energy concentrations up to target weight of 950g under rural condition	Confinement in rural areas.
8	Growth performance of *desi* chicken reared in confinement with diets of two energy concentrations from day old to target weight of 950g under farm condition	Floor management in confinement

Some pictorial views of project activities and trial

Figure 1: Training session in village farmers

Figure 2: Scavenging system in village

Figure 3: Intensive rearing of village flock on ME 2800 kcal/kg + CP 23%

Figure 4: 75% feed supplementation village flock

Figure 5: 50% feed supplementation of village flock

Figure 6: Grower chicken rearing in confinement

A total of eight feeding trials were conducted with diets of different nutrient combinations in both scavenging and confinement systems of rearing. Farmers were trained before the trials conducted in rural areas. Except the variations in nutrition, all other management practices including feeding, brooding, medication, debeaking, record keeping and statistical analysis etc. were similar for all feeding trials.

Results

A high nutrient density (HND) diet of 2800 ME kcal/kg and 23% CP would be required to achieve a target weight of 850g and 950g at 12 and 14 weeks of age respectively of desi chicks if reared in confinement. An improvement in meat yield characteristics in terms of dressed weight, breast meat, drumstick meat was possible during this period of growth. Feeding HND diet to such chicks was most profitable.

For scavenging indigenous chicks (3-14 week), 75% feed supplementation of a diet of similar composition (2800 ME kcal/kg and 23% CP diet), would be sufficient to optimize feed intake, chick's target weight of 950g and better meat yields in rural condition where birds are allowed for scavenging alongside supplemental feeding, thus enabling more saving of the cost of feed to maximize profit.

The results of present study also indicate that during the grower and pre-layer periods of indigenous female pullets (12-22 week), a nutrient density of 2700 ME kcal/kg and 17% CP (moderate nutrient density, MND) would be enough to optimize feed intake and growth rate if reared in confinement. But such chicks would mean for future laying purpose, not for immediate sacrifice for meat production, similar to that of high yielding layer strain.

Conclusion

Feed ingredients either home-grown or available in the market may be well utilized for the formulation of improved diets of indigenous chicks of Bangladesh. The chicks could be developed as a potential resource in terms of meat-type birds by feeding improved diets during the early growth period (day-old to 14 weeks). The birds may be reared under confinement and scavenging systems by providing improved diets to explore their meat production potentiality. Rearing under scavenging system with supplemental feeding will make the production more profitable.

Publication

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Development of Salt Tolerant Jute by Introducing Kat E gene

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Institution: Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000
Duration: Three Years (2011 - 2014)
Expenditure of the project: Tk. 2000000.00

Introduction

There is an urgent need to develop crops with a greater tolerance of environmental stress. This is more so for a fiber crop like jute which is being pushed into marginal lands in the regions where it grows best to make room for food crops. Therefore making jute resistant to salt stress is a pressing need in order to meet the growing demand for jute fiber in this changing environment. In this study, we have introduced kat E gene from Escherichia coli K12 into a tossa jute cultivar, Corchorus olitorius var O-72, a popular local cultivar in Bangladesh. The katE gene was cloned in a plant expression vector under CMV 35S promoter and transformed using a previously developed tissue culture independent Agrobacterium tumefaciens mediated protocol. Molecular analysis of transgenic plants viz. PCR, reverse transcription PCR and Southern blotting confirmed both the integration of the katE gene and its expression. Salt stress regimens of transgenic plants showed that these plants were more tolerant than the non-transgenic ones. The transgenic jute plants expressing the katE gene were able to grow and reproduce in the presence of 150 mM NaCl.

Objectives

To develop high yielding saline tolerant jute plants through introducing a bacterial kat E gene into a previously developed high yielding tossa jute variety O-72. kat E gene is known to confer salt tolerance when introduced into plants.

Methodology

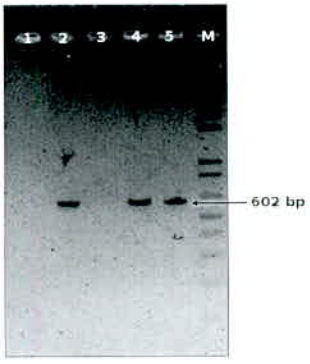
1) Amplification of kat E gene from *E. coli* by PCR
2) Insertion of kat E gene into TOPO TA entry vector
3) Insertion of constructed vector into *E. coli* DH5α cells
4) Confirmation and isolation of constructed vector
5) Recombination of TOPO TA entry vector with a the destination vector pH7WGF2
6) Insertion of the destination vector into *Agrobacterium* cells
7) In planta transformation of jute with *Agrobacterium*

Results

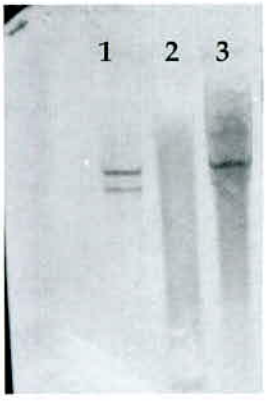
In this study, a high yielding jute variety *(C. olitorius var* O-72) with the E. coli (K-12) katE gene was transformed using a previously developed tissue culture independent transformation method apposite for recalcitrant plants like jute. The gene was cloned in the pH7WGF2 binary vector under the control of a constitutive promoter (35S promoter of cauliflower mosaic virus). Young plants (C. olitorius var O-72, 5 weeks old) were infected with A. tumifaciens LBA4404 harboring the construct. Seeds from these plants were transferred to a hygromycin selection media and later evaluated for katE expression, function, and salt tolerance.

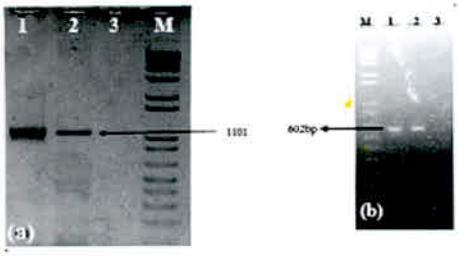
Molecular characterization of transgenic jute lines overexpressing the katE gene.

Full length ORF of Kat E was cloned in the binary vector pBI121 under the control of a constitutive promoter (p35S CAMV). After *Agrobacterium* mediated transformation of jute plants and selection with hygromycin, several transformants were identified. Integration of the kat E gene (602 bp) into the jute genome was confirmed by PCR analysis.



To determine the inheritance of the katE gene from T0 to the subsequent progenies and to estimate the copy number of the transgene integrated into the jute genome, Southern blot analysis using the hygromycin gene sequence as a probe was carried out. The observed hybridization signal pattern was indicating independent integration events at different locations of the genome for each plant. However, no hybridization signal was detected in untransformed control plants.

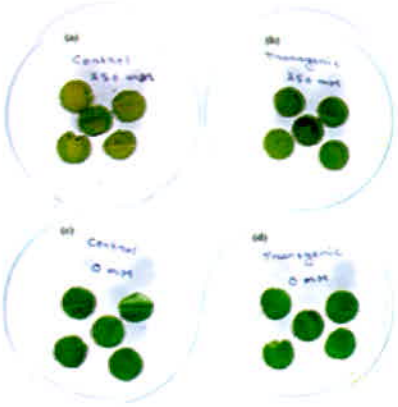




Expression of the transgene in engineered plants was confirmed by RT-PCR with mRNA isolated from leaves of the transgenic and the control plants. A 1101bps amplification product of the kat E gene and a 602 bps product in RT-PCR with *hyg*-specific primers indicated that both the genes (kat E and hyg) were stably expressed in T2 plants.

Increased salt resistance of jute plants expressing the Escherichia coli katE gene

In order to compare the relative salt tolerance, both the transgenic and non-transgenic jute plants were transferred to soil pots and watered with 150 mM NaCl. While the transgenic plants showed significant tolerance to salt stress and exhibited normal growth characteristics even after 4 weeks of treatment, the control plants showed typical yellowing of leaves as well as stunted growth. A total of 9 weeks of 150 mM NaCl treatment ultimately killed the control plants, while the transgenic plants were still growing normally without showing any noticeable change.



To estimate the salt tolerance potential leaf disk senescence assay was performed for both transformed and non-transformed plants. Leaf disks from both non-transgenic and transgenic plants were floated on 250 mM NaCl salt solutions for 10 days showed that the leaf disks from non-transgenic plants show extensive bleaching, which is a symptom of chlorosis, while the transgenic lines appear to have considerably less damage.

Conclusion

In this study we found that insertion and overexpression of the E. coli katE gene in jute plants improve tolerance to salt stress. This is the first transgenic approach for developing jute plants tolerant of an abiotic stress and hence could be considered as a significant achievement in the field of jute biotechnology.

Publication

Shahidul MI, Shafiul MA, Sazia S, Ashfaqur AS, Maksudul MA, Shamim MR, Rajib A and Haseena K 2013. Improved salt tolerance of jute plants expressing the katE gene from Escherichia coli. *Turkish Journal of Biology. Turk. J. Biol.*, 37, 206-211.

Exploring the Bioactivity of Medicinal Plants of the Sunderbans

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Institution: Pharmacy Discipline, Khulna University, Khulna-9208
Duration: Three years (2011 - 2014)
Expenditure: Tk. 1500000.00

Introduction

The Sunderbans is the single largest continuous mangrove forest of the world. At present at least 66 plant species are abundantly found in the forest. Drugs of natural origin play an invaluable role in the drug discovery process. The chemistry of mangrove plant, although little known, tends to establish that they may be a source of novel compounds along with providing a new source of many already known biologically active compounds. Moreover, numerous mangrove plants are recommended in traditional medicine as active against various diseases. But very little attempts have been made to investigate the veracity of these assertions in controlled *in vitro* and *in vivo* experiments along with to identify the chemicals directly responsible for the specific biological activity.

Majority of the people of Bangladesh live under the poverty line, modern allopathic treatments beyond their limit. Medicinal plants may be good alternative to them as they are safe, readily available and cheap. This project will authenticate the known traditional uses as well as unexplored biological activities of plants of the Sunderbans which will bring benefits to majority people of Bangladesh.

Therefore, this project aims to investigate different biological activities using various *in vitro* and in vivo models and to justify their traditional usage. At the same time different secondary metabolites present in these plant will also be explored.

Objectives

The specific objectives of this research are:

a) Collection of different plant samples from the Sunderbans;
b) Finding out the phytochemicals present; and
c) Exploring the bioactivity of these medicinal plants.

Methodology

Collection, drying & grinding

Plant samples were collected from the Sunderbans and identified. The samples were shade-dried, ground into a coarse powder.

Extraction

The plant materials were cold extracted by maceration in ethanol for at least seven days. After solvent evaporation, crude extracts were obtained.

Phytochemical Screening (Trease and Evans 1989)

Crude extracts were tested for the presence of different phytochemicals like reducing sugars, tannins, flavonoids, glycosides, saponins, gums, steroids, alkaloids, terpenoids *etc*.

Biological Activity testing

Qualitative antioxidant assay (*Sadhu et al.* 2003): Solutions of different extracts were spotted on silica gel TLC plates and the plates were developed in solvent systems of different polarities. The plates were sprayed with 0.02% w/v solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) in ethanol. Pale yellow color of the chromatogram indicated the presence of antioxidants.

Quantitative antioxidant assay using DPPH (Gupta *et al.* 2003): The aliquots of different concentrations (1-500 µg /ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH in ethanol. Absorbance at 517 nm was taken after 30 min, and IC50 was determined.

% inhibition = {(Blank absorbance - Sample absorbance) / Blank absorbance} x 100

Analgesic activity by acetic acid induced writhing (Dambisya *et al.* 1999): The extracts were tested for peripheral analgesic activity using the model of acetic acid induced writhing in mice (Figures 1 and 2). A comparison of writhing was made between positive control (diclofenac Na), negative control and extracts given orally 30 minutes prior to acetic acid injection (0.7% i.p.).

Figure 1: Swiss albino mouse

Figure 2: Acetic acid induced writhing in mouse

Analgesic activity by tail immersion method (Uma-Devi *et al.* 1999): The central analgesic responses of some extracts were evaluated using tail immersion method in mice.

Pain inhibition percentage (PIP) = $(T_1 - T) / T \times 100$. T_1 = Post drug latency and T = Pre drug latency

Antibacterial activity (Bauer *et al.* 1966): Antibacterial activity of extracts was evaluated by disc diffusion method. Sample possessing antibacterial activity would inhibit the growth of bacteria by forming 'zone of inhibition' together with standard Kanamycin 30µg/disc or Ciprofloxacin (5µg/disc).

Results

The tests carried out on different plant extracts revealed the presence of several important phytochemicals which might be responsible for their medicinal properties (Table 1).

Table 1: Phytochemicals in different plant extracts

Plant Samples / Phytochemicals	Reducing sugars	Tannins	Flavonoids	Saponins	Gums	Steroids	Alkaloids	Glycosides	Terpenoids
A. officinalis (leaves) extract	+	+	+	-	+	-	-	+	+
B. gymnorhiza (leaves) extract	+	+	+	+	+	-	+	+	-
A. aureum (whole plant) extract	+	+	+	-	+	-	+	+	+
H. fomes (leaves) extract	+	+	+	+	+	+	+	+	nd
S. caseolaris (leaves) extract	+	+	+	-	+	+	-	+	nd
S. caseolaris (fruits) extract	+	+	+	+	-	+	+	+	nd
X. moluceensis (bark) extract	+	-	+	+	+	nd	-	-	nd
E. agallocha (bark) extract	-	+	-	+	+	nd	+	-	-
V. monoicum (whole plant) extract	+	nd	-	+	+	nd	+	-	nd
C. inerme (leaves) extract	+	+	+	+	-	+	+	+	nd
D. trifoliata (whole plant) extract	+	+	+	+	+	nd	+	+	nd
B. tersa (leaves) extract	+	+	+	+	-	-	-	+	+
A.corniculatum (bark) extract	-	+	+	+	-	+	+	nd	nd
C. odollam (fruits) extract	+	+	-	+	-	+	-	nd	nd
C. odollam (bark) extract	+	+	+	+	+	+	-	nd	nd
P. foetidus (leaves) extract	+	+	+	-	-	+	+	+	nd
D. falcata (leaves) extract	-	+	+	-	-	+	+	+	nd
D. falcata (stem) extract	+	+	+	-	-	+	+	+	nd
C. bonducella (leaves and stem) extract	+	+	+	+	+	+	+	-	-
B. racemosa (leaves) extract	+	-	+	-	+	-	+	+	+
G. pentaphylla (leaves) extract	+	+	-	+	-	nd	+	+	nd

+ = present; - = absent; nd = not detected

Ext Std
Polar

Ext Std
Medium Polar

Ext Std
Non Polar (Mobile phase)

Figure 3: DPPH radical scavenging assay of extract (ext) and standard (std) on TLC plate

After applying DPPH on the TLC plate, pale yellow color on purple background was observed on the chromatogram which indicated the presence of antioxidant components in the sample extracts (Figure 3).

In the quantitative DPPH radical scavenging assay, most of the extracts displayed antioxidant activity which was comparable to that of ascorbic acid, a well-known standard antioxidant (Table 2). This assay may be used as a guide of fractionation and isolation of potential antioxidant components from these plants.

Table 2: IC50 of DPPH radical scavenging assay of different plant extracts

Samples	IC_{50} (approx) in µg/ml	Samples	IC_{50} (approx) in µg/ml
Ascorbic acid	18	*V. monoicum* (whole plant) extract	11
A. officinalis (leaves) extract	74	*C. inerme* (leaves) extract	28
B. gymnorhiza (leaves) extract	56	*D. trifoliata* (whole plant) extract	19
A. aureum (whole plant) extract	79	*C. odollam* (fruits) extract	>500
H. fomes (leaves) extract	26	*P. foetidus* (leaves) extract	64
S. caseolaris (leaves) extract	52	*D. falcata* (leaves) extract	38
S. caseolaris (fruits) extract	77	*D. falcata* (stem) extract	16
X. moluceensis (bark) extract	42	*B. racemosa* (leaves) extract	31
E. agallocha (bark) extract	43	*G. pentaphylla* (leaves) extract	32

In case of analgesic activity test using writhing inhibition model, we observed that the extracts of *A. officinalis* (leaves), *X. moluceensis* (bark), *E. agallocha* (bark), *B. tersa* (leaves), *C. odollam* (bark), *S. caseolaris* (fruits), *P. foetidus* (leaves), *D. falcata* (leaves), *D. falcata* (stem), *C. bonducella* (leaves and stem), *B. racemosa* (leaves) showed >60% writhing inhibition at a dose of 500 mg/kg body weight. In hot water tail immersion model, the extracts of *V. monoicum* (whole plant), *C. inerme* (leaves) and *D. trifoliata* (whole plant) showed considerable analgesic activity with time comparing to standard. Some of the extracts showed mild to moderate inhibitory activity as compared to standard against the bacterial species tested.

Conclusion

The study was conducted to assess phytochemical and selected pharmacological properties of some plants of the Sunderbans. Most of these plants are traditionally used for different therapeutic properties. In this study, we screened those for their antioxidant, analgesic and antibacterial activities. We also explored the phytochemicals they possess. These secondary metabolites are actually responsible for the traditional usage of medicinal plants. From this study, we were able to screen out some plants with potential bioactivities. The investigations were carried out using crude extracts of these plants. Further research might be conducted for finding out the bioactive molecules based on activity guided separation.

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Remedial Possibilities of Soil Arsenic Affected by Arsenic Contaminated Ground Water Irrigation Through Organic Amendments and Water Management

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Duration: Three years (2011 - 2014)

Expenditure of the project: Tk. 1500000.00

Introduction

In Bangladesh, Boro (the dry season rice) is the major recipient of irrigation water and more than 60% of this irrigation need are met from groundwater extracted by deep tube-well, shallow tube-well or hand tube-well (Imamul Huq, 2008). Rice is especially susceptible to arsenic toxicity compared to upland crops, because of increase in both the bioavailability and toxicity of As under the reducing condition of submerged soil in paddy fields. Management strategies to reduce As uptake by rice is therefore, very pertinent and urgent. Impact of various sources of organic matters in controlling the transfer of As from soil to plants and comparing the effectiveness of the different sources of organic matter as well as manipulating the water regime in the rice rhizosphere have been studied (Imamul Huq *et al*., 2006, 2008).

Objectives

The objective of the research project was to produce Arsenic-safe food, more particularly rice and vegetables by combining the manipulative processes of organic amendments and water regime management and to mitigate arsenic toxicity problem in Bangladesh by reducing arsenic (As) uptake/ accumulation in growing crops which are cultivated under irrigated condition with arsenic contaminated ground water.

Methodology

The research was conducted in two phases. In the 1st phase, water and soil samples were collected from an arsenic hotspot in the north-eastern part of the country. The reason is that no data or information on the extent and contamination level of this area is currently available to the community working or carrying out research on the problem of arsenic in the country.

The 2nd phase of the research comprised of growing BRRIdhan-11 on soils treated with various organic amendments and varying water regimes and analyzing the different growth parameters as well as chemical composition to ascertain the possibility of mitigating arsenic toxicity in rice grains.

1st Phase:

Sampling Site

Five locations, namely Selborash, Dakshin Sukhair Rajapur (DSR), Joysree, Paikurati and Dharmapasha union in Dharmapasha upazilla (sub-district) of Sunamganj district of Sylhet division of Bangladesh were selected as the sampling sites that covered a wetland/floodplain complex of some river basins and a part of the Haor Basin to the northeast of Bangladesh (latitude and longitude ranged between 24° 53′ & 25° 01′ and 90° 58′ & 91° 07′, respectively).

A total of 63 groundwater samples were collected from existing tubewells at different depths (90-140 ft) in the study areas in June 2010. The procedures followed during the collection of groundwater samples are described in Imamul Huq and Didar (2005).The approximate depth and age of each tubewell were noted from the record preserved by the well owners.

2nd Phase:

A macrocosm study in pot experiment was performed using the rice variety BRRI dhan-11 on a silty clay loam soil (background As level was 1.6 mg kg^{-1}). The treatments used were three water regimes viz., at 100%, 75% and 50% of field capacity (FC) and three organic matter treatments, viz., cow dung, poultry manure and a mixture of both, applied at a rate of 5 tons ha-1. Two sets of control experiments were also included where both As-laden and As-free water were used at the 3 levels of FCs with no added organic matter. All experiments were performed in triplicates. Seedlings of the rice variety were transplanted into the pots and all the pots were arranged in a completely randomized way. Fertilizers were added following the Fertilizer Recommendation Guide 2005 (BARC 2005).1 mg L-1 arsenic solution, in a combination of 80% arsenite (source: sodium meta-arsenite, $NaAsO_2$) and 20% arsenate (sodium arsenate, $Na_2HAsO_4.7H_2O$), was used as the As treatment. As accumulation in rice was analyzed using atomic absorption spectrometer (AAS) and the fate of *Asvis-à-vis* organic matter treatment was assessed by XRD analysis. All protocols concerning plant culture in this experiment were followed as described in Imamul Huq *et al.* (2011).

Results

1st Phase:

Arsenic in the groundwater and soil samples

The tube-well water samples were found to have As in the range of 2.70 to 282 µg/L, values much higher than either the WHO or Bangladesh permissible levels for drinking water. The data indicate that most of the tube-wells, 50 out of the 63 tube-wells, were contaminated with As higher than the Bangladesh standard. As in the soil samples were found to be in the range of 0.65 to 5.14 mg kg^{-1}.

2nd Phase:

Yield observation

The yield of rice grain was found to be the maximum for FC100, followed by FC75 and FC50 (table 1). Similar observations have also been reported in our previous experiments (Imamul Huq *et al.* 2006). The presence of As in the growth medium was found to have a negative impact on the vegetative growth of the plants as well as on the grain yield, for both organic matter treatments and control. This was also corroborated by Imamul Huq *et al.* (2008). However, organic matter application showed a more positive effect than no application and among the organic matter sources, the mixture of cow dung and poultry manure performed the best. Although AWD did not have a significant negative influence on yield parameters it however, promoted weed growth and delayed grain formation.

XRD analysis of the soils

XRD analysis of the treated soils indicatedthat there are significant differences in the XRD spectra indicating the fact that alleviation of arsenic toxicity has been the function of chemical changes. The organic matter could have entrapped the arsenic or there has been a biomethylation process to reduce the arsenic toxicity.

Arsenic accumulation in rice

Arsenic accumulation in different parts of rice including the rice grains has been assessed and the fate of the arsenic added through irrigation has also been evaluated. It was observed that regulating the water regime could reduce As accumulation in grain (table 2). So far the addition of organic matter is concerned, poultry manure could alleviate better than cow dung the accumulation of As in rice grains. The combined effects were yet better.

Table 1: Vegetative growth and yield of rice plants.

Treatments	Total weight of rice plant (Fresh wt., g/pot)	Height (cm)		Grain yield (g/pot)	Weight of unhusked grains (1000 grain wt, g)
		Shoot	Root		
PM FC100	47.49	81.67	29.00	21.38	21.10
PM FC75	45.00	66.00	21.33	13.90	19.93
PM FC50	20.31	51.67	18.67	9.00	18.30
CD FC100	37.11	80.00	23.67	18.02	19.90
CD FC75	25.74	62.17	16.25	7.29	18.75
CD FC50	17.86	52.67	19.00	2.55	16.30
No OM FC100	30.69	96.67	22.33	22.79	19.90
No OM FC75	23.85	64.67	13.67	15.87	18.93
No OM FC50	21.91	56.00	20.00	4.96	18.60
Control FC100	28.63	95.00	19.00	20.16	20.80
Control FC75	27.54	82.33	19.67	17.14	19.17
Control FC50	24.98	72.33	15.00	21.80	17.70
PM+CD FC100	26.50	80.00	20.67	23.01	20.03
PM+CD FC75	29.36	70.00	14.67	21.58	19.90
PM+CD FC50	24.91	65.00	17.67	19.16	18.47

PM = poultry manure; CD = cowdung, OM = organic matter, PM+CD = mixture of poultry manure and cowdung (ratio, 1:1)

Table 2: Concentration of arsenic in different parts of the rice plant including grain at different treatments.

Treatment	Root (ppm)	Shoot(ppm)	Husk (ppm)	Grain (ppm)	Total plant (ppm)
PM FC100	5.06	3.49	0.424	0.308	9.29
PM FC75	2.06	1.47	0.305	0.196	4.04
PM FC50	1.53	1.37	0.311	0.134	3.34
CD FC100	4.07	2.27	0.478	0.166	6.98
CD FC75	4.14	2.47	0.000	0.254	***6.87***
CD FC50	1.29	1.88	0.000	0.051	***3.22***
No OM FC100	2.28	3.23	0.161	0.318	5.99
No OM FC75	2.94	1.91	0.000	0.183	5.02
No OM FC50	1.58	2.11	0.373	0.111	4.17
Control FC100	2.04	0.64	0.000	0.257	2.93
Control FC75	1.98	0.36	0.000	0.155	2.49
Control FC50	0.43	0.46	0.000	0.181	1.07
PM+CD FC100	6.14	2.45	0.213	0.292	9.09
PM+CD FC75	19.09	1.40	0.000	0.238	20.73
PM+CD FC50	15.31	1.74	0.000	0.242	17.30

Conclusion

It is concluded from the present observations that manipulating the water regime in rice culture is helpful in alleviating As accumulation in rice grains. Using organic amendments is also helpful. Among the organic manures, poultry manure has a better performance than cow dung. Combining water regime management and organic matter management can be a better option for remedy of As accumulation in rice culture.

Publication

Imamul SMH, Roy S, Chowdhury MTA and Ahmed S 2012. Organic matter and water regime management to mitigate As-toxicity in rice. Paper presentedat the 4th International Congress on Arsenic in the Environment (As-2012) held in Cairns, Australia on the 22-27 July 2012.

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Gene Cloning for Increasing Production of Keratinase and Alkaline Protease by Bacillus licheniformis MZK05 for Biotechnological Application in Leather Industries of Bangladesh

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Duration: Three years (2011-2014)

Expenditure of the project: Tk. 2000000.00

Introduction

Alkaline protease and keratinase from *Bacillus* spp. has attracted attention of many researchers because of its tremendous technical applications in food, pharmaceutical and leather industries. Protease of about TK 5000 million is imported annually for leather industries in Bangladesh. Importing of enzymes takes long time during which the activity of the enzymes is decreased causing further expenses. On the other hand, local production of the enzyme will be cheaper not only due to its production on cheap raw materials but also enzyme preparation and uses in liquid state unlike dried enzyme being imported at high cost. In view of this reason, some *Bacillus* species were isolated from effluents of poultry farm and tannery and identified by 16S rRNA gene sequence analysis (Hoq *et al.* 2005). Among them the strains were tested for protease enzyme production in shake flask and bioreactor level and the enzyme preparations were found satisfactory in the various technical applications (Azad *et.al.* 2002; Uddin *et al.* 2006; Hossain *et al.* 2007 and Hossain *et al.* 2008). Since the production of the enzyme by natural *Bacillus licheniformis* MZK05 strain was not adequate.

Objectives

1. Cloning of *kerA* gene from *B. licheniformis* MZK05 into pGEX-6p-2 vector and its expression in *Escherichia* coli BL21 for increasing the production of keratinase;
2. To obtain hyper producer of the enzyme (alkaline protease), instead of gene cloning, classical mutation of the wild strain (*B. licheniformis* MZK05) was performed through treatment with different effective mutagens; and
3. Optimization of the enzymes production and their application in leather industries for eco-friendly leather processing.

Methodology

Isolation and identification of the microorganism: *Bacillus licheniformis* MZK05 (BlMZK05) was isolated from feather-decomposed soil of poultry farm and identified by both biochemical and 16S rRNA typing in the International Center for Biotechnology, Osaka University, Japan (Hoq *et al.,* 2005).

Cloning of KerA gene for overproduction of keratinase: *BlMZK*05 was used as the source of genomic DNA for *ker*A gene. The PCR product and vector were digested with *Bam*HI and *Xho*I and inserted into vector pGEX6P2 by ligation with T4DNA ligase. The recombinant vector, pGEX6P2kerA, was transformed into the chemically developed competent *E.coli* BL21 cells for the expression of GSTkerA fusion protein. The GSTkerA fusionprotein was expressed and optimized in different IPTG concentration and time. Protease and Keratinolytic activities of the purified *Ker*A were determined.

Mutation of BlMZK05 for enhanced production of alkaline protease: *BlMZK*05was treated with several different combinations of mutagens with various dose and exposure duration. Following treatments the hyper-proteolytic mutants were screened through clear zone ratio on Skim milk agar plates and production of enzyme titer in Alkaline Protease Producing Broth (APPB) containing (g/l): glucose 10, peptone 5, yeast extract 5, K2HPO4 5, MgSO4.7H2O 0.1.

Optimization of medium for production of alkaline protease by the mutant strain: After strain improvement, the optimization of the medium ingredients was carried out using statistical Plackett-Burman and Response Surface Methodology (RSM) based on Center Composite Design (CCD) in shake flasks followed by 7 liter bioreactor under control temperature, pH, and dissolved oxygen concentration.

Application of keratinase and alkaline protease in dehairing of goat skin: Freshly flayed goat skin obtained from the local slaughter house was cut into appropriate sizes and dipped into definite volume of water with 2.5% keratinase along with 2.5% alkaline protease solutions. The efficiency of the dehairing method to remove the hair from skin was evaluated by determining the area of the dehaired portion and the smoothness of grain surface of dehaired skin was examined by Scanning Electron Microscopy (JSM-6490LA, Jeol, Japan).

Application of Alkaline protease in bating of skin and hides: For determining the bating potentiality of the enzyme, alkaline protease from different mutant strains tested for hydrolyzing the nonstructural proteins without affecting the collagen itself (the leather component). Thus the suitable alkaline protease which was obtained was applied for bating of the animal hides (1000kg) in both laboratory (Institute of Leather Engineering and Technology, and Department of Microbiology, University of Dhaka) and industrial level (Samina Tannery Ltd and Bay Tannery Ltd., Hajaribagh, Dhaka).

Cattle hides (after deliming) emerged in water with the enzyme preparations were rolled in a drum for about 60 minutes. Then the quality of the bated leather by both our protease and commercial enzyme (Oropon K, TFL, UK) was tested in ISO laboratory, Institute of Leather Engineering and Technology, University of Dhaka.

Results

Cloning of kerA gene from Bacillus licheniformis MZK05: The pGEX-6p-2-kerA was transformed into E. coli BL21 and expression of GST-kerA fusion protein was observed within 1h of induction at 0.1 mM IPTG concentration (Fig. 1A). The optimum time and concentration for maximum expression of the desired protein were determined to be 3 h with 0.3 mM IPTG (Fig. 1B). From SDS-PAGE analysis, the molecular weight of the GST-kerA fusion protein was determined to be 58 kD (Fig. 1C). Glutathione Sepharose 4B batch purification followed by PreScission protease cleavage produced the KerA protein of about 39 kD (Fig. 1C). A corresponding increase in proteolytic (312 U/mL) and keratinolytic (196 U/mL) activities were obtained with the expressed keratinase.

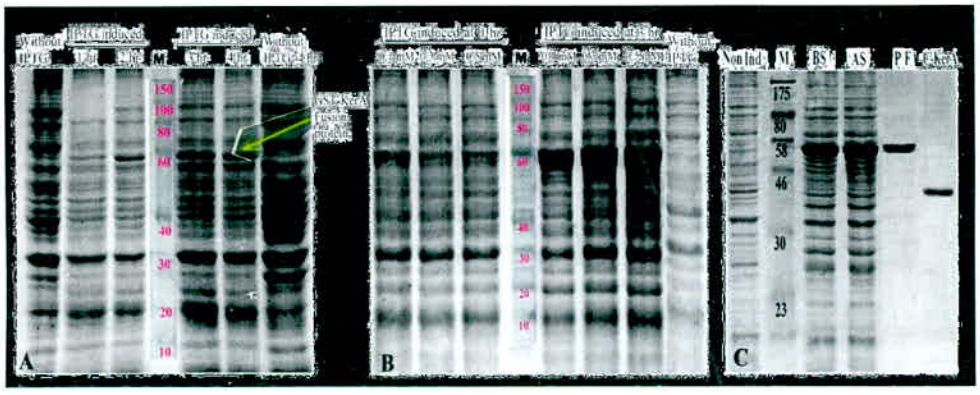

Figure 1: Expression of GST-KerA fusion protein. A) Time required for maximum protein expression at 0.1 mM IPTG. B) IPTG concentration required for maximum protein expression. C) Purification of recombinant protein. Non Ind: Non induced. BS: Before sonication, AS: After sonication, PF: Purified fusion protein, C- KerA: KerA protein after cleavage by PreScission protease free from GST tag. (M: Pre-stained protein Marker, NEB, USA).

Mutation and screening of the potential mutant: After testing many of the mutants for production of high level enzyme having properties for hydrolyzing nonstructural proteins without collagen itself *B. licheniformis* MZK05M9 revealed as one the potential strain. Therefore it was selected to develop bioprocess for the production of alkaline protease for bating of hides and skias in leather industry in Bangladesh.

Optimization of medium for production of alkaline protease by the mutant strain: To develop a cost-effective medium for production of the protease at high level by *Bl*M9, Soybean meal as nitrogen source and molasses as carbon source were selected by Plackett-Burman study for further optimization of the medium by RSM based on CCD and highest enzyme activity 765 U/ml was found in the statistically optimized medium in shake culture. From the fermentation in 7 L bioreactor with 3.5 L working volume it was found that the production of protease was growth associated with the highest protease activity 1020 ± 10 U/ml after 28 hrs at stationary phase which was 1.7 fold higher than that found in Molasses Soybean meal medium optimized by one variable-at-a time method. Thus, from the present study, it was found that strain improvement and optimization of the medium together resulted in 25 fold augmentation in the enzyme titer than that produced by wild strain (Figure 2).

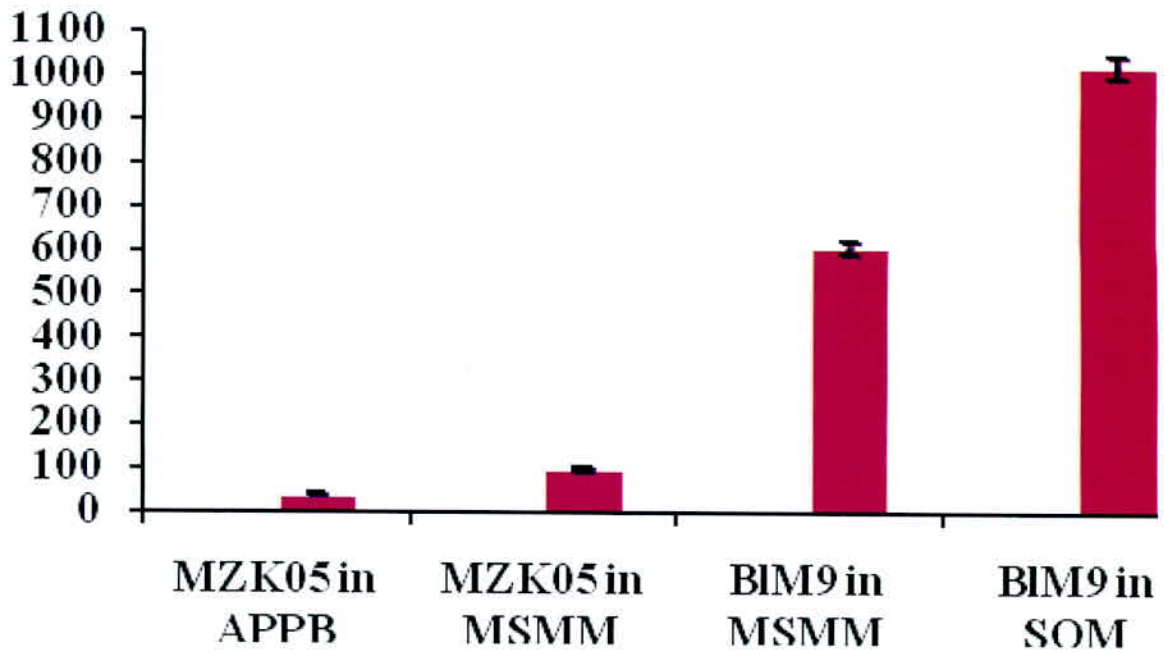

Figure 2: Development of protease production by strain improvement and medium optimization. **APPB -** Alkaline protease producing broth (Azad et al.2002), **MSMM -** Molasses soybean meal medium (Mamun et al. 2015), **SOM -** Statistically optimized medium.

Application of the enzymes in dehairing of goat skin: From the dehairing experiment it was found that the enzyme assisted method was comparable to conventional lime-sulfide method in complete dehairing. The surface of the skin dehaired by enzymatic method was smoother than that of the conventional method as revealed by Scanning Electron Microscopy.

Application of the BlM9 enzyme in bating of leather processing: The results of different tests of the enzyme treated leather (crushed leather) such as tensile strength, percent of elongation, stitch tear strength, water vapor permeability, grain crack strength (Lastometer) and tongue tear strength tests indicated that BlM9 bate was equally efficient to the commercial bate Oropon K. Also the bubble, thumb and cross section tests of the treated leather (pelt leather) met the requirement of quality bating performance and comparable to the commercial enzyme (Figure 3).

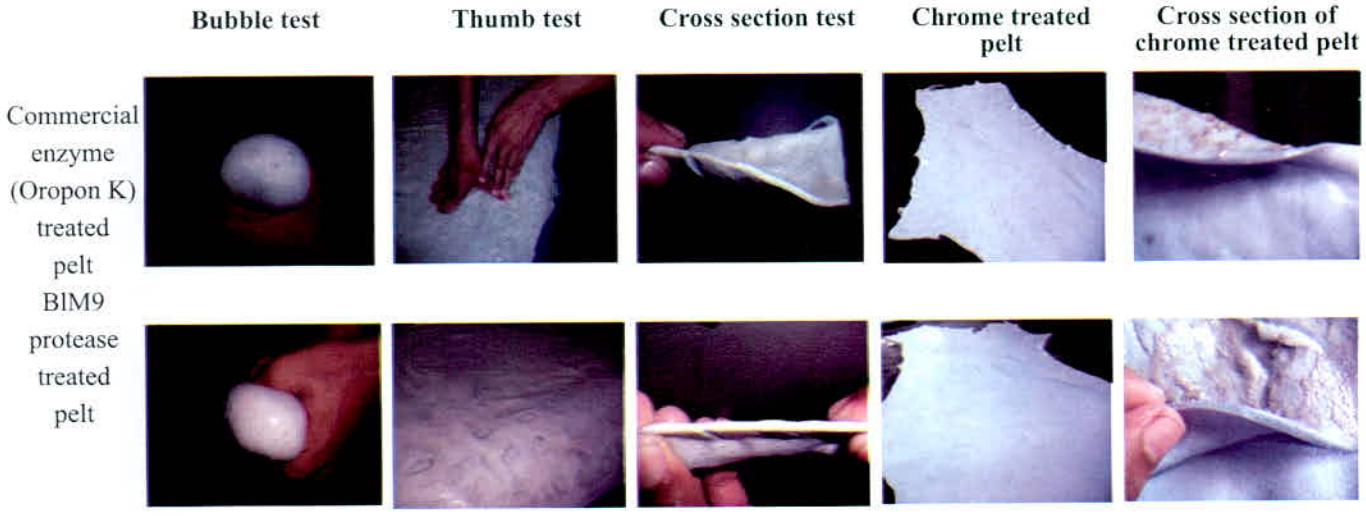

Figure 3: Different tests of pelt bated with BlM9 protease

Usages of the enzymes in different tanneries in Bangladesh: The technology of the production and application of the protease enzyme as bating agent of leather has been transferred to AFC Agrobiotech Ltd. through University of Dhaka. Although, the scale-up of the production is going on in AFC Agrobiotech Ltd. some large scale trial of the enzyme in bating step has been performed successfully in different leather industries.

Conclusion

In the present study, the over production of the keratinase and protease was achieved by both genetic manipulation of the bacterial strain and medium engineering. The major applications of the present enzymes were found in leather manufacturing industry where the results of different qualitative and quantitative tests suggested their potentiality in dehairing and bating of animal skins and hides. As Bangladesh leather industries still use conventional technique for leather processing, use of this enzyme assisted process can contribute to the reduction of environmental pollutions to the significant level and can be the good alternative in terms of the production of quality leather. Thus this will not only facilitate the eco-friendly hide processing in leather industries but also save foreign currency.

Publication

Mamun MAA, MM Khan, MNR Akand, SN Khan and MM Hoq. 2015. Characterization of an alkaline protease with high quality bating potential in leather processing from *Bacillus licheniformis* MZK05M9 mutant. *Int. J. Biol. Res.* Vol.3, Issue 1, 36-41.

Nahar M, Shishir MA, Waliullah S, Haque MS, Ilias M, Karim M, Khan SN and Hoq M M. 2016. Cloning, expression and structure simulation of keratinase from *Bacillus licheniformis strain* MZK05. *Malaysian J. Microbiol.* Vol 12, Issue 1.

Salaheen S, Mamun MAA, Khan SN and Hoq MM. 2015. *Improvement of Bacillus licheniformis* MZK05 by mutation for increased production of keratinase. *Dhaka Univ. J. Biol. Sci.* Vol. 24, issue 1, 17-23.

Mamun MAA, Hosain MA, Ahmed S, Zohra FT, Sultana R, Khan MM, Akhter MZ, Khan SN and Hoq MM 2015. Development of an alternative enzyme-assisted dehairing method of animal skins using proteases from *Bacillus licheniformis* MZK05M9. *Bangladesh J. Microbiol.*, Vol. 32, Issue 1&2, 33-37.

Hoq MM, Mamun AA, Shishir MA, Khan MM, Akand MNR and Khan SN. 2013. Bioprocess development for eco-friendly microbial products and impacts on bio-industry establishment in Bangladesh. Proceedings of international conference on biotechnology, 25-26 May, 2013.

Application: The enzymes were successfully applied in bating and dehairing of skins and hides in leather industries (Tanneries) in Bangladesh. This is under progress for industrial level production by AFC Agrobiotech Ltd.

Patent: Based on the above work, one patent BD/P/2013/000260 entitled " Development of a bioprocess for industrial production of protease by *Bacillus* DUH10 and its application in bating of hides and skins in leather industries" has been submitted to the Department of patents, Design and Trademarks, 91, Motijheel, Dhaka –1000, Ministry of Industries, Govt. of Bangladesh.

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Hossain MS, Ilias M, Paul HL, Karim MF and Hoq MM. 2008. Application of protease and keratinase from *Bacillus licheniformis* MZK03 in leather processing. *Dhaka Univ. J. Biol. Sci.* 17(2): 127-135.

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Study of Arsenic and other Trace Elements in the Silt, Borehole Sediments and Anions of the River Meghna-Jamuna Delta

A M Shafiqul Alam and Md Arifur Rahman

Institution: Department of Chemistry, University of Dhaka

Duration: Three years (2011-2014)

Expenditure of the project: Tk. 1000000.00

Introduction

The Bengal Delta Plain (BDP) in one of the biggest deltas in the world and Through which the rives Padma (Ganges), Jamuna (Brahmaputra) and Meghna pass into the Bay of Bengal. The Rivers generate large amounts of sediments each year and therefore alluvial sediments dominate the geology. The main part of the BDP is located in Bangladesh. The Bengal basin has one border to the north to the Shillongplateaa (on extension to the Himlayas) and another to the east through the Tripura Hills. The third border constituted by the Indian shield (a precambrian basement complex).

Many studies and surveys have been made on arsenic in the BDP. During 1998-99, BGS and DPHE performed a national survey where 3534 shallow wells were tested for arsenic. This study showed that most of the arsenic contaminated sediments are located in the medium shallow aquifers (10-150 m) whereas the shallower and the deeper sediments seem to be unaffected (BGS and DPHE, 2001)

Arsenic is a natural constituent in the earths crust and arsenic bearing minerds such as arsenides of iron, copper, nikel and lead are the primary natural sources of arsenic. The most common mineral is arsenopyrite (FeAsS). Arsenic can also enter the environment through anthropogenic activities. The metalloid has been used in a variety of products: herbicides, pesticides and wood preservatives are a few examples (Al and Ahmed, 2003). Arsenic is sensitive to mobilization at pH values typically found in ground water, i.e. Ph 6.5-8.5 and under both oxidizing and reducing conditions (Smedley and Kinniburgh, 2002)

Arsenic is harming the developing Countries like India and Bangladesh more vehemently owing to obvious reason. Ground water poisoning by this arsenic, positioned in the top rank as a carcinogenic agent has created a great health concern effecting at least 70 million people in Bangladesh and west Bengal. This staggering number and widespread mass poisoning is the pivotal factor for the underlying research that has received increasing attention recently. The diagenetically derived secondary amorphous iron, manganese, and copper hydroxides can adsorb arsenate. These arsenic adsorbed solid phases are transported, entrapped and deposited at the sediment water.

Interface in the sediments, So it is a greatg concern of the environmental chemists to estimate some other metals (such as Fe, Cu, Mn, Pb, Cd, Zn, Cr, Hg etc.) as well as As metal in the river sediments to the river Jumuna and Meghna in Bangladesh to find out whether there is any relation exists between the source of contamination of metals and/or their interference.

Objectives

The objective of this work is to see the levels of contamination of As in the sediments of the Jamuna and Meghan deltas in Bangladesh. The focal theme of this research is that the sediments with high arsenic content can play an important role in the ground water quality and consequently it also influences the concentration of the trace metals in both the water column and biota, if they are desorbed or become available to benthic organisms. So of the finding the As levels , of the sediment the specific objective of our work is to evaluate the concentration of three other metals (Fe, Mn and Cu) in the sediments of the same locations and to investigate the correlation of As with these three metals.

Methodology

Selection of sampling locations: One location with two sites (upstream and downstream) from Tista river and Dorla river were considered as sampling location Five locations with ten sites (upstream and downstream) from Jamuna river sites considered as sampling locations. The location were balashigh at near Gaibandha, Sariakandhi near bogra, Jamuna bridge near Sirajgong, Chowhli near Gangail, and Arichaghat near Manikgong for Jamuna river.

There are tow sub-rivers: Surma and Kushiara of Meghna river.Three locations with six sites upstream and downstream) form surma river were considered as sampling locations and two locations with four sites (upstream and downstream from Kushiara river were selected as sampling locations. Sampling locations were Zakiganj (Sylhet), Sylhet town and Chhatak Sunamganj) for Surm river and Bainibazer and Sherpur near Moulovibazer of Kushiara river. Four location with eight site (upstream and downstrea) from Meghnaa river were taken as sampling locations. The locations were Bhairab by of Kishorgong distric, Meghana bridge near Norsingdi, Mohonpur and lower stream of chandpur district, All the sediment samples were collected at several depths ranging from 1 to 6 meters using 1.5 inches (diameter) pipe through normal digging procedure.These were taken in plastic bottles and sealed with tapes and then taken to the laboratory for further analysis.

Digestion procedure of sediments:The sediment samples were digested following the HNO_3 and $HClO_4$ digestion method.

Spectrophotometric Method of Analysis of Arsenic:

Arsenic (AsH_3) generation followed by complication with silver diethyldithiocarbamate [$AgSCSN(C_2H_5)_2$]

Solution:

Arsenic reaction with solution of Ag-DDTC complex with morpholine in chloroformto form soluble andcomplex, which has an absorption maximum at 535 nm. This forms the basis of the methods.

Trivalent arsenic reacts with silver diethyldithiocarbamate [$AgSCSN(C_2H_5)_2$] to from a red colur complex, which absorbs at 525 nm.

Ag-DDTC + AsH_3 - As-DDTC (red color).
etermination in water at the ppb concentration level.

Valence As ion (As_3+) + HCl, KI, $SnCl_2$ -3 valance As ion (As_3+). By adding Zn to this solution the arsenic is reduced further to arsenic hydride.

Iron, manganese and copper were analyzed by AAS method and phosphate and sulphate were analyzed by UV spectrophotometirc method.

Results

The comparison between deeply flooded areas in Bangladesh and the areas having more than 10 and 50 percent of the tube wells producing water with more than 0.05 mg As (which is the present drinking water standard in Bangladesh) Shallow aquifers are the main As problem areas in Bangladesh, (Ahmed 2003).

The reducing soil environment in the deeply flooded areas appears to be conducive to the release of As from sediment. The tube wells tank in shallow aquifers in the Jumna and Meghna floodplains except in the coastal areas, are the worst affected, in the coastal areas. water supply is mainly based on manually operated deep tube wells as the water available in the shallow aquifer is saline. In Bangladesh, deep tube wells at depths greater than 500 ft have been found to be largely free from As contamination (DPHE and BGS 2001) Arsenic contamination the flooded areas on both sides of the Jamuna is relatively low. probably because of the dynamic nature of the river at a relatively higher energy level and deposition of sandy soil with low As content on the floodplains.In average both sulfate and phosphate concentration were higher in Meghna Delta compared to Jamuna Delta.

Conclusion

(i) The Jamuna and Meghna deltas consist of more rivers, Dorla and Tistariver is sub river of Jamuna and Surma and Kushiara river of the Meghna delta. Higher concentration of arsenic was found in the Jamuna delta compared to the Meghna delta.

(ii) Arsenic concentration was varied form upper middle and lower stream in every location due to the geochemical and geophysical aspect of sediments, rock and different areas present in soil. Arsenic concentration in upper soil was higher than the edge sediment of every location because river current kindness the deposition of arsenic in the edge sediments rather than the stagnant upper soil.

(iii) The study suggests that the distribution of As in the sediments is not only controlled by signal mineral phase, but As is partition into three phases: mental (Fe and Mn) hydroxides, Fe sulfides, and also organic matter. And also low amount of As was found in Meghnaghat region of Meghna delta due to strong current might swift As from the Suma and Kushiara river and high current of As was found in Bainibazer of Kushiara river because this region was received As form the next upstream Borak river coming from India.

(iv) Higher Fe/As ratio and relatively lower Mn/As ration were formed in the borehole sediment of both the Jamuna and Meghna delta which indicates As associated with Fe and Mn in the deeper sediments. Lower Cu/As ration was found in borehole sediments of different depth. It has been observed the Cu is transported from the water trace to sediments being rapidly re-mobilized form the solids and transfer back to the water stream. Higher ratio of Fe/As may be resulted form the presence of soluble Fe-oxides and hydroxide rather than in soluble Fe silicate in bore hole sediments of the Jamuna and Meghna delta.

(v) It is generally observed that the river Jamuna and Meghna changes its course of their path in every rainy season or during the flood as result, deposited As from the fresh land of the Jamuna& Meghna river might be leached to the shallow aquifers. The new tube wells were installed to these locations and the water content from these tube well contained higher amount of As.

(vi) These observed data which has been carried out for arsenic with other three metals in the borehole sediments may be treated as a reference data to the Government of the People's Republic of Bangladesh.

Publication

Shafiqul Alam AM 2013. Distribution of Arsenic with Iron, Manganese and copper in Borehole sediments of the River Tista and Jamuna, *Dhaka University, J. Sci.* 61(2);207-210.

Shafiqul Alam AM 2011. Mobilization of Arsenic with iron, manganese and copper in the brohole sediments of the River Jamuna. *J. of Bangladesh Chem. Soc,* 25(1) 30-37.

Conservation and Utilization of Rice Diversity Through Participatory Variety Selection for Increasing Productivity and Improving the Livelihood of Resource Poor Farmers in Southwest Coastal Areas of Bangladesh

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Institution: Agro technology Discipline, Khulna University, Khulna-9208

Duration: Three Years (2011-2014)

Expenditure of the project: Tk. 1400000.00

Introduction

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. Nearly 10,000 land races of rice are considered to exist in Bangladesh (Haque and Miah, 1990). 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.

The landraces (local varieties of cultivated rice) are usually poor yielder but they are superior to modern varieties in terms of stability, resistance to biotic and abiotic factors, quality characters, etc. A number of reports showed that indigenous rice cultivars from Bangladesh possess a wide diversity in ecological, morphological and physiological characteristics (Bhowmik *et al.*, 1990; Islam, 1990; Jahan, 2003). However, in comparison to a large number of landraces, only a few have been evaluated. These investigations are useful for analyzing genetic variations of a certain crop together with the geographical distribution. From these facts, the landraces are considered to be an important "cultural heritage". We have to consider how to advance the collection and conservation of genetic resources for the future.

Quality of rice grain has been an important factor in rice production. Jahan (2003) experimentally showed that cultivars from Khulna region are genetically more diverse in terms of grain quality characters than other regions and suggested that Khulna region can be a hot spot for future germplasm exploration.
Participatory Variety Selection (PVS) is the method where farmers have the freedom to choose suitable variety (s) to overcome their environmental and socio-cultural constraints. Therefore, evaluating rice diversity and selecting suitable varieties for their utilization by the resource poor farmers PVS will be conducted with locally available aus, aman and boro rice germplasm.

Objectives

The following objective was considered for the study:

i. To collect the indigenous rice germplasm from Khulna, Bagerhat and Satkhira districts of Bangladesh;
ii. To evaluate the collected rice cultivars for agronomic characters through PVS trials;
iii. To evaluate the collected rice cultivars for grain quality characters; and
iv. To conserve the collected germplasm for future utilization in breeding purposes. To engage farmers in decision making processes.

Methodology

To fulfill the above mentioned objectives three major studies were undertaken.

Study 1. Collection of indigenous rice cultivars for Aus, Aman and Boro seasons.

Study 2. Evaluation of the collected germplasm for yield and agronomic characters through PVS trials.

Study 3. Evaluation for quality characteristics of grains from rice germplasm collected.

Under the three studies a number of experiments were conducted in the field and laboratory.

Field trials were made in three locations. There were one mother trial and two baby trials. Mother trial was made in Khulna University campus and one baby trial was made at Bathiaghata, Khulna and other at Kaliganj, Satkhira.

Data on agronomic characteristics and grain physical and chemical parameters were taken.

Results

Forty six local varieties of rice (Aus-13, Aman-20 and Boro-13) were collected from the south-west coastal areas of Bangladesh. The varieties are very much different in morpho-physical characters, production potential and grain quality characters. In aman season, in 1st year, 20 varieties including 3 fine rice varieties were tested where, variety Horgoza produced the highest grain yield (4.51tha^{-1}), straw yield (9.43 tha^{-1}) and biological yield (13.94 tha^{-1}). Among the varieties, 14 varieties produced grain yield that was statistically similar to the highest yield of them varieties Ghunsi, Bashful balam, Jatai balam, Hoglapata, Boran, Kachra Rani selute and Chairo balam produced above 4.00 tha^{-1} grain yield. Variety Kalomona as a non-fine rice produced the lowest grain yield (1.82 tha^{-1}). Among the fine rice varieties Chini atap performed the best in respect of grain yield (2.03 tha^{-1}). In boro season, in the 1st year, grain yield did not vary significantly among varieties and it ranged between 3.17-5.01 tha^{-1}. In the 2nd year, grain yield varied significantly and it ranged between 3.86 - 4.69 tha^{-1}, the highest value was in Kajol lata and the lowest value was in Tere bale. Varieties Bere ratna, Ashan boro, Kajol lata, Koijore, Kaliboro, Choite boro and Sylhete boro produced above 4.00 tha^{-1} grain yield. Among three locations varieties did well in Kaligonj location. Mother trial for aus rice varieties was not successful due to various reasons but from baby trial at the farmer's field grain yields of 9 varieties were obtained and it ranged between 3.12 tha-^{1} and 5.20 tha^{-1}. Varieties Chandramoni, Shama and Abdul Hai performed better, where, Abdul Hai was the best. Variety Shada shate produced the lowest yield.

Grain physico-chemical analysis of the varieties indicated that, grain size was short to medium; grain shape was medium to bold; only 3 varieties had slender grain shape. Awn was present in 8 varieties. Varieties possessed low to intermediate alkali digestibility and soft to medium gel consistency.

Conclusion

- Forty six local varieties of rice grown in aus, aman and boro seasons were collected from the south-west coastal areas of Bangladesh.
- Performance study of local rice varieties indicates that a number of varieties have high yield potentiality.
- In aman season, varieties Rani selut, Ghunsi, Basful balam, Jatai balam, Hoglapata, Kachra, Horgoza, Chairo balam and Boran produced above 4 tha^{-1} grain yield. Among three fine rice varieties, Chini atap performed the best (2.03 tha^{-1}).
- In boro season, varieties Bere ratna, Ashan boro, Koijore, Choite boro and Sylhete boro produced above 4 tha^{-1} grain yield, where Koijore out yielded the others.
- Among aus rice varieties, Chandramoni, Shama and Abdul Hai performed better, where, Abdul Hai was the best.
- Grain size of the varieties was short to medium; grain shape was medium to bold, only 3 varieties had slender grain shape. Varieties possessed low to intermediate alkali digestibility and soft to medium gel consistency.

Publication

B. Sc. thesis: Growth and yield performance of local aman rice varieties collected from South-western region of Bangladesh.

M. S. thesis: Yield and quality characters of local boro rice varieties of South-west region of Bangladesh.

Scientific paper

Roy SK, Ali MY, Jahan MS, Saha UK, Ahmad-Hamdani MS, Hasan MM and Alam M A. 2014. Evaluation of growth and yield attributing characteristics of indigenous Boro rice varieties. *Life Science Journal,* 11(4): 122 – 126.

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Mono-sex Culture of *Macrobrachium rosenbergii* and its Impact on the Economy of Bangladesh

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Duration: Three Years (2011 - 2014)

Expenditure of the project: Tk.1000000.00

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.

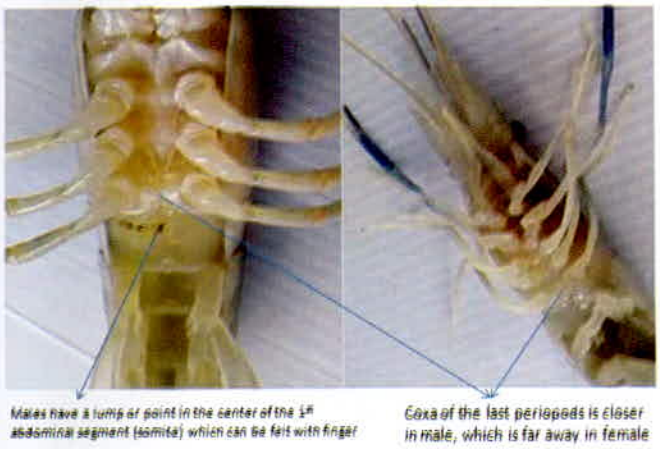
Objectives

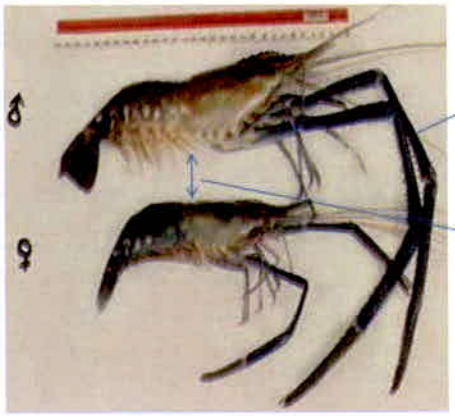
1. To compare growth, survival and production performance of mono-sex male with mono-sex female and mixed sex *Macrobrachium rosenbergii;*
2. To identify the suitable stocking density of mono-sex prawn culture and to evaluate its economic profitability; and
3. To increase farm production and to uplift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

Methodology

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 1.0-1.5 m.

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/m^2. 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/m^2 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.





1. Adult male has strong and large 2nd chelipeds
2. Male prawn is larger than female of the same age

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

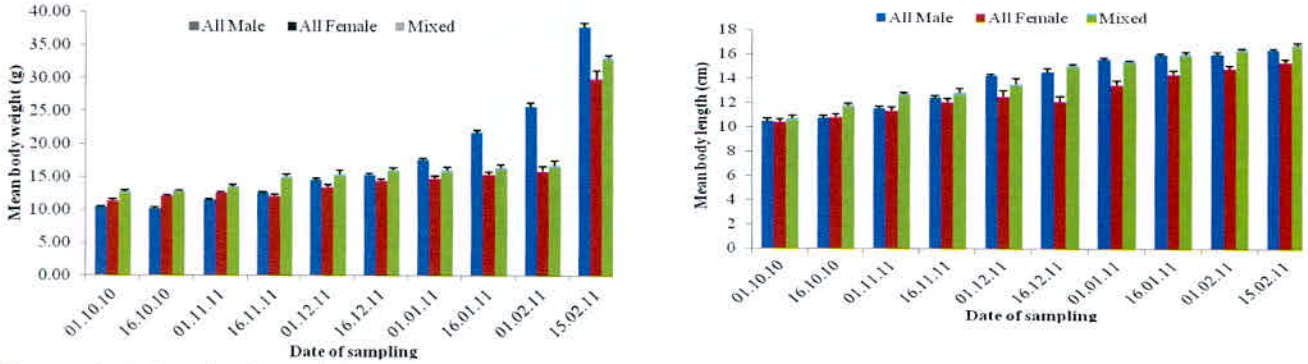


Figure 1: Mean body weight and mean body length of 3 different groups of prawn revealed by fortnightly sampling. The error bars indicating standard error of fifteen different samples.

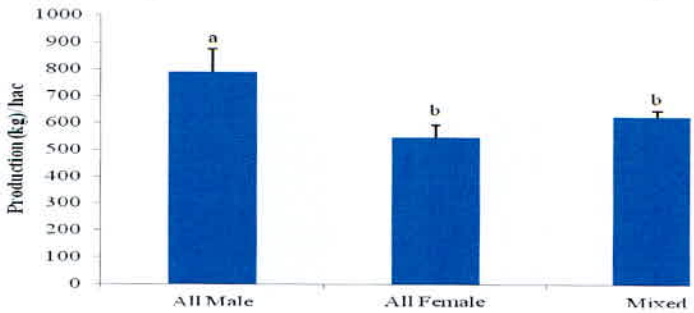


Figure 2: Production performace of all male, all female and mixed sex prawn after 150 days culture in earthen experimental ponds. The error bars indicating the standard deviations of three replicates. Significance test was done by one way anova using SPSS 16.0 version ($P < 0.05$).

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

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.

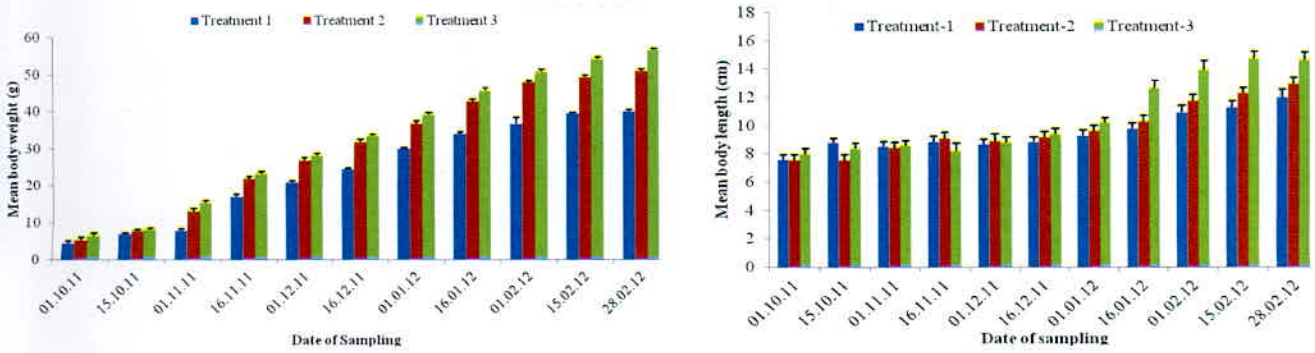


Figure 3: Mean body weight and mean body length of all male prawn at different stocking densities (Treatment 1 = 3/m^2, Treatment 2 = 2/m^2, Treatment 3 = 1/m^2). The error bars indicating the standard error of fifteen samples.

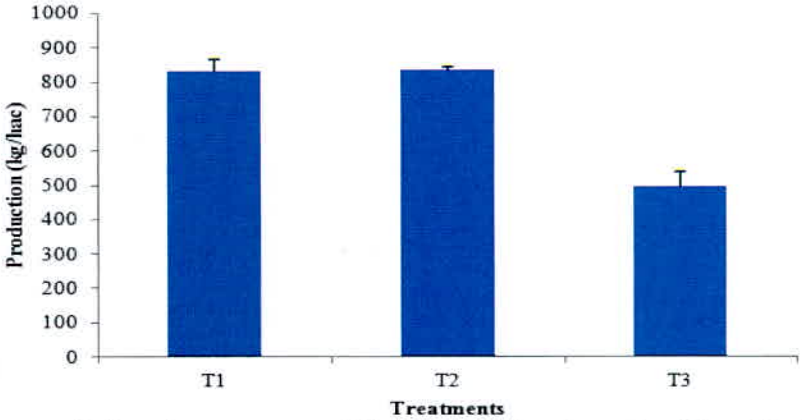


Figure 4: Production performace of all male prawns at different stocking densities (T1 = 3/m^2, T2 = 2/m^2, T3 = 1/m^2) after 165 days culture in earthen experimental ponds. The error bars indicating the standard deviations of three replicates. Significance test was done by one way anova using SPSS 16.0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

Publication

Two theses were made for the fulfillment of MS degree. The titles were-

1) Mono-sex culture of *Macrobrachium rosenbergii* and its impact on the economy of Bangladesh.
2) Morphological variation of Freshwater giant prawn at cultured pond and natural source in Batiagata, Khulna.

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Introduction of Kenaf *(Hibiscus Cannabinus* L.) as a New Crop in the Coastal Saline Soils of Khulna District and Exploring its Potentiality for Pulp and Oil Production

Md Monirul Islam and Md Sarwar Jahan

Institution: Agrotechnology Discipline, Khulna University

Duration: Three years (2011-2014)

Expenditure of the project: Tk. 1600000.00

Introduction

Kenaf *(Hibiscus cannabinus* L.), is a short-day, annual herbaceous and salt tolerant plant of the Malvaceae family.The kenaf stalk contains two distinct fibers, i.e., long, jute-like bast fibers in its bark and the short, balsawood like core fibers (Acreche *et al.,* 2005; Curtis and Lauchli, 1989 and Webber III, 1993.). The bast fibers have been used traditionally in the manufacture and trade of cordage products such as burlap cloth, twine, and ropes (Khatun, 2007). The core fiber provides raw materials for a growing number of products including paper, particle board, animal bedding and bioremediation aids (Cook and Scott, 1995.) Kenaf seeds yield a vegetable oil (21%) that is edible and high in omega antioxidants. The kenaf plant is considered one of the most promising alternatives to virgin soft and hard woods for paper production. The use of kenaf in paper production offers various environmental advantages over producing paper from trees (Atchison and Mc Govern, 1983; Karlgren *et al.,*1991).

In Bangladesh, about 1.0 million ha of arable lands are affected by varying degrees of soil salinity. Most of the lands remain fallow in the dry season (January-May). Crop yields, cropping intensity, production levels, and people's quality of livelihood are much lower in this region than other parts of the country. It is very important to provide farmers with alternative production systems with high land productivity for the enhancement of farmers' livelihood and sustainable development in the coastal zone.

Objectives

The objective of the project was to study the feasibility of kenaf cultivation with special reference to fiber, pulp and oil production in the southwest coastal region of Khulna District of Bangladesh.

Methodology

The project was implemented through a number of field and laboratory experiments. Standard practices and protocols were followed for kenaf production, processing, pulp and paper making and oil extraction.

Plant materials: Kenaf varieties HC-2 and HC-95

Seed source: Bangladesh Jute Research Institute, Dhaka

Design: RCBD with 4 replications (for fiber production),RCBD with 5 replications (for seed production)

Land selection: Medium high land ; Plot size: 4m × 5 m

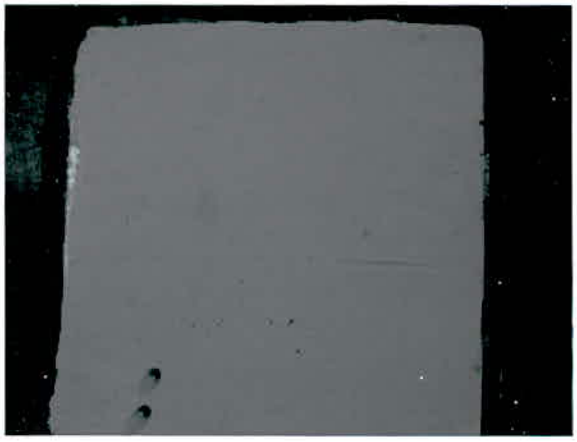
Field Preparation

Established Kenaf Field

Core Fiber of Kenaf

Bast Fiber of Kenaf

Oil Extracted from Kenaf Seed



Hand made paper from Kenaf pulp

Figure 1: Production of Kenaf

Land preparation: The land was ploughed and cross ploughed three to four times. The ploughing was followed by laddering. The ploughed field is made free of weeds and stubbles and leveled properly.

Seed rate: 11-12 kg ha^{-1}

Method and depth of sowing: Line sowing: 2.5 to 3 cm; Spacing: 30×10 cm

Intercultural operation: weeding and thinning is generally done simultaneously. The first weeding is done at the age of three weeks of crop and the second weeding was done after five weeks of age of the crop.

Harvesting: *130* days after sowing, harvesting was accomplished by cutting. The bundled kenaf plants were then kept 3-4 days in the soil for defoliation.

Retting: Ribbon retting method was practiced for fiber separation

Pulp production: Pulping was done by prescribed method with local technique

Oil extraction: Oil extraction was done by prescribed methodology

Data Collection: Growth and yield contributing data were collected as per prescribed agronomic rule.

Data Analysis: MSTAT-C software programme was used for data analysis

Results

Experiment 1. Yield potential of two kenaf varieties in 2010 and 2011

Experimental site: Field laboratory of Agrotechnology Discipline and Batiaghata, Khulna

Duration: April, 2010 to August, 2010 and April, 2011 to September, 2011

Plant height

In both the year the plant height of HC-2 variety was greater than HC-95 and it was 4.0 m in 2010 and 3.98 m in 2011. This may be due to its varietal characteristics. Result showed the resemblance with the recommended height of BJRI (2008).

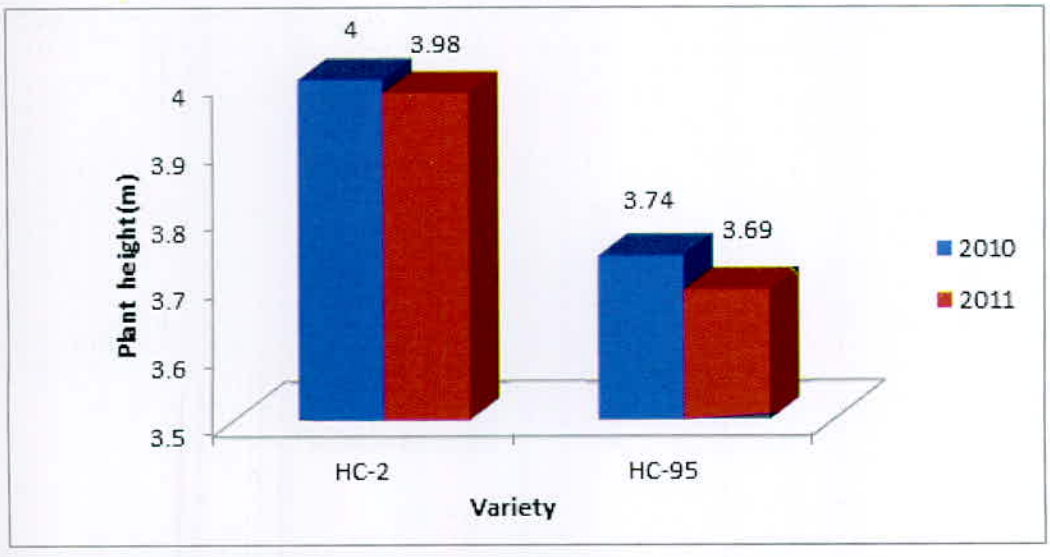


Figure 2: Plant height of two kenaf varieties in two different years

Stem base diameter

Stem base diameter of HC-2 was higher than HC-95 in both the year where the diameter of HC-95 was 27.66 cm and 29.60 cm for HC-2 in 2010. However, in 2011 it was found that the stem base diameter of HC-95 was 26.76 cm and 28.78 cm for HC-2.

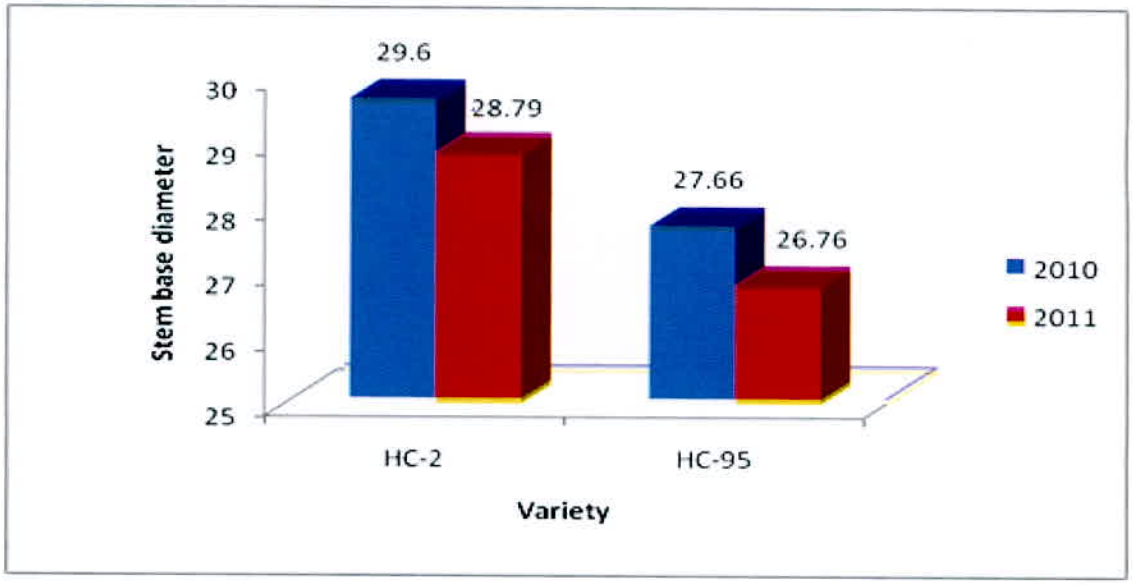


Figure 3: Stem base diameter of two kenaf varieties in two different years

Fiber yield

In both the year HC-95 yielded more fiber than HC-2. In 2010 the fiber yield of HC-95 was (4.07 tha^{-1}) and (3.81 tha^{-1}) was in 2011 where the yield of HC-2 was (3.265 tha^{-1}) in 2010 and (3.05 tha^{-1}) in 2011. BJRI (2008) recommended that the yield potential of HC-2 variety was greater than HC-95 but the present showed that the fiber yield of HC-95 is higher than HC-2.

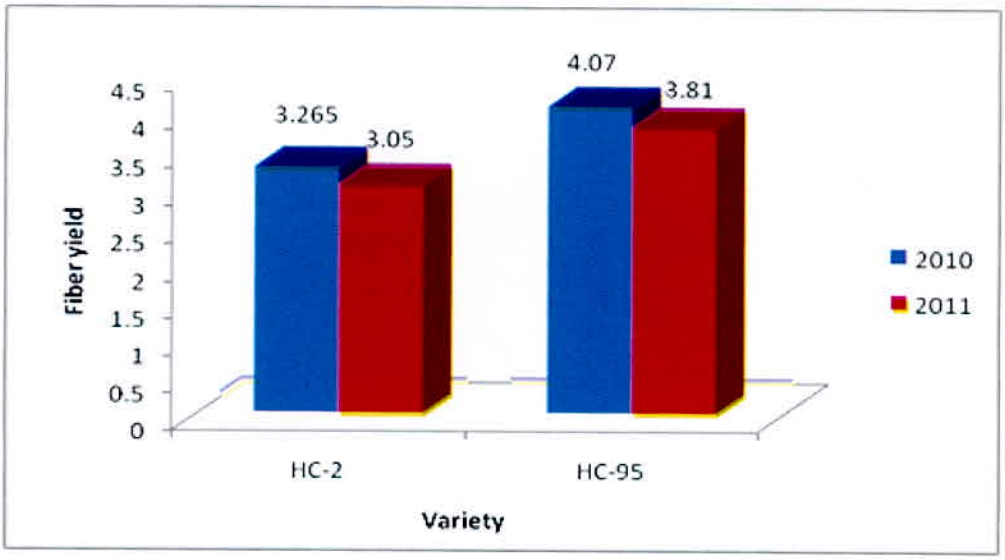


Figure 4: Fiber yield of two kenaf varieties in two different years

Stalk yield:

Stalk yield in two consecutive year showed that HC-2 variety produced higher stalk yield than HC-95. In 2010 the stalk yield of HC-2 was 11.251tha^{-1} and it was 10.09 tha^{-1} in 2011whereas the yield of HC-95 in these years was 10.131 and 9.98 t ha^{-1}.

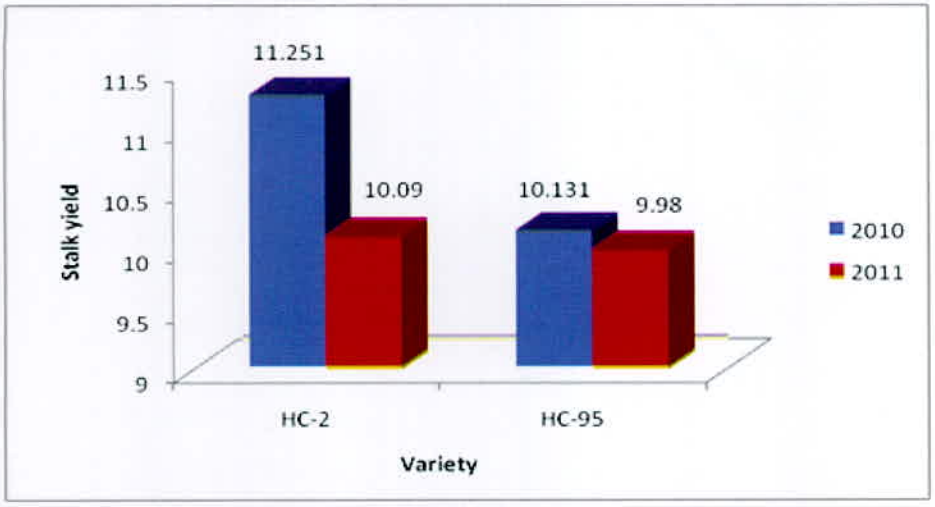


Figure 5: Stalk yield of two kenaf varieties in two different years

Experiment 2. Varietal performance in seed production of kenaf

Experimental site: Field laboratory of Agrotechnology Discipline

Duration: October 15, 2010 to May 20, 2011

Table 1. Varietal performance in seed production

Variety	Seed yield (tha^{-1})	Biological Yield (tha^{-1})
HC-2	0.182	1.69
HC-95	0.188	1.75

In 2010 the variety HC-95 produced the highest seed yield and biological yield in comparison to the variety HC-95. Yet, the yield was very low due to heavy shower of rain at the flowering stage and stagnant water hampered the growth of crops and ultimately yield was decreased (Table 1).

Experiment 3: pulp and paper making

In present study, when the bast and core fiber was chemically pulped separately, 39.06% yield of bast fiber and 37.80% yield of core fiber were achieved, whereas 57% and 41% pulp yield from bast and core fiber respectively was reported by other researchers. Low pulp yield might be lack of modern apparatus. Quality of paper sheets made from bast and core fiber pulps were tested in science laboratory, Dhaka and bast fiber paper was proved best in every quality parameter except brightness.

Experiment 4: oil extraction

Kenaf seed yield edible oil that is used for first class cooking oil and margarine production. In Bangladesh there are no reports on extraction of kenaf seed oil. We have attempted to extract seed oil in traditional oil extracting mill. On an average 7% oil could be extracted from both of the varieties of kenaf (HC-2 and HC-95) although it has been reported that kenaf seeds contain 21 % oil.

Conclusion

The results of the experiments showed that kenaf plant is highly salt tolerant andthe fiber yield potential of kenaf was satisfactory and the variety HC-95 performed better than the variety HC-2. So, the variety HC-95 may be included in the cropping patterns in coastal saline soils during dry season (March-August) for fiber purposes. Again, the seed production potential of kenaf was found lower in the first year of experiment due to unusual rainfall in the flowering stage but in the next year we got the satisfactory yield. But this crop is not suitable for cultivation as oil crop in Bangladesh due to its low oil content. Moreover, the plant is an excellent source of raw material for paper and pulp making.

Publication

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Establishing Medical Applications of Focused Impedance Measurement (Fim) - A Bangladeshi Innovation in Medical Physics

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Institution: Dept. of Biomedical Physics & Technology, University of Dhaka

Duration: Three years (2011 - 2014)

Expenditure of the project: Tk. 2200000.00

Introduction

The project was aimed at establishing various medical applications of a new method of electrical impedance developed by us, called, "Focused Impedance Method (FIM)".

Objectives

The objectives of the project were the following, using FIM:

(i) Development of single and multi-frequency equipment;

(ii) Development of a simple portable equipment that can be a screening tool for study and diagnosis of localized ventilation disorders of lungs;

(iii) Distinguishing FIM findings in pneumonia from other lung disorders;

(iv) Determination of abdominal fat thickness which is an indicator for risks of several diseases and disorders like heart disease, diabetes, gout, etc; and

(v) Development of special probes for detection of cervical cancer, a common disease of women in Bangladesh.

Methodology

i. Development of equipment: A dual frequency FIM system was designed and developed. A multi frequency electrical impedance equipment was purchased which was adapted for measurements of FIM.

ii. Localized lungs ventilation study: A probe was designed for measuring FIM at localized regions of the lungs. The electrode separation is important as this determines the depth sensitivity. Difference between measurements taken from healthy individuals and patients with lungs ventilation disorders were recorded and used to assess values to represent conditions of health and disorder.

iii. Diagnosis of Pneumonia: For this a dual frequency FIM system was designed and fabricated. A multi-frequency impedance measurement system was also procured for this purpose. However, actual measurements on patients could not be performed due to logistic problems and time limitations.

iv. Determination of abdominal fat thickness: Empirical modeling was performed in order to understand the physical system, which involved measurement using suitably designed phantoms, and to develop a practicable method for fat thickness measurement using dual electrode separation of FIM. Later data be collected from human subjects correlated well with measurements using clippers.

v. Detection of Cervical Cancer: A group in Sheffield, UK reported success of using electrical impedance measurement in the early diagnosis of cancer of cervix. However, this method needs several measurements by placing the probe at different places in order to ascertain that it has not missed any cancerous tissue. We plan to use FIM concepts in designing a multiple electrode probe that can localize the site of the lesion within a short time.

Results

Three modes of FIM have been innovated by us, using 8, 6 and 4 electrodes respectively. We have obtained numerical analysis to verify the focusing effect (Islam *et al.* 2010, Saha *et. al* 2013, Abir and Rabbani 2014).

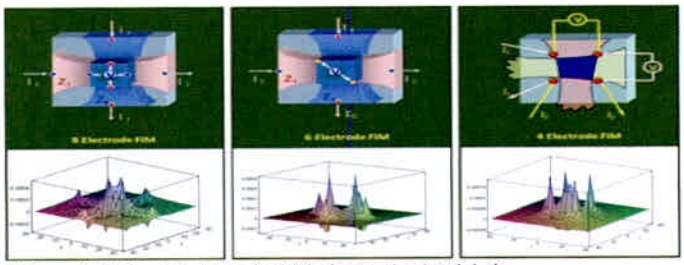

Figure 1: 8, 6 and 4 electrode FIMschemes (top)and their respective sensitivities (bottom)

i. Developing/procuring dual and multifrequency FIM equipment

Two devices - a dual frequency FIM and a multi-frequency FIM equipment with manual switching for frequency have been designed and fabricated by us (the latter with the help of Warwick University, UK) Aktharuzzaman *et al.* 2011. A commercial multi-frequency impedance measuring device (Maltron Bioscan II) has also been procured.

ii. Study on Lungs Ventilation and Respiration using FIM

TTo use FIM for breathing and respiration, we had developed a spring loaded electrode probe and carried out several studies (Kadir *et al.* 2010, Ferdous 2013). To measure respiration rate in babies without them crying (since crying changes respiration rate) we have designed a special flexible rubber pad with all the electrodes in it which a mother will wear in her palm and then place her hand on the thorax of the baby (Figure-2). We already taken data from several babies and children and none of them cried.

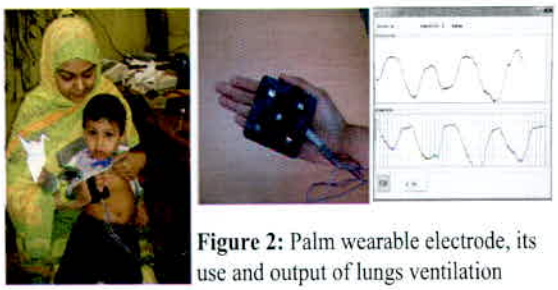

Figure 2: Palm wearable electrode, its use and output of lungs ventilation

iii. Abdominal Fat Thickness Measurement (under the skin)

We used an innovative dual electrode separation technique to achieve this objective (Haowlader *et al.* 2010, Al-Quaderi *et al.* 2014. Measurement on an innovative phantom designed by us provided us with a new technique to measure abdominal fat thickness (Figure-3). This technique was then used on live human subjects. The results based on FIM correlated well with the measured fat thicknesses using calipers on several subjects (Figure-4) (Surovy *et. al.* 2012).

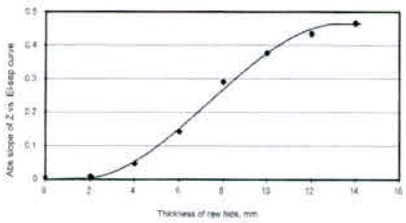

Figure 3: An impedance parameter plotted against different simulated fat thickness.

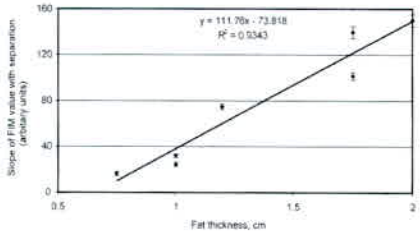

Figure 4 : Abdominal fat thickness measured using FIM against that using calipers.

iv. Breast Cancer Characterization

For this we made a 4 electrode FIM probe and used the commercial equipment to perform measurements on 20 breast tumour patients. Applying signal processing techniques we could get some distinction between patients with malignant tumours and those with benign tumours (Figure-5) (Al Amin *et al.* 2014). However, the technique needs further development.

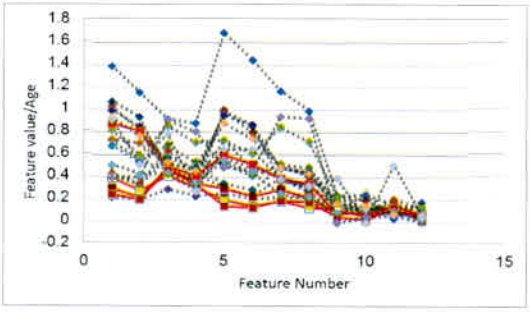

Figure 5: Feature plot of different electrical impedance parameters from breasts of patients. The red plots are for confirmed malignancy cases.

v. Cervical Cancer Detection

Cancer of the cervix of the uterus is a very common cancer of women in Bangladesh. Using a pencil like probe (Figure-6) a group in Sheffield University, UK has developed an electrical impedance based technique to detect the cancer early.

Figure 6: Sheffield group's probe for cervical cancer detection

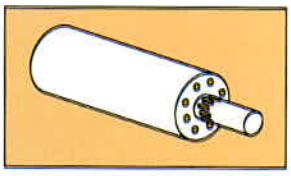

Figure 7: Proposed multi-electrode probe to cover the whole of the cervix

However, this needs multiple measurements which may be unacceptable to patients. We are planning to develop a multi-electrode probe system (Figure-7) which will allow measurement over the whole of the cervical surface simultaneously, reducing time and making it easy for the operator and the patient. We are carrying out numerical simulation studies first and also developing the hardware.

vi. Determination of Organ Volume

We also carried out work to determine the volume of an organ inside the body using simulation and phantom experiment (Iquebal and Rabbani 2013, Ahmed *et al.* 2014).

Conclusion

The effort increased the understanding of FIM in relation to different abnormalities of the human body and the way to detect and diagnose these disorders. This has also increased the experience and expertise of our group in translating these results to devices that can be used by the people all over the world for improved healthcare.

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Polymerase Chain Reaction (Pcr), a Genome Based Diagnostic Assay and Immuno-histopathology of White Spot Syndrome Virus (Wssv) - Strong Weapon for the Sustainable Shrimp Production in Bangladesh

Ghausiatur Reza Banu

Institution: Fisheries and Marine Resource Technology, Khulna University, Khulna
Duration: Three years (2011 - 2014)
Expenditure of the project: Tk. 1200000.00

Introduction

The black tiger shrimp known as Bagda (*Penaeusmonodon*) has emerged as one of the most important aquatic resources in Bangladesh. Attracted by the high demand in the both domestic and international markets, subsequently it played a major role in our national economy. Shrimp has been cultured broadly in the southern part of Bangladesh, especially in the districts of Khulna, Satkhira and Bagerhat, Chittagong, Noakhali, Barisal, Bhola, Pirojpur, Patuakali (Karim, 1986). Southeast Asia is an area of great significance in world shrimp culture. However, in recent years, the production of cultured shrimp has markedly decreased as the result of serious viral diseases. Disease is one of the major limiting factors caused by various pathogens, which lead serious loss in the sustainable production in our fisheries sector. Among the pathogens, White Spot Syndrome Virus (WSSV), rod-shaped double-stranded DNA virus a devastating, highly virulent pathogen that can cause high mortality rates of 100% in black tiger shrimp (*Penaeus monodon*) throughout the world. Lo *et. al.* (1998). Till date no substantial research work has been conducted in this regard in Bangladesh. Realizing this point an intensive investigation was planned to detect the pathogen WSSV using viral genome based detection methods (PCR, RT-PCR, Nested PCR).

Objectives

1. Detection of White Spot Syndrome Virus (WSSV) by a technique of Polymerase Chain Reaction (PCR); and
2. To investigate the tissue tropism of the virus.

Methodology

I. Detection of White Spot Syndrome Virus (WSSV)

To select the area for sampling an intensive field work had been performed in all the Thanas covering the entire Khulna and Bagerhat districts. Based on the intensity of the shrimp culture and vulnerability of the disease infection as well, three thanas of Batiaghata, Dumuria, Paikgacha and Ramapal, Mongla, Morrelgonj from Khulna and Bagerhat district had been selected respectively. After that a group of shrimp (*Panaeusmonodon*) samples were collected randomly from each of the gher and organ samples were aseptically prepared for DNA extraction to detect the virus of WSSV. For DNA extraction DNAzol reagent (Invitrogen) was used, after extraction, DNA pellet was re-suspended in 80 µl of TE-RNAse (10mM Tris, 1mM EDTA). PCR reactions were carried out in 50ul of reaction mixture that consist of 10 mMdNTP, 1.5 mM $MgCl_2$, 1 mM each primer, 0.02 U Taq DNA polymerase (Invitrogen) in a PCR buffer. Diagnostic PCR for WSSV was carried out using the primers of Lo 1-2 (1447bp), 146 R2-F2 (941bp) and Lo3-4 (298bp)Lo et. al.(1996). The PCR wasperformed in a thermocycler for 35 cycles. Each cycle consists of three steps of denaturation, annealing and elongation, and final extension for 10 min.

II. To know about the Tissue Tropism

A group (80) of specific pathogen of WSSV free healthy juvenile of *P. monodon*(12 – 15 gm of BW), were collected from farm located in Batiaghatathana, at Khulna district. The tiger shrimp *P. monodon* were kept in 1.5ft x1ft x1ft size aquarium tank at room temperature (28-30°C) with salinity between 5 and 6 ppt. Then the experimental shrimps were screened for white spot virus using nested-PCR. After screening, a total of 80 healthy shrimps (SPF) were used in the experiment. Virus (0.1 ml of inoculum) was injected intramuscularly into the second abdominal segment of the experimental shrimp. A mock infected control groupkept inanother aquarium.Sampling was done in a subsequent time intervals of 6hr, 12hr, 18hr, 24hr (day1), 36h, 48hr (day2), 72hr (day3) and daily upto 7 days. Each time of sampling, 3 individual shrimps were sacrificed to collect the organ of gill, eyestalk, abdominal muscle, tail muscle, pleopod and heamolymph and tissue tropism was investigated using PCR test.

Results

From our result it can be seen that the virus was found to be present in the apparently healthy shrimp by powerful techniqueof PCR. The presence of WSSV in cultured shrimps was quite high in both Khulna andBagerhat district which was 69% and 73% respectively by the one step PCR reaction using different primers of Lo 1-2 (1447bp), 146 R2-F2 (941bp) and Lo3-4(298bp).

A total of fifteen ghers were sampled from the Khulna district and 45 samples were analyzed for WSSV and the result showed that the maximum intensity was observed with band of 298 bp followed by the amplified product of 941bp. In our results we detected WSSV from 31out of 45 samples examined. Prevalence of white spot syndrome disease (WSD)was in Dumuria showed the highest prevalence of 95%, followed by Paikgacha (93%) and Batiaghata (28%).

From our results, it was also seen that the presence of WSSV in cultured shrimps in Bagerhat district was quite high (73%) in the one step PCR reaction using different primers where the virus was detected from 33 samples out of 45 samples.Prevalence of white spot syndrome disease (WSD) was in Mongla showed the highest prevalence of 80%, followed by Morrelgong (73%) and Rampal (66%).Out of 33 positive samples only 4 samples reacted positive with all primers of 1447, 941 and 298bp indicating severe infection and the rate was 10% and another 29 samples reacted in a distinguish manner.

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 primersare +ve then moderate infection and if all three band are produced then high infection occurred.

Based on the total number of shrimp examined for PCR test positive, the prevalence of WSSV infection was found to be avg. of 71% (64/90) and area wise prevalence of infection was more than 75% prevalence of infection was found in Dumuria (95%) and Paikgacha (93%) of Khulna and Mongla (80%) thana of Bagerhat district (vide Table).

Table 1: Prevalence of infection of WSSV in different thanas under Khulna and Bagerhat district

Khulna District (69%) (n=45)		**Bagerhat District (73%) (n=45)**	
Name of the Thana	**Prevalence of Infection**	**Name of the Thana**	**Prevalence of Infection**
Dumuria (14/15)	95%	Mongla (12/15)	80%
Paikgacha (13/15)	93%	Morrelgong (11/15)	73%
Batiaghata(04/15)	28%	Rampal (10/15)	66%

To know the tissue tropism, a total of 30 samples were taken to observe whether WSSV can replicate in all the organ samples or not of shrimp body. The results revealed that none of the sample was showed positive result at one step PCR test using the primer of 1447bp. The samples were then subjected to 2 step PCR using 1µl of one step amplified product as a DNA template and the results of PCR analysis showed that the presence of WSSV in all the organs of gill, hemolymph, abdominal muscle, pleopods and the tail muscle were found.

From the result, it can be seen that WSSV in the injected shrimp was particularly prevalent in the gills (at 1d), followed in order of decreasing prevalence by hemolymph, abdominal muscle, pleopods, and the tail muscle respectively. During the experimental period, WSSV prevalence was highest in gill (60%) then at hymolymph (57%) where aspleopod showed lower magnitude of prevalence of 46% (vide Figure).

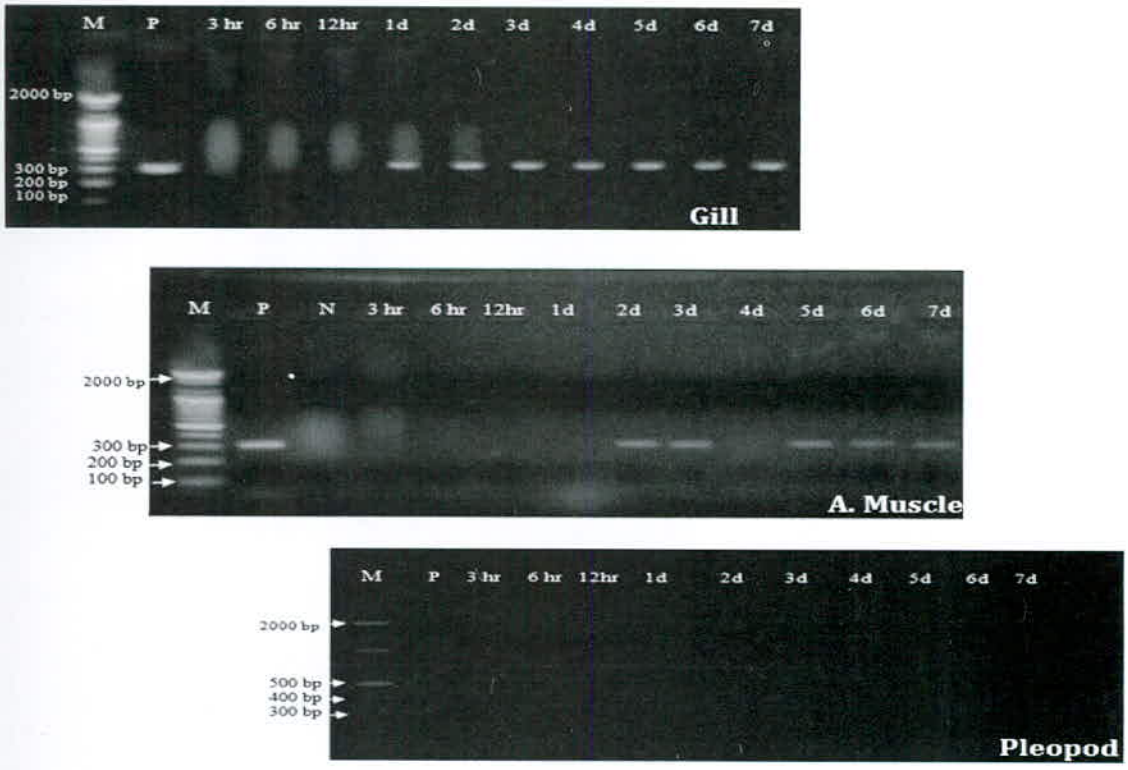

Figure 1: WSSV tissue tropism of intra-muscularly infected P.monodon as revealed by 2- step WSSV diagnostic PCR

Though virus was injected at tail region however, tail muscle showed positive result at very high prevalence rate (100%) up to 12hpi and a significant decline was observed which implies that the injected virus are not capable of infecting all types of cells or organs. This result also suggesting that tail muscle is not suitable for early screening for WSSV infection.According to the study it was found that no clinical disorder was observed before 48 hpi., indicating that the WSSV infection could be occurred before the manifestation of any clinical sings of disease. In mock infected control groups, the animals were normal and healthy, and no mortality was observed after 6 hpi.

From our tissue tropism result, as virus was first detected in the gill and hemolymph (non-injected organ). It might suggested that the injected virus was transported to gill (within 24h) through heamolymph indicating systemic infection and gill is the possible route of entrance for this viral infection as well.

Conclusion

From the result it is clearly found that 71% of the collected samples became virus carrier and it was prominent that Dumuria andPaikgacha from Khulna and Monglathana from Bagerhat district were more flooded (more than 75%) by WSSV rather than other thanas of Batiaghata as well as Rampal respectively. By the tissue tropism experimental result, it might be concluded that the injected virus can be transported through haemolymph and gill was the possible route of entrance for this viral infection.The present observations on the sequential study in the tissues of the gill revealed that these tissues could be used for an early diagnosis of WSSV infection. In the present in vivostudy it was found that about 10% shrimp died within 48 hours after infection. Farmer could not find any clinical sign of the disease within 48 hours after outbreak of the disease. If the sign of disease will be found very early after infection then the treatment will be started as early as possible. It decreases the loss of the farm.At present, the main goal of the shrimp industry is to meet the growing demand in a sustainable manner without damaging the environment. So, further studies on the development of environmental friendly treatment for white spot disease are severely needed.

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Survival Modeling and its Applications to Data Emerging from Medical and other life sciences

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Institution: Chittagong University, Chittagong, Bangladesh
Duration: Three years (2011 - 2014)
Expenditure of the project: Tk. 550000.00

Introduction

The phenomena in life sciences are dynamic and time dependent. In survival analysis two important topics are getting attention. First to describe the process of the development of a disease and second to identify the risk and prognostic factor of the disease. Markov chain is one of the appropriate process for time dependent longitudinal study study. The longitudinal surveillance and recording of these events is therefore a natural mode of study to obtain a complete picture of disease causation. The essence of longitudinal studies in epidemiology is the identification of a group of individuals who are followed forward over a specified period of time, to ascertain the occurrence of an event or condition of disease of interest at different time points. The information about them is then gathered at different points and categorized accordingly and thus we get repeated ordinal measurements. Longitudinal data sets therefore, comprise of repeated observations of a disease and a set of risk factors for each subject of a certain population. So the statistical analysis of these types of data presents special opportunities. Because of the richness and flexibility in the analysis of many types of data, longitudinal studies are becoming popular day by day and prominently being undertaken in the Health Sciences. Increased power and robustness to model selection are the two most important advantages of longitudinal studies.

Objectives

The objectives of the project is to model the data with Markov chain and estimate the parameter by classical and Bayesian approaches and compared with the confidence and credible interval and posterior risk.

Methodology

A repeated measures data on Maternal Morbidity in Bangladesh is used. The survey was conducted from November 1992 to December 1993 by the Bangladesh Institute for Research for Promotion of Essential and Reproductive Health and Technologies (BIRPERHT). The subjects comprised of pregnant women with less than 6 months of duration and were followed on regular basis (roughly at an interval of 1 month) throughout the pregnancy. The data of first four consecutive antenatal visits is considered from 548 such women's information for the analysis. Women are considered as morbid if in the pregnancy period at least one of the antenatal complications such as, hemorrhage, edema, excessive vomiting, headache, pelvic pain, anxiety and weakness, cough or fever more than three days, burning maturation, fits and convulsion are identified. The explanatory variables considered are age at marriage, economic status and any miscarriage. The dependent variable is categorized as, no complicacies and having complicacies. One of the appropriate procedure for analyzing repeated observations is by Markov Chain Modeling. This study focuses on a logistic marginal model with Markovian dependence proposed by Azzalini (1994) and Rubinstein(1985). The parameters of the model were estimated by both classical and Bayesian approaches.

Assume that each individual is observed at T occasions. Then the binary random variable

$$Y_t = \begin{cases} 1, \text{if the ith individual succeed at time t} \\ 0, \text{if the ith individual failed at time t.} \end{cases}$$

To obtain the estimates for the regression of yt on xt using binary Markov chain, a first order two state Markov model represented by, where, Pr (Yit|Yij¬; j>t) = Pr (Yit|Yit-1).

The associated covariates xt = (x1, x2, ... xr) are recorded for each subject at each occasions. Transition count from state '0' to '0' is 1338 , transition count from state '0' to '1' is 250, transition count from state '1' to '0' is 250 and transition count from state '1' to '1' is 354.

Results

This study modeled the pregnancy data with Markov chain where transition probability are modeled with logistic regression. Two approaches of the Markov chain with covariate dependence are employed for modeling and comparison purpose. Bayesian and Classical approaches are applied for estimating the parameter of that model. In Bayesian approach, different types of loss function were used namely squared error loss function, LINEX loss function and MLINEX loss function. The outcomes of the analyzed data are presented in the Tables below.

For State 0 to 0 transition

Table 1a: Confidence interval for maximum likelihood estimators for Muenz's model

Covariates	Point Estimate	Odds ratio	Interval Estimate (95%)		
			Lower	Upper	Length
Constant	1.7752	--	1.4532	2.0972	0.6439
Any miscarriage	0.1603	1.1739	0.0471	0.2736	0.2265
Economic Status	-0.3065	0.7360	-0.4587	-0.1542	0.3045
Age at Marriage	0.0244	1.0247	-0.0977	0.1464	0.2441

Table 1b : Credible interval for Bayesian estimator under squared error loss function for Muenz's model

Covariates	Point Estimate	Odds ratio	Credible Interval (95%)		
			Lower	Upper	Length
Constant	1.7264	--	1.4176	2.0353	0.6177
Any miscarriage	0.1606	1.1742	0.1319	0.1894	0.0575
Economic Status	-0.3075	0.7353	-0.3624	-0.2525	0.1100
Age at Marriage	0.0448	1.0458	0.0368	0.0528	0.0160

For State 1 to 0 transition

Table 2a: Confidence interval for maximum likelihood estimators for Muenz's model

Covariates	Point Estimate	Odds ratio	Interval Estimate (95%)		
			Lower	Upper	Length
Constant	-0.7020	--	-1.2956	-0.1083	1.1872
Any miscarriage	0.2854	1.3303	0.0603	0.5104	0.4501
Economic Status	0.2354	1.2654	-0.0339	0.5047	0.5387
Age at Marriage	-0.1635	0.8492	-0.3966	0.0696	0.4662

Table 2b: Credible interval for Bayesian estimator under squared error loss function for Muenz's model

Covariates	Point Estimate	Odds ratio	Credible Interval (95%)		
			Lower	Upper	Length
Constant	-0.7257	--	-1.0468	-0.4045	0.6423
Any miscarriage	0.3033	1.3543	0.1691	0.4375	0.2684
Economic Status	0.2717	1.3122	0.1515	0.3919	0.2405
Age at Marriage	-0.1787	0.8364	-0.2577	-0.0996	0.1581

Table 3: Confidence interval for Maximum Likelihood Estimate based on Azzalini's model

Covariates	Point Estimate	Interval Estimate (95%)		
		Lower	Upper	Length
Constant	0.09938452	0.08689621	0.11187283	0.02497662
Any miscarriage	0.10014495	0.09726278	0.10302712	0.00576434
Economic Status	0.10027485	0.09187339	0.10867631	0.01680292
Age at Marriage	0.10003594	0.09733139	0.10274049	0.00540909
λ	0.09999810	0.09958594	0.10041026	0.00082431

Table 4: Credible interval for Bayes estimator function based on Azzalini's model

Covariates	Point Estimate	Credible Interval (95%)		
		Lower	Upper	Length
Constant	0.09933129	0.0981074	0.1005552	0.0024479
Any miscarriage	0.09999754	0.0987654	0.1012297	0.0024643
Economic Status	0.10008238	0.0988492	0.1013156	0.0024664
Age at Marriage	0.09988351	0.0986528	0.1011142	0.0024615
λ	0.09985544	0.0986251	0.1010858	0.0024608

Table 5: Comparison of the two models under Bayesian framework

Covariates	Posterior risk			Minimum Risk	
	Azzalini	Muenz-Rubinstein		For 0 to 0	For 1 to 0
		For 0 to 0	For 1 to 0		
Any miscarriage	3.9519E-07	0.000215	0.004690	Azzalini	Azzalini
Economic Status	3.9586E-07	0.000787	0.003763	Azzalini	Azzalini
Age at Marriage	3.9429E-07	0.000017	0.001627	Azzalini	Azzalini

The study observed that the performance of Bayesian estimate under LINEX and MLINEX loss function for different values of C (not shown in the table). We have seen that, posterior risk under squared error loss function has smaller than LINEX and MLINEX loss function and also LINEX loss function are better than MLINEX loss function except for C=1. This study also observed that Bayesian approach under squared error loss function give better result than under LINEX and MLINEX loss functions. This study then compared Bayesian approach under squared error loss function with the classical maximum likelihood method with their respective interval estimation and found that smaller length of Bayesian credible interval than maximum likelihood confidence interval for all covariates in two types of transitions.

Finally we got the Baysian estimate under squared error is the best of all other approach. Again when we compared between two type of model, Azzalini's model is found more preferable than Muenz and Rubinstein's model by T.K. approximation than by Lindley's approximation. Non classical Bayesian inference involves complex integration which we solved by using R-Programming Language and we established that Non classical Bayesian estimate is preferable estimate than Classical maximum likelihood estimate. Also, pregnancy related complications are very common in rural areas of Bangladesh. Health status although improved a lot but still are not up to the mark in rural Bangladesh which is responsible for causing pregnancy complications.

Publication

Mahanta J, Biswas SC, Roy MK, M. and Islam A. 2015. A Comparison of Bayesian and Classical Approach for Estimating Markov Based Logistic Model. *American Journal of Mathematics and Statistics,* 5(4): 178-183.

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Streptococcus pneumoniae, a Pathogen of Childhood Pneumonia: its Serotyping and Antimicrobial Resistance

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Institution: Department of Microbiology, Jagannath University
Duration: Three years (2011-2014)
Expenditure of the project: Tk. 1000000.00

Introduction

Streptococcus pneumoniae (*Pneumococcus*) is one of the most common bacterial pathogens in humans. It causes severe, life-threatening infections such as pneumonia and meningitis, and is often associated with bacteremia that also occurs without these conditions, especially in children. *Streptococcus pneumoniae* has 90 serotypes of which only a few contribute to disease formation. The pneumococcal infection in children results in 1 million annual deaths worldwide most of them are in under developed countries. *S. pneumoniae* a childhood pathogen exists as part of normal nasopharyngeal flora and nasopharyngeal carriage is considered as the main reservoir of pneumococcus. Acquisition of nasopharyngeal carriage is the first step toward pneumococcal disease. A child typically encounters several different serotypes during the first years of life and with increasing age pneumococcal carriage decreases with a change of colonizing serotypes as seen in adults . The incidence of pneumococcal diseases in different regions of the world is not caused by some specific serotypes/serogroups rather they are randomly distributed in population.

To understand the epidemiology and pathogenesis of pneumococcal diseases, it is necessary to understand the ecology and epidemiology of pneumococcal carriage as the reservoir of the infection. Since the burden of serious pneumococcal diseases is heaviest in the developing world, such studies are particularly needed . Serotyping is the most important method for characterizing pneumococcal isolates.

The recent introduction of vaccine that reduces not only pneumococcal disease but also carriage in a serotype-specific way has created a need for serotyping in rational planning and surveillance of vaccination programs. Serotyping is usually performed through modified of the quellung method for serotyping of pneumococci. Appropriate vaccination and treatment with antibiotic therapy render the reduction ofmorbidity and mortality due to pneumococcal infections, but the success depends on the serotypes distributed in the community. Different serotypes often have different virulence and carriage properties. and it focuses on the need of serotype study of pneumococci. The antipneumococcal conjugate vaccines are in use and are in trials have different coverage of the disease causing invasive serotypes of Streptococcus pneumonia. Frequency of pneumococcal carriage is highest in developing countries including Bangladeshi families.

Efficacy of these vaccines depends on the distribution and stability of the disease causing pneumococcal serotypes in the population. Recurrence of invasive serotypes in population focuses the importance of isolation, identification and serotyping. Study of serotype stability in population is important factor for vaccine efficacy trial. Antimicrobial resistance acquired by bacterial strain causes treatment failure with conventional antibiotics that emphasizes on the need of study on the sensitivity pattern of the S. pneumoniae strains. In Bangladesh, in case of serious and often fatal infections due to pneumococcal disease, instead of taking enough primary care, most of the patients are treated at the tertiary stage in hospitals leading the increased rate of death. A limited number of hospital based reports and a few community based study in Bangladesh focuses on the need of extensive study of pneumococcal serotypes in large number of population to know the existing serotypes for selecting appropriate type of pneumococcal vaccines. And also to facilitate the treatment, the antibiotic sensitivity pattern of the pneumococcal isolates in crucially important. Study concerning isolation of the pathogen from human population and identifying their serotypes indicates the justified use of vaccines (already developed and under trial) in our population. So, the concern of the present study was to emphasize on the basic important study (isolation, identification and serotyping of the isolates) followed by antibiotic sensitivity testing needed for both prevention and treatment of pneumonia.

Objectives

1. Strengthening the microbiology laboratory for health research especially for pneumococci;
2. Laboratory investigation for isolation and identification of the pneumococcal strains in the samples collected from the subjects suspected for pneumococcal infection;
3. Serotyping of the isolates to know the serotypical distribution of *S. pneumoniae* strains in population; and
4. Promoting research and practical training on applied medical microbiology for the students.

Methodology

The study was conducted by the project investigator of the Department of Microbiology, Jagannath University for the stipulated time period (2009-2012) of the research project. The activities were performed in several steps (i) sample collection: nasopharyngeal secretion as sample was collected according to the procedures followed in SOP, using calcium-alginate swab and skim milk-tryptone-glucose-glycerol (STGG) medium in screw cap tube in cold box (the collected samples were transported to laboratory within six hours of sampling using insulated box containing cold chargers), (ii) culturing of samples on 5% sheep blood agar with gentamicin (5 ug/ml) at 36°C for up to 48 hrs incubation, (iii) observation of characteristic colonial growth for Streptococcus pneumoniae and sub-culturing the selected desired colonies for obtaining pure-culture, (iv) identification of the cultured organism Streptococcus pneumoniae, was confirmed by alpha-haemolytic colony and optochin resistivity, (v) serotyping of the isolates was performed using chessboard modification of quelling method with two sets of antisera pools (A-I and P-T) and omniserum (Staten Seruminstitut, Copenhagen, Denmark) . Penicillin sensitivity was checked by using oxacillin biodisk (1 µg) based on standard

microbiological methods described in NCCLS and CLSI guideline. Fresh pure-culture of isolated pathogen was used for sensitivity testing according to Kirby-Bauer method.

Results

A total number of 412 nasopharyngeal swab samples were cultured according to SOP and the isolation of pneumococci (PNC) were done. The positive isolates were characteristically identified as *Streptococcus pneumoniae.*

The growth positive isolates with alpha-haemolytic colony on gentamicin blood agar and optochin resistivity confirmed the isolates as pneumococci. The tests for quelling reaction with different antisera for the isolates resulted in specific serotypes of Streptococcus pneumoniae. From a total of 412 cultured samples, positive pneumococcal growth was found for 102 samples (24.76%) (Table-1). The distribution of the serotypes among different age groups (Table-2) shows that the age groups below 15 years and over 60 years are more susceptible to streptococcal infections. The serotypes found in the study subjects were 20, 33, 6, 19, 23, 15, 11, 10, 14 among which the predominating ones were 20 (17.6%, n=18), 33 (16.7%, n=17), 6 (15.7%, n=16), 19 (14.7%, n=15), and 23 (10.8%, n=11) (Table-3). The isolates of higher frequencies are under the coverage of pneumococcal vaccines under trail but it does not reflect the sufficient argument for vaccine use rather it emphasizes on the extensive study with larger population size of the community and hospitals. As the findings reflect the presence of variability of different serotypes in samples, it importantly emphasizes the selection of serotypes to be functional for using in vaccine preparation applied to our population.

Table 1: Pneumococcal samples distribution in different sexes:

	Total Sample	Source (Male)	Source (Female)
Total collected	412	218 (52.9%)	194 (47.1%)
Positive	102	58 (56.9%)	44 (43.1%)

Table 2: Distribution of positive pneumococcal cases in different age groups:

Age group (Years)	Male Number (%)	Female Number (%)
0 - 15	22 (37.9%)	15 (34.1%)
16 - 30	12 (20.7%)	8 (18.2%)
31 - 45	4 (6.9%)	4 (9.1%)
46 - 60	8 (13.8%)	6 (13.6%)
>60	12 (20.7%)	11 (2 5.0%)
Total	58 (100%)	44 (100%)

Table 3: Different serotypes of pneumococcal isolated:

Serotype	Number (Frequency)	Serotype	Number (Frequency)
20	18 (17.6%)	15	7 (6.9%)
33	17 (16.7%)	11	8 (7.8%)
6	16 (15.7%)	10	7 (6.9%)
19	15 (14.7%)	14	3 (2.9%)
23	11 (10.8%)	Total	58 (100%)

Conclusion

The research project undertaken reveals the number and types of Streptococcus pneumoniae strains and their serotypical distribution pattern in the population investigated. The findings are helpful to indicate most vulnerable subjects in population and the effectiveness of vaccines by coverage of different serotypes causing the disease. But to have a conclusive remark, it is needed to conduct the study considering a higher number of populations of different groups. The research findings bear positive impact on health care and create concerns for selecting prevention and treatment measures.

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Trace Element Level in Biological Sample of Healthy Subjects and Patients with Chronic Kidney Disease

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Duration: Three years (2011-2014)

Expenditure of the project: Tk. 2500000.00

Introduction

Metal ions are essential to maintain biological activities. Kidneys normally balance the tissue levels of metallic ions. In chronic kidney disease (CKD) different metals either increases like aluminum and magnessium (Al, Mg) in toxic level or decrease like iron, zinc and selenium (Fe, Zn, Se) to subnormal level due to impaired renal function, dietary restrictions, various prescribed drugs and dialysis therapy.

Objectives

- To measure the selected metal Mg, Zn and Fe levels in blood of healthy subjects;
- To observe the variations of these selected trace metal levels in blood among chronic kidney disease patients; and
- To see the association of these metal level with different appropriate clinical and biochemical parameters.

Methodology

Study type: Cross sectional study

Subjects: 351 study subjects were recruited from those attending BIRDEM and NIKDU outpatient department and dialysis units. They were divided as following groups-

1) Gr 1: 126 renal failure patients not requiring dialysis (predialysis) (DN)
2) Gr 2 : 80 diabetic patients without nephropathy (DM)
3) Gr 3 : 56 renal failure patients on maintenance hemodialysis (HD)
4) Gr 4: 89 healthy controls (diabetes, kidney disease free) (Cont)

Inclusion criteria: Predialysis chronic kidney disease patients with stable renal function for at least 3 months. The maintenance hemodialysis patients were those who were on dialysis through permanent access.

Exclusion criteria: Acute or chronic liver disease, heart failure, active infections, malignancy or grossly edematous.

Sampling:

Blood collection: blood samples were taken in the morning from the patients and controls after an overnight fasting and in dialysis group before dialysis session in the morning or afternoon.

Figure 1: Blood sample collection

Figure 2 : Sample processing

Urine collection: Urine samples were collected in thoroughly rinsed polyethylene containers. A 24 hrs urine samples or spot urine sample was collected as convenient.

Clinical evaluation: Medical history of both past and present illness was recorded. All data recorded in a pre-designed data collection sheet.

Anthropometric evaluation: Height, weight and body mass index (BMI) was measured.

Laboratory investigations: Complete blood count (CBC); Glycaemic status: fasting blood sugar (FBS), after breakfast (AFB); Renal function: urine R/M/E, urinary albumin, ACR, urinary total protein (UTP), creatinine (Cr) and creatinine clearance rate (CCR); Liver function: Albumin, aminotransferase (ALT); bone markers: Ca, PO_4, Alk Phos and inflammatory marker C reactive protein and ferritin.

Metal analysis: Meticulously steps are observed for preparing blood samples for measuring iron (Fe), zinc (Zn) and magnesium (Mg).

Specimen collection: tips, tubes and sample cups were cleaned by soaking into nitric acid solution (5%) for 24 hours followed by soaking into de-ionized water for another 24 hours.

Results

Total 351 subjects of four groups as predialysis diabetic CKD (DN) 36%; diabetics on dialysis (HD) 16%; diabetics without nephropathy (DM) 22% and healthy control (Cont) 26% were selected. The male female ratio was 54:46.

Table 1: Comparisons of Clinical and Laboratory Parameters

	Cont	DM	DN	HD	Significance
Age	44±12	56±9	57±7	53±12	0.000
BMI	24±3	26±4	25±2	25±4	0.001
Systolic BP	114±12	128±16	1130±12	148±23	0.001
Diastolic BP	86±11	86±11	86±11	78±71	0.001
S Creatinine	0.9±2.9	1.1±0.4	1.5±0.5	9.9±2.7	0.001
FBS	4.5±0.7	7.7±5.1	7.1±2.1	7.5±3.1	0.001

Clinical parameters like BMI, blood pressure; hemoglobin and creatinine were measured. The control group was lower in age. Other parameters like BMI, blood pressure; blood sugar and S Creatinine were altered most in dialysis group and DN than DM and controls as expected from the inclusion criteria (table1).

Serum Ca, PO_4, K and Fe was be measured by ion sensitive electrode; Zn and Mg by auto analyzer and crosschecked by flame AAS. SRM was used for quality control. Biochemical tests were done in lab of contractual concerns.

Comparisons of other ions between predialysis, dialysis and only diabetic groups showed serum potassium, and phosphate level were increased and calcium decreased more in dialysis group. The iron (Fe) level was highest in dialysis group (75±58, 83±58 and 125±29 mg/dl, $p<0.001$) when compared between DM, DN and HD groups.

Table 2: Comparisons of Zinc and Magnesium among groups

	Cont	DM	DN	HD	Significance
Zinc	190±97	186±88	152±77	227±85	0.000
Magnesium	2.2±0.6	2.3±0.6	1.9±2	2.4±0.4	0.001

Mg (normal value 1.5-2.4 mg/dl) and Zn (normal value 60-110 µg/dl) have been measured among cont, DM, DN and HD. Serum Zn (µg/dl) was 190±97, 186±88, 152±77 and 227±85, ($p<0.001$); and Mg (mg/dl) 2.2±0.6, 2.3±0.6, 1.9±2 and 2.4±0.4 ($p<0.001$) respectively in four groups. Results showed zinc level is higher in control and DM than the DN group and highest in HD. The magnesium value is highest in HD group and lowest in DN group (Table-2).

There was a positive correlation of creatinine with PO_4, Fe, Mg and negative with Ca. No apparent correlation between Zinc and Creatinine.

Conclusion

In conclusion diabetic chronic kidney disease patients have altered serum ion and mineral levels. The serum Fe level is sufficiently high resulting from probable therapeutic iron supplementation. Serum Mg and Zn levels are lowest in diabetic predialysis CKD patients. The optimum level of Mg and Zn in dialysis patients is attributable to dialysate composition. The relation of Fe, Mg and Zn with diabetes and altered renal function needs further exploration.

Publication

A paper submitted to the 15th Asian Pacific Conference of Nephrology (APCN) & 52nd Australia New Zealand Society

of Nephrology (ANZSN) Annual Scientific Meeting (ASM), 17 September to 21 September, 2016.

Flow Investigation Through a Curved Duct with Various Cross-sections

Rabindra Nath Mondal and Md Sharif Uddin

Institution: Mathematics Discipline, Khulna University, Khulna
Duration: Two Years (2011 - 2013)
Expenditure of the project: Tk. 400000.00

Introduction

Fluid flow through curved ducts and channels has been extensively used in many engineering applications, such as in turbo-machinery, refrigeration, heat exchangers, internal combustion engines and blade-to-blade passages in gas turbines. Dean first formulated the problem into mathematical terms for a curved channel flow. After that, lots of investigations have been made; here the article by Berger *et al.* may be referenced to.

Flow through a curved rectangular duct has been investigated, both experimentally and numerically, by many authors. Yana Se *et al.* investigated flow in a curved duct and classified the flow range into three different regimes; steady-stable, periodic and chaotic. Wang and Liu performed numerical as well as experimental investigations of periodic oscillations for the fully developed flow in a curved square duct. Recently, Mondal *et al.* investigated spectral numerical study for non-isothermal flow through a curved rectangular duct of aspect ratios 1 to 3, and showed that the steady-state flow turns into chaotic flow through various flow instabilities if the aspect ratio is increased. However, transient behavior as well as effects of aspect ratio on unsteady solutions is not yet resolved, in detail, for the non-isothermal flow and the present study fills up that gap. In this study, we investigate transitional behavior of the unsteady solutions for the non-isothermal flow through a curved rectangular duct of various aspect ratios by using the spectral method, and show an enhancement of convective heat transfer by secondary flows.

Objectives

The objective of the present study is to establish the precise solution that gives rise to the predicted flows in curved rectangular ducts. The flow in curved ducts shows similarities with a number of other centrifugally unstable systems. The present study, therefore, focuses on the time-dependent solutions of the flows through a curved rectangular duct for various aspect ratios. In this study, a spectral-based numerical result will be presented for the fully developed two-dimensional (2D) flow of viscous incompressible fluid. The main objective of the present study is to investigate pattern variation of secondary flows with transitional behavior of the unsteady solutions in detail.

Methodology

In this study, spectral method is used as a basic tool to solve the system of non-linear partial differential equations. Then Chebyshev polynomial and collocation methods are used. Finally, Crank-Nicolson and Adams-Bashforth methods together with the function expansion and the collocation methods are applied to obtain unsteady solutions.

Basic Equations and Numerical Methods: Consider a fully developed 2D flow through a curved rectangular duct. The x, y and z axes are taken to be in the horizontal, vertical, and axial directions respectively. The flow is uniform in the z-direction, which is driven by a constant pressure gradient as shown in Figure 1. The variables are made non-dimensional.

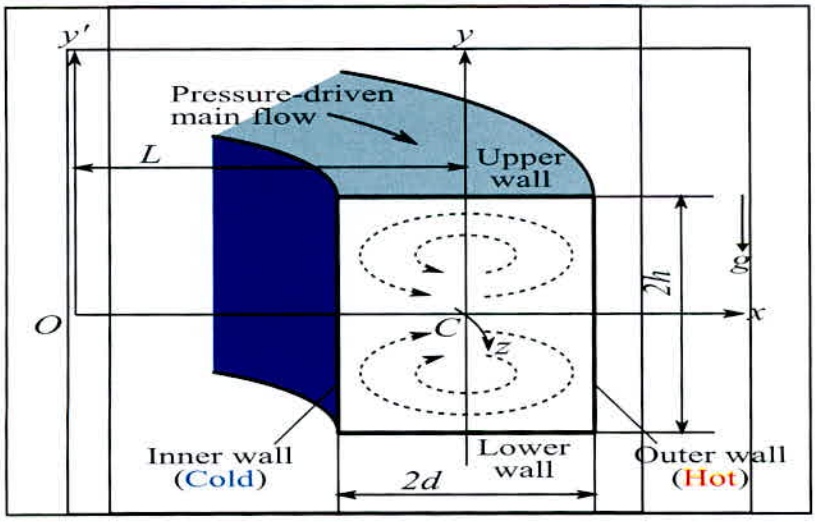

Figure 1: Coordinate system of the curved duct.

For uniform flow in the direction, the sectional stream function is introduced as

$$u = \frac{1}{1+\delta x}\frac{\partial \psi}{\partial y}, \quad v = -\frac{1}{1+\delta x}\frac{\partial \psi}{\partial x} \tag{1}$$

Then, the basic equations for w, ψ and T are expressed as

$$(1+\delta x)\frac{\partial w}{\partial t} + \frac{1}{l}\frac{\partial(w,\psi)}{\partial(x,y)} - Dn + \frac{\delta^2 w}{1+\delta x} = (1+\delta x)\Delta_2 w - \frac{\delta}{l(1+\delta x)}\frac{\partial \psi}{\partial y}w + \delta\frac{\partial w}{\partial x}, \tag{2}$$

$$\left(\Delta_2 - \frac{\delta}{1+\delta x}\frac{\partial}{\partial x}\right)\frac{\partial \psi}{\partial t} = -\frac{1}{l(1+\delta x)}\frac{\partial(\Delta_2\psi,\psi)}{\partial(x,y)} + + \frac{\delta}{l(1+\delta x)^2}\left[\frac{\partial \psi}{\partial y}\left(2\Delta_2\psi - \frac{3\delta}{1+\delta x}\frac{\partial \psi}{\partial x} + \frac{\partial^2 \psi}{\partial x^2}\right.\right.$$

$$\left.\left. + \frac{\partial^2 \psi}{\partial x^2}\right) - \frac{\partial \psi}{\partial x}\frac{\partial^2 \psi}{\partial x \partial y}\right] + \frac{\delta}{(1+\delta x)^2} \times \left[3\delta\frac{\partial^2 \psi}{\partial x^2} - \frac{3\delta^2}{1+\delta x}\frac{\partial \psi}{\partial x}\right] - \frac{2\delta}{1+\delta x}\frac{\partial}{\partial x}\Delta_2\psi \tag{3}$$

$$+\frac{1}{l}w\frac{\partial w}{\partial y}+\Delta_2{}^2\psi-G_r\,(1+\delta x)\frac{\partial T}{\partial x},$$

$$\frac{\partial T}{\partial t}+\frac{1}{(1+\delta x)}\frac{\partial(T,\psi)}{\partial(x,y)}=\frac{1}{\mathrm{Pr}}\left(\Delta_2 T+\frac{\delta}{1+\delta x}\frac{\partial T}{\partial x}\right). \tag{4}$$

Here, l is the aspect ratio defined as $l=h/d$, and Dn, Gr and Pr are defined as:

$$Dn=\frac{Gl^3}{\mu\upsilon}\sqrt{\frac{2l}{L}},\ Gr=\frac{\beta g\Delta T l^3}{\upsilon^2},\ \mathrm{Pr}=\frac{\upsilon}{\kappa}.$$

The rigid boundary conditions used here for w and ψ are

$$w(\pm1,y)=w(x,\pm1)=\psi(\pm1,y)=\psi(x,\pm1)=\frac{\partial\psi}{\partial x}(\pm1,y)=\frac{\partial\psi}{\partial y}(x,\pm1)=0 \tag{5}$$

and the temperature T is assumed to be constant on the walls as

$$T(1,y)=1,\ T(-1,y)=-1,T(x,\pm1)=x. \tag{6}$$

In order to solve Eqs. (2) - (4) numerically, the spectral method is used. By this method, the expansion functions $\Phi_n(x)$ and $\Psi_n(x)$ are expressed as

$$\Phi_n(x)=(1-x^2)C_n(x),\ \Psi_n(x)=(1-x^2)^2C_n(x), \tag{7}$$

and $w(x,y,z)$, $\psi(x,y,t)$ and $T(x,y,t)$ are expanded in terms of $\Phi_n(x)$ and $\Psi_n(x)$ as

$$\left.\begin{aligned}w(x,y,z)&=\sum_{m=0}^{M}\sum_{n=0}^{N}w_{mn}(t)\Phi_m(x)\Phi_n(y),\\ \psi(x,y,t)&=\sum_{m=0}^{M}\sum_{n=0}^{N}\psi_{mn}(t)\Psi_m(x)\Psi_n(y),\\ T(x,y,t)&=\sum_{m=0}^{M}\sum_{n=0}^{N}T_{mn}(t)\Phi_m(x)\Phi_n(y)+x,\end{aligned}\right\} \tag{8}$$

The resistant coefficient λ is used as the representative quantity of the flow state, and is used in fluids engineering, defined as

$$\frac{P_1^*-P_2^*}{\Delta z^*}=\frac{\lambda}{dh^*}\frac{1}{2}\rho\left\langle w^*\right\rangle^2 \tag{9}$$

λ is related to the mean non-dimensional axial velocity $\langle w\rangle$ as

$$\lambda=\frac{8l\sqrt{2\delta}Dn}{(1+l)\langle\omega\rangle^2} \tag{10}$$

Finally, to calculate the unsteady solutions, the Crank-Nicolson and Adams-Bashforth methods together with the function expansion (8) and the collocation methods are applied.

Results

Case I: Aspect Ratio 0.5

We investigated time evolution of λ for various Dn over a wide range of Gr for the aspect ratio 0.5. Figure 2(a) shows unsteady solutions for Dn = 10000 and Gr = 100, and we see that the unsteady flow is a steady-state solution. Typical contours of secondary flow patterns, temperature profiles and axial flow distribution are shown in Figure 2(b) for Dn = 10000 at Gr = 100, where we see that unsteady flow is an asymmetric two-vortex solution.

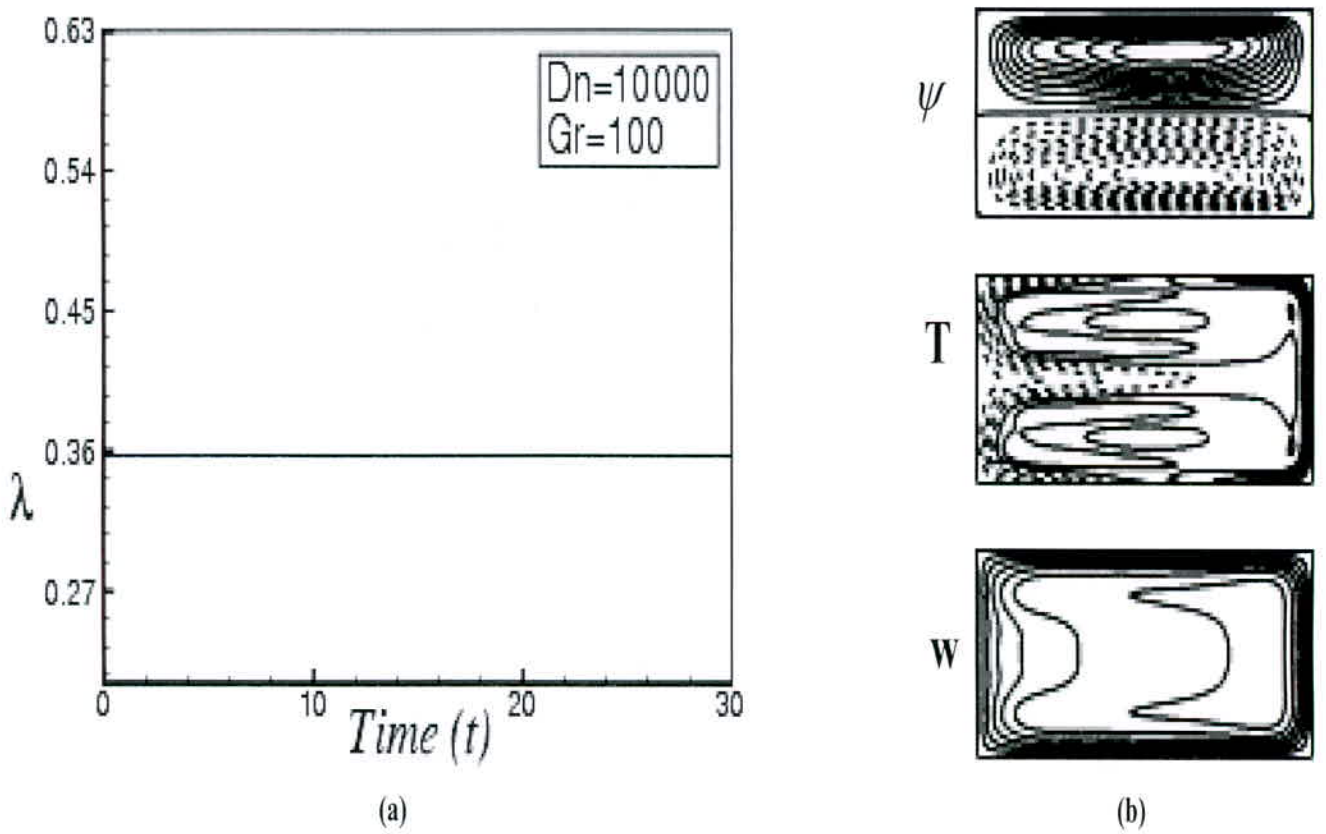

Figure 2: Results for Dn = 10000 and for the aspect ratio 0.5. (a). Time evolution, (b) Secondary flow (top), temperature profile (middle) and axial flow distribution (bottom).

Case II: Aspect Ratio 2

We investigated time evolution for Dn = 100, 500 and 1000 over a wide range of Gr for the aspect ratio 2. Figure 3(a) shows time evolution result for Dn = 100 and Gr = 1000, which shows that the flow is periodic, which is well justified by drawing the phase space as shown in Fig. 3(b). Contours of secondary flows and temperature profiles are shown in Figure 3(c).

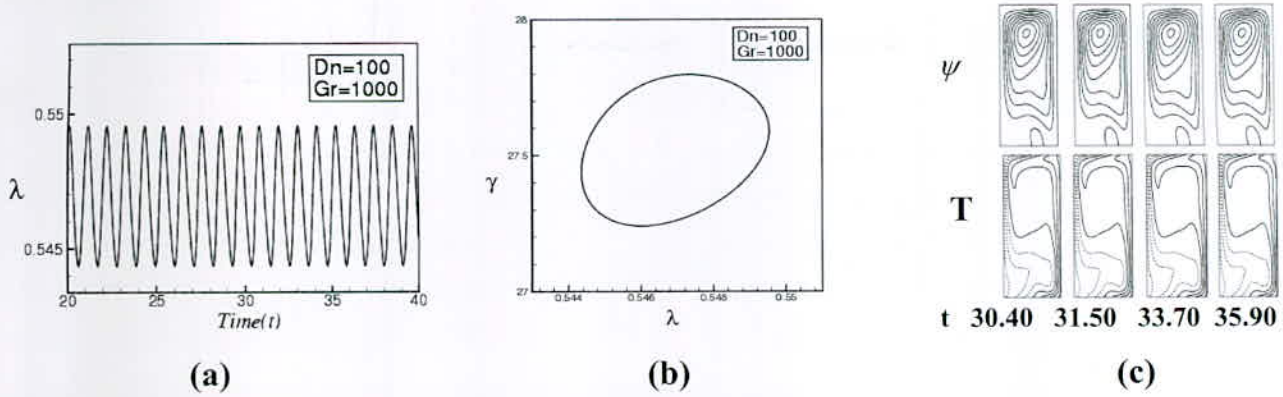

Figure 3: Results , for the aspect ratio 2. (a) Time evolution (b) Phase space (c) Secondary flow pattern (top) and Temperature profile (bottom)

Case III: Aspect Ratio 4

We performed time-evolution of for various values of Dn and Gr for the aspect ratio 4. Figure 4(a) shows time evolution result for Dn = 500 and Gr = 1000, and it is found that the flow is chaotic. This chaotic oscillation is well justified by drawing the phase space as shown in Fig. 4(b). Contours of secondary flow and temperature distribution is shown in Figure 4(c) for Dn = 500, Gr = 1000. It is found that for the large aspect ratio with large Gr, the flow becomes chaotic at small Dn's. It is also found that as the number of secondary vortices increase, the temperature distribution occurs more frequently and consequently convective heat transfer is significantly enhanced.

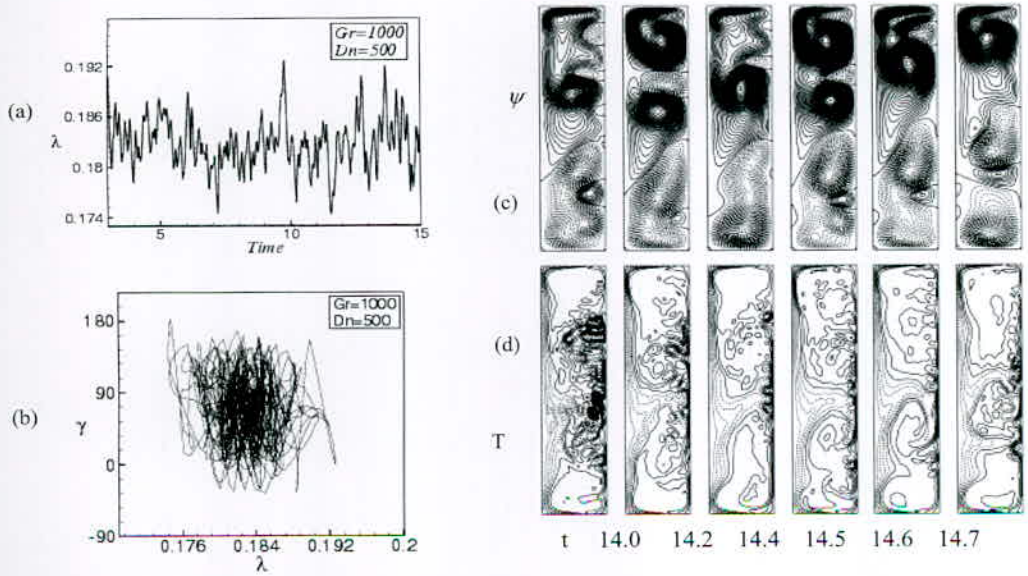

Figure 4: Results for Dn = 500 and Gr = 1000 for the aspect ratio 4. (a) Time evolution (b) Phase space (c) Secondary flow pattern (top) and Temperature profile bottom)

Conclusion

A spectral-based numerical study is presented for the flow through a curved rectangular duct of various aspect ratios. Time evaluations calculations as well as their phase spaces show that the steady-state flow turns into chaotic flow through periodic and multi-periodic flows, if the Dean number (Dn) is increased. It is found that for large Dn's, the solution becomes chaotic whatever the Grashof number (Gr) is. For large Gr, the unsteady flow undergoes through various flow instabilities in the scenario "steady-state periodic steady-state periodic multi-periodic chaotic". It is found that the unsteady flow is a two-vortex solution for the steady-state and periodic solutions, while two- to four-vortex for the chaotic solutions at small aspect ratio. For large aspect ratio, however, two- to multi-vortex solution is obtained. It is found that Dn is increased, the number of secondary vortices also increases, which enhances heat transfer in the fluid.

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Chapter 3

Funding Year

2012-2013

Studies on the Fisheries Potentials in Hilly Areas of Bangladesh

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Institution: Department of Zoology, University of Dhaka.
Duration: Three years (2012-2015)
Expenditure of the project: Tk. 2800000.00

Introduction

The hilly areas of Bangladesh occur in the northern and eastern areas of the country in Khagrachari, Rangamati, Bandarban, Chittagong, Cox's Bazar, Mymensingh, Sylhet, Moulavibazar and Netrokona, districts. The hills contain a number of creeks, small rivers, waterfalls, caves, lakes and a large reservoir. There are a remarkable number of waterfalls in the hilly area which has created large, medium and small streams and pools. Among these, the Madhabkundo waterfalls of Sylhet, the Himchori and Barochara waterfalls of Cox's Bazar, the Shailopropat Waterfall of Bandarban, and the Chittagong University campus waterfalls of Chittagong are remarkable. There is an unusual cave pool near Teknaf, the Kudong Cave.

These streams, rivers and reservoirs are assumed to contain a great diversity of fish and shellfish fauna, although they have not been properly inventoried. Therefore, the present study was initiated to assess the biodiversity potentials of fisheries resources in relation to the diverse habitats of hillstreams of Bandarban, Cox's Bazar, Chittagong, Sylhet and Netrokona districts for preparing a checklist of hillstream fish and shellfishes of the area with information on their taxonomy, biology and habitat preferences.

Objectives:

The specific objectives of the research were to study- the occurrence and distribution of the hill stream fauna, especially the fish and shellfish in relation to habitat diversity; the present status and diversity of fish, shellfish (mollusks, prawn and crabs), including turtle and tortoises; the taxonomical aspects of hillstream fish and shellfish species, different aspects of biology including habits, habitat, breeding, growth and meristic characteristics of these fauna i.e. the autecology of hillstream fauna; the contribution of hillstream faunal resources to the fisheries sector as well as to the sustainable livelihood of tribal people living near the hillstream; major threats to the degradation of faunal diversity of hillstream and to find the mitigative measures to protect the hillstream faunal resources.

Methodology

The study was conducted in the different hillstreams and rivers of north, north-east and south-eastern part of Bangladesh during May 2011 to 30 March, 2014. The 11 major sampling stations included are Himchori, Borochra and Kudong Cave of Cox's Bazar; Chittagong University Waterfall of Chittagong; Shailopropat Waterfall and Sangu River of Bandarban; Piyang and Sari Rivers of Sylhet and Madhabkundo Waterfall of Moulovibazar; and Kangsha and Someshwari Rivers and Gopalpur Hill Stream of Netrokona District.

Barilius bendelisis

Danio dangila

Danio rerio

Devario asamensis

Garra gotyla

Psilorhynchus rahmani

Lepidocephalichthys guntea

Pangio pangia

Hara hara

Akysis prashadi

Erethistes pusillus

Olyra longicaudata

Parambassis lala

Badis chittagongis

Trichogaster lalius

Melanodies tuberculata

Paludomus ornatus

Atyopsis spinipes

Macrobrachium lamarrei

Macrobrachium lanchesteri

Macrobrachium lar

Sartoriana trilobata

Labothelphusa wood-masoni

Episesarma versicolor

Figure 1: Some attractive hillstream fish and shellfishes of Bangladesh

Field surveys were conducted in these 11 (+1) selected sites 2 times a year covering the major seasons. Live specimens were collected from the selected areas and the samples were preserved in 4% (for shellfish) and 10% (for fish) formalin for detail taxonomic and morphometric studies. Sampling methods included hooks and line, seines, push nets, dip nets, cast-nets, traps and electric shockers. For collection of mollusks hand picking and push nets were important techniques. At each location field notes were taken with basic locality data, including latitude and longitude with GPS unit, date, time, habitat data, water quality information and species list. Photographs of live and preserved specimens and habitats were also taken.

Socioeconomic surveys to know the role of hillstream faunal resources to the sustainable livelihood of local people were also conducted. For the livelihood studies several approaches and techniques such as House hold survey; Focus group discussion; Interview with Key informants; and Questionnaire survey was conducted. An appropriate database program was developed and the pertinent data were transformed and subsequently analyzed by Computer package program SPSS (Version 12).

Results

During the study period a total of 118 fish species of 29 families under 9 orders were recorded from the 11 sampling sites. Among them Cyprinidae (37 %) were found to be the most dominant family followed by Balitoridae (5%), Channidae (3%), Sisoridae (5%), Notopteridae (2%), Engraulidae (1%), Psilorhynchidae (3%), Cobitidae (7%), Bagridae (9%), Schilbeidae (4%), Amblycipitidae (1%), Erethistidae (1%), Clariidae (1%), Heteropneustidae (1%), Olyridae (1%), Aplocheilidae (1%), Synbrachidae (1%), Ambassidae (3%), Nandidae (1%), Siluridae (3%) Badidae (2%), Mugilidae (1%), Gobiidae (2%), Osphronemidae (2%), Mastacembelidae (3%), Belonidae (1%), Hemiramphidae (1%), Tetraodontidae (1%) and Akysidae (1%).

A checklist of the 118 hillstream fishes of Bangladesh has been developed for the first time with their occurrence and abundance; and sampling station wise distribution of the fishes in 9 different tables. Brief description of all the fishes with their scientific names, synonyms, English and local names, taxonomic features, biometric data (in 23 tables), habitat and ecology, global and local distributions, population status and colour photographs (in 5 photo plates) has been furnished.

Similarly a checklist of shellfish and other associated aquatic hillstream fauna of Bangladesh have also been prepared. The list includes 9 species of molluscs, 14 species of prawn, 7 species of crabs, 2 species of frogs and 2 species of turtle. Those 34 species of shellfishes have been properly described like the fishes with their biometric data and colour photographs (in 5 plates).

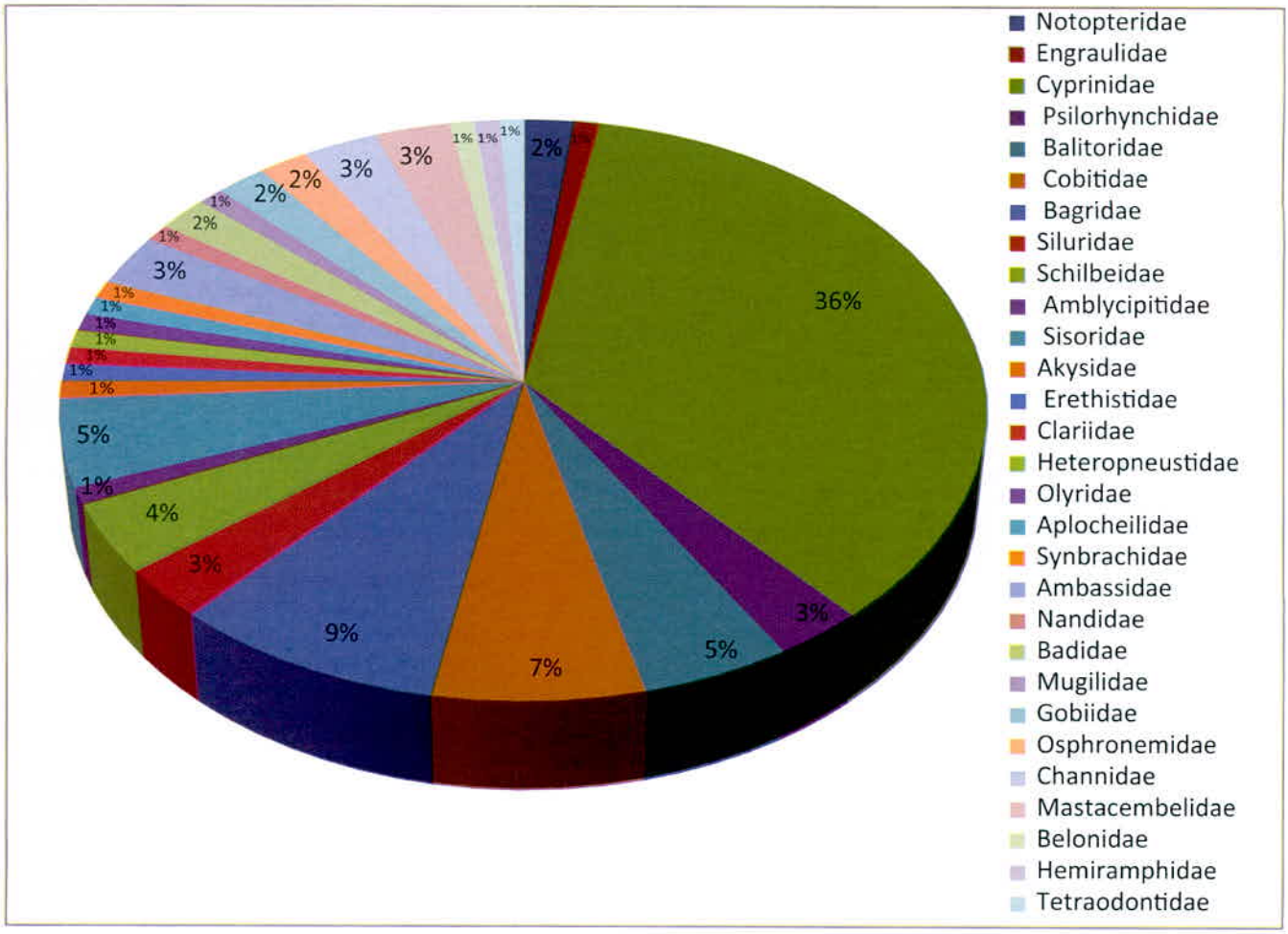

Figure 1: Showing the species diversity of hillstream fishes among the families

A total of 44 tables, 7 figures and 20 photo plates have been produced to present data and for illustration. Major Threats to the Hillstream Faunal Resources and biodiversity like overexploitation of hillstream faunal species, habitat modification, loss and fragmentation, degradation of the aquatic ecosystem and climate change have been identified and overall conservation and management approaches for hillstream faunal resources have been suggested.

The socioeconomic objective of the study was to find out the contribution of hillstream faunal resources to the fisheries sector as well as to the sustainable livelihood of hilly people living near the hillstreams. After completion of this research work, suggestive measures have been made for the sustainable use of

the resources and conservation of aquatic faunal resources of hillstream, which ultimately contribute to the sustainable environment and national economy of Bangladesh.

Based on the nature of the study and the socioeconomic context of the target respondents, qualitative and quantitative data were collected from the selected hillstream sites of Cox's Bazar, Bandarban, Netrokona, and Sylhet.

Outcomes from the Household Surveys present data and information on 1) Area wise Household distribution, 2) Population by origin, 3) Population by religion, 4) District wise population ratio, 5) Area wise Age and Sex distribution, 6) Gender of Household head , 7) Education level of study population, 8) Occupation and yearly income, 9) Food intake criteria, 10) Protein intake criteria, 11) Area wise protein intake criteria, 12) Food preparations for Bangali and Ethnic population, 13) Range of area wise fish intake, 14) Frequency of shellfish intake, 15) Other ethnic sources of protein, 16) Evaluation of availability: fish/ shellfish, 17) Fishing methods and 18) Reason of threat/extinction of various fish.

Seven public consultation meetings were held in Cox's Bazar, Bandarban, Netrokona and Sylhet where local male and female stakeholders were consulted through the meetings using participatory tools, Conventional Questionnaire, Secondary Data Collection etc. and summarized in a table. The Key Findings of the survey are-

- Most of the sample population is Bangali and Marma. Some are Garo and Bawn. Only few of them are Khasia and Hazong.
- Population ratio of Bandarban and Cox's Bazar are comparatively higher, whereas the ratio of Shylhet and Netrokona are lower. Majority percent people are Muslim.
- Most of the households of hilly area are headed by male. Comparatively, a greater number of female headed households were found in Kechinghat (Bandarban), on the contrary, no female headed households were found in Shoilopropat (Bandarban).
- The highest number of population enter for primary education, but dropout rate increases at an exponential rate as they move towards higher studies. Madrasa based education is not popular in the study area.
- The population is heavily dependent on rice for their regular intake of carbohydrate. Another interesting choice of food is bamboo shoots. After that, potato, sugar, wheat, puffed rice are their optional choices respectively. Shidal/Nappi seemed to be a popular as well as rational protein preference in addition to fresh fish and shellfish for these people.
- Dry or fresh fish are the most preferred protein choice for the sample population. Consumption rate of meat and shellfish is comparatively lower than other protein items.
- The population of Cox's Bazar prefers fresh fish compared to dry fish. They have never eaten Shidal/Nappi, which was found to be very popular in the regions of Bandarban. People of Bandarban also like all sorts of dry fish. People of Durgapur (Netrokona) are rather interested in fresh fish compared to dry fish, but fresh fish intake rate is the highest in Sylhet compared to any other regions of the study area.

- Bangali food preparation is similar for all the protein items. Food preparation methods of ethnic households are comparable to Bangali methods for similar items. Ethnic (tribal) households have some additional variety of protein like turtle, snake, monitor lizard, frog, etc. Frog and fox are more popular among these items.

- Surma, Hilsha as well as other marine fish are commonly taken in the households of Cox's Bazar. Cyprinid (Punti, chela etc.) are common fish intakes in Bandarban in addition to frog, crab, snails etc in some regions. Telapia, Bainefish, etc. were found popular in Netrokona and Cyprinids and Cat fish are popular in Sylhet.

- Cyprinid, catfish, loach, prawns, mollusks crabs, etc. are named that were very common about 10 years back in these regions. Unfortunately, only Baila are named to be still common .All the rest of the range is now rare according to the participant household survey.

- The most common threats to the species are manmade. Post disaster issues are involved in some cases, but they can be considered minor compared to climate change issues. Turtles, mollusks and sucker fish are more affected by climate change, whereas manmade issues are the major reason of extinction of prawns and various Cyprinids.

Conclusion

The present investigation indicates that the streams, lakes, pools, caves, rivers, tributaries and creeks of the hilly areas of Bangladesh contain large diversity of fishes and shellfish that have neither been inventoried to any appreciable degree nor adequately described for science before. The present study is a preliminary investigation and so far 118 species of finishes and 34 species of shellfish (9 snails, 14 prawns, 7 crabs, 2 frogs and 2 turtles) and other associated aquatic fauna could be identified. Some are yet to identify. Extensive survey is needed to assess the faunal resources of the hilly areas covering all the major hillstreams and lakes for proper conservation and future use of the aquatic biodiversity of the hilly area.

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Butterfly Colonization, Farming and Ecotourism Industry

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Institution: Department of Zoology, University of Dhaka
Duration: Three years (2012 - 2015)
Expenditure of the project: Tk 2800000.00

Introduction

Conservation ensures the rational optimum use of the biological resources in accordance to the demand and need to protect them for future use. Insect-plant relationship and host-plant selection strategies are based on insect's plant-recognition abilities and adaptations in an ecological condition suitable for both of them (Jermy 1988). To enhance species assemblage and species richness both for plants (first trophic level) and animals (successive trophic levels) butterfly-colonization stands as an important key factor in a terrestrial ecosystem (Bashar 2013). This hypothesis is much more practicable especially in the tropical rain forests like the forests of Bangladesh (Bashar *et al.* 2006). Butterfly-colonizing process is characterized by some special biotic interactions. Butterflies require species specific host-plants, selective egg-laying supports, pupating and resting plants; they need to remain within the choice of their range of behavioural activities and adaptabilities (Bashar 2012). But, foraging behaviours are many and highly characteristics in the different families of butterflies (Bashar 2014). They need large volume of plant variety in connection with suitable ecological conditions. These requirements stand essential for the normal life-building of butterflies (Bashar 2010). The maintenance of such combinations provides an optimum colonizing environment as "in-situ" conditions for the butterflies, i.e. the sound "home-place" for them. The butterfly colonization is a process of establishing butterfly-plant interaction in an open area under the presence of all required necessaries (plants, butterflies, water-channel, optimum humidity, temperature, light and other abiotic factors) in a channel which can play as a vital role model for the sustenance of an ecosystem (Bashar 2012, Islam *et al.* 2014).

Objectives

- To find out the way to colonize the different butterflies for farming; ways of conservation of endangered butterfly species, conservation of endangered plants associated with butterflies; and
- To create consciousness of biodiversity conservation and a strong biodiversity conservation working group.

Methodology

Under the following three steps activities were undertaken.

1) Procedure of butterfly colonizing centre frame-work

The Forest Department of the Government of Bangladesh has allotted ten-acres of land to the Department of Zoology, University of Dhaka for forest conservation technique innovation. Three-acres area of the land has been selected for establishment of an open butterfly park. This area is designed with four area-components as hedge-boundary (10%), canopy-tree area (30%), jungle-bush hedges (30%) and multimorphic beds-area (30% of total experimental area) (Fig. 1). The hedge boundary has been prepared with the composition of approximate 30±5 essential different natural floral species. The Canopy-tree area has been designed and prepared with tall-trees and their associated vines and climbers. It is a typical area with canopy-covering and man-height supportive bushes. The Jungle-bush hedge is a bushy area that has been prepared with biotic composition of vines, herbs, shrubs, climbers, trees, grasses and also with the canes population. The area ensures safe pupation and quick sheltering (due to sudden extreme changes in weather) for the butterflies. The above biotic conditions constitute a suitable assemblage for the survival of considerable number of fecund butterflies in the park area.

a) Land preparation and plantation by organic farming practices:

An accommodation of plants in the colonizing centre was to provide nutrition, sheltering, safe pupation and egg-laying support. There were successive stages for land preparation, viz. i. Bed modeling preparation; ii. Sand analysis, sand collection and application; iii. Organic manure preparation and application; and iv. Plant culture and plantation.

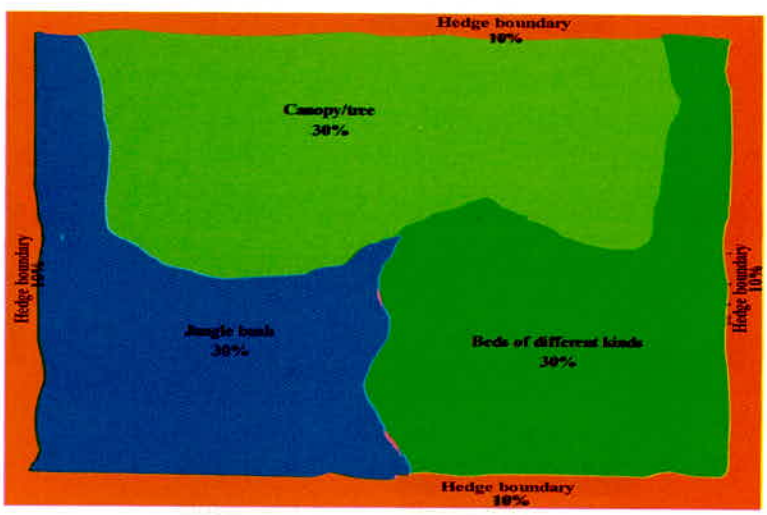

Figure 1: Butterfly colonization centre frame-work with demarcated biotic component-parts.

b) Colonization process-success and butterfly activities:

Establishment of a sound relationship between butterflies and their related plants were assessed by studying different activities. The activities are: foraging, puddling, resting, gene-flow activities, territorialities, pre-mating, mating, egg-laying behaviours, larvae and larval activities, pupating characters, emerging behaviours, predator activities and life cycle of butterflies.

The behavioural activities like biotic-biotic interactions; and abiotic-biotic interactions were examined by the methods of Jermy (1984, 1988); Dethier (1970); Ehrlich and Murphy (1988); Slama (1987) and Bashar (2010b, 2014ab).

Figure 2: Butterfly

2) Enhancement of plant population

The EBBL has innovated a process of examining healthy plant-seed production by operating a technique called "Cassette–ribbon system".

The plants that have been selected to cover their flowering period with a view to keep them "not exposed to butterflies" in the Butterfly Colonization Centre (BCC). These were taken under the 'cassette-ribbon' system. In this system, a device with the plastic cassette-ribbons was practiced. The ribbons were used to make a net-trap. With the net-trap, the experimental plant area was covered in such a way that the plants under the covered-area had every facilities of receiving light, humidity, air flow, water-flow, temperature and the optimum plant-nutrition. These facilities were also equally available to the plants those were not covered by the cassette-ribbon system. Under this system the covered plants were not visited by butterflies because the butterflies in the field were obstructed by the ribbon-system vibration. This vibration created disturbance to the flying-butterflies; and then they could not reach to the covered flowers. Under this way the plants named "not visited". On the other hand, the designated "treated plants" were exposed to butterflies, and the butterflies could freely visit the plants without facing any disturbance.

3) Butterfly colonization and Successive trophic levels conservation

The BCC provides inter-linkage of various biotic-biotic; and biotic-abiotic interactive factors together. The researchers found gradual increase in vertebrate populations, such as amphibians, reptiles, birds and mammals. The innovative technique was practiced for four years long (2010 to 2013). Sampling was made three times per year with an interval of four months.

Results

Establishment of butterfly colonizing centre frame-work:

Figure 1 shows the greater area of the BCC that has been framed by dividing into four major vegetation areas. The species richness in each of the four areas has brought the entire BCC into a suitable ecosystem for the butterfly-populations. The floral arrangements together with the proportional abiotic supports (water, air and sunlight) provided services for the butterflies. Services provided by the hedge boundary (10% area) were to protect the butterflies and the vegetation of entire planned butterfly park premises. Canopy tree area (30%) provides both sheltering plants and host plants. The jungle bush area (30%) ensures safe pupation and sudden sheltering for butterflies. Bed areas (30%) gave services provided various flowers in different seasons. Accommodation of the plants were made by taking them from different forest areas of Bangladesh. More than 50 forest areas have been studying in our grant project on "Butterflies and their related plants". Required plants have been collected from different forest areas of the country.

Major plants were accumulated in the BCC taking from Sylhet (viz. Lawachara, NoorJahan, Anarashbari, Borshijoor, Chautali, Satchori and Rema-Kalenga), Chittogong (viz. Karerhat, Mirsarai, Sitakunda, Chunati, Fashiakhali, Eidgaon) and Madhupur. The plants were accumulated by plantation, transplantation, cutting and seedling methods. The process was practiced for a long duration (about 8 years). The whole process was divided into two sections viz. "ex-situ plantation" and "in-situ plantation" (Table 1). In the ex-situ plantation process, total 37,500 plants belonging to 50 species were exercised. Out of the experimental plants, 4,500 were planted; 3,000 were transplanted and 11,000 plants were processed through cutting methods. The seedling process was followed for 19,000 plants. Among the ex-situ accommodation, the highest survival rate resulted 80% and lowest 52%. Average survival rate was 65% among the plants examined in accommodation process. All the ex-situ plants were brought from long distances and from different remote forest areas of Bangladesh.

Table 1: Plant accommodation structure in the BCC by plantation, transplantation, cutting and seedling methods in the Bhawal National Park, Bangladesh.

	Number of plants accommodated by different methods in ex-situ plantation (50 species examined)					
No. of beds in the BCC	Planted method	Transplanted method	Cutting plantation	Seedling process	Total plants examined	% survived (in average)
27	4,500	3,000	11,000	19,000	37,500	65%

	Number of plants accommodated by different methods in in- situ plantation (100 species examined)					
In all four major components in the BCC	Planted method	Transplanted method	Cutting plantation	Seedling process	Total plants examined	% survived (in average)
1. Hedge boundary 2. Canopy layer 3. Jungle-bush 4.Flower-beds	4,000	2,500	2,400	5,000	13,900	More than 95%

Examining the colonization process-success by the butterfly activities:

The colonization-success was determined by recording data on the ethological behaviours, like foraging, puddling, resting, gene-flow activities, territorialities, pre-mating, mating, egg-laying behaviours, larvae and larval activities, pupating characters, emerging behaviours, predator activities and life cycle of butterflies (Bashar 2015). Our success was to maintain the population of species ranging about 120 species (±10) in the optimum season favourable for butterflies. But the variation in the size of butterfly population is evident when we analyzed their abundance

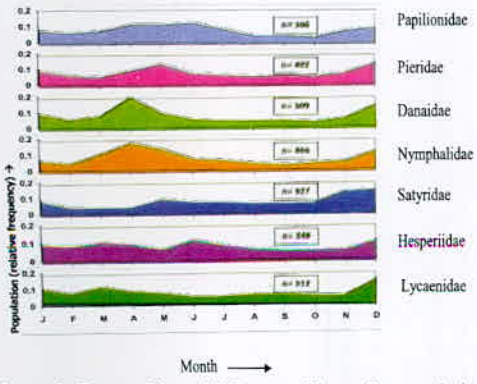

Figure 3: Successful establishment of butterfly population (colonization): family-wise abundance of the butterflies throughout the year (2010-2011) in the butterfly

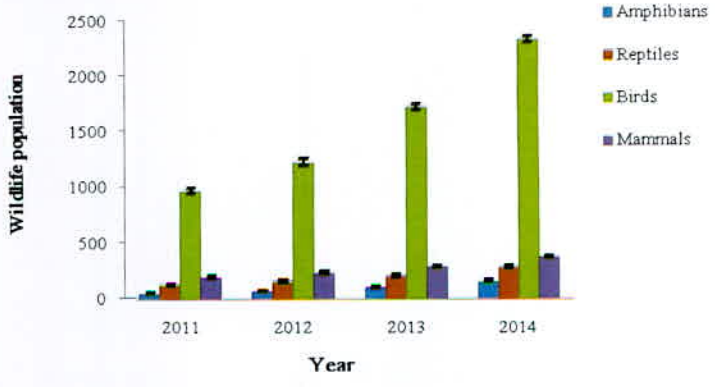

Figure 4: Concomitant increase of wild vertebrates with the progressive success of the butterfly colonizing mechanism in the butterfly colonization centre (4-years duration of study: 2011-2014).

Enhancement of plant population sustenance:

The selected plants were Asclepias curassavica, Aristolochia indica and Duranta plumieri. The productivity and frutiings were found significantly remarkable.

The Table 2 shows the experimental results. The Asclepias sp. is related with butterflies both as host and nectar plants. The plants exposed to butterflies are found to produce more fruits and seeds than those of the plants not exposed to butterflies. On the other hand, the non-exposed plants produced seeds with low quality and less weight compared to the seeds produced by the plants exposed to the butterflies. Similar results were also obtained when experiments were conducted with other two plants, viz. Aristolochia indica and Duranta plumieri. From the analysis of the results it is evident that butterflies can bring significant results to the case of healthy seed production. The healthy seed-production can enhance the production of genetically more viable plants also and can sustain good population size in the ecosystem where the colonizing process is practiced. As each of the butterfly species was related with its respective plants for foraging, egg-laying, resting and pupating, and other activities, and if through the activities they (butterflies) bring healthiness to the related plants, they can help in the questions of their conservation in the same ecosystem.

Table 2: Assessment of fruiting and seed production of three experimental plants in the BCC when these were exposed (Treated) and not exposed (Non treated) to the butterfly activities (Example of 10 samples)

Experimental plants (cluster) (10 Samples)		**No. of branches per sample**			**No. of effective branches per sample**			**No. of fruits /seeds per sample**			**Wt. (gm) of 1000 seeds per sample**		
Names	Expt. type	Max	Min	Av±SD	Max	Min	Av±SD	Max	Min	Av±SD	Max	Min	Av±SD
Asclepias curassavica	Treated	17	9	12.9± 2.601	14	7	10.3± 2.45	142	85	109.1± 18.76	4.46	4.10	4.334± 0.17
	Non treated	17	9	13.2± 2.66	15	7	10.2± 2.52	89	49	70.2± 13.62	3.68	2.56	3.046± 0.352
Aristolochia indica	Treated	17	9	12.8± 2.66	14	7	10.5± 2.368	114	87	105.4± 7.961	4.13	4.1	4.148± 0.026
	Non treated	15	9	11.9± 1.852	13	6	8.2± 2.043	105	75	90.3± 12.06	4.15	3.8	3.847± 0.197
Duranta plumieri	Treated	18	9	14.5± 2.635	15	8	12.0± 2.054	2489	2377	2443.3 ±42.06	57	43	50.9± 4.605
	Non treated	17	11	13.6± 1.955	13	8	0.2± 1.549	2419	2309	2374.3 ±44.22	50	40	44.7± 3.233

Wildlife conservation and the butterfly colonization :

The gradual assemblage of the wild vertebrates in the BCC has been recorded regularly from the year 2007-2014. Wild vertebrate population increase was regularly observed from the year 2007 and it was estimated in four groups, such as amphibians, reptiles, birds and mammals (Fig. 3). Amphibians belonged to 9 species. In 2011, the size of population was 47±5 and it stood 158±12 in 2014. Reptiles belonged to 11 species. The size of reptilian population was 122±9 in 2011 and it increased up to 277±13 in 2014. Birds belonged to 22 species. In 2011, the size of population was 970±21 and it stood 2333±21 in 2014. Mammals belonged to 11 species The mammalian population was 194±11 in 2011 and it increased upto 372±9 in 2014.

Conclusion

We observed the concomitant increase of wild vertebrates with the progressive success of the butterfly colonizing mechanism in the BCC from 2011 to 2014. It is evident that the insect interaction with plants (especially the phytophagous pollinating insects and the flowering plants with entomophilous pollens) establishes strong gene-flow mechanism in the forest ecosystem. Then the ecosystem becomes healthy and gave more functional services. Consequently the ecosystem becomes suitable and compact home for the successive trophic levels which provided fruitful services to all the wild animals living in the forest ecosystem. The fact remains that the forest can serve as a home of "in-situ" conservation site for wildlife fauna (Bashar 2011).

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Synthesis and Characterization of Mixed-metal Clusters for Nanoscale Electronic Devices and Catalysts

Kazi Ali Azam, Arzu Miah and Shishir Ghosh

Institution: Department of Chemistry, Jahangirnagar University
Duration: Three years (2012-2015)
Expenditure of the project: Tk. 2500000.00

Introduction

Nanoparticle research is currently an area of intense scientific research, due to a wide variety of potential applications in biomedical, optical, and electronic fields. Nanocrystals and nanotechnology are rapidly developing into a stand-alone industry that covers a host of applications and industries. As the science and technology grows, it will reach several stages of commercial success over the next five to ten years. Most of the current commercial activity has been centered on marginal to significant improvements of existing consumer and industrial products. However, nano-only type application exists today in many academic, governmental, and industrial laboratories around the world, and many are beginning to emerge while many more are being conceived. In the decades to come, nanocrystals and nanotechnology will permeate many of the products and technologies we use in our daily lives, either directly or indirectly.

Objectives

The term "nano-cluster" refers to an aggregate of atoms, molecule or ions which have at least one dimension between 1 and 10 nanometers. Nanoclusters could be easily obtained from traditional organometallic clusters by controlled decomposition or crystal growth of the later. However, most of the nanoclusters so far reported contain single metal atom. Little attention has been paid to construct nanoclusters build up of more than one type of metal atoms. In view of this, we have extended our research vista to synthesize mixed-metal nanoclusters. Our aim was to prepare mixed-metal nanoclusters i.e. clusters which have at least one dimension between 1 and 10 nanometers and a narrow size distribution and consist of more than one-type of metal atoms. We had divided our research into three parts.

(i) Firstly, we will synthesize and characterize some homonuclear metal complexes that can be used as precursors for the synthesis of mixed-metal clusters.

(ii) Secondly, we will synthesize and characterized the desired mixed-metal clusters.

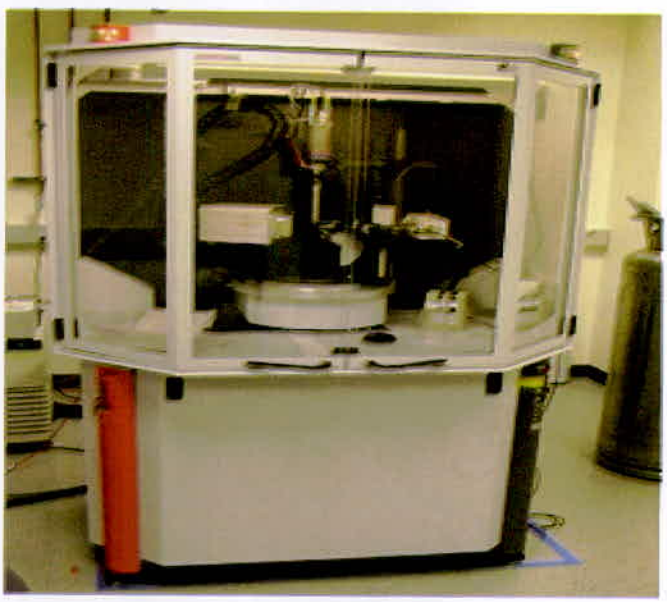
(iii) Finally, we will study the physical and chemical properties of these mixed-metal clusters to reveal the potential use of the resultant clusters in nanoscale electronic devices and as nanocatalysts.

Methodology

(i) Synthesis and characterization of homonuclear complexes (precursors for mixed-metal clusters): The reactivity of pyridine-2-thiol, pyrimidine-2-thiol, thiazole, 4-methylthiazole, thiazolidine, thiomorpholine, benzothiazole, and benzimidazole-2-thiol with osmium and ruthenium carbonyl clusters have been reported in literature, which show that in most cases the metal triangle remains intact in the resultant complexes which are thermally stable and the M—N and M—S bonds (M = Ru, Os) in these complexes are pretty strong. However, the reactions of these heterocyclic thiols with manganese and rhenium carbonyls furnished di-, tri-, and tetrametallic complexes depending on the structure of the heterocyclic ligands and the intrinsic reactivity of the metal carbonyls. Although the metal atoms in these complexes are apparently strongly held by sulfur and nitrogen bridges and are thermodynamically very stable, these bridges are easily cleaved which make them useful precursors for the synthesis of mixed-metal clusters. We had followed this methodology in order to synthesize homonuclear complexes of Group 7 metals. The synthesized homonuclear complexes were characterized by a combination of spectroscopic data and single crystal X-ray diffraction studies.

(ii) Synthesis and characterization of mixed-metal clusters: Reactivity studies of the di- and tetra-nuclear complexes of rhenium and manganese containing heterocyclic thiols revealed that the facile M—N and M —S bond cleavage (M = Mn, Re) of these complexes during reactions generate [M(CO)3(L)] species. Taking this advantage, a series of mixed-metal clusters have been synthesized. Following this strategy, we have synthesized a number of Group 7/8 mixed-metal clusters of higher nuclearity taking the advantage of strong M—M bonds (M = Ru, Os) in Os3(CO)12 and Ru3(CO)12.

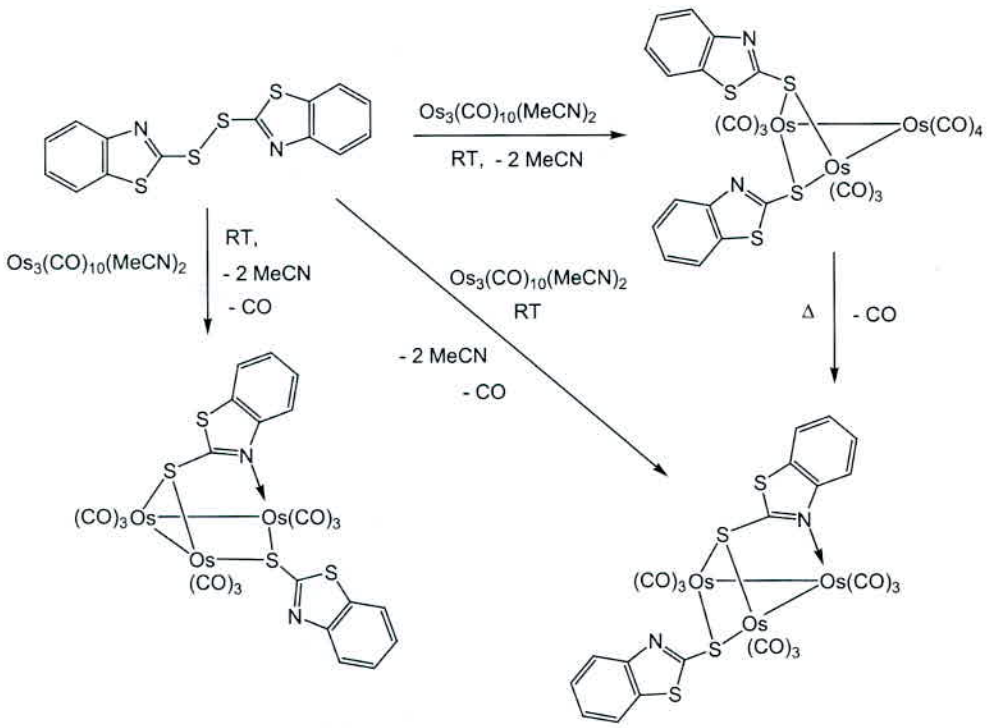
(iii) Preparation of mixed-metal nanoclusters and investigation of physical and chemical properties: During our investigation we aimed to apply controlled crystal growth



(iv) Technique to generate nanoclusters using "electrosynthesis technique" (for use as electronic device) or "diffusion technique" (for catalytical use). Theoretical (e.g. DFT calculations) and electrochemical (e.g. Cyclic Voltammogram) studies would have been carried out to understand electronic properties of the resultant nanoclusters for the preparation of potential nanoscale electronic devices. We also aimed to conduct chemical reactions to find out the catalytic activity of the resultant nanoclusters with special emphasis on alkyne oligomerization.

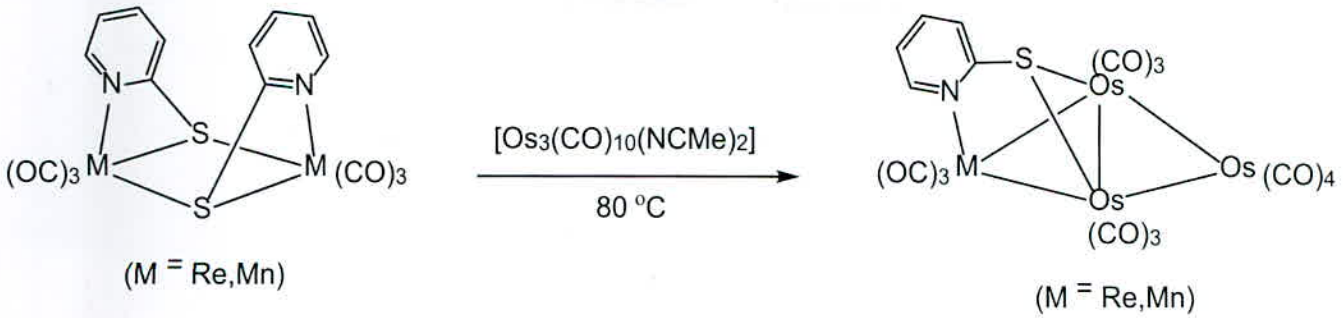
Results

A number of homonuclear complexes of rhenium, manganese other transition metals have been synthesized and characterized which are used as precursors for the synthesis of mixed-metal clusters. For examples, reactions of $M_2(CO)_{10}$ (M = Re, Mn) with pyridine-2-thiol (pySH) in the presence of Me_3NO afford the dinuclear complexes $[M_2(CO)_6(\mu\text{-pyS})_2]$, while a similar reactions between $M_2(CO)_{10}$ (M = Re, Mn) with pyrimidine-2-thiol (pymSH) lead to the formation of tetranuclear square-type complexes $[M_4(CO)_{12}(\mu\text{-}\kappa3\text{-pymS})_4]$. On the other hand, treatment of the labile cluster $Os_3(CO)_{10}(MeCN)_2$ with a stoichiometric amount of 2, 2'-benzothiazyl disulfide at room temperature in dichloromethane lead after chromatography to the isolation of four compounds; the known hydride complex $Os_3(CO)_{10}(\mu\text{-H})(\mu\text{-}S_2NC_7H_4)$, $Os_3(CO)_{10}(\mu\text{-}S_2NC_7H_4)_2$ and two isomers of $Os_3(CO)_9(\mu\text{-}S_2NC_7H_4)(\mu3\text{-}\eta 2\text{-}S_2NC_7H_4)$ (Scheme 1).



Scheme 1: Synthesis of homonuclear Group 8 metal clusters

These di- and tetra-nuclear complexes then treated with $M'_3(CO)_{12}$ clusters to synthesize tetra-nuclear Group 7/8 mixed-metal clusters. For instance, tetra-nuclear mixed-metal butterfly clusters $[MOs_3(CO)_{13}(\mu 3\text{-pyS})]$ were obtained when $[Os_3(CO)_{10}(NCMe)_2]$ and $[M_2(CO)_6(\mu\text{-pyS})_2]$ (M = Re, Mn) were heated together in benzene (Scheme 2).



Scheme 2. Preparation of Group 7/8 mixed-metal clusters

The precursor complexes and the synthesized mixed-metal clusters were characterized adequately by a combination of analytical and spectroscopic data together with single crystal X-ray diffraction analysis. For example, the solid-state molecular structures of the mixed-metal clusters described in Scheme 2 are shown in Figure 1.

Figure1. The solid-state molecular structure of $[ReOs_3(CO)_{13}(\mu 3\text{-pyS})]$ (left) and $[MnOs_3(CO)_{13}(\mu 3\text{-pyS})]$ (right).

Conclusion

The research program initiated under this project contributes significantly in the area of low-valent transition metal mixed-metal clusters. A number of new mixed-metal clusters have been synthesized and structurally characterized together with various homonuclear complexes used as precursor materials. We had also investigated the catalytic applicability of some of the resultant mixed-metal clusters towards alkyne oligomerization and other organic transformations, but the results were not promising.

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Molecular Characterization of the Pathogens Associated with Export Quality Frozen Shrimp to Determine Antibiotic Resistant Genes and Virulent Properties

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Institution: Stamford University Bangladesh and University of Dhaka
Duration: One year (2012-2013)
Expenditure : Tk. 1200000.00

Introduction

In shrimp cultivation and export, Bangladesh holds a huge economic importance. After the garment industry, shrimp production ranks second in Bangladesh in terms of the sector's ability to earn valuable foreign exchange. Over the last two decades, shrimp export has undergone rapid expansion. Currently, 36 shrimp species are harvested and cultivated in Bangladesh, of which bagda (shrimp, Penaeus monodon) and the golda (prawn, Macrobrachium rosenbergii) are the two main varieties. Chittagong, Cox's Bazar, Khulna, Shatkira and Bagherat regions account for approximately 95% of the total area dedicated to shrimp cultivation.

However, being into the category of seafood, shrimps are prone to microbial attack resulting in various food-borne diseases upon consumption. Though viruses are the most common cause of seafood related infections, most of the hospitalization and deaths are due to bacterial agents. As a consequence, food safety and quality aspects in trade became important, since fresh food is more susceptible to microbiological contamination. Microbiological proliferation in the shrimp is mainly dependent on the condition of transport, handling and processing. Thus, the quality of shrimp and frozen fish of desirable food standards has to be ensured with necessary measures. Frozen shrimps are normally subject to pre-shipment inspection based on physical and sensory characteristics followed by microbiological quality. Therefore, with the growing importance of shrimp as one of the major export items from Bangladesh, it is important to arefully maintain the microbiological quality of the exported item through appropriate measures.

Methodology

Export quality frozen shrimp samples were collected from processing industries located at Cox's Bazar and Khulna area. Hatchery shrimps were collected from Shatkhira, Khulna. Market shrimps were ollected from Malibagh bazaar, Shantinagar market and Agora departmental store.

Isolation of pathogenic microorganisms from shrimp samples

Isolation of Escherichia coli and Klebsiella spp.:

To isolate Escherichia coli and Klebsiella spp. Mac Conkey agar medium and Eosin-Methylene Blue (EMB) agar medium were used.

Isolation of Salmonella, Shigella and Vibrio spp.:

For Salmonella, Shigella and Vibrio spp. XLD and TCBS media were used.

Isolation of Clostridium perfringens:

Perfringens agar medium was used for the isolation and detection of Clostridium perfringens.

Isolation of Listeria monocytogens: Listeria isolation medium was used to detect Listeria monocytogenes.

Identification of the bacterial isolates:

According to the Manual of Methods for General Bacteriology (ASM, 1981), a series of biochemical tests were performed to identify the bacteria of interest.

Determination of antimicrobial susceptibility of the isolates:

Isolates were tested for antibiotic susceptibility on Mueller-Hinton agar against different antibiotics by Standard Kirby-Bauer method.

Tests for antimicrobial activity:

Mueller Hinton agar was used for this test. In the final phase of export quality shrimp samples, the investigation of the antibacterial activity was performed by using agar well diffusion method and also by spot test.

Extraction of genomic DNA:

Genomic DNAs from the bacterial isolates were extracted through modified boiling method. Different primers were used to detect respective genes by PCR.

Amplification of target genes through PCR: PCR amplification of the target DNA was carried out in a thermal cycler (Bio-Rad). The reaction mixture was subjected to amplification of 35 cycles at different denaturing and annealing temperatures.

Detection of amplified DNA:

The successful amplifications of the primers of specific genes were examined by resolving the PCR products in 1.2% agarose gel.

Results

Table 1: Comparison among the pathogenic load of the shrimp samples from market, hatchery and from export quality frozen shrimps

Isolated Pathogenic Bacteria	Count (cfu/g)							
	MB	**SM**	**ADS**	**ES-1**	**ES-2**	**ES-3**	**ES-4**	**HS**
E. coli	$8x10^5$	$1.20x10^6$	$1.00x10^5$	0	0	$1x10^4$	$1x10^4$	$1.2x10^7$
Kliebsiella spp	$1x10^5$	0	0	0	0	$4x10^4$	$6.5x10^5$	$2.0x10^7$
Vibrio spp.	$1.15x10^6$	$1.26x10^7$	$6.50x10^5$	0	$1.4x10^5$	$3x10^4$	$4.5x10^5$	$2.9x10^5$
Aeromonas spp.	0	0	0	0	0	0	0	$2.7x10^5$
Pseudomonas Spp.	0	0	0	0	0	0	0	$5.5x10^7$
Shigella spp.	$3.00x10^6$	$2.00x10^5$	$1.02x10^7$	0	0	0	0	0
Salmonella spp.	0	0	0	0	0	0	$2x10^4$	0
Clostridium spp.	0	0	0	0	0	0	0	0
S. aureus	$4.58x10^7$	$6.57x10^7$	$2.09x10^7$	0	0	$1.50x10^7$	$1.01x10^7$	0
Listeria spp.	0	$7.85x10^7$	0	0	0	$3x10^4$	$4.5x10^5$	$3x10^7$

MB: Malibagh Bazar, SM: Shantinagar Market, ADS: Agora Departmental Store, ES: Export Quality Frozen Shrimps HS: Hatchery shrimp

Table 2: Average Microbial load (cfu/g) in the export quality shrimp samples

Sample fraction	**Total aerobic bacteria**	**Total fungi**	***Escherichia coli***	***Staphylococcus*** spp	***Listeria*** spp
Head	1.5×10^7	1.8×10^5	1×10^3	9.1×10^6	1×10^5
Body	1.5×10^8	5.4×10^4	-	4.1×10^5	-
Tail	7.6×10^6	4.1×10^5	-	2×10^6	5.3×10^4

Growth of the pathogenic isolates on selective media

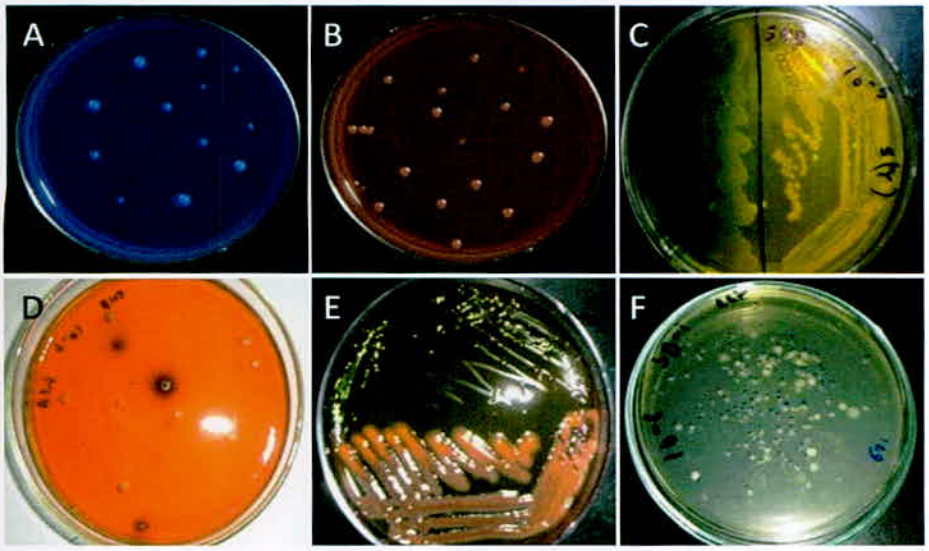

Figure 1: Growth of the pathogenic isolates on selective media: A. Fecal coliform colonies on mFC agar, B. Shigella colonies on XLD medium, C. Aeromonas colonies (left) and Vibrio (right) on TCBS agar, D. Colonies of Listeria monocytogenes on Listeria agar, E. E.coli colonies (Green metallic sheen) and Klebsiella colonies (pink) on EMB agar, F. Pseudomonas colonies on Cetrimide agar.

Among the samples of the 4 companies studied, 3 were found to contain E. coli 1x104-1.2x107 cfu/g, Klebsiella spp. 4x104-2x107 cfu/g, Vibrio spp. 3x104-4.5x105 cfu/g, Salmonella spp. 2x104 cfu/g, Staphylococcus aureus 1x107-1.5x107 cfu/g, Listeria spp. 3x104-3x107 cfu/g while one sample was completely free of pathogens. Samples from hatchery were found to be contaminated with E. coli, Klebsiella spp. Vibrio spp., Aeromonas spp., Pseudomonas spp. and Listeria spp. in the range of 105-107 cfu/g. Shrimps collected from markets also showed E. coli, Klebsiella spp., Listeria spp. and Vibrio spp. along with Shigella spp. and Staphylococcus aureus ranging from 105-107 cfu/g. Most of the pathogens isolated showed resistance against commonly used antibiotics. Interestingly, antibacterial activity was observed in case of one export quality shrimp sample indicating the presence of some sort of residual antimicrobial agent present in the sample. Gene specific polymerase chain reaction (PCR) study revealed the presence of eae gene in E. coli, sodB gene in Vibrio spp., and stx1 gene in Shigella spp., Listeria spp. specific gene 16s rRNA and gyrB2 gene in Klebsiella spp. indicating their pathogenicity and public health risk. The findings therefore, suggest proper sanitary and hygienic measures be taken in order to have a microbiologically safe and healthy produce and emphasize the importance of proper implementation of the HACCP system.

Publication

Rashed N, Hasan F and Rahman MM 2014. Molecular characterization of the virulent microorganisms along with their drug resistance traits associated with the export quality frozen shrimps in Bangladesh, Springer Plus, 3:469. http:/www.springerplus.com/ content/3/1/469.

Rashed N, Hasan F, Munna S, Rahman MM 2015. Demonstration of virulent genes within Listeria and Klebsiella isolates contaminating the export quality frozen shrimps. International Aquatic Research. DOI: 10.1007/s40071-015-0097-7.

Bioactive Principles from Some Medicinal Plants of Bangladesh

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Institution: Department of Pharmaceutical Chemistry, University of Dhaka
Duration: One year (2012 to 2013)
Expenditure : Tk. 800000.00

Introduction

Natural products have been, and continue to be, an invaluable source of novel drug compositions for the treatment of various diseases. Of the 1073 small molecules approved as drugs between 1981 and 2010, 59 (6%) are compositions containing natural products. Another 299 (28%) are derived from natural products, 177 (16%) are synthetic molecules that are derived directly from natural products and 146 (14%) are synthetic structures that are modeled on a natural product.

Bangladesh is a rich repository of medicinal plants, many of which are widely used in the Ayurvedic, Unani, Herbal and other traditional systems of medicines. The study programs were initiated to investigate some of the traditionally used medicinal plants of Bangladesh, including *Kalanchoe pinnata* Lam., *Melocanna baccifera* (Roxb.) Kurtz ex Skeels, *Syzygium cumini* L. and *Quisqualis indica* L.

The use of plant preparations in the treatment of various diseases is an age-old practice. In the present days, the World Health Organization (WHO) is also giving emphasis on concomitant use of traditional formulations which are largely based on plant materials to ensure the total health coverage.

Objectives

The principal objectives of the study program included-

1) Phytochemical and pharmacological (antimicrobial, antidiabetic, cytotoxic, antioxidant activities) investigations of the selected plants;
2) Isolation and characterization of the active principle(s) from these plant species;
3) Determine the efficacy of the isolated compounds against various diseases;
4) Discover new drug candidates from natural sources;
5) Assists the local drug- manufacturing units to attain knowledge on herbal drugs;
6) Authenticate our work by publishing the outcome of research findings in reputed national and international journals, seminars and symposia; and
7) Train our students for the masters and higher education programs.

Methodology

The respective part(s) of K. *pinnata* (whole plant), *M. baccifera* (fruit) and *S. cumini* (seed) and *Q. indica* (stem bark) were collected and identified by the experts of Bangladesh National Herbarium. The collected plant materials were separated from undesirable parts, air-dried and ground into coarse powder using high capacity grinding machine. The powder was stored in airtight container and kept in a cool, dark and dry place until analysis commenced.

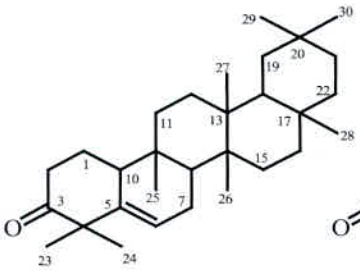
About 500 g of powder of each of the plant material was separately macerated with methanol for 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered through cotton plug followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated below 40^0 C with a rotary evaporator. An aliquot (5.0 g) of the concentrated extract was subjected to fractionation by the modified Kupchan partitioning protocol. Repeated chromatographic separation and purification of the crude extract and/or partitionates through colum chromatography (CC), vacuum liquid chromatography (VLC) and high performance liquid chromatography (HPLC) over silical gel of appropriate grade(s) allowd to isolate 18 secondary metablites. The structure of the isolated molecules were solved by extensive spectroscopic studies, including high resolution 1H and 13C NMR (nuclear magnetic resonance spectroscopy and mass spectrmetery (MS), by comparison of their spectral data with published values and co-TLC with authentic samples when available.

The crude extracts were subjected to screening for biological activities such as antimicrobial screening by disc diffusion method, antioxidant activity by free radical scavenging assay, cytotoxicity through brine shrimp lethality bioassay and antidiabetic activity. The safety and efficacy of *S. cumini* (seed) in treating diabetes was evaluated through analysis of the liver enzymes (SGPT, SGOT, alkaline phosphatase) and histopathological studies of the liver and kidney of the extract/fraction treated Long Evan's rats.

Results

The whole plant of *K. pinnata*, fruits of *M. baccifera*, seeds of *S. cumini* and leaves and stem bark of Q. indica have been collected from authentic sources, dried and then ground into coarse powder and extracted separately with methanol. The concentrated extracts were then partitioned with n-hexane/ pet-ether, carbon tetrachloride and chloroform/ dichloromethane. Extractives obtained from enlisted plants have been investigated for biological activities with special emphasis to antimicrobial, antioxidant, cytotoxic and antidiabetic activites. Few extractives revealed promising bioactivities whereas other showed mild to moderate activities which prompted us for chemical investigations.

Repeated chromatographic fractionation and purification of *K. pinnata* afforded a total of six compounds, glut-5(6)-en-3-one (1), taraxerone (2), 3β-friedelanol (3), β-amyrin-3-acetate (4), 3,5,7,3',4'-pentahydroxyflavone (5) and β-sitosterol (6). M. baccifera provided two triterpenens and two aromatic compounds identified as isochavicinic acid (7), eugenol (8), oleanonic aldehyde (9) and 3β -acetoxyoleanolic



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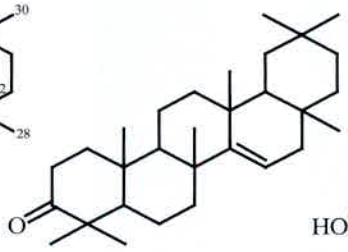

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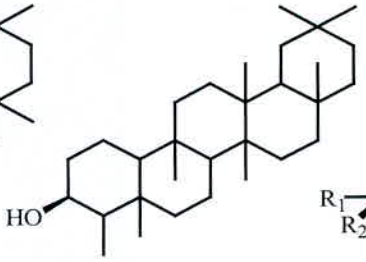

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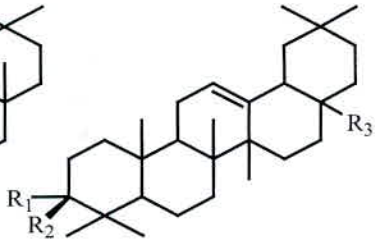
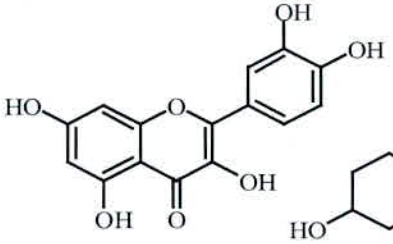


4: $R_1 = H$ $R_2 = OCOCH_3$ $R_3 = CH_3$

9: $R_1 = R_2 = O$ $R_3 = CHO$

10: $R_1 = H$ $R_2 = OCOCH_3$ $R_3 = COOH$

13: $R_1 = H$ $R_2 = OH$ $R_3 = COOH$



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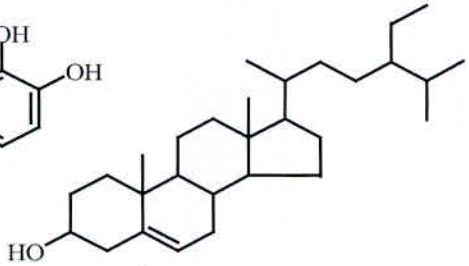

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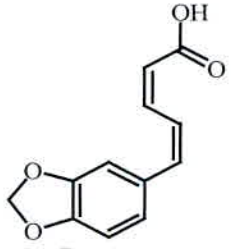

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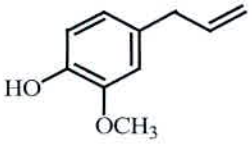

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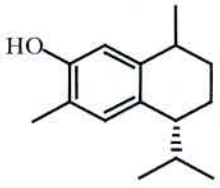

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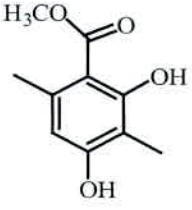

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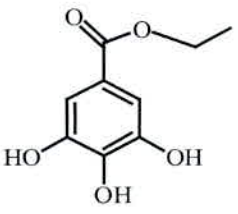

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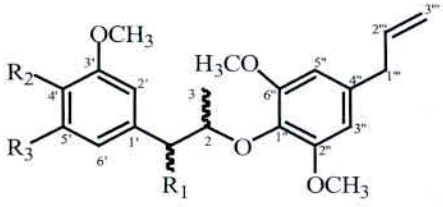

15: $R_1 = OH$ $R_2 = OH$ $R_3 = H$

16: $R_1 = OH$ $R_2 = OCH_3$ $R_3 = H$

17: $R_1 = OCOCH_3$ $R_2 = OCH_3$ $R_3 = H$

18: $R_1 = OH$ $R_2 = OH$ $R_3 = OCH_3$

acid (10). On the other hand, *S. cumini* yielded five compounds namely 7-hydroxycalamenene (11), methyl-β-orsellinate (12), β-sitosterol (6), oleanolic acid (13) and ethyl gallate (14). The fourth plant, *Q. indica* gave a total of four phenylpropanoids which were identified as 1-(4-hydroxy-3-methoxy-phenyl)-2-(4-allyl-2, 6-dimethoxy-phenoxy)propan-1-ol (15), 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-di-methoxy-phenoxy)propan-1-ol (16), 1-(3,4-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxyphenoxy) -propan-1-ylac-etate (17) and 1-(4-hydroxy-3,5-dimethoxyphenyl)-2-(4-allyl-2,6-dimethoxy-phenoxy)propan-1-ol (18). These phenylpronanoids exhibited significant anti-staphylococcal activity.

The crude extract and some partially purified fractions exhibited significant antimicrobial, antioxidant, cytotoxic and antidiabetic potential. In addition, the phenyl propanoids (15-18) isolated from *Q. indica* showed antistaphylococcal activity.

The project has led to the isolation and characterization of 18 compounds, including some structurally unique and biologically active molecules from the *K. pinnata, M. baccifera, S. cumini and Q. indica*. A total of 7 papers, including 6 in international journals have been published.

It has enabled to train several post-graduate students to carry out higher research in advanced laboratories as well as lead research team.

It has allowed upgrading the existing research facilities to some extent, which will help the researchers to carry out research projects and train the students in future.

Continuation of this type of projects will, one day, lead to the discovery of new drug candidates from the traditionally used medicinal plants of Bangladesh. This type of funding of scientific projects should be continued to facilitate research programs at the university level.

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Development of Probiotic Products for Human and Poultry

Khondoker Moazzem Hossain and S M Mahbubur Rahman

Institution: Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna
Duration: One year (2012-2013)
Expenditure of the project: Tk. 1000000.00

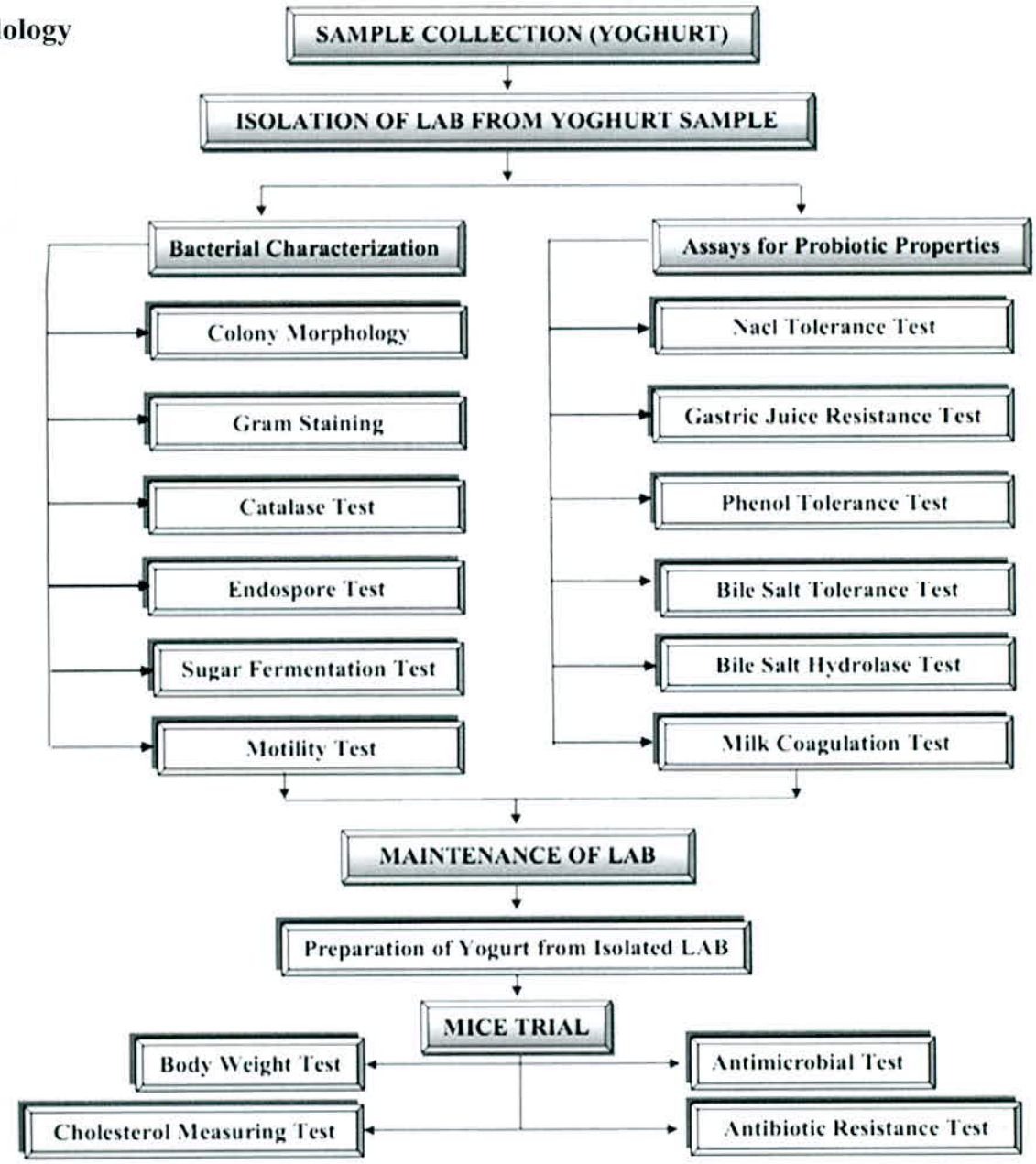
Introduction

"Probiotics are live microorganisms which, when ingested in adequate amounts as a single strain or as a combination of strains, confer one or more specified health benefits to the consumers." Probiotics are considered beneficial and are sometimes referred to as "friendly" bacteria. According to FAO and WHO, probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Parker defined 'probiotic' as 'substances and organisms which contribute to intestinal microbial balance', a regular intake of the organisms found in yoghurt can have a beneficial effect to the digestive tract. The concept of probiotic (which means, "for life") was introduced in early 20th century by Elie Metschnikoff, however it gained momentum only recently with considerable and significant advances in functional and health food market. Bangladesh is fast emerging as a potential market for probiotic in food. Probiotics, particularly *Lactobacillus* and *Bifidobacterium* have been associated with alleviation of lactose intolerance; prevention and cure of viral, bacterial and antibiotic-or radiotherapy-induced diarrheas; immunomodulation; anti-mutagenic and anti-carcinogenic effects; and even blood cholesterol reduction. A variety of probiotic supplements are now available for human and poultry uses in many developed and developing countries. These range from fermented milks to lyophilized forms, containing both single and multiple strains of probiotic bacteria.

Objectives

- Isolation and characterization of potential probiotic bacteria from selected regional yoghurt and poultry;
- Study of probiotic properties of isolated bacteria;
- Development of yoghurt using isolated bacteria;
- Laboratory based mice trials for in vivo studies for measuring effects of yoghurt on blood cholesterol level, antibiotic resistance, antimicrobial activities and body weight gain;
- Molecular characterization of selected isolated potential probiotic bacteria; and
- To suggest the strategies for the development of probiotic products for human and poultry.

Methodology



Yoghurt samples were collected from six different regions. Poultry feces were collected from eight different regional poultry farms. Besides, two broiler chickens were collected from one broiler farm of Khulna district.

Molecular Identification of Probiotic Bacteria

Biochemically characterized 60 isolates obtained from different regional yoghurt were used as the sample source in this experiment.

Feasibility of Nematode (*Panagrellus sp.*) Culture in Bangladesh and its Suitability as Live Feed for Larval Rearing of Giant Prawn, *Macrobrachium rosenbergii*

Mohammad Mostafa, Md Golam Sarower, Khandaker Anisul Huq and Abul Farah Md Hasanuzzaman

Institution: Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna
Duration: One year (2012-2013)
Expenditure: Tk. 1000000.00

Introduction

Fisheries sector in Bangladesh is very potential for food safety and economical development of the nation. However, the prawn larvae production in Bangladesh is solely dependent on costly live food "Brine Shrimp", Artemia nauplii for their nutrition and growth. But this Artemia has some prominent negative aspects. The most common ones are: high costs, a highly variable hatching rate, quick growth, the varying nutritional quality and the consuming of algal feed and therefore to compete with the cultured species for food (Biedenbach *et al.,* 1989; Lavens and Sorgeloos, 2000). Therefore, searching of an alternative live feed and coping of that particular feed for the larvae in the hatcheries could be a very good plausible solution in order to save the growing prawn industry.

The free-living soil nematode, *Panagrellus redivivus* is known as the microworm which has been popular in many countries as an alternative live feed for first feeding fish and prawn larvae (Guillaume *et al.,* 2001; Lee *et al.,* 2005). It is ecologically and logically believed that the nematode *Panagrellus sp.* will be found available in Bangladesh soil, and a local success of a mass culture of this low costs species could deliver a permanent available live food throughout the larval rearing period of the prawns. And this could provide a breakthrough in the prawn and shrimp culture sector.

Objectives

The main objectives of the present study were to identify the nematode, *Panagrellus redivivus* as well as establish a feasible culture technique of the species for ensuring its mass production and use as an inexpensive live feed in prawn hatchery.

Methodology

Searching of *P. redivivus* and its habitat: Ten sites of soil ground in different areas of Khulna University campus were selected randomly to search the availability of the species and thus to trace out the habitat characteristics of the species. The characteristics of the species habitats (soil moisture; pH; temperature and soil depth) were recorded.

Collection and identification of the species: Raw potatoes were placed at different sites and depths (ranging from 5-15 cm depth) of soil ground randomly and checked for the species after 7 to 14 days using high resolution microscope. The nematode *P. redivivus* identification was done following the morphological characteristics as described by Stock and Nadler (2006). And for a further confirmation of the identity of the species a molecular technology (PCR based method) was used.

Preparation of the culture media: The starter culture media was prepared with white oat and baker's yeast as described by Ricci *et al.* (2003). The mixer was then cooled in room temperature (26 ± 1°C) and incubated for 24 hours at 37°C temperature, and then used as starter media for the *P. redivivus* culture in room temperature. After getting the starter culture the key experiments were started by replacing with oatmeal, oatmeal with sunflower oil (added 5% sunflower oil), cereal (used paste of baby cereal), raw potato (used blended paste of peeled raw potato) and boiled potato (used blended paste of boiled potato) based media following the guideline describe by Ricci *et al.* (2003). The efficiency of the media was observed (through estimating the abundance of the produced species). For each experiments (with a specific media) three replications were used.

Culture of the starter species: The collected P. redivivus was placed on the culture media kept in plastic containers. The containers were then placed in incubator at different temperatures (22, 24, 25, 26, 27 and 28°C). Three replications were done for each of the temperature tests.

Harvest and counting of the cultured species: The *P. redivivus* was harvested from the inner wall of the culture container with scalpel. Live *P. redivivus* move very frequently and difficult to handle it. Hence, it was fixed with 10% formaldehyde for 5 minutes in order to fix them (Ted Pell, Inc., 2010). The number of the cultured species was counted by using "rafter cell" under light microscope.

Results

Identification of *P. redivivus:* For identification, the collected species were studied carefully under light microscope. An adult was observed with a clear layer of cuticle and flagella like tail. Male tail was found short while the female had comparatively long and spiky (Figure 1, 2). Mouth was located at the front of the body tip (Figure 3). The intestine was observed straight and ends shortly before the end. In male nematode there was an opening just before the end of the body and common body output for digestive and reproductive organs (Figure 5). For female, new young birth canal and excretory opening observed separate. On the edge of the opening, in two pockets two hook-like structures (Spicula) were present in the male (Figure 5). The vagina found oriented by muscular sheath and this genital opening was located at the middle part of body (Figure 6). The organ is then extended into a sex tube in which the fertilized eggs develop and provides shelter to developing nematodes; thereby helps to produce young nematodes (Figure 4). These results were similar to the findings of Stock and Nadler (2006) and Kumlu and Fletcher (1997) that confirmed the morphological identification of the species. However, through a molecular technology (PCR based method) the identity of the species was further confirmed.

Figure 1: Tail of male *P. redivivus*

Figure 2: Tail of female *P. redivivus*

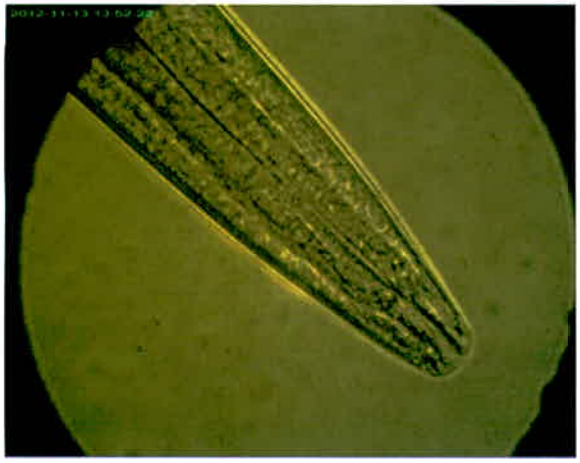


Figure 3: Mouth opening of *P. redivivus*

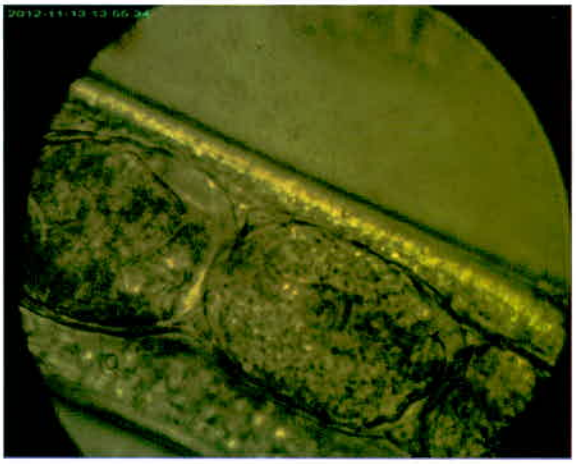


Figure 4: Fertilized egg in female *P. redivivus*

Figure 5: Genital organ of male *P. redivivus*

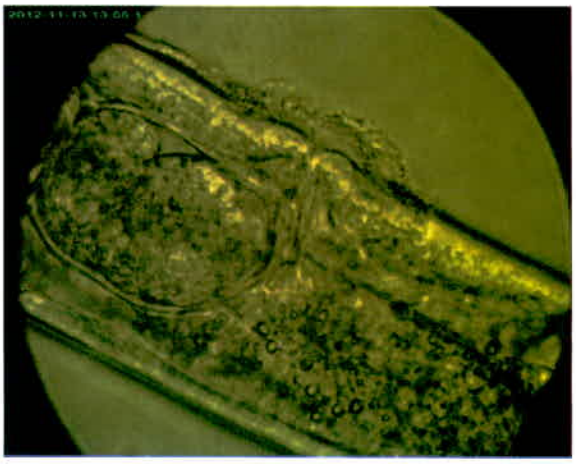


Figure 6: Genital organ of female *P. redivivus*

Habitat of P. redivivus: It was revealed that P. redivivus resides under some specific condition of the environment. The environmental parameters found in the habitats of the species in the local area are presented in the following Table 1.

Table 1: Soil parameters found suitable for the nematode, *Pangrellus redivivus* species.

Soil parameters	Species found available	Species found few
Moisture	30±6%	16-24%
pH	7±0.5	>7.5
Temperature	20±2^{0}C	>24^{0}C
Depth in soil	5±1cm	>6cm

Suitable culture media for the species: Out of the five media used for culture, oatmeal and boiled potato paste were found better for culturing the species. The efficiency of different media for P. redivivus culture is summarized below in the Table 2.

Table 2. Efficiency of different media used for Panagrellus sp. culture

Types of culture media	Species produced available
Oatmeal	+++
Sunflower oil enriched oatmeal	+
Cereal	+
Raw potato paste	-
Boiled potato paste	++++

Most feasible media for the culture: The primarily identified suitable media, boiled potato paste and oatmeal media were tested further for mass culturing. The calculated number of the produced P. redivivus individuals obtained from per gm of boiled potato media was 1,50,032±9,285 while the number was 1,24,622±6,722 from the oatmeal media. However, locally available boiled potato media was considered as the most feasible one for P. redivivus culture as it produced the highest number of individuals as well as due to its low preparation costs in comparison to the oatmeal media .

Suitable temperature and time-length for the culture: Figure 7 represents the number of P. redivivus individuals produced with regard to temperature regimes and incubation periods. The highest production was found at 26 0C temperature with the incubation period of around 14th day that ensured the maximum harvest in this study; although, from the day 7 the newly grown species found started to climb up the container wall which were even ready to harvest. The result showed that a 5 gm weighted boiled potato can produce approximately 0.75 million of nematodes (Figure 7).

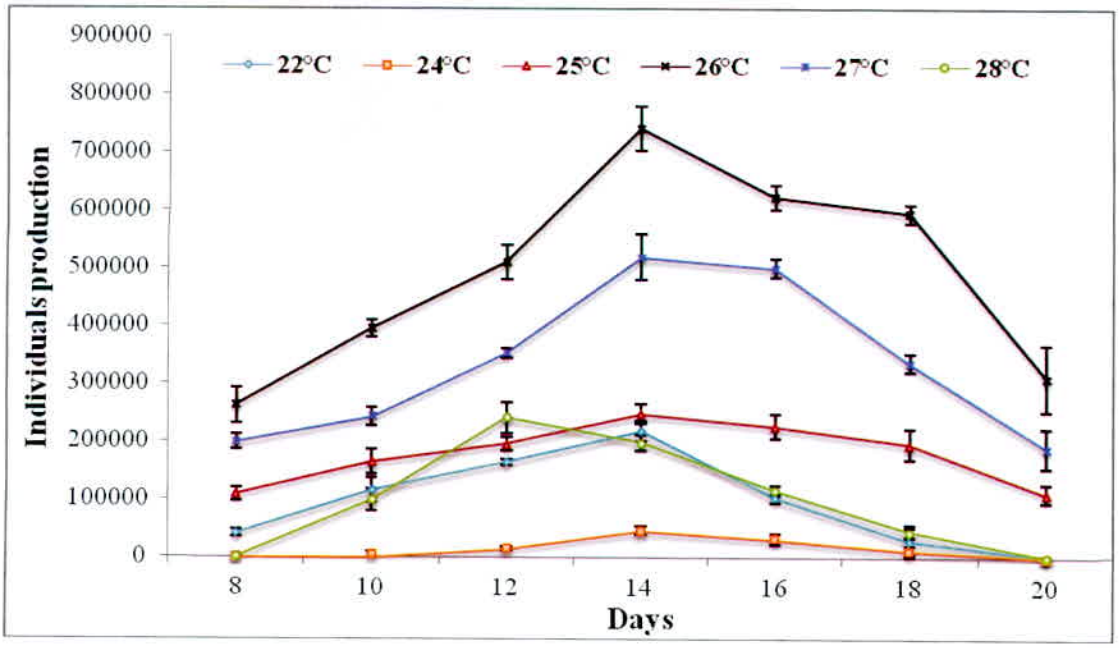


Figure 7: Production of *P. redivivus* at different temperature on boiled potato based media.

The production of *P. redivivus* found in this study could be say satisfying. Thus, this boiled potato media technique which costs very low as well can be used for the production of *P. redivivus*. The present study revealed that approximately 15 million nematodes can be produced just from 100 gm of boiled potato media in small scale basis that is enough to use as food for at least 3000 larvae in a day.

Conclusion

Availability of live food is one of the major bottlenecks for the hatchery sector worldwide. The nematode species, *Panagrellus redivivous* recently used as an inexpensive live feed in prawn hatchery industry in some countries is assumed to be a very potential one in Bangladesh as well. The present study has been successful in developing a suitable and inexpensive culture technique of *P. redivivus* which can make the fisheries sector entered into a new domain specially in case of shrimp and prawn hatchery. However, further research is needed for the development of the culture media to increase more the production and nutritional value of the nematodes; comparative study of *P. redivivus* with Artemia on the factor of feeding rate, survivability, growth and nutritional value of larvae.

Publication

Two manuscripts on the research results of the project have been submitted for publication in "Bangladesh Journal of Veterinary and Animal Sciences" (titled: Detection and culture feasibility of a soil nematode *(Panagrellus redivivus)*, a potential live feed for prawn larvae in Bangladesh) and in "Khulna University Studies" (titled: Molecular Identification of a soil nematode *(Panagrellus redivivus)*, a potential live feed for prawn larvae in Bangladesh).

Rapid Genotyping of Mrsa Isolates Using Oligonucleotide Arrays and Multiplex PCR

Sabita Rezwana Rahman and Housne Ara Begum

Institution: University of Dhaka

Duration: One year (2012 - 2013)

Expenditure of the project: Tk. 1500000.00

Introduction

Burn patients are more readily colonized and infected than other patient groups. Disruption of the normal skin barrier and depression of immune responses caused by extensive burn injuries are particularly susceptible to infection. Despite remarkable advances in burn care units Staphylococcus aureus infection remains an increasing problem for higher morbidity and mortality in burn patients. MRSA, ***(methicillin-resistant staphylococcus aureus),*** is a form of bacterial infection that is resistant to numerous antibiotics including methicillin, amoxicillin, penicillin and oxacillin, thus making it challenging to treat the infection successfully.

We sought to determine frequency of methicillin-resistant S. aureus (MRSA) in burn wound patients and inspect pattern of antimicrobial susceptibility together with molecular basis of methicillin/oxacillin resistance among the isolates. Samples were collected (from August 2010 to October 2011) from burn unit of Dhaka Medical College Hospital, Bangladesh. S. aureus was identified by conventional culture based methods. For antimicrobial susceptibility testing Kirby-Bauer disc-diffusion method in accordance with NCCLS guideline was followed. The methicillin resistance determinant gene mecA was confirmed in the isolates and sequenced for computational analysis. A total 180 burn wound samples were tested, from where S. aureus was confirmed in 44.44% cases. Among these isolates 22.5% were resistant and 2.5% were borderline resistant to oxacillin. Resistance for other commonly used drugs like amoxicillin, azactum, erythromycin, azithromycine etc. were noted for 90%, 82.5%, 57.5% and 55% of the isolates respectively. The basis of drug resistance was found to be linked to chromosomal DNA since plasmid was absent in these isolates. The gene mecA, internal control gene femA and insertion sequence geneIS431 were amplified. We sequenced the mecA gene from MRSA isolates and found homology to S. aureus 'penicillin binding protein 2a' (PBP2A) (Accession no. NC 002952.2).

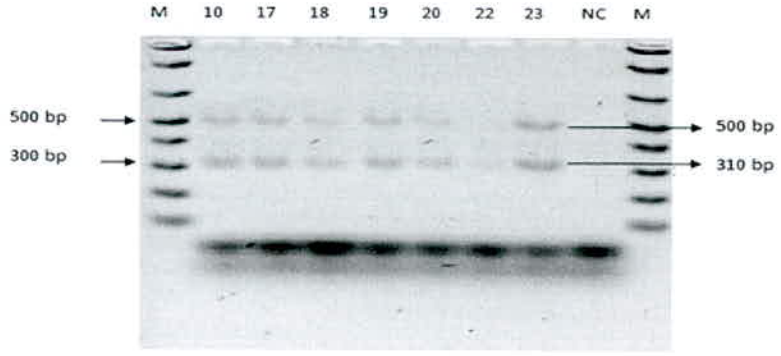


Figure 2: PCR products for detection of mecA and 16S31 responsible for methicillin resistance resolute on 2% agarose gel. Lane M indicate marker, lane NC indicate negative control and lane 10,17,18,19,20,22,23 indicate the sample ID. The lengths of amplified fragments and molecular size markers (in base pairs) are indicated on the right and left, respectively.

Staphylococcus aureus species were further confirm by the detection of species specific gene femA primer F-1 and F-2. The 686-bp amplification product of femA fragment was obtained from all of the Staphylococcus aureus and positive control *(Staphylococcus aureus* ATCC No. 6538) where as such amplification product did not occur in lane NC, used as a negative control *(Salmonella typhimurium ATCC No 13311).*

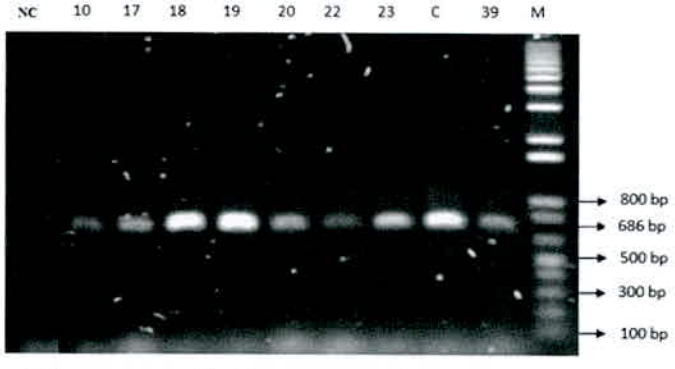


Figure 3: PCR products for detection of S. aureus specific femA gene resolute on 2 % agarose gel. Lane M indicate marker, lane C indicate positive control, NC indicate negative control and lane 10, 17,18,19,20,22,23,39 indicates the sample IDs. The product size of femA is 686 bp.

Sequencing for approximately 145 bp was performed (Macrogene, Korea) and the sequence alignment was carried out for the 6 isolates using ClustaW 1.8, showed homology with the mecA of *S. aureus* (Accession no NC 2952.2). The analysis of the mecA sequence by BLASTX displayed homology with the altered penicillin binding protein (PBP2a) of S. aureus (Accession no NC 2952.2). Figure 4 shows the comparative picture of the genes from the MRSA isolates of this study with other MRSA sequence from the gene bank. Isolate 1 also were in the same branch of mecA gene however Isolate 2 and 3 aligned in a different branch showing there independent identity (figure 5) and it should be investigated further to understand the extent or reason of divergence among these isolates. Finally majority of MRSA were isolated directly from boiling pus samples for 5min followed by PCR for establishing rapid detection of MRSA. This study demands for complete genomic sequence of MRSA which has not been done for Bangladesh. Due to fund constrain, it could not be done in this project.

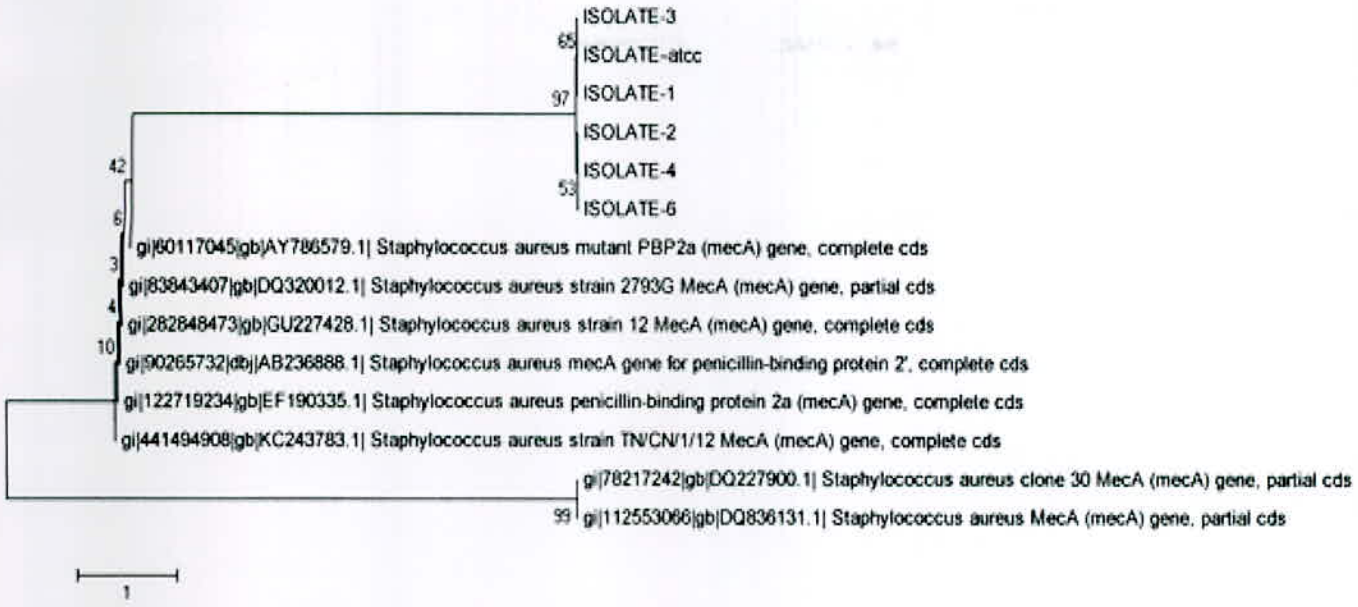


Figure 4: Phylogenetic tree showing a distinct group of the mecA gene sequenced from the experimental organism.

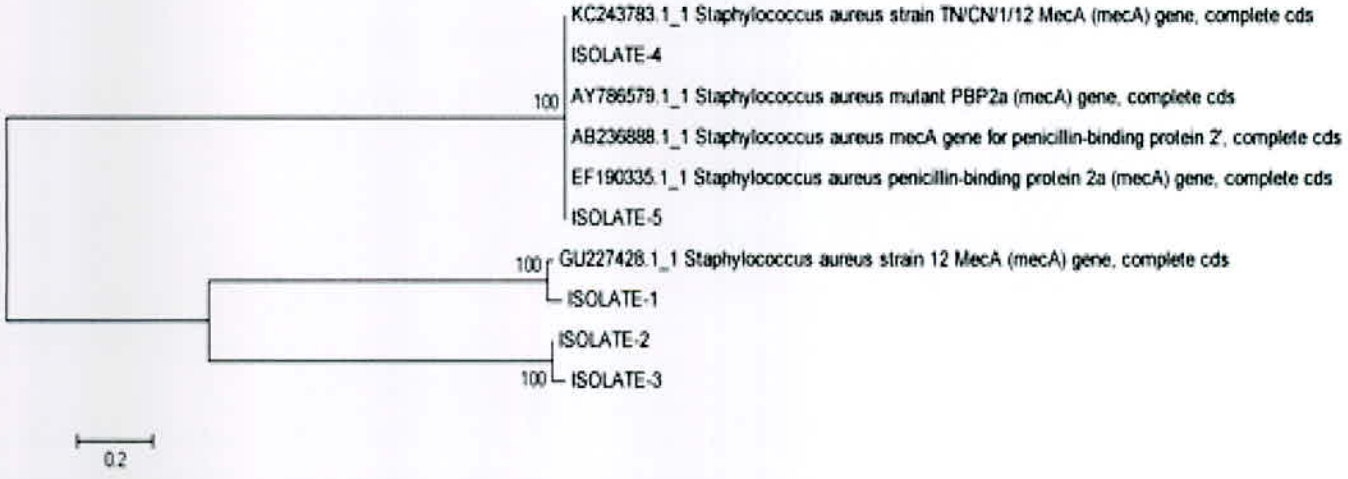


Figure 5: Phylogenetic tree showing various branches of the mecA gene product of the test organism.

Conclusion

The aggressive behavior of different pathogenic microbes in various health care centers are very common therefore detection system for those pathogens should be rapid, sensitive, specific, informative and comparative. We determined chromosomal basis of resistance inheritance among the MRSA isolates and hence suggest thorough and periodic monitoring of the isolates at genome level. To resist the succession of MRSA in burn wound patients we recommend drugs blocking PBP2A synthesis and/or its function to be added with other drugs in practice. These approaches may not culminate the pathogens totally but can indeed resist their progression to a deadlier version.

Effectiveness of Different Classes on Molecular Marker for Classifying and Revealing Variation in Some Available Growing Cucurbitaceae Vegetables in Bangladesh and Their Application to Improve the Quality of Cultivars

Biswanath Sikdar and Md Khalequzzaman

Institution: Department of Genetic Engineering and Biotechnology, Rajshahi University, Rajshahi
Duration: Three Years (2012-2015)
Expenditure of the project : Tk. 2000000.00

Introduction

The botanical family Cucurbitaceae, commonly known as cucurbits and gourds, includes many economically important cultivated plants, such as cucumber *(Cucumis sativus* L.), melon (*C. melo* L.), watermelon *(Citrullus lanatus* Thunb.), squash, bitter gourd *(Momordica charantia* L.) and pumpkin *(Cucurbita spp).* Cucurbitaceae family contains about 90 genera and over 700 species of economic importance. The family is distinct morphologically and biochemically from other families and is therefore considered monophyletic. As the plants of this family produce unisexual flowers, crosspollination is a regular feature.

Taxonomy:
Kingdom- Plantae
Division- Magnoliophyta
Class- Magnoliopsida
Order- Cucurbitales
Family- Cucurbitaceae
Genus & Species- *Lagenaria siceraria, Cucurbita maxima, Cucumis sativus, Benincasa hispida, Luffa acutangula, Luffa cylindrica, Trichosanthes cucumerina, Trichosanthes dioica, Momordica charantia, Momordica dioica* and *Coccinia cordifolia*

Name of the species, their common names and chromosome numbers of the present study

Name of the species	Common name	Chromosome no (2n)
Lagenaria siceraria	Bottle gourd	22
Cucurbita maxima	Pumpkin	40
Cucumis sativus	Cucumber	24
Benincasa hispida	Ash gourd	24
Luffa acutangula	Ridge gourd	26
Trichosanthes cucumerina	Snake gourd	22
Luffa cylindrica	Spongy gourd	26
Trichosanthes dioica	Parble	22
Momordica dioica	Tisal gourd	28
Coccinia cordifolia	Ivy gourd	24
Momordica charantia	Bitter gourd	22

Objectives

- To minimize risks involved in the introduction of Cucurbitaceae as vegetable producing crop in our country;
- To focus on productivity in relation to genetic resources, environmental setting and crop management;
- To integrated a wide range of global experience and global collection of different species cucurbitaceae and their analysis;
- To compare the efficiency of Isozyme, RAPD, SSR and ISSR markers for genetic diversity assessment on some members of cucurbitaceae family;
- To characterize and compare with some members of cucurbitaceae family of agronomic interest using isozyme tests;
- To determine the genetic similarities based on RAPD, SSR, and ISSR markers;
- To detect the unique/ specific/ major band or gene; elute the identify band; clone the eluted band and send it for sequencing;
- To clone the major gene with suitable vector and transfer it to suitable explants; and
- To observe the bacterial and viral diseases through 16S rDNA, cDNA and PCR application.

Methodology

General protocols of plant tissue culture, Cell lyses protein extraction, DNA extraction following CTAB method, Trizol method for RNA extraction.

Results

(i) We have been established a Laboratory the in Dept. of Genetic Engineering & Biotechnology with some modern equipments.

(ii) We have been established the in vitro regeneration protocol using different explants of *Lagenaria siceraria, Cucurbita maxima, Cucumis sativus, Benincasa hispida, Luffa acutangula, Luffa cylindrica, Trichosanthes cucumerina, Trichosanthes dioica, Momordica charantia, Momordica dioica* and *Coccinia cordifolia.*

Among them, meristem shoot tips culture techniques were established successfully to produce disease and viruses free plantlets e.g. in case of Momordica charantia recommended media combination was 1.5 mg/l BAP + 0.1 mg/l GA3

- We have been successfully established genetic transformation protocol in different species of cucurbitaceae through *Agrobacterium* – mediated genetic transformation system (GUS gene & *YCF1* gene).
- Genetic diversity of the different species of cucurbitaceae family was done using different biochemical and molecular markers.

Figure 1: A. Direct shoot multiplication from cotyledonary node on MS having 2.0 mg/l BAP + 0.2 mg/l GA3; B&C. Direct regeneration in media having 2.0 mg/l BAP; D. showing roots formation on half strength MS + 0.5 mg/l IBA in *Cucumis sativus*

Biochemical markers

Alcohol dehydrogenase, Amino peptidase, Malate dehydrogenase, Peroxidase, β-galactosidase, β-glucosidase, Fumarate dehydrogenase, Superoxide dismutase, Hexokinase, Alkalyne phosphatase, Estarase, Catalase, Tyrosinase, Aspartate amino transferase, Fructose bisphosphate , Formate dehydrogenase and Acid phosphatase isozymes were tested. In isozyme tests ACP, FBP, HEX, GAL, AMP, FUM and AAT showed highest genetic polymorphism as well as genetic diversity in most of the species of *Momordica.*

Molecular markers

RAPD and ISSR markers were used as molecular markers. From this following conclusion are to be taken among the species:

(i) This dendrogram shows more similarity to ISSR based dendrogram with some uniqueness.

(ii) Jaccard similarity coefficient ranges from 0.2 to 0.80 with a mean similarity of 0.5. The dendrogram divided the taxa into 4 main groups.

(iii) The first subcluster was formed by *Lagenaria siceraria, Cucumis sativus, Momordica dioica* and *Momordica charantia.*

(iv) The second subcluster was formed by *Cucurbita maxima, Trichosanthes dioica*

(v) The third subcluster *Trichosanthes cucumerina, Luffa acutangula* and *Luffa cylindrica* formed.

(vi) The fourth subcluster was formed by *Benincasa hispida and Coccinia cordifolia.*

- Among the genotypes/varieties of a species were also used to characterize / identify / relationship etc for different genetic quality improvements.
- Some bacterial and virus diseases were characterized by biochemical and molecular marker systems (16s rDNA and cDNA methods)

Conclusion

Developing countries like Bangladesh need more of the applied subjects like biotechnology which has high demand in home and abroad and that can contribute significantly in the development of our nation by supporting and improving our agricultural system. The project work was proposed to develop and screening our existing some vegetables of cucurbitaceae family as well as their cultivars. Farmers will be benefited to produce more disease (bacteria/virus) free cultivars through this project. Due to the consistency in the expression of DNA/RNA-based molecular markers from banding profiles could be expressed at all times of same characteristics of those cultivars/variety which have been released as high yielding variety in respective species of cucurbitaceae family.

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Kausar M, Parvin S, Haque ME, Khalekuzzaman M, Sikdar B and Islam MA. 2013. Efficient Direct Organogenesis from Shoot Tips and Nodal Segments of Ash Gourd *(Benincasa hispida L.). J Life Earth Sci,* 8: 17-20.

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Evaluation of Molecular Based Methods in Diagnosis of *Mycobacterium tuberculosis* in Suspected Tuberculosis Patients

Mahmuda Yasmin, Choudhury Rafiqul Ahsan and S M Mustafa Kamal

Institution: Department of Microbiology, University of Dhaka
Duration: Three years (2012 - 2015)
Expenditure of the project: Tk 2000000.00

Introduction

Tuberculosis is a common disease, which is associated with significant morbidity and mortality. Timely administration of anti- tuberculosis treatment would prevent complications and spread of disease. The commonest site of infection is the lung, which accounts for more than 90% of cases. *Tubercle bacilli* are conventionally detected by smear examination and culture. Smear examination is a rapid, low cost method but has low sensitivity. Culture is much more sensitive than smear examination, but takes an average of six to eight weeks to give positive results. Amplification of a highly conserved insertion sequence, IS6110 present in multiple copy numbers in the genome is a rapid, sensitive and specific method of detection of tubercle bacilli in clinical specimens.

Bangladesh ranks as the fourth highest tuberculosis (TB) burden country in the world and it is estimated that 4-5% of the isolated *Mycobacterium tuberculosis* is multi-drug resistant. The inappropriate use of anti-TB drugs has resulted in the emergence of drug-resistant strains which must be monitored closely to control their spreads. Multi drug resistant tuberculosis is a specific form of the drug resistant tuberculosis. When the tuberculosis bacteria are resistant to Isoniazid and Rifampicin, the two most powerful anti-tuberculosis drugs, with or without resistance to other anti-tuberculosis drugs, they are known as multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB).

Objectives

In the proposed project, we evaluated PCR based molecular diagnosis method for the detection of M. tuberculosis from suspected AFB positive casesas a routine method for early diagnosis of tuberculosis. We also investigated multiple drug resistance (MDR) mutation by multiple allele specific PCR (MAS-PCR) and sequencing.

Methodology

Samples: Within three years work time we have collected more than 1200 MTB suspected patient, some of which were multiple drug resistant. The study included collection of sputum sample only from routine patient.

Identification of *Mycobacterium tuberculosis*

Microscopy and Conventional Culture method

Acid-fast stain was performed using Ziehl-Neelsen staining method and the slides were examined under the oil immersion lens. The presence in Acid-fast staining of acid-fast bacilli in the culture was confirmed. *All the sputum* samples after being processed were subjected to cultivated onto L-J media. After 6 to 8 weeks of incubation, *M. tuberculosis* appeared as yellow, dry, irregular and raised colonies on the L-J medium. Drug susceptibility of the isolates to isoniazid (INH) and rifampicin (RIF), was performed by standard proportion method (Strong and Kubica, 1981), where diluted samples were inoculated on both the control and drug containing media and incubated at 37°C. The first reading was taken on 28th day of incubation and the second on 40th day.

M. tuberculosis was also detected using molecular based method by amplifying IS6110 sequence.

Molecular based method for analysis of drug resistance mutations

Eleven hundred and thirty-five sputum samples were collected from suspected patients attending the NTLR, NIDCH, Dhaka over 3 months period. A portion of each decontaminated sputum sample was used for initial identification and conventional drug susceptibility test.The other portion was preserved at -20°C for later molecular analysis.

For DNA sequencing purpose, DNA was extracted from clinical isolates grown on L-J medium. On the other hand, for IS6110 PCR assay and nested MAS-PCR assay, DNA extraction was done directly from sputum samples. Insta-Gene Matrix (Bio-Rad, Hercules, CA) was used to extractDNA from isolates grown on L-J medium and RTP- Mycobacteria Kit was used to extract DNA from sputumsamples following anufacturer's instructions. DNA was eluted in a final volume of 100 ll and stored at -20°C.

PCR amplification of IS6110, RRDR and DNA sequencing

To confirm presence of *M. tuberculosis* in all sputum samples, the IS6110 PCR assay was performed using specific primers.The RRDR (81 bp) of the rpoB gene was amplified from template DNA extracted from isolates using primer pairPR1 and PR2. For DNA sequencing, the PCR products were then purified with the AccuPrep-PCR Purification Kit (Bioneer,Daejeon, South Korea) according to the provide dinstructions.

Nested multiplex allele-specific PCR

A previously established MAS-PCR assay was adapted to suit RIF-associated mutation detection directly from sputum samples. First-round PCR was performed using primer set PR1 and PR2. First-round PCR products wer ethen used as template for next round of nested MAS-PCR.To detect RIF-associated mutation by absence of bandin electrophoresis gel (AB-nMAS-PCR), three allele-specific forward primers (rpoB516, rpoB526 and rpoB531) andone common reverse primer (RIRm) were combined into a single PCR reaction mixture. These allele specific primers amplify only if the targeted loci are wild type.Molecular based method was also used for the amplification of KatG gene and the mutations responsible for isoniazid resistance were analyzed by sequence analysis.

Results

Our main objective was to establish molecular based method as a tool for detection of TB bacilli as early as possible upon admission to hospital so that a physician can start therapy otherwise it takes 6-8 weeks to grow the TB bacilli by conventional culture method.

In the first phase of our study, we compared the molecular method with the conventional diagnosis procedures (N=135), where L-J medium culture results have been used as the "gold standard."The direct smear staining yielded 44(32.6%) of the sputum samples to be acid-fast positive, and fluorescence microscopy using auramine O staining further increased the number of positive samples to 67(49.6%). The culture and biochemical tests showed 75(55.6%) of the sputum samples to be culture positive, positive. On the other hand, PCR yielded 93(68.9%) positive results, 20(21.5%) of which were culture-negative sputum specimens. The PCR positive and culture negative samples were further confirmed by solution hybridization test.

In the second phase, emphasis was given to rapid detection of multi-drug resistant tuberculosis (MDR-TB) bya modified nested multiplex allele-specific polymerase chain reaction (MAS-PCR) method. For this we analyzed 1135 sputum samples.

All sputum samples were tested for the presence of M. *tuberculosis* complex (MTBC) using BACTEC-MGIT-960 liquid culture system followed by SD Bioline TB Ag MPT64RAPID assay. Among the 1135 clinical samples, 323 were positive for MTBC. These 323 MTBC positive liquid cultures were then subcultured on solidL-J media. Conventional culture based DST was performed for all 323 isolates grown on L-J slant and the results showed that 54 samples contained multidrug resistant (MDR) strains of *M. tuberculosis.* RIF resistance was shown by 56 samples (54 MDR and 2 rifampicin mono-resistant samples) and 267 were susceptible to both INH and RIF.

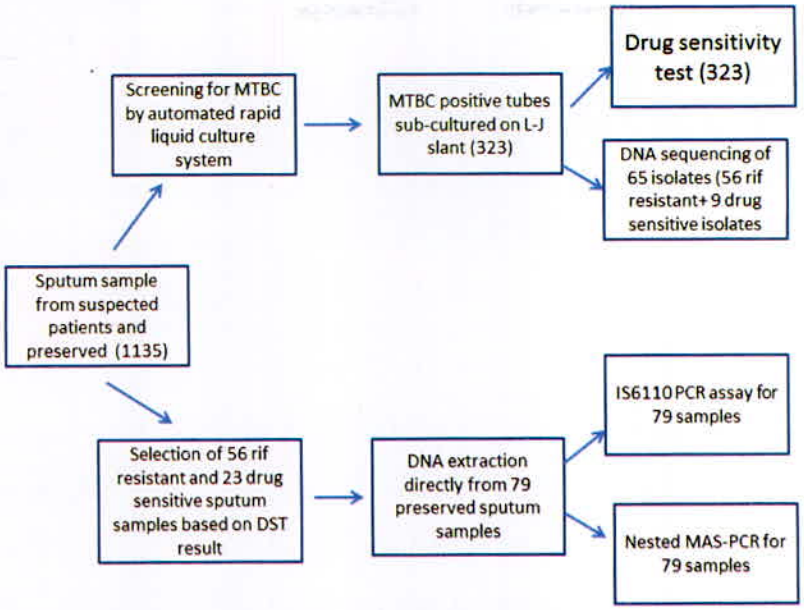

Fig-1 Flow chart showing available drug susceptibility testing, sequencing, and nested multiplex allele-specific PCR results

Based on DST result of 323 isolates, 79 sputum samples (54 MDR samples and 2 rifampicin mono-resistant samples and 23 drug sensitive samples), that were preserved immediately after sputum decontamination, were selected for nested MAS-PCR. Of the 79 samples, 65 including all 56 rifampicin resistant samples were sequenced. DNA sequencing detected point mutations in the RRDR of rpoB gene. According to DNA sequencing results, 54 out of the 56 samples contained point mutation in the RRDR, which accounted for 96.43% of the RIF-resistant samples. Two types of Nested MAS-PCR were performed for all 79 sputum samples. In case of nested MAS-PCR where absence of band indicates mutation (AB-nMAS-PCR), distinct patterns of bands were obtained for different mutation profiles at the three targeted loci. The concordance between nested MAS-PCR and DNA sequencing was found to be 96.3%. When compared to DST, the sensitivity and specificity of the nested MAS-PCR assay for RIF resistance-detection were determined to be 92.9 and 100% respectively.

The analytical sensitivity of nested MAS-PCR protocol was measured and compared with IS6110 PCR protocol. The IS6110 PCR protocol was able to produce visible and on electrophoresis gel when cell concentration per ml of sputum was as low as 50. The nested MAS-PCR (first and second round combined) protocol showed sensitivity similar to that of IS6110 PCR protocol detecting 50 cells ml^{-1} of sputum. On the other hand, MAS-PCR assay without nesting could not even produce visibleband from 5 x105 cells ml^{-1} of sputum. This meansthat, in case of sputum samples, the nested MAS-PCR approach would show better sensitivity than conventional MAS-PCR (without nesting) method. When applied to 79 sputum samples, the first round of nested MAS-PCR using PR1 and PR2 primer pair produced visible band in electrophoresis gel for 65 samples. Although, rest of the14 samples did not produce any visible band in first round PCR, visible bands were produced after second round of MAS-PCR.

Conclusion

Molecular based diagnosis of tuberculosis is fast, easy, sensitive and specific system for detection of pathogen as well as drug resistance. The AFB negative cases can also be detected by Molecular method. The turn around time can be reduced to 24 to 48 hrs. Early detection can help early treatment, thereby reduce mortality and as well as hospital staying. Chance of transmission to others can be reduced. Ultimately overall TB burden will be reduced.

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Inventory of Threatened Vascular Plants of Bangladesh for Conservation and Production of a Red Data Book

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Institution: Department of Botany, Faculty of Biological Sciences, University of Chittagong

Duration: Three years (2012 - 2015)

Expenditure of the project: Tk.1000000.00

Introduction

The IUCN has given a grim statistics that over 12.5% of the world's vascular plants are threatened at the global scale. It is being a global concern to protect and save the threatened biodiversity for restoring the environment. Most of the developed countries including India have made complete inventory of their threatened biodiversity and published Red Data Books.

Bangladesh is endowed with rich plant resources on which we depend variously for our existence. This plant resource is impoverished by continuous loss and degradation of the plant diversity from the nature due to several biotic and abiotic interferences, but no concrete attempts have been made to check the process until Professor M. S. Khan of Dhaka University took the initiative in 1991 and published a list of threatened vascular plants of Bangladesh for the first time (Khan 1991). Khan et al (2001) then finally produced a Red Data Book of vascular plants of Bangladeshfrom Bangladesh National Herbarium with 106 threatened species which is the first of its kind in Bangladesh. As per IUCN estimation about 12.5% of the total species of Bangladesh estimated to be under threatened categories. It is, therefore, considered to be the first and foremost attempt of scientists of natural sciences and the government of Bangladesh to take initiative to identify the remaining threatened species of plants and to assess their status in the wild as per IUCN's direction for implementing appropriate conservation measures, and hence, this project was proposed to the Ministry of Education and conducted under GRANTS FOR ADVANCED RESEARCH IN EDUCATION with a view to make a complete inventory of the threatened vascular plants of Bangladesh and production of a Red Data Bookfor taking conservation management.

A complete inventory of the threatened species of 13 angiosperm families has been made under this project. A total of 211 species are recognized as IUCN Red List Categories and presented in this volume of RED DATA BOOK OF FLOWERING PLANTS OF BANGLADESH with necessary information for proper conservation management. The families are: Anacardiaceae, Annonaceae, Apocynaceae, Asclepiadaceae, Begoniaceae, Boraginaceae, Cucurbitaceae, Magnoliaceae, Menispermaceae, Myrsinaceae, Periplocaceae, Rubiaceae and Vitaceae, and represented in Bangladesh by a total of 497 species of which 211 (about 42.5%) are recognized as threatened. Of these, 52 species are extinct, 128 endangered, 20 vulnerable and 11 other categories. The species which are recognized as extinct have not been collected or no report of collection after their first collection from the area of Bangladesh for about 80 to 200 years. There are no reports of second collection of 5 species since Roxb. (1832), 12 species since Wallich (1828-1849), 1 species since G. Mann in 1828 (Barbhuiya&Gogoi 2010), 17 species since Hook.f.(1872-1890), 1 species since Kurz (1877), 1 species since Gamble in 1880 (Das 2010), 2 species since Badal Khan in 1885 & 1887 (Das 2010), 3 species since Prain (1903), 2 species since Brandis (1906), 1 species since Kanjila in 1920 (Barbhuiya & Gogoi 2010), 5 species since Heinig (1925) and 1 species since Cowan (1932). The threatened categories and conservation status of plants have been determined through intensive field investigations throughout the flora, survey of the herbarium specimens available at different Herbaria and consultation of floristic literature relevant to the flora of Bangladesh. Intensive survey of the flora and collection of specimens have been conducting since independence of Bangladesh initially under the active participation and guidance of Prof. M. S. Khan from the Herbarium of the Department of Botany, Dhaka University (presently DUSH: Dhaka University Salarkhan Herbarium) and there after Bangladesh National Herbarium, Dhaka, Bangladesh Forest Research Institute Herbarium at Chittagong (BFRIH), Bangladesh Council for Scientific and Industrial Research Herbarium at Chittagong (BCSIRH) and the Herbarium of Chittagong University (HCU).

M.S. Khan *et al.* (2001) for the first time published a Red Data Book of Vascular Plants of Bangladesh with 106 threatened species and stated the importance of making complete inventory of the threatened plants of the flora for conservation management. Later Rahman (2004) and Rahman *et al.* (2010) reported altogether 75 species under threatened categories to include in the Red Data Book of Bangladesh.

Objectives

The project aimed at -

1) making a complete inventory of the IUCN threatened categories of plants of Bangladesh (rare, vulnerable, endangered and extinct species);
2) producing a Red Data Book of flowering plants of Bangladesh for taking actionplan and setting up conservation priorities for endangered species by the Government of Bangladesh; and
3) producing a trained research team/manpower development in the field of biodiversity conservation and environmental management of Bangladesh.

In order to achieve these principal objectives both extensive and intensive field trips have been made throughout the flora of Bangladesh.

i) to investigate and identify the threatened categories of plants and to assess their status in the wild
ii) to collect and preserve the voucher specimens in the herbarium
iii) to collect propagating parts of threatened plants for ex-situ conservation
iv) to determine the degree of threatening and status of occurrence for setting up conservation priorities
v) to record the frequency of distribution and places of occurrence of threatened species in the flora
vi) to determine the causes of threatening of individual species for suggesting appropriate conservation measures
vii) to suggest specific conservation measures for specific categories of species
viii) to suggest specific site or ecosystem for in-situ conservation

To make assessment of Red List Categories of plants, information data have been collected by

ix) studying the herbarium specimens available in both national, local and international herbaria
x) surveying relevant floristic literature for distribution and occurrence in the flora, phenology and other botanical information

To make a trained and expert team in the relevant field, training workshops and seminars have been organized for

xi) M. Phil. and Ph. D. Research Fellows
xii) Junior teachers and researchers of Botany from CU and other colleges (field trip participants).

Methodology

The entire research has been conducted in 4 phases by adopting standard taxonomic methods as well as by applying techniques of assessment of Red List Categories of plants following field investigation, literature survey and herbarium examination.

Phase 1: Botanical Exploration

i) botanical explorations have been made through repeated field trips toeach of the botanical divisions of the flora of Bangladesh by separate expert teams in different flowering seasons of the year
ii) red list categories (rare, vulnerable, endangered, critically endangered and extinct) of species have been determined through investigationsfollowing the IUCN method 'Global Standard of Threat assessment'
iii) collection, herbarium preparation and preservation have been made following the standard taxonomic method/herbarium technique
iv) propagating parts of some endangered species have been collected and planted in the conservatory block of Chittagong University Botanic Garden as an ex-situ conservation

Phase 2: Identification

Identification of collected specimens have been made in the Herbarium of Chittagong University by using Long Arm Stereo-microscope and updated nomenclature applied consulting World Plant List, Kew Plant List and other internet sources.

Phase 3: Consultation of Herbaria

Examination of Herbarium specimens and preparation of data sheets have been made by consulting Bangladesh National Herbarium (BNH), Dhaka University Salarkhan Herbarium (DUSH) and the Herbaria of Chittagong University (HCU), Bangladesh Forest Research Institute (BFRI) and Bangladesh Council for Scientific and Industrial Research Herbarium (BCSIRH).

Phase 4: Organization of training workshops and seminars: A training workshop on research methodology have been organized at thebeginning of the project activities to make a trained and expert team in order to

i) conduct botanical exploration
ii) make assessment of Red List Categories
iii) make documentation of information in data sheets
iv) make collection and identification of voucher specimens

Results

Outcome of this three years research project are as follows:

1. Complete Inventory of threatened taxa of 15 families of flowering plants have been made, and first volume of a Red Data Book of Flowering Plants of Bangladesh with the threatened plants of 13 families has been produced as per RDB Format (see App. II).
2. Field Data have been documented and Voucher specimens collected andpreserved at the Herbarium of CU.
3. Propagules of some rare and endangered species have been collected and grownin CU Botanic Garden as ex-situ conservation.
4. A Trained and Expert Team in the field of biodiversity assessment and conservation management has been produced.
5. A Data Bank is created and complete information data of the plants of 15 families (Anacardiaceae, Annonaceae, Apocynaceae, Asclepiadaceae, Begoniaceae,Boraginaceae, Cucurbitaceae, Magnoliaceae, Menispermaceae, Myrsinaceae, Periplocaceae, Rubiaceae, Vitaceae) and preliminary data of another 7 families (Acanthaceae, Araceae, Celastraceae, Euphorbiaceae, Fabaceae, Verbinaceae, Zingiberaceae) have been stored for the second volume of the Red Data Book.

Conclusion

The present project of conducting family wise inventory of threatened plants of Bangladesh under Higher Education Research Project of the Ministry of Educationis for the first time. The status of occurrence of the plants of 15 families occurring in the flora of Bangladesh has been inventoried completely (App. I). Initially the first volume of Red Data Book of Flowering Plants of Bangladesh is published with the result of inventory of 13 families consisting of 235 threatened plants suggesting for immediateconservation management. These 13 families are represented in Bangladesh by 497 species of which 52 species (more than 10%) have no report of collection after their firs tcollection from the area for about 80 to 200 years which are supposed to be extinct, andsecondly, 159 species (about 32%) occur under different threatened categories which is alarming to the environmental health of the country. Significant achievement is, therefore, made through this research project which indicating the urgency of conducting complete inventory of the threatened plants of Bangladesh immediately through such projects for taking proper conservation management before disappearing the vulnerable especies from the flora.

Publication

An international standard Red Data Book with colored field photographs of threatened plants. Red Data Book of Flowering Plants of Bangladesh, vol. 1, pp. 235.

Inventory of threatened plants of Bangladesh and their conservation management. *International Journal of Environment* 3(1): 141-167 (2014).

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Effectiveness of an Intervention Program on Risk Pregnancy Among Reproductive Aged Group of Couples in Rural Community to Achieve Millennium Development (Goals-5)

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Institution: National Institute of Preventive and Social Medicine (NIPSOM)Mohakhali, Dhaka-1212.

Duration: One Year (2012 - 2013)

Expenditure of the Project: Tk.500000.00

Introduction

Bangladesh has a high maternal mortality ratio, with 320 deaths per 1000 live births and about 11,000 to 12,000 women die from pregnancy and childbirth related complications every year in Bangladesh. Many of those who survive often suffer from diseases or health problems carried from childbirth. Official estimate says 32 Bangladeshi women out of a thousand die during childbirth, a high maternal mortality rate blamed on the dependence on unskilled midwife. Nearly 90% of deliveries are done at homes assisted by illiterate and unskilled women who work as "dai". Eighty percent of the deaths occur at home. The process of becoming a mother from conception to delivery involves a mixed feeling, it's a time of pangs as well as joy. Many women in Bangladesh die in the process of becoming mother. That is not something unusual in a country where only 14% women, who live in cities, have access to health care, while 86% do not. The figures are staggering. At least 24 lac women in Bangladesh are at risk of suffering pregnancy related complications a year. In 2001, 400 out of one lac women died from such complications.

Maternal mortality in Bangladesh is alarmingly higher than in other developing countries. At right time correct decision is very much important issues to perform safe delivery. Maternal death and disability are the leading cause of healthy life years lost for developing country women of reproductive age, accounting for more than 28 million disability-adjusted life years (DALYs) lost and at least 18% of the burden of disease in these women. For each woman who dies, an estimated 100 women survive childbearing but suffer from serious disease, disability, or physical damage caused by pregnancy related complications. Long-term consequences of pregnancy-related complications include uterine prolapse, pelvic inflammatory disease, fistula, incontinence, infertility, and pain during sexual intercourse.

Intervention on risk pregnancy and childbirth complications can sensitized to the reproductive age group of women to do need based action, for good pregnancy out-come. Intervention programme directly influences and motivate them no doubt to take wise decision to make pregnancy safer in rural community. There is limited information regarding intervention strategies on safe motherhood in Bangladesh. Hopefully this, small study will collect qualitative data to give further information about intervention strategy on safe motherhood. The document of the research findings and experiences will be analyzed particularly in relation to study objectives. In future, planners, researcher will do action plan, policy and research in this field for successful safe motherhood. Finally the research findings will be disseminated globally through seminars, workshops and publications for fruitful action oriented further research to reduce maternal mortality, morbidity and childbirth complications in developing countries like Bangladesh to achieve Millennium Development Goal (MDG-5).

Objectives

General objectives:

The objective of the study was to assess the awareness of risk pregnancy and childbirth complication among the reproductive age group of rural couple before and after intervention

Specific objectives : Divided into three stages. These are:

First phase Objectives

- To assess he awareness of married reproductive age group of couple about risk factors in pregnancy and their consequences;
- To assess awareness about the importance of prenatal care for detection of risk pregnancy and childbirth complications; and
- To find-out the socio demographic status of the respondents.

Second phase Objectives

- Apply intervention strategy by counseling of the respondents

Third phase Objectives

- To explore the awareness after intervention;
- To compare the awareness before and after intervention; and
- To compare the awareness with different variables.

Methodology

Study design

The study was an experimental study due to intervention (quasi-experimental-"pre test-post test") study. The study was conducted in four (4) unions of one upazilla under one district from one division of the country-Chittagong (purposively selected).

Pretest – Post test design

Study site

Four (4) unions were selected (randomly) out of 14 unions under one selected upazilla, Comilla Sadar Daxin upazilla, Comilla. Two villages were selected from each union (randomly). Total eight (8) villages were the study site.

Four union were	*Villages were*
Perul South	Aliswar, Chaknondi.
Perul North	Vora, Khalilpur
Vuluin	Jadabpur, Golachou
Bagmara South	Dattapur, Jaikanta

Sample size determination: by Z2 Formula

Procedure of data collection

a) Baseline data collection 1st phase

b) Intervention Phase 2nd phase

c) Post test data collection 3rd phase

d) Edit data, analysis and report writing

Results

Table1 : Age of respondents (wife)

Classification of Age	Number	Percentage (%)
17-22	24	16
23-28	58	38.37
29-34	30	20
35-40	38	25.33
Total	**150**	**100**

Table 2: Place of Delivery of last child (wife)

Characteristic	Number	Percentage (%)
Home	98	65.33
Govt. Hospital	16	10.67
Privet Clinic	36	24
	150	100

Table 3 : Knowledge: Problem during Pregnancy (wife)

	Number	Percentage (%)
Excess vomiting	124	
Bleeding /vagina	18	
swelling hands &legs	48	
High Blood Pressure	8	
Convulsion	36	
Multiple answer		

Table 4 : Religion of the client (both husband & wife)

Characteristic	Number	Percentage (%)
Islam	64	42.67
Hindus	32	21.33
Buddhist	54	36
Total	150	100

Table 5 : Client's education level (wife)

Education of level	Number	Percentage (%)
Illiterate	16	10.67
Only sign	26	17.33
Class: I-V	23	15.33
Class: VI-X	52	34.68
Class: XI-SSC	14	9.33
Up to HSC	8	5.33
HSC +	11	7.33
	150	100

Table 6 : Occupation of the client (wife)

Characteristic	Number	Percentage (%)
House wife	98	65.33
Service	24	16
Small business	28	18.67
Total	150	100

Table 7: Knowledge: About danger signs of Pregnancy (wife)

Knowledge danger signs	Number	Percentage (%)
Yes	85	56.67
No	65	43.33
	150	100

Table 8 : Correct answer about knowledge of Danger (N=150)

Answers	Number	Percentage (%)
Correct answers	61	59.33
Incorrect answers or No answer	89	40.67
		100

Table 9 : Husband's knowledge: about danger signs of pregnancy

Knowledge of danger signs	Number	Percentage (%)
Yes	43	28.66
No	107	71.34
	150	100.00

Table 10 : Husband's knowledge: Problem during pregnancy

Characteristics	Number	Percentage (%)
Excess vomiting	85	56.66
Swelling hands & legs	65	43.34
	150	100.00

Table 11 : Standard guide line Table: Danger sign of pregnancy

Sl. No	Danger signs
1	Bleeding per vagina
2	Obstructed labour
3	Severe headache / blurring of vision
4	Legs or Hands comes out / vagina
5	Convulsion

Table 12: After intervention: Result of effectiveness

	Number	Attend: education		Remarks
Husband	150	98	50% Effective	Effectiveness
Wife	150	132	75% Effective	Scoring method

Conclusion

Among the couple less than 50% had knowledge regarding risk pregnancy but knowledge was not correct except few. After intervention (education program) knowledge had increased up to the satisfactory level.

Production Performance of Over-wintered Freshwater Giant Prawn *(Macrobrachium rosenbergii)* in Polyculture with Common Carp *(Cyprinus carpio)* for an Early Crop

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Duration: One year (2012-2013)
Expenditure of the project: Tk. 500000.00

Introduction

In greater Khulna region, paddy-prawn-fish culture system is being increasingly practiced under the backdrop of negative social and environmental consequences of mono culture of prawn/shrimp. Such technological shift has been creating a very positive impact in terms of various socioeconomic and environmental indicators (Ahsan, 2010). Production from crop rotation technology of paddy-prawn/fish farming could be substantially increased if it were possible to raise an early crop, particularly prawn in the first half of the year (Karim 2006, Karim 2010). However, both monsoon fed water and freshwater prawn *(Macrobrachium rosenbergii)* fry are unavailable or scarce during the first quarter of the year whereas the common carp fry can be found as early as February. While the scarcity of water issue can be addressed through the use of irrigation waters or tidal waters to some extent, availability of hatchery produced prawn fry remains to be the major obstacle. Besides, shifting of monsoon periods towards the third quarter of the year in recent years, apparently due to climate change, further limits the grow out period for aquaculture. It was, therefore, necessary to investigate the technical and economic feasibility of raising an early crop from over-wintered prawn with common carp *(Cyprinus carpio)* during the first half of the year. Since hatchery reared prawn fry are not available during the first quarter of a year, the study investigated the feasibility of using over wintered prawn stocked at the end of previous year for growing out along with common carp from February of the following year, the beginning of the culture period.

Objectives

The objectives of the research were:

i) to investigate the technical and commercial feasibilities of raising an early aquaculture;
ii) crop with over-wintered late season prawn and common carp juveniles; and
iii) to conduct a preliminary survey to identify ideal locations with suitable water sources for raising an early prawn-fish mixed aquaculture crop besides a traditional aquaculture crop.

Methodology

The study was carried out in two overlapping phases:

Field study to compile knowledge on the state of affairs:

Table 1: Distribution of sample f or data collection from field

District	Number of FGD	Number of Interview
Khulna	01	40
Bagerhat	01	40
Narail	-	40
Gopalgonj	-	40
Total	**02**	**160**

Compilation of knowledge on the management of fish-prawn-crop rotation/integration practice in the greater Khulna region with particular reference to the vulnerability context of the farmers and the existing coping strategy towards seed supply and water resources use was carried out. Data were collected essentially through using participatory approaches viz. Focus Group Discussion (FGD) with farmers and questionnaire

Table 2: Pond experimental design

Stocking density/decimal		Over wintered prawn (1.2 g size)		
		40	60	80
Common carp (8 g size)	15	40+15 Treatment-1 (T_1)	60+15 Treatment-2 (T_2)	80+15 Treatment-3 (T_3)
	20	40+20 Treatment-4 (T_4)	60+20 Treatment-5 (T_5)	80+20 Treatment-6 (T_6)

Pond experiment to investigate the technical feasibility of raising an early crop:

The pond experiment was carried out at on-campus Experimental Pond facility of FMRT Discipline. Six treatments each with three replication of species composition were investigated for about six months starting in March 2013 (Table 2). The ponds were properly treated beforehand following standard liming and fertilization regimes.

The fish were given supplementary feed of proven quality (30% final protein level) twice daily at a food ration equivalent to 8% of their total body weight/day for two months, 6% for another two months, and 5% until final harvest.

The fish were sampled fortnightly to monitor various growth and survival parameters. Key water quality parameters. A simple economic analysis was performed to estimate the net profit (total returns from harvest - total cost of production) and cost-benefit ratio (CBR = total benefit – total cost) from prawn with common carp for different treatments, separately. One-way ANOVA test was performed to identify any significant difference among treatment means.

Results

Field study: The field study gathered various information on current prawn culture practices amongst which the source of water and timely availability prawn PL were most crucial. Most of the farmers stocked prawn PL during August for over wintering. The PL's were transferred to the canal in winter season when paddy had been growing. The paddy cultivation used to continue until February. During this period, the last year's PL experience overwintering and become juveniles in the beginning of the following year (Feb./March). Though there was availability of common carp fry, the farmers didn't stock them with prawn because of their lack of knowledge and lack of culture technology. However, farmers showed interested in polyculture of prawn and common carp if an early harvest can be realized.

Pond experiment: The pond experiment was carried out to optimize prawn and common carp stocking density to achieve the highest production performance. The prestocking management included liming and fertilization prior to stocking while post stocking management included supplementary feeding at a fixed rate. The water quality indices among the treatments appeared to be within the optimal range for aquaculture and thus had no significant effects on the dependent variables.

Table 3: Combined growth performance of prawn and common carp

Parameters	T_1*	T_2	T_3	T_4	T_5	T_6
Prawn yield (kg/ha/180 days)						
Yield	343.61 ± 11.06^{a}	474.87 ± 10.97^{b}	490.76 ± 80.50^{c}	315.85 ± 10.86^{d}	445.01 ± 20.28^{e}	451.33 ± 10.37^{e}
Common carp yield (kg/ha/180 days)						
Yield	2262.75 ± 68^{a}	2307.98 ± 80^{a}	2072.12 ± 87^{cd}	2103.85 ± 58^{cad}	2195.69 ± 70^{a}	2005.51 ± 66^{d}
Combined yield (kg/ha/180 days)						
Yield	2606.37 ± 70.95^{a}	2782.86 ± 107^{b}	2562.88 ± 172^{ad}	2419.70 ± 76^{e}	2640.70 ± 98^{a}	2456.83 ± 82^{cd}
FCR	1.93 ± 0.01^{ac}	1.83 ± 0.02^{b}	1.94 ± 0.03^{ac}	1.99 ± 0.05^{cd}	1.87 ± 0.04^{ab}	2.05 ± 0.08^{d}
* Data in the same row having different superscripts are significantly different ($p<0.05$)						

Production performance was found to be different for overwintered prawn and common carp in six different treatments. The specific growth rate (SGR/day) of prawn ranged from 2.10 to 2.28 where the highest weight gain was found in T_2 (52.44±0.62 g) and the lowest in T_6 (40.31±0.40 g.) On the other hand, common carp registered a sharp growth performance during the same period. The SGR of common carp ranged from 2.29 to 2.58% where the highest weight gain was observed for the same treatment i.e., T_2 (628.70±3.44 g) closely followed by T_3 and T_1 whereas the lowest weight gain was recorded from T_4 (432.35±2.06 g). Considering data for both prawn and common carp T_2 appeared to be best in terms of weight gain, SGR and other growth parameters (Table 3).

Conclusion

The study was undertaken for investigating the technical and commercial feasibilities of raising an early aquaculture crop with over-wintered late season prawn and common carp juveniles. From field survey, farmers' existing prawn farming practice with regard to wintering of PL was identified by field survey. The pond experiment optimized the stocking density of prawn with common carp in polyculture. In the experiment, stocking density of 60 PL/decimal for prawn with 15 carp/decimal for common carp produced the best result.

Shifting of monsoon periods towards the third quarter of the year in recent times, apparently due to climate change, limits the grow out period for aquaculture. It is, therefore, highly recommended to produce an early crop of prawn using over wintered prawn juveniles in poly culture with common carp. This is particularly relevant for the coastal communities because the risks and vulnerabilities of the poor who live in insecure places and need to build their resilience to cope with climatic fluctuations will be immensely benefited by being equipped with the results of this study.

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