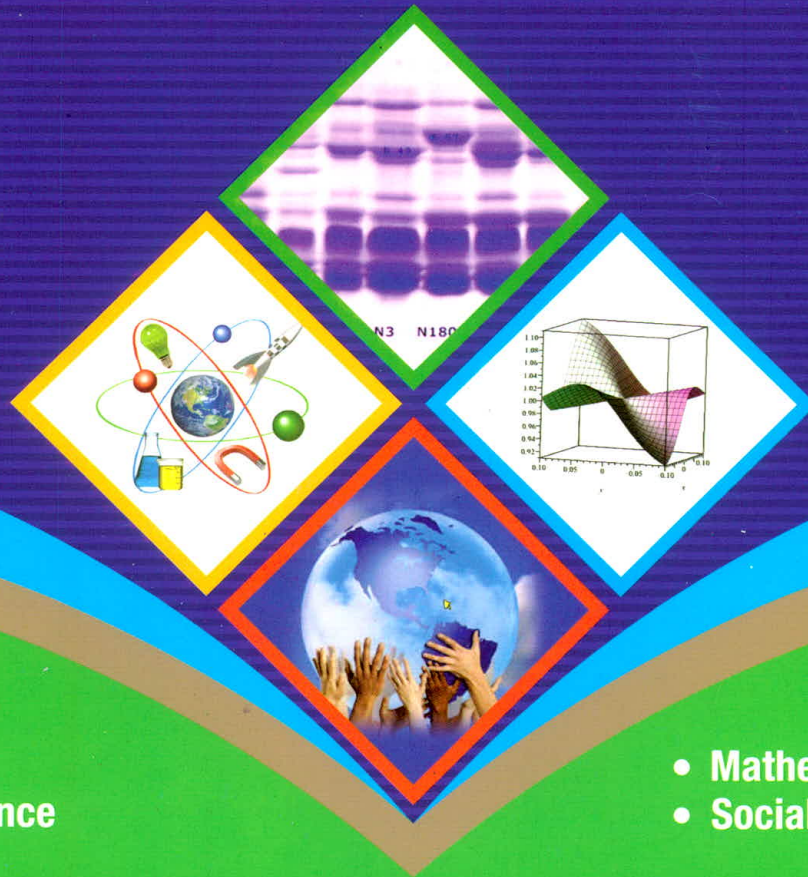




Volume 2

*Report on
Advanced Research*
2013-2015



- Life Science
- Physical Science

- Mathematical Science
- Social Science

Grants for Advanced Research in Education



**Secondary and Higher Education Division
Ministry of Education**



Ministry of Education



Report on Advanced Research 2013 - 2015

Grants for Advanced Research in Education (GARE)

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



PRIME MINISTER
GOVERNMENT OF THE PEOPLE'S REPUBLIC OF
BANGLADESH

Message

I am happy to learn that Ministry of Education is going to publish the 2nd report on its completed research projects under the program Grants for Advanced Research in Higher Education (GARE) during past two years.

The Awami League government has relentlessly been working to turn Bangladesh into a middle income country by 2021 and a developed one by 2041. In this regard, we have attached top priority to education sector. A time-befitting and scientific 'National Education Policy' has been enacted to ensure quality education. We adopted 'Private University Act 2010' to boost up higher education facilities. We need extensive research in all branches of knowledge especially in the field of science and technology. Our government has been providing funds to the university teachers and researchers 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 researches carried out under GARE will contribute to develop our socio-economic condition and inspire others to embark upon researches in newer areas.

I hope, these types of research programs will play an important role in building a Golden Bangladesh as dreamt by the greatest Bangalee of all time, Father of the Nation Bangabandhu Sheikh Mujibur Rahman.

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

Bangladesh has witnessed tremendous growth and development in all its sectors during the past decade. It is extremely important that the education sector is also constantly upgrading to complement and cater to the national and international demands.

In order to focus on cultivating the right value systems among the youth and skills development alongside communication, critical thinking is now necessary to contribute effectively to the productive outputs of the country. The time has come when the world is actively replacing laborers with automation. So the capital-intensive industries are on the rise. There is an ever urgent need to hunt down specific skills that can co-exist with automation and robotic of the future.

Thus under the Grants for advanced Research in Education (GARE), we strive to provide facilities for research to achieve excellence. This could be attained better in the future if our talented young generation of researchers come up and do their best.

Here in the Second Report for Advanced Research, we have presented abridged versions of all projects funded so far. This publication represents the efforts, achievements and potential of the eminent scientists of our country. I hope that the publication may serve as a representation of all the work put forward by the promising and budding scientific community.

The GARE now hopes to go forward achieving dynamic results in the next phase of the project and thus contribute in improving the fate of the growing population.

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

Bangladesh is gradually becoming a growth and development model in all sectors during the past decade. The improvements in the social development indicators were even more impressive, making Bangladesh a leader from a laggard in relation to per capita income in cross-country comparisons. Despite less public expenditure on the education sector compared to the demand, the achievements of the government are appreciable, which is a matter of pride.

Successful businesses thrive due to research and development. Federally funded research helps the economy to grow because it opens up job opportunities. The country can benefit, too if researchers and their right creative minds get together and an idea can become visible using fund to pursue research. Some of the talented academics in higher education were supported by the government and govt. needs to continue to do so in order to let the next set of brilliant youths accomplish their research goals. The research grant selection is done carefully so that the scientists requesting for these grants can prove that the research they wish to conduct is actually worth paying for and will ameliorate society in some way. In order to achieve better healthcare, technology or just a more advanced society, supporting government research funding should be a top priority. I believe the achievements of our researchers will be demonstrated through publication of their research findings in this report.

স্বাক্ষর

Md. Sohorab Hossain



Mesbahuddin Ahmed
President

Editorial committee
Grants for Advanced Research in Education (GARE)

Preface

I am happy to find that we have successfully brought out the Second Report on Advanced Research (2013-2015). This edition contains valuable articles from the researchers who have completed their projects with financial support from 'Grants for Advanced Research in Education (GARE), Ministry of Education. These projects encompass basic science to agricultural science and life sciences.

One of the greatest assets of Bangladesh is its people. We must now focus our attention to develop human resources. The government of Bangladesh has taken the right steps in funding research. It is widely known that economic growth in this modern era largely depends on the welfare of its people and development of science and technology in the country. Our country now requires skilled and efficient manpower. We have been exporting mostly un-skilled labour abroad to work to earn our much needed foreign exchange to meet the import bills. The earning from human resources abroad could be multiplied if we could export skilled technicians, expert farm workers and educated manpower. The government of the People's Republic of Bangladesh has undertaken the initiative to fund generously the research projects. The innovative researchers are now getting opportunity to do research and contribute to the development of the country.

I am confident that this effort will bear fruits for our future generation.

A handwritten signature in black ink, appearing to read 'M. Ahmed'.

Mesbahuddin Ahmed



Md. Fashiullah
Member Secretary
Editorial Committee &
Director General
BANBEIS

Foreward

This is a great pleasure for me that we are going to publish the Second Report on completed Advanced Research Projects, 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 several programmes, advanced research is one of them. Recently, the Government of Bangladesh is providing its remarkable budget in education research sector. As a consequence, we may hope that research sector in Bangladesh will be able to achieve its goal.

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

Md. Fashiullah

Executive Summary

Introduction

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. The Ministry took initiatives for the Grants for Advanced Research in Education (GARE) program and laid down policies on 24 June 2008 and subsequently the funding started in the Fiscal Year 2009 - 2010. 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 relevant fields, and befitting funding made available based upon the potential of the proposals and the credential of researchers submitting the proposal.

Aims and objectives

The GARE is funding on competitive basis for research in all public and private Universities, Post-Graduate Colleges of National and Open Universities, Public Medical Colleges and Universities and Engineering Universities since its inception. The aims and objectives of this research program are:

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

Areas of research

- Mathematical Sciences
- Life Sciences
- Physical Sciences
- Social Sciences
- ICT
- Marine Sciences
- Sustainable Development Goals and research to achieve 7th Five Years Plan Targets.

Eligibility for research grant

- The applicants must have sufficient research papers published in reputed journals.
- The research team/individual researcher should have experience on presenting papers in International/ National Seminar/Symposium/Workshop.
- On-going number of research programs of the researchers and physical facilities and international relationship of receiving organization must be satisfactory.
- The topic of the research should reflect the national need, 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.

Reports included in this publication

This publication includes total 36 Project Completion Reports (PCR) submitted by Principal Investigator of the projects and covers different areas of research under the purview of the Grants for Advanced Research in Education. There are 19 PCRS from life science, 14 from physical science, 2 from mathematical science and 1 from social science in this publication. The reports are of satisfactory standard and based on original work of the researchers. The learned and reputed Principal Investigators presented their study in brief indicating the outcomes/findings of their research.

Conclusion

Our expectation is that more talented researchers with innovative ideas would become interested and submit project proposals in the designated areas for GARE funds. The MoE and the GoB are generous to make more funds available for creative and need based research. We are also hopeful that our researchers will publish their research outputs in renowned journals home and abroad to highlight Bangladesh. We trust the technologies developed by our learned researchers using GARE fund would get IPRs and potential outcomes would go for commercial applications and thus industrial development of Bangladesh.

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Chapter 1

Funding Year

2013-2014

Synthesis, Characterization and Biological Activities of Some New Macrocyclicligands and Their Metal Complexes

Tapashi Ghosh Roy and Debashis Palit

Location: Department of Chemistry, University of Chittagong, Chittagong

Duration: Two years (2013-2015)

Expenditure of the project: Tk. 1782000.00

Introduction

14-membered tetraazamacrocyclic ligands and their metal complexes continue to attract significant attention owing to i) the relative ease of derivatization, and ii) their applications in a number of disparate fields. Recent investigations into their coordination chemistry have focused on a variety of considerations such as examining electron transfer processes, kinetics, magnetic properties, and structural characteristics. Metal complexes have also been used in crystal engineering [1] and in analytical chemistry [2]. In the realm of biological applications [3,4], metal complexes of compounds related to cyclam have been explored for their use in magnetic resonance imaging (MRI) [5] and radioimmunotherapy [6]. Further, a number of metal complexes have also been explored for their anti-fungal [7] and anti-bacterial [8] activities. It is the latter application that motivates much of our interest in this area and which has led to a number of structural studies. The vital functions exhibited by natural macrocyclic complexes like vitamin B12, chlorophyll, haemoglobin etc. can be mainly determined by the nature of the metal ions as well their confinement in the enclosed cavity of the macrocyclic ligand. But a clear understanding of the metal ion chemistry in these systems is still lacking due to their structural complexities. Moreover transition metals like Cu, Ni, Co V, Zn, Cd, Mo etc. are also very much important from biological viewpoint. So it appears interesting to investigate the behaviour of these metal complexes in macrocyclic environment as well as in linear analogues.

Objectives

So considering the above great importance, our research proposal was undertaken to achieve the following objectives:

- Synthesis of different new macrocycles and separation of isomers of ligands;
- Synthesis of N-pendent derivatives by the reaction of these ligands with different alkylating agents;
- Synthesis of their complexes by the reaction of the ligands with some transition metal salts;
- Characterization of the new ligands and their complexes by IR, NMR, EA, conductometric data, magnetic measurement and by X-ray crystallographic analysis;
- Study of the antifungal, antibacterial, antiviral & anticancer activities of the ligands and their complexes;
- Physico-chemical studies on the macrocyclic compounds;
- Preparation and characterization of linear analogues and same studies on them for comparison.

Methodology

All the ligands were prepared by the methods adopted in literature [7-10]. These ligands were allowed to react with different metal salts to prepare corresponding metal complexes. Then they were characterized by different physical methods (color, melting point, solubility etc), microanalysis (C,H,N,S, metals etc.), spectroscopic methods (IR,UV-VIS, NMR, MASS) and X-ray crystallography in some cases. Antifungal and antibacterial activities of the compounds were performed against some fungi and bacteria respectively under suitable conditions.

Results

In this study different new macrocycles, their reduced isomers and N-pendent derivatives of selected ligands, and different metal complexes of these ligands were synthesized. The macrocyclic ligands, Me6[14]diene (L) and its isomeric ligand tet-a (Fig-1) and tet-b; Me8[14] diene (L1) and its isomeric ligand LA, LB (Fig-2) & LC ; dimethyl tetrabenzyl substituted ligand Me2Bz4[14]diene(L2) (Fig-3) and N-pendent derivatives, LBZ (Fig-4), LCZ & LCZ1 of isomeric ligands LB & LC were prepared. Then the metal complexes of these ligands were prepared using different metal salts of Cd(II), Cu(II), Ni(II), Zn(II) and Co(II). On the other hand four non-cyclic diaza ligands designated as L3, L4, L5 & L6 and their complexes with Ni(II), Cu(II), Co(II) salt were also prepared. All newly prepared ligands and complexes were characterized using different analytical techniques such as, physical methods (color, melting point, solubility etc), microanalysis (C,H,N,S, metals etc.), spectroscopic methods (IR,UV-VIS, NMR and MASS) and X-ray crystallography in some cases. The molecular structure of one cobalt complex, two copper complexes and a non-cyclic ligand were confirmed by X-ray crystallography. The antibacterial and antifungal activities of these ligands and complexes were tested against different gram positive and gram negative bacteria and fungi. The activities of these compounds were remarkable in some cases and for some compounds the activities were more than the commercial standards. From this project one Ph. D and five MS degrees were awarded from this department and five good papers were published where the grants from Ministry of Education, Peoples' Republic of Bangladesh under GARE is acknowledged. The research works done under this project were presented in different national and international scientific conferences.

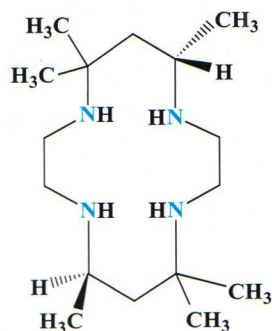


Fig.1: tet-a

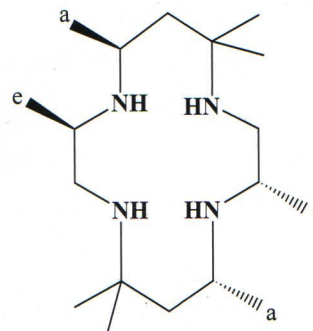


Fig.2: L_B

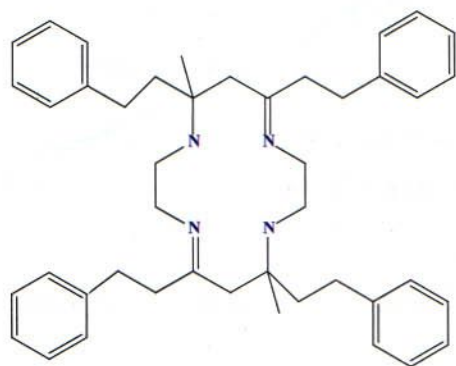


Fig.3: Me₂Bz₄[14]diene

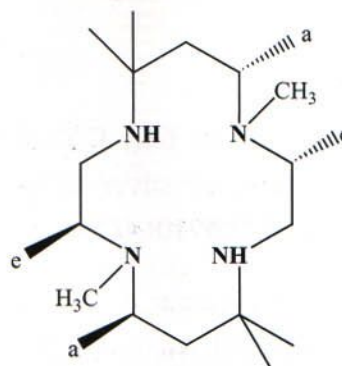


Fig.4: LBZ

Conclusion

As a whole, this successfully completed research has enriched the research quality of the department as well as country to a large extent. The achievement in the biological part will be informative for the drug discovery section. And further studies are warranted in this sector in future for its higher applicability and importance in the different sector. The successful completion of this research enhanced the present level of knowledge in this field. The newly produced compounds may be used as drugs or a component of drugs which may help nation in various ways. So this study will contribute greatly to the socio-economic development of our country and the knowledge of human being will also be improved in this area.

Publications

- Yasmin, S., Rabi, S., Biswas, F. B., Roy, T. G., Olbrich, F., Rehder, D. 2017. Synthesis, characterization and antimicrobial studies of zinc(II) complexes with hexamethyl-tetraazacyclotetradecadiene Me₆[14] diene (L) and C-chiral isomers of its reduced analogue. Crystal and molecular structure of [(ZnL)₂(NO₃)₂](ClO₄)₃. *J. Inclu. Pheno. Macro. Chem.*, 87: 239-250.
- Biswas, F., B., Roy, T. G., Islam, R. S. 2016. Synthesis, characterization and electrolytic behavior of cadmium (II) complexes of 5,7,7,12,14,14-hexamethyl-1,4,8, 11-tetraazacyclotetradeca-4,11- diene and isomers of its saturated analogue. *Eur. Sci. J.*, 12(12): 186-200.
- Rabi, S., Habib, A. Z. M. A. and Roy, T. G. 2016. Synthesis, characterization and antimicrobial activities of copper(II) complexes of a tetraazamacrocyclic ligand. *BGC Trust University Journal*, 2:17-30.
- Roy, T. G., Hazari, S. K. S., Miah, H. A., Gupta, S. K. D., Roy, P. G., Behrens, U and Rehder D. 2014. Synthesis and antimicrobial activities of copper(II) complexes of N(4),N(11)-dimethyl (LBZ & LCZ) and N(4) monomethyl (LCZ1)-3,5,7,7,10,12,14,14- octamethyl-1,4,8,11-tetraazacyclotetradecane. Crystal and molecular structure of [CuLCZ1](ClO₄)₂. *Inorganica Chimica Acta*, 415:124–131.
- Biswas, F. B., Roy, T. G., Rahman, M. A and Emran, T. B. 2014. An in vitro antibacterial and antifungal effects of cadmium (II) complexes of hexamethyltetraazacyclotetradecadiene and isomers of its saturated analogue. *Asian Pac. J. Trop. Biomed.*, 4(Suppl 2): S618-S623.

References

- Suh, M.P., Moon, H.R., and Lee, E.Y., Jang, S.Y., 2006. A redox-active two-dimensional coordination polymer: preparation of silver and gold nanoparticles and crystal dynamics on guest removal. *J. Am. Chem. Soc.*, 128:4710–4718.
- Singh, A.K., Panwar, A., Kumar, S. and Baniwal, S. 1999. Chromium(III)-selective electrode based on a macrocyclic compound. *Analyst*, 124:521-525.
- Singh, D., Kumar, K., Kumar, R. and Singh, J. 2010. Template synthesis and characterization of biologically active transition metal complexes comprising 14-membered tetraazamacrocyclic ligand. *J. Serb. Chem. Soc.*, 75(2):217–228.
- Shiekh, R.A., Shreaz, S., Khan, L.A. and Hashmi, A.A. 2010. Development and characterization of bioactive macrocyclic metal complexes, use as a potential drug. *J. Chem. Pharm. Res.*, 2(2):172-185.
- Silvio, A., Mauro, B., Simonetta, G.C., Giovanni, B.G., Giancarlo, J., Roberto, P. and Massimo, S., 1995. MRI Contrast agents: macrocyclic lanthanide(III) complexes with improved relaxation efficiency *J Chem. Soc., Chem. Comm.*, 18:1885-1886.
- Konig, B., Pelka, M., Zieg, H., Jones, P.G. and Dix, I. 1996. A new synthetic route to substituted cyclam macrocycles. *Chem. Commun.*, 4:471.
- Biswas, F.B., Roy, T.G, Rahman, M.A. and Emran, T.B. 2014. An in vitro antibacterial and antifungal effects of cadmium(II) complexes of hexamethyltetraazacyclotetradecadiene and isomers of its saturated analogue. *Asian Pac. J. Trop. Biomed.*, 4 (suppl2):618-623.
- Roy, T.G., Hazari, S.K.S., Dey, B.K., Miah, H.A., Olbrich, F. and Rehder, D. 2007. Syntheses and antimicrobial activities of isomers of N(4), N(11)-dimethyl-3,10-C-meso-3,5,7,7,10,12,14,14 octamethyl-1,4,8,11-tetraazacyclotetradecane and their nickel (II) complexes. *Inorg. Chem.*, 46:5372-5380.
- Hay, R.W., Lawrance, G.A., and N.F., Curtis. 1975. A Convenient Synthesis of the Tetra-aza-macrocyclic Ligands trans-[14]-Diene, Tet a, and Tet b. *J. Chem. Soc. Perkin Trans.*, 1:591–593.
- Bembi R, Sondhi, S.M., Singh, A.K., Jhanjii, A.K., Roy, T.G., Lown, J.W. and Ball, R.G., 1989. Polyazamacrocycles V. Structure of isomeric 3,5,7,7,10,12,14,14-octamethyl-1,4,8,11-tetraazacyclotetradecanes. *Bull Chem. Soc. Jpn.*, 62:3701.

Morpho-Molecular Characterization, *in Vitro* Regeneration and Quality Plantlet Production in Ginger (*Zingiber Officinale* Rosc.)

Md Ekramul Hoque

Location: Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Duration: One year (2013-2014)

Expenditure of the project: Tk. 1000000.00

Introduction

Ginger (*Zingiber officinales*) is one of the most important major spice crops of Bangladesh. It is valued for its culinary preparation and also for its medicinal properties. It is a herbaceous perennial plant having tuberous root or rhizome. Little attention had been made in the past for its improvement and morpho-molecular characterization. The traditional clonal propagation methods of ginger cultivation are unable to produce disease free and healthy planting material. Supply of quality planting materials (rhizome) is a great challenge to horticulturist for large scale production of ginger. *In vitro* micropropagation of ginger is one of the best alternatives to solve the problem. Clonal multiplication of ginger through shoot has been reported (Balachandran *et al.* 1990). Ilahi (1987) reported that it is possible to obtain a multiplication of 7×10^5 plants per rhizome per year. *In vitro* regeneration technique offers an efficient method for rapid propagation and pathogen free planting material production. The DNA base marker has several advantage over morphological markers. It has abundance through out the whole genome, not affected by environment and have high power of discrimination between very closely related individuals. Hence, the research proposal was design to develop regeneration protocol and to characterize the ginger germplasm on the basis of molecular marker.

Objectives

- *In vitro* regeneration protocol development in ginger
- Pathogen free plantlet production in ginger
- Rapid multiplication of planting material in ginger
- Molecular characterization of ginger germplasm
- DNA fingerprinting of ginger genotypes

Methodology

In vitro propagation of ginger: The best existing variety (BARI Ada-1 and China Ada) were used as experimental materials. The initiated sprout of ginger was excised carefully and washed thoroughly under tap water and sterilized by 70% ethanol. Afterwards, it was sterilized with mercuric chloride for 2/3 min. and then washed several times with autoclaved distilled water and finally it was incubated in culture vial. The vials were incubated at 23-25°C temperature under approximate 4000 lux light intensity by cool-white fluorescent tubes for 16:8 light: dark photoperiod cycle. Different shoot and root inducing hormonal *viz.*, 2, 4-D, KIN, and IBA were supplemented with MS media (MS, 1962) in different concentration (0.5, 1.00, 1.5 and 2.00 mg/L) to find out the best combination and suitable concentration for shoot regeneration and root induction. Data were recorded on- percent of shoot regeneration, percent of callus induction, number of multiple shoot, days to root induction, number of root per explant, survivable rate etc.

Molecular diversity study: Thirteen ginger genotypes were collected from different areas of Bangladesh. Genomic DNAs were extracted from sprouted rhizome. DNA extraction was done from the sprouted rhizome of ginger genotypes. DNA quantification was done by 1% agarose gel. The extracted DNA was visualized by UV transilluminator and the gel photo was captured by gel documentation. PCR reaction was performed using BIONEER KIT-Korea, GeneOn-Taiwan, Geneaid- Taiwan, Biolab-Canada, Paglab- Germany. The PCR reaction mixture was 10.0 µl having all components. The reaction mixture was subjected to thermal cycler for DNA amplification. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining with ethidium bromide. The amplified bands were scored as present (1) and absent (0) for each primer. The score of bands were pooled to create a single data matrix.

Results

A. Tissue culture experiment

Two Ginger genotypes *viz* BARI Ada-1 and China Ada were used to study the regeneration potentiality of Ginger. MS+1.0 mg/L of 2,4-D took minimum time (9 days) for callus induction and it was maximum (15 days) at 2.0mg/L of 2,4-D application in BARI Ada-1 . Greenish shoot development was noticed (Fig. 01) within 15 day in the treatment MS+0.5 mg/L 2,4-D. Chaina Ada took comparatively more time (25 days) for shoots development . Highest 48% callus induction (Fig 02) was recorded in BARI Ada-1 which was followed by China Ada (42%). Maximum 7 number of shoots (Fig. 03) were generated from China Ada and it was minimum in BARI Ada-1 on the treatment application of MS+0.5 mg/L of 2,4-D. The treatment MS+1.5 mg/L KIN took minimum 7 days for shoot regeneration in both the genotypes under studied. Multiple shoot were developed from a single explant and it was upto 7 number in different treatments (Fig 03). Shoot length was increased by the course of time and 3-5 leaf were developed from each shoot. The treatment MS+ 2.00mg/L IBA showed best result within 25 days after sub-culture. The well developed root and shoot bearing plantlets were acclimatized under natural field condition. It was observed that, the survival rate of BARI Ada-1 was 80% and it was 69% in China Ada.

B. Molecular diversity experiment

Molecular diversity and polymorphism analysis were done in 13 ginger genotypes through RAPD primer. Seven primers *viz.* OPG-19, OPJ-13, OPM07, OPP-12, OPP-13, OPW-03, and OPX-04 showed distinct polymorphic DNA profiles. Some total of 44 bands were obtained from these primers with an average of 6.28 bands per primer. The polymorphic DNA fragments ranged from 1-3 in different RAPD oligomer. The primer OPJ13 produced the highest (3) number of polymorphic DNA band and it was the lowest (1) in OPW-03 and OPX-04 primers. The percent of polymorphic DNA fragment was 19.85 under this present investigation. The maximum DNA fragment (10) was generated by the primer OPJ-13 and it was minimum (3) in OPP-12. The DNA profile of 13 ginger genotypes are shown in Fig 05-07, respectively. The number of polymorphic bands was considered appropriate to assess the genetic divergence of Ginger genotypes. The cultivar identification using RAPD markers is well documented in studies of molecular characterization (Bianchi *et. al*, 2003). Fingerprinting based on RAPD marker type was used for identification and characterization of potato cultivars in North America (Sosinski and Donches, 1996). Sawy *et. al.* (2007) reported that, RAPD technique can be successfully applied to determine the genetic fidelity of plant species. The results of present study revealed the existence of low level of genetic diversity among the studied 13 Ginger genotypes grown in Bangladesh.



Fig. 1: Callus induction in explant



Fig. 2: Shoot development from callus



Fig. 3: Multiple shoots from callus



Fig. 4: Mature plantlet of Ginger

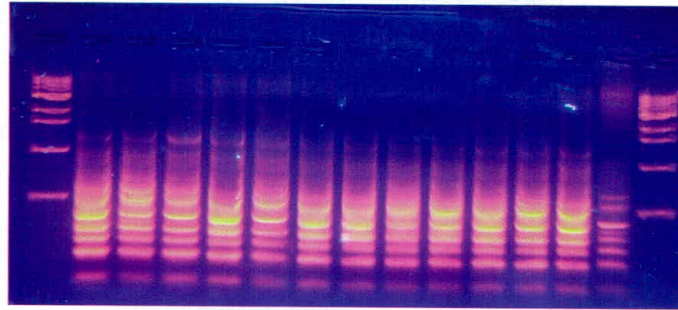


Fig. 5: PCR amplification with RAPD primer OPG 19 in ginger

M1 & M2 = 1 kb DNA ladder, 1 = Kurigram local, 2 = Tangail local, 3 = Jamalpur local, 4 = Modupur local, 5 = B. Baria local, 6 = Rangamati local, 7 = Thakurgoan local-1, 8 = Thakurgoan local-2, 9 = Lalmonirhat local-1, 10 = Lalmonirhat local-2, 11 = Lalmonirhat local-3, 12 = Rangpur local and 13 = Kaptai local.

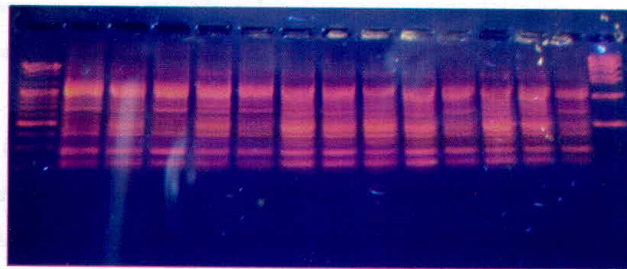


Fig. 6: PCR amplification with RAPD primer OPJ 13 in ginger

M1 = 100 bp DNA ladder, M2 = 1 kb DNA ladder, 1 = Kurigram local, 2 = Tangail local, 3 = Jamalpur local, 4 = Modupur local, 5 = B. Baria local, 6 = Rangamati local, 7 = Thakurgoan local-1, 8 = Thakurgoan local-2, 9 = Lalmonirhat local-1, 10 = Lalmonirhat local-2, 11 = Lalmonirhat local-3, 12 = Rangpur local and 13 = Kaptai local

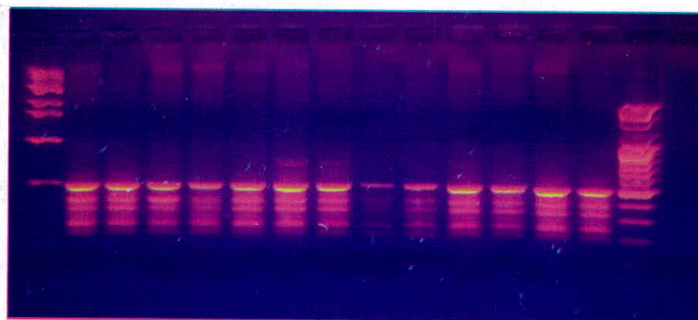


Fig. 7: PCR amplification with RAPD primer OPW 03 in ginger

M1 = 1 kb DNA ladder, M2 = 100 bp DNA ladder, 1 = Kurigram local, 2 = Tangail local, 3 = Jamalpur local, 4 = Modupur local, 5 = B. Baria local, 6 = Rangamati local, 7 = Thakurgoan local-1, 8 = Thakurgoan local-2, 9 = Lalmonirhat local-1, 10 = Lalmonirhat local-2, 11 = Lalmonirhat local-3, 12 = Rangpur local and 13 = Kaptai local

Conclusion

As a vegetatively propagated crop, huge amount of seed-rhizome is needed for commercial production of ginger. *In vitro* regeneration of seedling is the best alternative for continuous supply of planting material in ginger cultivation. Hence, the project is designed to develop a suitable *in vitro* regeneration protocol for large scale production of ginger. The best existing variety (BARI Ada-1) and elite genotype (China Ada) were used as tissue culture works. Multiple shooting was noticed by the application of shooting hormone KIN. Maximum number of shoot (7) was induced by the application of 2.00 mg/L of KIN (Fig.03.). Huge number of roots were developed in most of the cultured explants. The combined effect of shooting and rooting hormone gave good quality plantlets in most of the combination. DNA fingerprinting and molecular diversity analysis can help to identify diverse germplasm for its improvement. The extracted DNA was visualized by 1% agarose gel and captured by Gel Documentation system. The PCR amplification, DNA band scoring, data analysis, and DNA fingerprinting were done to study the molecular diversity analysis of ginger genotypes. The polymorphic DNA fragments ranged from 1-3 in different RAPD oligomer. It was observed that the primer OPJ13 produced the highest 3 number of polymorphic DNA band and it was the lowest (1) in OPW-03 and OPX-04 primers (Fig. 05-07). The results of present study revealed that low level of genetic diversity were present among the 13 Ginger genotypes grown in Bangladesh.

References

- Balachadram, S.M., Bhat, S.R. and Chaddel, K.P.S. 1990. *In vitro* clonal multiplication of turmeric (*Curcuma sp.*) and ginger (*Zingiber officinale* Rosc). *Plant Cell Reports*, 8: 321-324.
- Bianchi, V.J., Fachinello, J.C. and Schuch, M.W. 2003. RAPDs na caracterizacao genetica- molecular enoestudo da variabilidade genetica de cultivares de ameixeira. *Revista Brasileira de Fruticultura*, 25:272-274.
- Ilahi, H. and Jabeen, N. 1987. Micropropagation of *Zingiber Officinale* Rosc. *Pakistan J. Bot.* 19: 61-65.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15:473-497.
- Sawy, A. E., Bekheet, S. and Aly, U.I. 2007. Morphological and molecular characterization of potato microtubers production on Coumarin inducing medium. *Int. J. Agril. Bio.* 9-5: 675-680
- Sosinski, B. and Douches, D.S. 1996. Using polymerase Chain reaction-based DNA amplification to fingerprint North America potato Cultivars. *Hort. Science*, 31;130 133.

Search For Bioactive Natural Products With Antibacterial Activity From Medicinal Plants of Bangladesh.

Firoj Ahmed and Biplob K Das

Location: Department of Pharmaceutical Chemistry, University of Dhaka.

Duration: Two years (2013-2015)

Expenditure of the project: TK. 1000000.00

Introduction

Natural products have long been an indispensable source in the field of new drug discovery and have also made significant contributions in promoting basic studies of life science. Recent studies have revealed that medicinal plants from various parts of the world can provide a rich source of antibacterial activities.

Although for more than a century, extensive studies have been made on isolation and identification of antibacterial agents from medicinal plants, chemistry of natural products is still of great importance as a basic science. As such, we are particularly interested to work on unexplored medicinal plants of Bangladesh, which are traditionally used for treating different diseases. It will be an effective way to find out the new antibacterial agents.

Objectives

The aim of the research is to preparation of an extract library, screening of extract library, find out the bioactive chemical constituents with antibacterial activity and characterization & purification of the isolated constituents.

Methodologies

The overall methodology of the proposed study can be divided into the following sub groups:

1. Preparation of Extracts Library
2. Screening for antibacterial activity
3. Antibacterial activity guided separation

Preparation of Extracts Library

Collection and Identification: About 35 plants were collected from Natore Medicinal plant village and were authenticated by Mr. Mustafizur Rahman, Lecturer, Department of Botany, Rajshahi University.

Drying and grinding: The collected plant parts were washed with water, separated from undesirable materials or plants or plant parts. Cutting into small pieces, they were sun-dried for one week. After a final drying in dryer at 40°C for one hour, the plant parts were pulverized into coarse powder and kept in airtight containers. The containers were stored in a cool and dry place. Necessary precautions were taken to prevent any type of contamination within the plant samples.

Cold extraction: The powdered plants were taken in glass containers, immersing in alcohol (methanol and ethanol) in such a way that the solvent surface should remain 3 cm above the powder surface. The containers were tightly sealed and kept for overnight with occasional shaking and stirring. The solvent was decanted through a cotton cloth and finally filtered through a cotton plug. Maceration process was carried out three times for the same powder for complete extraction.

Evaporation of the solvent: The filtrate (alcoholic extract) obtained by repeated maceration, was evaporated under reduced pressure at 40°C using rotary evaporator. The concentrate was designated as crude extract.

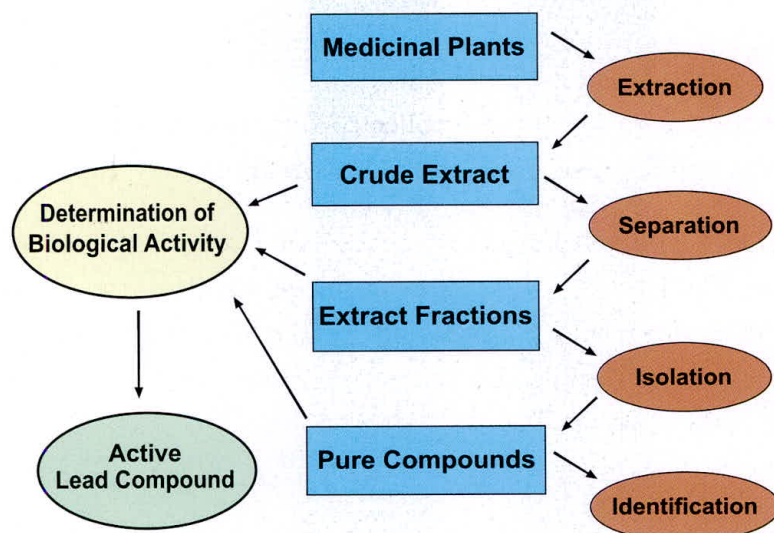
Preparation of library: Extracts library was prepared by suspending crude extracts in 50% EtOH to make a concentration of 50 mg/ml and then transferred in a 1.5 ml Eppen tube. These tubes were then preserved in refrigerator at -8°C. These collection of extracts are called extracts library.

Screening for antibacterial activity

The extracts library was screened for antibacterial activity against a number of pathogenic microorganisms by disk diffusion method (Ahmed *et al.*, 2003).

Antibacterial activity guided separation

The active samples will subsequently undergo activity guided separation. In short, the crude extract will be suspended in 10% MeOH and then partitioned between hexane, BuOH, and ethylacetate. Then each fractions will be checked for the activity, and active fractions will be undergone column chromatography, and preparative TLC to give the single compound. Activity guided separation process is schematically represented bellow:



Result

Collection of Plants

Following plants were collected from Natore Medicinal plant village, Natore.

Table 1: List of Collected Plants

Sl. No.	Scientific name	Local Name	Parts used
1	<i>Casurina littorea</i>	Jhao	Leaves
2	<i>Clerodendron viscosum</i>	Boti, Getu	Leaves
3	<i>Trigonella toenum</i>	Methi	Whole plant
4	<i>Moringa oleifera</i>	Shajna	Leaves
5	<i>Aphanamixis</i>	Pitraj	Leaves
6	<i>Withania somnifera</i>	Arshawgandha	Roots
7	<i>Oxalis corniculata</i>	Amrul	
8	<i>Ipomoea paniculata</i>	Bhuikumra	Tuber
9	<i>Bombax ceiba</i>	Shimul	Leaves & Small
10	<i>Abroma augusta</i>	Ulot kombol	Leaves
11	<i>Asparagus racemosus</i>	Shotomul	Roots
12	<i>Cadariocalyx matorius</i>	Bonchandal	Leaves
13	<i>Crataeva nurvala</i>	Boron	Leaves& stem
14	<i>Aristolochia indica</i>	Ishwarmul	Stem
15	<i>Andrographis</i>	Kalomegh	
16	<i>Mikania cordata</i>	German Iota	leaves
17	<i>Piper longum</i>	Pipolti morich	Arial parts
18	<i>Cassia alata</i>	Bichandal	Leaves & small
19	<i>Mucuna pruriens</i>	Alkusi	Leaves
20	<i>Scoparia dulcis</i>	Misridana	Leaves

21	<i>Saraca asoca</i>	Asok pata	Leaves
22	<i>Leea macrophylla</i>	Hosti palas	Leaves
23	<i>Curcuma amada</i>	Am-ada	Leaves
24	<i>Hemidesmus indicus</i>	Onontomul	Arial parts
25	<i>Curculigo orchioides</i>	Talmul	Leaves
26	<i>Phlogacanthus</i>	Kalo Bashak	Leaves
27	<i>Pterocarpus santalinus</i>	Rokto chandal	Arial parts
28	<i>Centalla asiaticac</i>	Thankuni	Leaves
29	<i>Vitex negundo</i>	Nishindha	Leaves
30	<i>Glycyrrhiza glabra</i>	Jostimodhu	Arial parts
31	<i>Withania somnifera</i>	Arshawgandha	Root & stem

Screening for antibacterial activity

Table 2 showed the antibacterial activity of the collected plants. *A. polystachya*, *C. littorea*, *O. corniculata*, *W. somnifera*, *C. allata*, *Curcuma amada*, *H. indicus*, *A. paniculata* and *A. indica* showed antibacterial activity against *S. aureus* and *E. coli*.

Table 2. *In vitro* Antibacterial activity of *Casuarina equisetifolia*

Sl. No.	Scientific name	Conc .Used (µg/disk)	Diameter of zone of inhibition	
			<i>S. aureus</i> (mm)	<i>E. coli</i>
1	<i>Casurina littorea</i>	250	7	7
2	<i>Clerodendron viscosum</i>	500	-	-
3	<i>Trigonela toenum</i>	500	-	-
4	<i>Moringa oleifera</i>	500	-	-
5	<i>Aphanamixis polystachya</i>	500	12	13
6	<i>Withania somnifera</i>	500	-	-
7	<i>Oxalis corniculata</i>	500	8	7
8	<i>Ipomoea paniculata</i>	500	-	-
9	<i>Bombaxceiba</i>	500	-	-
10	<i>Abroma augusta</i>	500	-	-
11	<i>Asparagus racemosus</i>	500	-	-
12	<i>Cadariocalyx matorius</i>	500	-	-
13	<i>Crataeva nurvala</i>	500	-	-
14	<i>Aristollochia indica</i>	500	7	6
15	<i>Andrographis peniculata</i>	500	7	6
16	<i>Mikania cordata</i>	500	-	-
17	<i>Piper longum</i>	500	-	-
18	<i>Cassia alata</i>	500	11	11
19	<i>Mucuna pruriens</i>	500	-	-
20	<i>Scoparia dulcis</i>	500	-	-

21	<i>Saraca asoca</i>	500	-	-
22	<i>Leea macrophylla</i>	500	-	-
23	<i>Curcuma amada</i>	500	7	8
24	<i>Hemidesmus indicus</i>	500	15	14
25	<i>Curculigo orchioides</i>	500	-	-
26	<i>Phlogacanthus</i>	500	-	-
27	<i>Pterocarpus santalinus</i>	500	-	-
28	<i>Centalla asiaticac</i>	500	-	-
29	<i>Vitex negundo</i>	500	-	-
30	<i>Glycyrrhiza glabra</i>	500	-	-
31	<i>Withania somnifera</i>	500	-	-

Screening for antioxidant activity

Antioxidant activity of the extracts was determined on the basis of their scavenging potential of the stable DPPH free radical in quantitative assay on the basis of modified method of Gupta *et al.*, 2003. Stock solutions (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100 and 500 g/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC₅₀ was calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound, drug, crude drug or a plant extract. In the present study, it was noticed that the MeOH extracts of *Withania somnifera* and *Oxalis corniculata* showed strong antioxidant activity, while MeOH extracts *Ipomoea paniculata*, *Abroma augusta* and *Bombax ceiba* showed moderate activity. Remaining MeOH extract of *Mikania cordata* showed mild free-radical scavenging activity. The free radical scavenging property may be one of the mechanisms by which this drug is effective in traditional medicine.

MeOH extracts of leaves of *Aphanamixis polystachya* showed potential DPPH free radical scavenging activity where the IC₅₀ was 31.18 g/mL compared to that of ascorbic acid, used as standard, where the IC₅₀ was 6.68 g/mL. *Casuarina equisetifolia* showed strong antioxidant (DPPH free radical scavenging activity) activity where the IC₅₀ = 25.71 g/mL.

Table 3. DPPH free radical scavenging activity of the collected plants

Sample	IC ₅₀ (µg/ml)
<i>I. paniculata</i>	86.48
<i>W. somnifera</i>	126.20
<i>A. augusta</i>	101.4
<i>B. ceiba</i>	58.60
<i>O. corniculata</i>	19.98
<i>C. equisetifolia</i>	25.71
<i>A. polystachya</i>	31.18
<i>A. indica</i>	223.63
Ascorbic acid	7.95

Brine Shrimp Lethality Bioassay

The Brine shrimp Nauplii were obtained by hatching eggs in artificial sea water after incubation at 37 °C for 48h with continuous oxygen's supply. The nauplii were allowed to stand for another 48 h in seawater to ensure survival and maturity before use. Six conc. of plant extract (10, 20, 40, 80, 160, and 320 µg/mL) in DMSO. Each extract preparation was dispensed into clean test tube 10 ml volumes and tested in triplicate. The concentration of DMSO in the vials was kept below 20 µL/mL. For control, same procedure was followed except test sample. After marking the test tubes properly, 10 living nauplii were added to each of the 20 vials with the help of the Pasteur pipette. The test tube containing the sample and control were then incubated at 37°C for 24 h in a water bath, after which tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration (Ahmed *et. al.*, 2008; Meyer *et. al.*, 1982).

Methanolic extracts of leaves of *Casuarina equisetifolia* showed moderate Cytotoxic Activity comparable to standard sample in Brine Shrimp lethality bioassay test where the LC50 was found at 95.87 µg/mL.

MeOH extract of *A. polystachya* showed lethality against the brine shrimp nauplii. The extract showed different mortality rate at different concentrations where the LC50 was found at 77.98 µg/mL.

Methanolic extracts of the *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn) showed strong antioxidant activity where the IC50 was 31.18 mg/mL, against DPPH free radical.

Table 4. Cytotoxic activity of the collected plants

Sample	LC 50 (µg/mL)
<i>C. equisetifolia</i>	95.87
<i>A. polystachya</i>	77.98
<i>A. paniculata</i>	18.45
<i>A. indica</i>	36.31

Conclusion

Medicinal plants were collected and extract library was prepared. Extracts library was screened for antibacterial activity followed by bioassay guided separation of one of the active plant. But failed to isolate any active principle. The extracts library was then screened for antioxidant and cytotoxic activity. Some plant extracts showed moderate to strong antioxidant and cytotoxic activity. However, search for antibacterial agents would be continued for further results.

Publication from this Research

Hossen, S. M. M., Hossain, M. S., Islam, J., Pinto, M. N., Nur-E-Jannat, Ahmed, F.. Comparative preliminary phytochemical and biological investigations on *Andrographis paniculata* (Nees) and *Aristolochia indica* (Linn). *Der Pharma Chemica*, 2014, 6, 332-338.

Hossain, M. S., Islam, J., Alam, M. S., Ahmed, F., Kabir, A. K. L., Hossen, S. M. M., Preliminary phytochemical, antioxidant, antibacterial and cytotoxic activity evaluation of MeOH extract of *Aphanamixis polystachya* (Wall.) leaf. *J. Adv. Pharm. Res. Biosci.* 2014, 2, 50-53.

References

Ahmed, F., Das, P. K., Islam, M. A., Rahman, K. M., Rahman, M. M., Selim, M. S. T., 2003. Antibacterial activity of *Cordyline terminalis*. Kunth. leaves. *J. Med. Sci.* 3, 418-422.

Ahmed, F., Amin, R., Shahid, I. Z., Sobhani, M. M. E., 2008. Antibacterial, cytotoxic and neuropharmacological activities of *Cerbera odollam* seeds. *Orient. Pharm. Exp. Med.* 8, 323-328.

Gupta, M., Mazumdar U. K., Sivahkumar, T., Vamis, M. L. M., Karki, S., Sambathkumar, R., Manikandan, L. 2003. Antioxidant and anti-inflammatory activities of *Acalypha fruticosa*. *Nig. J. Nat. Prod. Med.* 7, 25-29.

Meyer, B. N., Ferrigni, N. R., Putnam, J. B., Jacobsen, L. B., Nichols, D. E., Mc Laughlin, J. L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 45, 31-34.

Development of Anticancer Drug-Design Laboratory in Advanced Level in a View to Formulate Novel Anticancer Drugs

Jahan Ara Khanam and M Shahjahan

Location: Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi

Duration: Two years (2013-2015)

Expenditure of the project: Tk. 1000000.00

Introduction

Cancer is one of the most important health problems in the world including Bangladesh. According to WHO, Bangladesh is bearing about 10 million cancer patients. About 2 million extra cancer patients are added to this feature per year. Perfect cure is not to be brought into the world medicine. The major drawbacks are undesirable side effects and active drugs are highly expensive. Hence, there is a need to find out novel anticancer alternative drugs at nontoxic doses, inexpensive and affordable to common people. Bangladesh is rich in natural sources. Hence this research project work has been designed to find out novel anticancer drugs from plant source.

Objectives

To design the research laboratory with equipments and chemicals

To isolate ingredients from plants *Eucalyptus camaldulensis*

To isolate, separation and purification of compounds from plant extract

To study anticancer activity against EAC cells both in vivo and in vitro

To study mechanism of action of isolated compounds on EAC cells -To study effect of isolated compound on cell cycle

Methodology

Extraction, isolation and purification of ingredients from stem bark of *Eucalyptus camaldulensis* was done as per Islam *et al.*, 2015.

Anticancer activity of isolated compound (EC-1) was measured using the following parameters:

1. Determination of cell growth inhibition (Islam *et al.*, 2014a)
2. Determination of survival curve (Islam *et al.*, 2014a)
3. DNA fragmentation assay (Islam *et al.*, 2015)
4. Reverse transcriptase polymerase chain reaction (Benyahia, 2005)
5. Apoptosis study (Islam *et al.*, 2014a)
6. Cell cycle analysis (Islam *et al.*, 2015)

Results

Inhibition of cancer cell growth with EC-1 in vitro

The cytotoxic effect of EC-1 on EAC cell was assessed by MTT colorimetric assay. The compound inhibited cancer cell growth in a dose-dependent manner (Fig. 1A). EC-1 showed maximum cancer cell growth inhibition (85%) at the concentration of 100 μ g/ml when compared with the growth of cancer cells in the control solvent ($p < 0.01$). The minimum suppression of cancer cells growth (12.5%) was observed at 10 μ g/ml compared with control cells (treated with solvent only). The IC₅₀ value of the compound was determined as 32 μ g/ml against EAC cell (Fig. 1B).

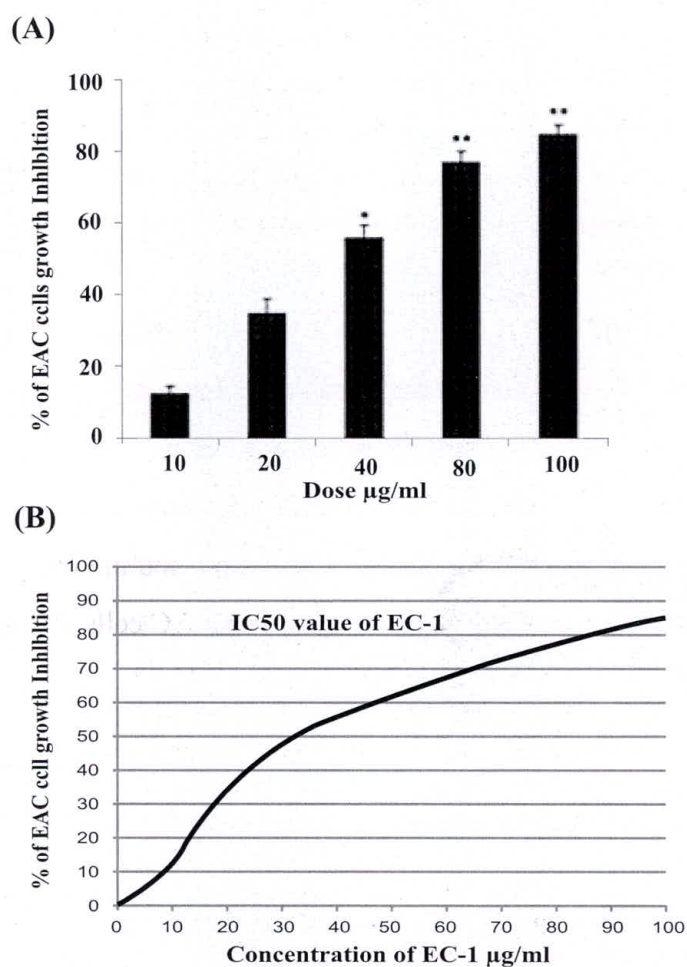


Fig. 1: (A) The Ehrlich ascites carcinoma (EAC) cells were treated with various doses of EC-1 for 24hr in RPMI-1640 medium. The growth inhibition was measured by MTT assay ($n=3 \pm$ standard error of mean). (B) IC₅₀ value of EC-1 was calculated from the dose-response curve. Level of significance * $p < 0.05$ and ** $p < 0.001$ when compared with that of control group.

Cell growth inhibition of EC-1 *in vivo*

Effects of EC-1 and bleomycin on EAC cells growth after tumor inoculation are shown in Fig. 2A and B. Treatment with EC-1 resulted in significant reduction of cell growth *in vivo*. The maximum cell growth inhibition with EC-1 was noted at doses of 2 and 1 mg/kg/day (73% and 57%, respectively) compared with the control mice ($p < 0.01$). Meanwhile, the bleomycin treated mice showed cell growth inhibition by 87.62% (0.3 mg/kg/day).

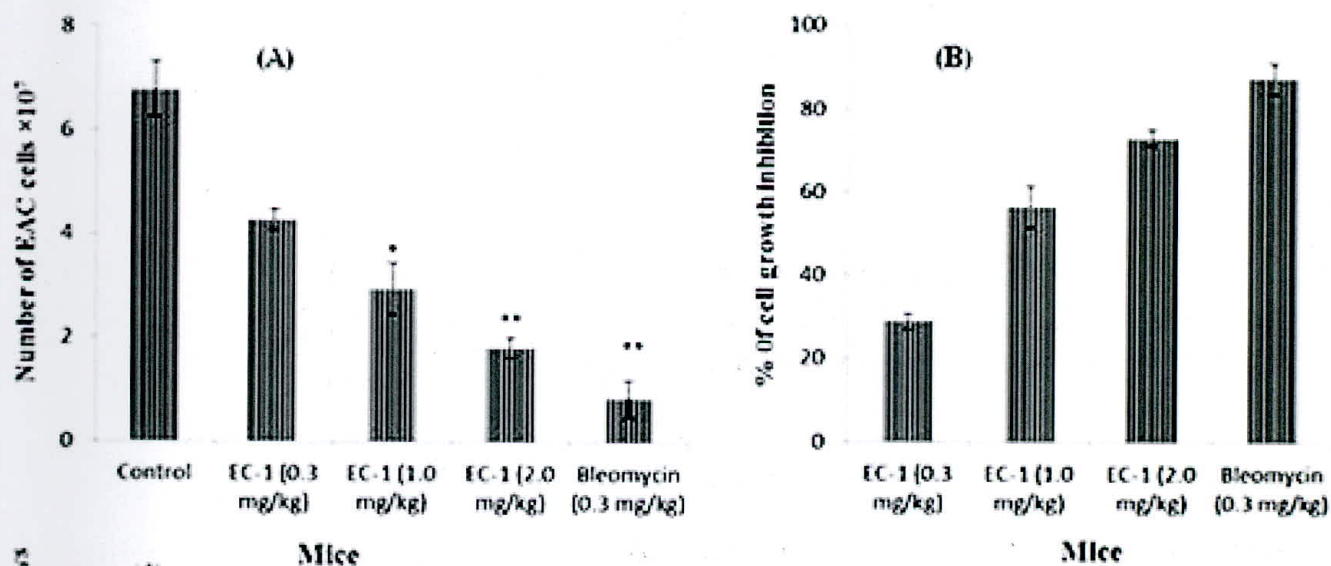


Fig. 2: (A) Number of Ehrlich ascitescarcinoma (EAC) cells in experimental mice, counted by inverted microscope in presence and absence (control) of EC-1 at different doses on day 6 of tumor inoculation. (B) Percentage of cell growth inhibition at different doses in comparison to control mice (n=6). Level of significance * $p < 0.05$ and ** $p < 0.01$ when compared with that of control mice group.

DNA fragmentation analysis

The activation of the endogenous Ca²⁺/Mg²⁺ dependent endonuclease is the most distinctive biochemical hallmark of apoptosis (Islam et al., 2014a). This activated endonuclease will mediate the cleavage of inter nucleosomes and generate oligonucleotide fragments of about 180– 200 base pairs. DNA isolated from EC-1-treated EAC cells showed ladder-type DNA that is a characteristic feature of apoptosis induction; whereas in the control group, a smear-like DNA degradation was obtained (Fig. 3A).

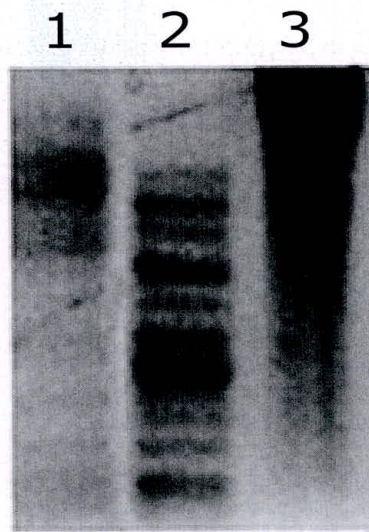


Fig. 3: DNA was extracted from EC-1-treated and control mice and run in (1.5%) agarose gelelectrophoresis. Lane (1) DNA from EC-1-treated EAC cell, lane (2) ladder and lane (3) DNA from control EAC-bearing mice (DNA fragmentation was observed in EC-1 treated mice).

Altered expressions of cancer-related genes in EC-1-treated cells

Reverse transcription PCR was used to study the mRNA expression levels of several tumor related genes in control and EC-1-treated EAC cells (Fig. 4). The control cells showed high expressions of Bcl-2 and Bcl-X genes. Also, the Bax mRNA expression was not found in the control cells. When treated with EC-1, EAC cells showed reduced Bcl-2 and Bcl-X mRNA expressions, whereas the expression level of Bax gene was increased remarkably. In addition, the p53 and PARP-1 genes showed increased expressions in EC-1-treated cells. On the other hand, no expression of these genes were found in control mice EAC cells (Fig. 4)

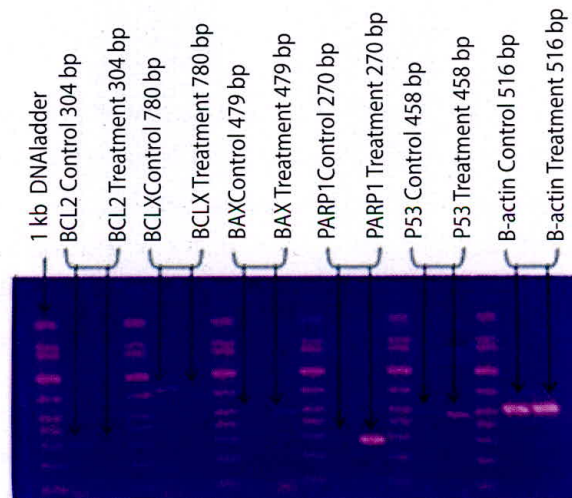


Fig. 4: RNA was extracted from the experimental mice on day 6 and level of mRNA expression of antiapoptotic genes (Bcl-2 and Bcl-X) and proapoptotic genes (p53, Bax, and PARP-1) were studied. In EC-1-treated mice, all the three proapoptotic genes expression increased remarkably, and the expression of Bcl-2 and Bcl-X were not observed. In control group, opposite results were observed.

Conclusion

1. Anticancer activity has been observed more or less with all the compounds at different doses.
2. Promising effects have been reflected with new isolated compound p-menth-1-ene-4,7diol (EC-1).
3. A new compound has been isolated from Eucalyptus.
4. Further works with human cancer cell line with higher animals are necessary to develop novel anticancer drug in future.

Output of the project

A drug design laboratory has been set up

A new compound p-menth-1-ene-4, 7diol (EC-1) has been isolated from Eucalyptus camaldulensisstem bark which posseses anticancer activity.

Utilization of Shrimp Waste in the Ration to Optimize Growth and Egg Production Performance of Layer Chickens

Sarder Safiqul Islam and Bidhan Chandro Sarker

Location: Agrotechnology Discipline, Khulna University, Khulna

Duration: Two years (2013-2015)

Expenditure of the project: Tk. 2442000.00

Introduction

In Bangladesh, poultry and poultry industries are contributing significantly in our national economy through providing food and nutrition and creating job opportunity. But very recently prices of eggs and poultry meat become very high due to high prices of poultry feeds in local as well as international market. Feed costs accounts 60 to 70% of total operation cost for poultry production (Qunaibet *et al.*, 1992; Mian, 1994). Therefore, to keep the prices of poultry and poultry products within the capacity of resource poor people, it is important to formulate low cost balanced ration utilizing unconventional feed resources. Traditionally soybean meal (SBM) is the major protein source in commercial layer chicken ration elsewhere in the world. But it is prime concern yet because of its scarcity as well as its expensiveness. Shrimp head meal (SHM) may be an excellent source of cheap and abundant substitute of soybean meal or fishmeal. It contains, 24.03% CP, 5.14% fat, 26.89% CF and 25.60% ash on the basis of dry matter (Mahata *et al.*, 2008). To make layer chicken as a profitable enterprise by reducing the cost of feed, SHM may be used as a cheaper protein source of feed ingredients which are locally available and can be used as a replacement of soybean meal.

Objectives

a. Broad objective

Formulation of low cost rations for grower and layer chickens utilizing shrimp waste.

b. Specific objectives

To investigate the effect of different levels of shrimp head meal in the ration on growth rate and egg production performance of layer chicken.

Nutritional evaluation of shrimp head meal.

To find out the economic feasibility of using shrimp head meal in the poultry ration.

Methodology

A field survey was conducted in Batiaghata and Dacope upazila of Khulna district during the period from August to October, 2013 to observe the socioeconomic conditions of poultry farmers, existing feeding and management practices of poultry farms and performances of 5 different layer hybrids. An experiment was carried out at Dr. Purnendu Gain Field Laboratory, Agrotechnology Discipline, Khulna University, Khulna. The objective of this study was to measure the effect of substituting soybean meal in the ration of growing layer birds by different levels of shrimp head meal (SHM). A control corn-soybean layer grower diet and four different levels of SHM substituted diets were fed to Hisex White chicks from 5th to 18th weeks of age. The SHM replaced at the rate of 0, 25%, 50%, 75% and 100% of soybean meal (SBM). The experiment was assigned at random to birds under 5 treatment groups having 3 replications in each. Number of birds in each replication was 20. The second experiment was continued with similar treatments to observe the egg production percentage, feed intake, mortality percentage and egg weight. Data of body weight was collected in every 15 days interval.

Results

Results of the feeding experiment on growing chickens showed that the survivability of chickens improved with increasing level of shrimp head meal (SHM) in the ration. Feed intake increased significantly ($p < 0.01$) in treatment group fed 100% SHM ration. No significant difference ($p > 0.05$) was observed in feed cost. However, numerically the lowest feed cost for live weight gain was observed in treatment group fed 100% SHM. Weight at maturity was significantly ($p < 0.05$) highest when 25% SHM was used in the ration. Daily feed consumption per bird increased significantly ($p < 0.01$) with increasing shrimp head meal in the ration.

Fig. 1 showed that feed cost decreased with increasing SHM in the ration and expressed by the equation of $y = -3.24x + 121.1$ ($R^2 = 0.493$) and 49% variation observed in feed cost due to level of SHM.

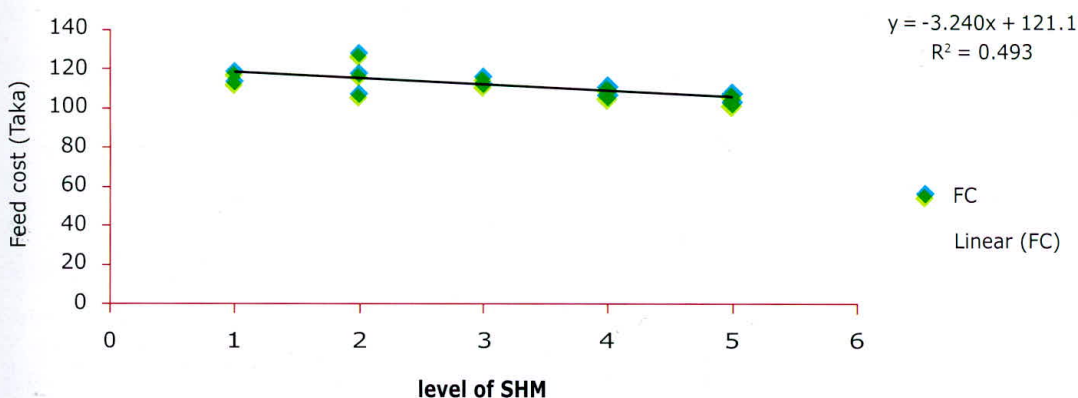


Fig. 1: Relationship between level of SHM and feed cost of layer grower birds

Fig. 2 Showed that mortality rate decreased with increasing level of SHM.

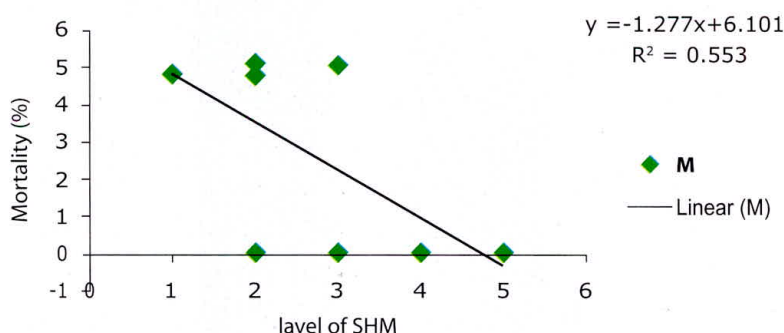


Fig. 2: Relationship between level of SHM and mortality of layer grower birds

Results of another experiment on layer chickens showed that there were no significant differences ($P > 0.05$) in daily feed intake, egg production percentage, body weight, egg weight, feed conversion ratio, feed cost per egg and mortality. The highest daily feed intakes per bird of 119.33, 117.67, and 117.67g were found in 31st, 36th, and 37th weeks respectively in birds fed 100% SHM and the lowest feed intakes of 115.00, 115.67 and 116g were found in 100% SBM (soybean meal) group. The effect of shrimp head meal on egg production percentage of layer hens was found non significant but the numerically highest egg production percentage of 94.10, 95.33, 95.27, 94.40, 95.27, 93.87, 97.43, 94.70, 92.40, 91.13 and 88.90 were found in 23rd, 24th, 25th, 26th, 27th, 28th, 29th, 30th, 31st, 34th and 35th weeks of age respectively in treatment group fed ration in combination with 25% SHM and 75% SBM. In most of the age categories the lowest feed conversion ratio was found in treatment group fed ration in combination with 25% SHM and 75% SBM. The highest feed cost per egg production was found in treatment group fed 100% SBM and the lowest feed cost per egg was found in treatment group fed 100% SHM.

Conclusion

From the study with grower layer chicks, it was revealed that the use of shrimp head meal (SHM) in the ration had significant effect on feed cost per kg live weight gain. The substitution of 25% soybean meal by SHM was suitable for body weight gain and feed conversion ratio. On the other hand, the survivability of chicken was improved with increasing the level of SHM in the ration.

Results of the feeding trial with layer chickens revealed that overall the highest egg production percentages were observed in layer birds fed diet in combination with 25% SHM and 75% SBM. Better egg weight was also found in treatment group fed 100% SBM which was more or less similar to the treatment group fed ration in combination with 25% SHM and 75% SBM. The highest feed cost per egg was found in treatment group fed 100% SBM and the lowest feed cost per egg was found in treatment group fed 100% SHM. It can be concluded that the ration in combination with 25% SHM and 75% SBM is suitable for better egg production, egg weight and lower feed cost.

Recommendations

Due to high fiber contents of shrimp head meal it may not be suitable to substitute cent percent protein sources (soybean meal) of the ration by it.

Combination of 75% soybean meal and 25% shrimp head meal as protein source in layer ration may be suitable for better growth, egg production, egg weight, lower mortality and feed cost.

Low cost ration can be formulated using shrimp head meal at the rate of 25% of protein source (soybean meal).

Further study is necessary to determine the digestibility of shrimp head meal in poultry.

Publication from this Research

Islam, S. S., Paul, C. and Sarker, B. C., 2013. A comparative study on the performances of layer hybrids in some selected areas of Khulna region. *Bangladesh Journal of Animal Science*, 42 (2): 114-122.

Paul, C. 2014. Effect of different levels of shrimp head meal in poultry ration on the growth performance of growing pullets, Undergraduate Thesis, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh.

Islam, M. B., 2016. Effect of different levels of shrimp head meals in the ration on egg production performance of layer chickens, Undergraduate Thesis, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh.

Yeasmin, S. 2016. Effect of shrimp head meal on the nutritional and physical quality of egg and meat at different production stages of layer. Undergraduate Thesis, Agrotechnology Discipline, Khulna University, Khulna, Bangladesh.

Yeasmin, S., Islam, M.S., Islam, S.S. and Nath, S.D. 2017. Effect of shrimp head meal on physical and nutritional quality of egg and meat at different production stages of layer. Proceedings of 10th International Poultry Show and Seminar, 2-4 March, 2017, International Convention City, Bashundhara, Dhaka, pp. 296-298.

References

Mahata, M. E., Dharma, A., Ryanto, H.I. and Rizal, Y. 2008. Effect of substituting shrimp waste hydrolysate of penaeus merguensis for fish meal in broiler performance. *Pakistan Journal of Nutrition*, 7(6): 806-810.

Mian, M. A. 1994. Poultry Production. In; Animal Husbandry. National Book Foundation, Islamabad. 294 pp.

Qunaibet, M. H., Elwafa, E.A. and Mansour, M.M. 1992. Improving the competitive status of Saudi broiler industry. *Journal of King Saud University of Agricultural Science*, 4(2):164-184.

Microbial and Physico-Chemical Characterization of Supply Water for the Sustainable Development of Water Supply and Sanitation in Khulna City

Molla Mohammad Shafiqur Rahman and Md Nazmul Ahsan

Location: Environmental Science Discipline, Khulna University, Khulna

Duration: One year (2013-2014)

Expenditure of the project : Tk 1000000.00

Introduction

Groundwater has become the main freshwater source in Bangladesh as it requires less treatment. However, in 1993 arsenic contamination was discovered in the groundwater.

Khulna City is a major urban conglomerate situated on the southwest coastal region of Bangladesh where total water supplied to a growing mass of about 1.5 million is groundwater. Khulna WASA established in 2008 is supplying water among the city dwellers through a network system. This water is mainly being used in domestic purpose. Although some community and private tube wells are found in the city, a large number of people directly depend on the supply system. According to Khulna WASA (kwasa) the present status of water supply is satisfactory but the peoples' perception in some areas is reverse. At present kwasa is supplying 110 million liter water /day as the situation was in 2008. Development project works are going on to improve the situation. The outcome would be found in 2018.

Water samples were collected for physico-chemical and microbial parameter determination from 21 pump houses with respective nearby house connections.

To find out the seasonal variation among of the ground water physico-chemical parameters sampling had been carried out during pre-monsoon, monsoon and post-monsoon period. The obtained data is being analyzed by using different analytical methods.

In order enlighten the current situation, development measures and peoples' perceptions, interviews with key informant personnel, focus group discussions etc. were arranged.

This research work has been carried out to provide with data based scientific information to set up networks among the stake holders.

Objectives

- To analyze and characterize the geochemical (solute load: the major cations and anions), microbial parameters of ground water of the area;
- To estimate the potable water needs of the urbanites and the volume of available waters from the aquifers;
- To identify the route of pollution/contamination of ground water and to suggest suitable measures to contain such pollution/contamination; and
- To develop a guideline for eco-friendly sustainable management of water sources based on the new information.

Methodology

The research has covered the following activities:

- (a) Collection of secondary source materials: (i) Topographic maps (ii) Khulna City Corporation (KCC) and Khulna Development Authority (KDA) maps, (iii) Spot Imagery, (iv) Meteorological, hydrological, geological and environmental data along with demographic reports and (v) City plans.
- (b) Selection of sampling stations depending on spatial and temporal variability: Khulna city is comprised with 31 wards. Among them ward no.1 to 8 and ward no.15 have no pump house to feed network system. However pump houses in ward no.13 only pump house at Khalishpur Charerhat and in ward no. 25 at Habelibag are out of order. As a result 21 wards have one or more pump houses. Water samples were collected for physico-chemical and microbial parameter determinations from 21 pump houses with respective nearby house connections so that one sample was collected from each ward. Samples were collected in pre-monsoon, monsoon and post-monsoon period to find out the seasonal variation.
- (c) Physico-chemical and microbial parameters of ground water samples: Study the chemistry of ground water has both spatial and temporal variations. The following parameters were:
 - (i) Electrical conductivity (Ec) and pH were measured with Ec-Meter (HANNA) and pH-Meter (HANNA) respectively.
 - (ii) The major cations (Ca, Mg, Na and K);
 - (iii) The major anions (HCO₃, Cl, SO₄, PO₄);
 - (iv) Microbial parameters (Coliform) etc. were analyzed in the laboratory with Standard Methods (APHA, 1995)

(d) Data analysis and interpretation:

(i) Data analyses were done on by using standard statistical and some special software, (ii) Analyzed data and interpretations were compiled for preparation of elemental profile of the waters from aquifers; (iii) The data were interpreted to decipher the source and controlling mechanism of the nature of solute load, the route of pollution/contamination of waters, the potability of waters, volume of the available consumable waters and the water needs of the area etc.

Results

Chemical composition of groundwater is the combined result of the composition of water that enters the groundwater reservoir and reacts with minerals present in the rock that may modify the water composition. The major components of groundwater are Na⁺, K⁺, Ca₂⁺, Mg₂⁺, Cl⁻, HCO₃⁻, SO₄²⁻, PO₄³⁻, H₄SiO₄²⁻ and the trace elements (Berner and Berner, 1987). The major component represents the chemistry of the water. For this reason pH, EC, Na⁺, K⁺, Ca₂⁺, Mg₂⁺, Cl⁻, HCO₄⁻, SO₄²⁻, PO₄³⁻, NO₃⁻ parameters have been analyzed.

Table 1: WHO (World Health Organization) and Bangladesh standards for drinking water

Serial No.	Water quality parameter	Unit	Bangladesh standard	WHO standard (1993)
01	pH	-	6.5-8.5	7-8.5
02	EC (Electrical Conductivity)	μ s/cm	-	-
03	TDS (Total Dissolved solids)	ppm	1000	1000
04	Sodium (Na^+)	ppm	200	200
05	Potassium (K^+)	ppm	12	
06	Calcium (Ca^{2+})	ppm	75	75-200*
07	Magnesium (Mg^{2+})	ppm	30-35	50-150*
08	Chloride (Cl^-)	ppm	150-600*	200-600
09	Bicarbonate (HCO_3^-)	ppm	-	-
10	Dissolve silica ($\text{H}_4\text{SiO}_4^{2-}$)	ppm	-	-
11	Phosphate (PO_4^{3-})	ppm	6	-
12	Sulphate (SO_4^{2-})	ppm	400	250
13	Fluoride (F^-)	ppm	1	0.5-1

Source: Ahmed and Rahman, 2000

- For coastal areas of Bangladesh, in case of non-availability of alternative sources value is 1000 ppm. (Ahmed and Rahman, 2000)

Table 2: Ward Numbers at which samples from pump houses and house connections are found higher of WHO (World Health Organization) and Bangladesh standards for drinking water

Seasons	Pre-Monsoon		Monsoon		Post-monsoon	
	Pump house	House connection	Pump house	House connection	Pump house	House connection
pH	All ground water samples satisfy the requirement and suitable for drink.					
Sodium	29	22	9,10,14,29	Nil	9, 10, 11, 12, 14, 16, 17, 19, 20, 21, 22, 23, 29	24, 25, 26, 27, 28
Potassium	26	26	29	18, 22, 23	Nil	Nil
Calcium	Nil	Nil	Nil	Nil	Nil	Nil
Magnesium	Nil	Nil	Nil	Nil	Nil	Nil
Chloride	Nil	Nil	29	29	Nil	Nil
Phosphate	Nil	Nil	Nil	Nil	Nil	Nil
Sulphate	Nil	Nil	Nil	Nil	Nil	Nil

According to the WHO and Bangladesh standard the comparative study concludes that most of the water is suitable for drinking except in post-monsoon when the sodium content is high in most of the water samples.

Conclusion

The study area characterized by tropical monsoon climate with seasonal temperature variation and often excessive humidity.

The present study deals with the seasonal variation of groundwater chemistry in Khulna City. The major cations (Ca, Mg, Na and K); the major anions (HCO_3 , Cl, SO_4 , PO_4); and microbial parameters (Coliform). were analyzed in pre-monsoon, monsoon and post-monsoon seasons. Comparing to the WHO and Bangladesh standard the study concludes that most of the water is suitable for drinking except in post-monsoon when the sodium content is high in most of the water samples. But the microbial analyses show that tap water is not safe for drinking. The city dwellers are to pay extra charge to collect or to filter water to drink.

Khulna WASA is now supplying 110 million liter water per day through 282 km long pipeline network among some 18000 households. At the end of 2018 it is expected that 75000 households would be brought about in this network with 650 km long pipeline. Then 240 million liter water per day would be supplied. Although kwasa claims their service is satisfactory, due to some technical reason people are to suffer. Regular monitoring and surveillance is required.

References

- Ahmed, M. F., and Rahman, M. M. 2000. Water Supply & Sanitation. ITN-Bangladesh. Publ. 1st Edition: 301-338p.
- APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 19th Edition
- Berner, Kay, E., and Berner, R.A. 1987. The Global Water Cycle. Prentice-Hall, Inc., Englewood Cliffs. New Jersey 07632: 185-235p.

The Impact of Cultural Diffusion and Globalization: Basic Study on Garo Ethnic Community of Bangladesh

Abu Ibrahim Mohd Nurul Huda and Srijon Sangma

Location: Department of Sociology, Eden Mohila College, Dhaka

Duration: One Year (2013 - 2014)

Expenditure: Tk 530000.00

Introduction

Garo is one of the major ethnic communities living in Bangladesh. Usually these people prefer the name 'Mandi' instead Garo. In Bangladesh, out of many other linguistic and cultural groups the Abeng, the Attongs, the Braks, the Chiboks, the Duals, and the Megams – are the six sub-tribes are found to live. The specific sub-tribe's residing area could be traced where these community people have been usually residing generation to generation. For more than four thousand years the Garos are nourishing their ethnic cultural tradition, but in spite of that distinctiveness their cultural assimilation is going on due to cultural diffusion and cultural globalization. As Garos are closely staying with Bengalese for a long period and due to cultural hegemony of western nations the Garo ethnic community is facing and experiencing some sort of changes in their social structure. This study has inclined to identify the factors affecting the cultural changes among the Garos living in Bangladesh. The study has focused some changes on Garo marriage system, family structure, Garo customs, Garo social and political organization, Garo Sangsarek religious beliefs, values, rituals and change, higher education and mobility in Garo society, satellite culture and information technology on Garo society.

Objectives

There are some basic objectives of this study. These are:

- to investigate the changing process of family structure among the Garos, if any;
- is there any change in the customs related to clothing and daily behavior and conduct;
- is there any distinctly marked changing process in social and political organizations of Garos;
- is there any impact of capitalism and western culture on matriarchal and matrilineal family structure;
- is there any impact of Bengali and Western culture on their ethnic religious beliefs, values, rituals;
- to study the impact of higher education (mobility) among the Garos.

Methodology

For the purpose of the study three sub-districts (upazilla) of Netrokona and Mymensingh districts have been selected. The sub-districts are Durgapur and Kalmakanda of Netrokona and Dhobaura of Mymensingh district. The six sub-tribes of Garo community live dispersedly in these sub-districts. Total 30 HHs from each sub-tribe have been brought under the interview process. So a grand total of 180 HHs (6 sub-tribe x 30 HHs from each = 180 samples) have been total samples. These samples have followed Deliberate or Purposive sampling procedure. Some elderly people of this community have been brought under in-depth study by the principal investigator himself. Five Unions, one Pouroshobha and six villages have been selected on the basis of Simple Random sampling procedure. Information has been collected from both sources - primary and secondary. To collect some previous secondary information, historical method has been applied. To collect factual primary information, social survey method has been conducted. The questionnaire has been compiled with open ended and close ended questions and some sorts of unstructured questions have been included. Six FGDs have been conducted with each sub-tribe.

Results

The tradition was to select the bridegroom from maternal side. But at present this tradition is not in vogue. The educated bridegroom doesn't want to settle at in-law's house. So the tradition doesn't persist. They bring their bride at their own houses breaking the tradition. Endogamy is being practiced frequently. As traditionalism has been broken, none is being forced to abide by the traditional marriage system. Due to education, the females of new generation are enjoying individualism, freedom of speech, and freedom of spatial mobility. Changes have commenced in inheritance system too. Usually the younger daughter of the family inherits the property. At present, sons are also receiving the shares of their properties. The leadership of the family goes through sharing process. Both husband and wife take the decisions of their family matters. The respondents have informed that Western and Bengali culture have profound influence over their family and social lives. Once, the Garo male used to put on nengty, a small piece of cloth to cover his genital organ, but now they are putting on shorts, pants, t-shirts, dhuti, Panjabi etc. Though the females usually put on traditional dress at home but when they move outside of their houses they put on jeans, t-shirts, shares, blouses, shalwar, kamij etc. Earlier, the females used to put on un-sewed cloths covering up to the knee, use big ear rings with small rings on the upper side of the ears, these system is not being viewed anywhere. Anybody can find profound change among the Garo societies due to their migration and looking for jobs outside their villages especially to the urban areas. Those who are job holders are purchasing land properties in their villages and erecting their old traditional houses in accordance with urban style. They have the change in their life-styles.

Table 1: Selection of Process of Bride and Bride-Groom

Is there any change on traditional selection process of bride and bride-groom	Frequency	%
Yes	170	94.44
No	10	5.56
Total	180	100.0

Table 2: Reasons behind this

If yes, what are the reasons behind this process	Frequency
Impact of Christian religion	159
Impact of Bengali culture	170
Impact of Western culture	131
Attitude towards disobeying the tradition or individualism	162

Table 3 : Whether any change on traditional dress customs

Whether any change on traditional dress customs	Frequency	%
Yes	180	100.0
No	0	0
Total		100.0

Table 4 : If yes, how the changing process is proceeding

If yes, how the changing process is proceeding	Frequency
Children are putting on jeans pant and shirt following western fashion	173
Putting on pant, shirt, panjabi, fatua following Bengali fashion	177
Putting on dhuti at home but pant-shirt in outside	160
Putting on lungi-shirt at home but pant-shirt in outside	171
Increasing attitude towards traditional dress	0
Others (females are putting on t-shirts, jeans, shalower , kamij and share)	150

Table 5 : If yes, how the changing process is proceeding

Attitudes towards Bengalese	Frequency
Bengalese are aggressive	18
Bengalese are oppressors	10
Bengalese are land grabbers	35
Bengalese are violators	12
Bengalese are friendly	160
Bengalese are benevolent	73
Bengalese are sympathetic	87

Table-6: Whether the financial conditions are changing in the village lives due to the urban based education and profession of the children

Whether the financial conditions are changing in the village lives due to the urban based education and profession of the children	Frequency	%
Yes	180	100
No	0	0
Total	180	100

Conclusion

The study has successfully investigated the changing process of family structure among the Garos. Some reasons behind this process have been identified. The respondents and the ethnic community members have opined that education, mobility, non-government profession, Christianization, and influence of Bengali and Western culture have inverted the traditional social lives. Changes in their social and political organizations have commenced due to the impact of cultural assimilation and diffusion. The impact of capitalism is not directly visible, but it has profound impact on the socio-economic lives of the Garos. They are struggling for moving towards the middle class status from the poor marginalized class with the help of education, non-agricultural professions, social, spatial mobility. The younger generation is migrating to urban areas for higher education and jobs for enhancing their financial conditions. Some are purchasing land in their villages and erecting their houses in accordance with the pattern of modern Bengali culture or western culture. Matrilineal family structure still exists but influence of the females or mothers are gradually decreasing due to westernization and globalization process. There is western cultural impact on their religious beliefs, as more than 98% of the Garos living in Bangladesh have been converted to Christianity. The Garos have gone through the changing process due to spatial mobility, education, conversion to Christianity, urban based non-agricultural jobs, business etc. They are connecting with modernity, modern information technology, individualism, and freedom of speech, freedom of taking personal decisions, freedom of attitude, and freedom of spatial mobility. They think all these are happening due to cultural diffusion and aggression of Bengali and Western cultural. The Garos are also embracing change due to the Christianization. Their religious beliefs have brought profound change over all aspects of their life-style and they have come to the outer world and communicating with influential cultures.

Mainstreaming of Eccentric Aquatic Production System as a Climate Adaptive Alternative Livelihood Strategy for the Coastal Community

Md Nazmul Ahsan and Sk Mustafizur Rahman

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

Duration: One year (2013-2014)

Expenditure of the project: Tk. 800000.00

Introduction

The coastal region of Bangladesh has, until recently, supported the livelihoods of millions mainly through subsistence fisheries activities. However, unplanned and rapid expansion of shrimp culture has resulted in grave socio-economic consequences, which have been passed on to rural poor communities (Guhathakurta, 2003). Loss of employment has led to the displacement of thousands of people from lands used traditionally towards the adjacent cities in search of alternative livelihoods involving harvesting and selling of unconventional aquatic products such as aquatic fruits and vegetables, snails etc. While such eccentric aquatic production system is creating a niche in the economic activities with a notable potential for new livelihood opportunities, the problem remains with apprehending such realities with adequate research based market information and policy supports. It was, therefore, essential to identify, elaborate, discuss and consolidate the socio-economic and livelihood situation of coastal communities involved in eccentric aquatic production system in rural and rural-urban areas of coastal districts.

Objectives

The broad objective of this project was to know the socio-economic and livelihood condition of fisher folks evicted from traditional coastal communities, their role and perception about eccentric aquatic production system in the vicinity of coastal cities and to analyze the potentials such unconventional aquatic production as alternative livelihood strategy.

Methodology

A total of fifteen (15) markets enriched with non-conventional aquatic products from nine (09) upazillas under three (03) districts (Khulna, Satkhira and Bagerhat) located at the south-western coastal region of Bangladesh were selected following established scientific procedures. Selected markets were visited and surveyed with a structured questionnaire for the identification and characterization of non-conventional edible aquatic products and the marketing channel of those products. Identification of the plants were done by consulting with local experienced persons and further confirmed through consulting relevant botanical reference books. To know the socio-economic and livelihood conditions 90 women vendors involved in marketing of such aquatic products were interviewed using a semi-structured questionnaire. In addition, proximate composition of four selected aquatic plants was carried out following established protocol (AOAC, 2000; Raghuramulu *et. al.*, 2003).

Results

The questionnaire survey revealed a holistic overview of 27 specified indicators belonging to five broad aspects of socio-economic and livelihood conditions of the respondents. The five aspects included i) Demographic aspects: age, religion, marital status, family type, family size, academic qualification, type of occupation; ii) Social aspects: housing condition, size of family land holding, restriction from family, problem faced in home/family, housing condition, size of family land holding; iii) Health and sanitation aspects: type of illness, type of treatment received, knowledge about family planning; iv) Economic aspects: source of credit, income per month, expenditure per month, savings per month, source of credit and v) Other aspects including type of seller, type of selling place, type of products of sold, collection time of aqua product, method of preservation. The analysis of data revealed that the collection and marketing of aquatic vegetables and fruits are carried out exclusively by women most of whom are widow or divorced and family to feed. It was the primary source of income for about two-third of the respondents and most women have maintained a minimum livelihoods by being involved with this trade. They appeared to be poorest of the poor with no knowledge on market integration of such products and as such earn a nominal livelihoods by selling these products in the urban fringe markets.

Identification and characterization of aquatic products

A total of 35 non-conventional aquatic products belonging to 27 families were identified and characterized from the study areas. Out of the 35 aquatic products, 32 species were aquatic plants while the remaining three were animal species (snails). Of the 32 aquatic plants, only 12 species under eight families were found to be edible by the local people. The remaining 20 plant species belonging to 16 families were found to be used as cattle feed, as herbal medicine, agriculture manure etc. Table 1 summarizes the edible plants with their classification and other information.

Table 1: List of non-conventional edible (by human or other animal) aquatic products

Local Name	Common/English Name	Scientific Name	Photographs
Edible (for human) aquatic local plants found in the study areas			 <p>Fig 1: Malanacha</p>  <p>Fig 2: Panifal</p>
Malancha/Gira	Alligator weed	<i>Alternanthera philoxeroides</i>	
Sacchi	Sessile joyweed	<i>Alternanthera sessilis</i>	
Brahmi	Water hyssop	<i>Bacopa monnieri</i>	
Kochu	Green arum leaves	<i>Colocasia esculenta</i>	
Kochu shak	Taro	<i>Colocasia nymphae</i>	
Kochur loti	Green taro	<i>Colocasia esculenta</i>	
Helencha	Water cress	<i>Enhydra fluctuans</i>	
Kolmi	Morning glory	<i>Ipomoea Iquatic</i>	
Shapla	Red and blue water lily	<i>Nymphaea nouchali</i>	
Poddo	Lotus	<i>Nelumbo nucifera</i>	
Pani fol	Water chestnut	<i>Trapa bispinosa</i>	
Cathali	Broadleaf cattail	<i>Typha latifolia</i>	
Edible for other animals but not human			 <p>Fig 3: Kochu</p>  <p>Fig 4: Helencha</p>  <p>Fig 5. Shapla</p>
Chand Mala.	Arrow-Head	<i>Sagittaria guyanensis.</i>	
Naja Grass.	Water-Nymph	<i>Najas graminea.</i>	
Rashchora	Rashchora	<i>Hydrolea zeylanica.</i>	
Zanji	Bladderwort	<i>Utricularia flexuosa</i>	
Chosuri Pana	Water Hyacinth.	<i>Echhornia erassipes</i>	
Pani Kochu.	Smile Aurum.	<i>Monochoria vagiralis</i>	
Ghechu.	Ghechu	<i>Aponogeton appendiculatus</i>	
Jalpippali.	Longleaf Pondweed	<i>Potamogeton nodusus.</i>	
Ghagra Shak	Common Cocklebur	<i>Xanthium indicum</i>	
Edur Kani Pana	Rootless Duckweed	<i>Wolffia arrhiza</i>	
Choto Kuth	Arrowhead	<i>Sagittaria sagittifolia</i>	
Sushni Sakh	Sushni	<i>Marsilea quadrifolia</i>	
Panikola	Duck-Lettuce	<i>Ottelia alismoides</i>	
Janglidal	Unknown	<i>Hygroryza aristata</i>	
Bish Katali	Water-Pepper	<i>Polygonum hydropiper</i>	
Pani Chach	Purple Spike Rush	<i>Eleocharis atropurpurea</i>	
Nima	Frog Fruit	<i>Phylla nodiflora</i>	
Tilok Pana	Floating Watermoss	<i>Salvinia natans</i>	
Topa Pana	Water Lettence	<i>Pistia strotiortes</i>	
Pati	Fringe-Rush	<i>Fimbristylis acuminata</i>	
Jhinuk	Duck mussel	<i>Anodonta anatina</i>	
Shamuk	Apple snail	<i>Pila globossa</i>	
Kuiche	Swamp eel	<i>Monopteris albus</i>	

Marketing channel and value chain

The marketing system of non-conventional aquatic plants was very simple compared to traditional crops and fish. Price is determined by direct bargaining but the women vendors were found to accept any price they are offered due, perhaps, to the centric nature of the products. In some cases, marketing channel involved one to two intermediaries as shown in Table 2.

Table 2: Four different supply chains identified in the study areas

Category	Flow of products			
Supply chain-1	Collector	—————→		Consumer
Supply chain-2	Collector	————→	Retailer	————→ Consumer
Supply chain-3	Collector	————→ Paiker	————→ Retailer	————→ Consumer
Supply chain-4	Collector	————→ Mediator	————→ Paiker	————→ Retailer ———→ Consumer

Proximate composition of four selected plants

All four species subjected to proximate composition analysis were found to be nutritious in terms of protein, lipid, ash and moisture content. Among the four species *Enhydra fluctuants* was found to be the most significant and nutritious. Nutrition contents in leaves were higher than in stems (Table 3).

Table 3: Proximate composition of selected edible aquatic plants

Proximate composition	Helencha		Malancha		Kochu		Shapla	
	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf*
Moisture %	71.03±1.0	67.16±1.0	70.19±1.0	60.19±1.0	77.01±1.0	59.41±1.0	83.03±1.0	
Protein%	19.64±0.6	20.58±0.5	16.50±0.4	19.97±0.4	6.34±0.4	9.04±0.2	12.62±0.8	
Lipid%	2.02±0.2	2.96±0.1	1.81±0.1	1.12±0.2	1.11±0.3	1.79±0.4	2.00±0.1	
Ash%	17.28±0.5	16.13±0.5	14.73±0.5	13.93±0.5	14.91±0.5	13.39±0.5	14.29±0.5	

*Not determined. Data shown are as dry weight basis

Conclusion

Collection from peri-urban waterbodies and selling in urban hinge markets of unconventional aquatic fruits and vegetables is the primary source of income for poor women who do not have any other livelihood options. While there is great potentiality of mainstreaming of eccentric aquatic vegetables for creating sustainable livelihood option for these women, lack of education, family restriction, discrimination and misbehave by buyers, in absence of technical assistance and market information are constraints to the sustainability of aquatic vegetable trading. Moreover, poor livelihood assets, common diseases problem, weak transportation system and knowledge gaps regarding aquatic vegetable trading are identified as constraints for mainstreaming these otherwise valuable products. It is therefore essential to afford institutional, organizational and government support for sustainable aquatic vegetable trading.

This study has for the first time identified and characterized several edible aquatic weeds in this region with notable market demand. Amongst the aquatic plants found, *Enhydra fluctuants* (Helencha) appeared to be the most nutritious, regular consumption of which could help in alleviating the problem of malnutrition at negligible cost. However, further studies are necessary to determine the micronutrients contents of the studied plants and other edible aquatic plant species.

Notwithstanding this, cultivation of these aquatic vegetables in managed landscape should be encouraged. Instead of collecting from the wild, farmers can increase the production by growing aquatic plants in their unused wasteland, which can provide an additional income generating avenue.

Unlike fish, shrimp and traditional crop items, a predominantly short and simple supply chain with none or a few intermediaries was observed for aquatic vegetables. The marginally poor people were the primary linkers, who collect aquatic vegetables from pond, ditches, marshy lands, beels and riversides, to the edible aquatic products marketing channels. Accessibility to the market and lack of transport facility has been identified as a major obstacle to sell and obtain accurate price of the products.

While a detailed study involving value chain analysis and characterization of all nonconventional aquatic products are necessary the findings of the present study, nevertheless, provides a holistic overview of the eccentric aquatic production system and its potential to become a climate adaptive alternative livelihood for the poor women in the three coastal districts of Bangladesh. The results of the present study might pave the way for orientation of policy and program towards mainstreaming of such eccentric aquatic production system so as to develop a climate adaptive sustainable alternative livelihood option for the otherwise vulnerable poor women community of the coastal and other districts of Bangladesh.

Publication from this Research

Hasan, M. M., Nahar, S., Arafat, S. T., Debnath, S., Parvez, M. S., Rahman, S. M. and Ahsan, M. N. 2016. Proximate composition of edible aquatic vegetables: A preliminary assessment of four species from Bangladesh. *Khulna University Studies*, 13 (1): 49-53.

References

- AOAC, (2000). Official Methods of Analysis. Association of official Analytical Chemist, EUA.
- Grivetti, L.E., and Ogle, B.M., 2000. Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. *Nutrition Research Reviews*, 13: 31-46.
- Guhathakurta, M. 2003. Globalization Class and Gender Relations: The Shrimp Industry In South-Western Bangladesh, In: Globalization, Environmental Crisis And Social Change In Bangladesh, Motiur Rahman (ed.), The University Press Ltd., Dhaka, Bangladesh.
- Raghuramulu, N., Madhaban, N.K. & Kalyanasundaram, S. 2003. A Manual of Laboratory Techniques. National Institute of Nutrition. *Indian Council of Medical Research*, Hyderabad, India. pp. 56-58.

Crystal Structures and Growth Mechanisms of Nanoparticles Prepared by Polyol Method with Different Couples of Metal

Md Jahangir Alam and Fahmida Sultana

Location: Department of Agronomy and Agriculture Extension, University of Rajshahi

Duration: One year (2013-2014)

Expenditure of the project: Tk. 750000.00

Introduction

Nanomaterials have received a great interest over the past two decades due to their unique electronic, physical, and chemical properties with dimensionality and size confinement, which are often substantially and sometimes radically different from their constituents or bulk counterparts. Chitosan–Silver nanocomposite is one of the rare composite materials that is seen to possess a capability of being used as a biosensor as well as in the treatment of cancer as the chitosan present in the nanocomposite is very specific to the cancer cells. It prolongs the action of silver on the affected cells while preventing the normal cell from the effect of silver. One more advantage of this nanocomposite is that it is biodegradable i.e., it can be degraded by the enzymes present in the body making it suitable for the treatment of cancer. The influence of the salt concentration on the optical properties, structures and morphologies of CS-Ag nanoparticles was characterized by Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and Ultraviolet-visible spectroscopy (UV-Vis).

Objectives

Nanoparticles are fundamental to modern science and technology. Mastery over the shape of a nanocrystal enables control of its properties and enhancement of its usefulness for a given application. The specific objectives of the proposed project are as follows:

- Development of a new technique for synthesis of more active nanoparticles.
- Improvement of knowledge for growth mechanisms of nanocrystals.

Methodology

Preparation of chitosan-silver nanoparticles: 0.05g of chitosan was dissolved in 20 mL of HCl (10 mole/L) solution and used as stock solution. 5 mL stock solution, 5 mL DMF or polyol and 0.05g of SDS were mixed for 30 min using a magnetic stirrer to get homogeneous solution. 4.90 mM silver nitrate salt was added in 10 mL DMF or polyol and mixed for 30 min using magnetic stirrer to get homogeneous solution.

The total volume of solution was 20 mL and final concentration of AgNO_3 was 2.45 mM and these placed in a three neck round bottom flask. Out of three neck of the round bottom flask, one neck was kept for solution upload, the other neck was used for thermometer and the middle one was used for condenser setup. When the effect of concentration of Ag precursor salts was examined, the concentration of AgNO_3 was changed from 2.45 mM to 5 mM for the preparation of CS-Ag nanoparticles. The Ag/SDS molar ratio was kept at the same values as those used at initial condition. The solution temperature was increased from room temperature to 120°C by heating for 10 min.

Results

Freshly prepared nanoparticles were centrifuged with ethanol, followed by casting onto glass slide for film and subsequent dried in open air at room temperature. Figure 1 depicts SEM images of CS-Ag nanoparticles with different metal ion concentrations such as 2.45 mM, and 5 mM.

SEM observation: The surface morphology of synthesized CS-Ag nanocomposite was analyzed using SEM technique. The SEM image of CS depicted that the particles are in the form of bundles with a leaf morphology in Figure 1a. The SEM images of CS-Ag nanoparticles depicted that the shape of particles are nearly spherical or plate like shaped in Figure 1b for 2.45 mM. Figure 1b shows a mixture of CS and Ag wherein Ag nanoparticles are seen to be enveloped by the chitosan (CS) polymer indicated in Figure 1b [1]. In case of 5 mM concentration, the synthesized particles are in the form of aggregates or larger in size than that of 2.45 mM CS-Ag particles in Figure 1c. It is observed that Ag nanoparticles are embedded in a matrix of chitosan. The morphology of CS-Ag nanoparticles was predominantly nearly spherical or plate like in Figure 1c and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph of Figure 1d under heating or storage.

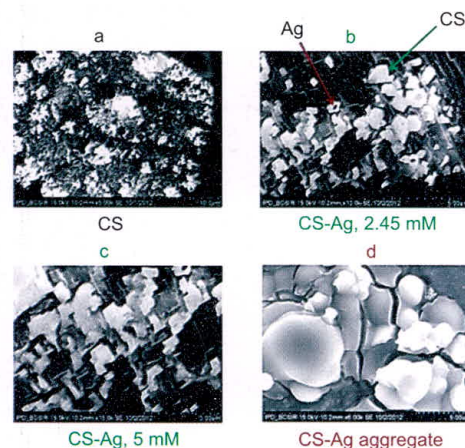


Fig. 1: a, b, c and d represent SEM micrograph of freshly prepared chitosan, CS-Ag nanoparticles for 2.45 mM, 5 mM and aggregated nanoparticles under heating respectively.

The peak at 2835 and 2831 cm^{-1} correspond to O-H stretch carboxylic acids of CS-Ag nanoparticles and chitosan itself respectively. The peak at 1402 and 1383 cm^{-1} correspond to C-N stretching of aromatic amine group of CS-Ag nanoparticles and chitosan itself respectively. The other bands are of C-O stretch alcohols and carboxylic acids observed at characteristics spectral shifts. Therefore the synthesized CS-Ag nanoparticles are surrounded by chitosan having functional groups of amines, carboxylic acids, alcohols and esters.

UV-Visible spectroscopy analysis: The UV-Visible spectral measurements were carried out using UV-Visible spectroscopy. Figure 3 depicted the UV-Vis spectra of Ag nanoparticles prepared from two different concentrations of AgNO_3 (2.45 mM, and 5 mM) with chitosan (5 mL) for 10 min heating. All spectra exhibit an absorption band in the range of 300–400 nm with a typical plasmon resonance band of CS-Ag nanoparticles. The absorption against wavelength curves at various concentrations is shown in Figure 3. As the salt concentration increases, possibly more and more of hydroxyl groups are converted to carbonyl groups by air oxidation, which in turn reduces more Ag^+ at a constant chitosan concentration. A single strong peak with a maximum around 295 nm was observed in the UV-Vis spectra. The intensity of the absorption of solutions increased with increase in the concentration of AgNO_3 up to 5 mM at constant heating time 10 min. Ag nanoparticles with nearly spherical morphology are embedded in the chitosan matrix which arise a small peak at around 380 nm in Figure 3. As the particles increase in size, the absorption peak usually shifts toward the red wavelength [2].

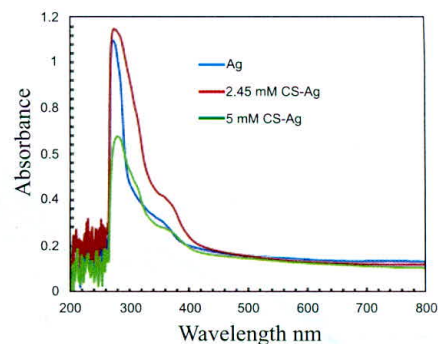


Fig. 2: UV-visible spectra of pure Ag salt solution, CS-Ag nanocomposite obtained from 2.45 mM and 5 mM of AgNO_3 concentrations.

Mechanism: The growth mechanisms of CS-Ag nanostructures under different preparation routes are summarized in Figure 4. In this process a large amount of CS-Ag

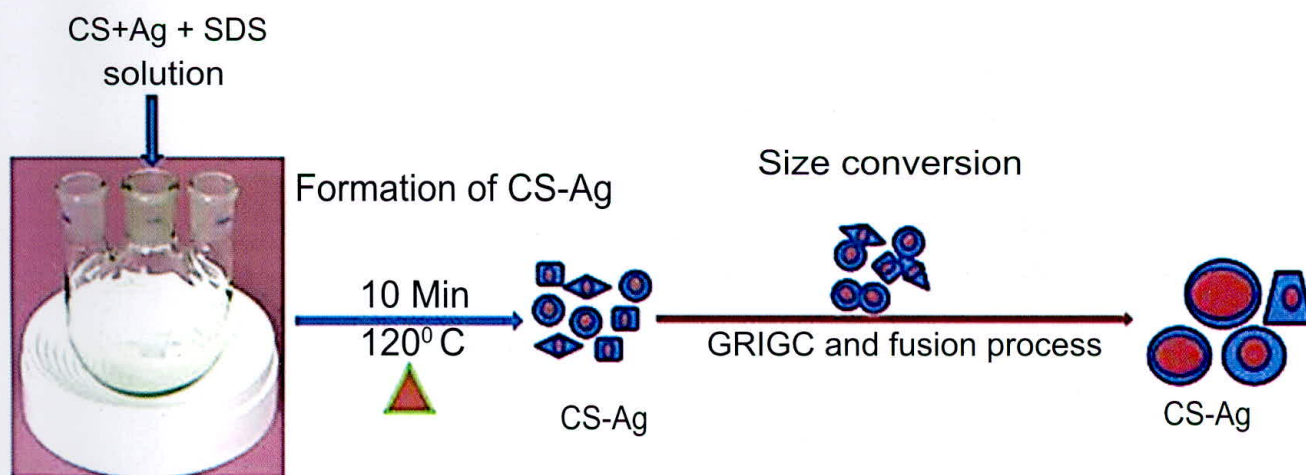


Fig. 3: Schematic diagram of CS-Ag nanocomposite formation and their shape and size conversion at 120°C heating for 10 min.

nanoparticles are prepared. Mechanism in Figure 4 is the grain-rotation induced grain coalescence (GRIGC) and fusion mechanism [3]. According to this model, the rotation of grains among neighboring grains results in a coherent grain grain interface which leads to the coalescence of neighboring grains via the elimination of common grain boundaries, thus forming a larger grain. In our case, single crystal, particles having well defined faces were lost after the formation of large polycrystalline spherical particles. Thus, during GRIGC process fusion of polygonal crystals leading to polycrystalline spherical or plate like particles. . In the Ostwald-ripening mechanism, the atoms from one particle undergo dissolution and then they are transferred to another particle. There is a net atomic transport from the particles with sizes smaller than the average value to larger particles. Particles smaller than the average value will shrink or even disappear.

Conclusion

Silver nanoparticles were successfully introduced into chitosan using non aqueous thermal reduction method. This was confirmed by the maximum surface resonance peak at 295 nm. Topographical heights of the CS-Ag nanoparticles were revealed to be around 295 nm for most of the particles made from 2.45 mM and 5 mM of silver nitrate (AgNO_3) salt. In SEM micrograph nanoparticles with nearly spherical or plate like morphology are embedded in the chitosan matrix which is arise a small peak at around 380 nm. From FTIR spectra it was revealed that the synthesized nanoparticles are surrounded by chitosans having functional groups of amines, carboxylic acids, alcohols and esters. Wide particle size distribution of nanoparticles synthesized here depicts that the parameter, concentration of AgNO_3 , which is responsible for the morphology and stability, should be optimized, suggesting lower salt concentration is favorable.

References

- Alam, M. J., Tsuji, M., and Matsunaga. M., 2010. Shape changes from polygonal gold nanocrystals to spherical nanoparticles induced by bubbling n_2 or o_2 gas in polyol synthesis of gold nanostructures. *Bull. Chem. Soc. Jpn.*, 83(1): 92-100.
- Xia, Y. and Halas, N. J. 2005. Shape-controlled synthesis and surface plasmonic properties of metallic nanostructures. *MRS Bulletin*. 30: 338 – 343.
- Yoshizuka, K. Lou, Z. Inoue, K. 2000. Silver-complex chitosan microparticles for pesticide removal. *React. Funct. Polym.*, 44 (10): 47–54.

Approaches of Optimizing the Production and Quality of Tomato in the Adverse Climatic Condition of Summer Season

Mohammad Humayun Kabir and Khairul Kabir

Location: Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka

Duration: One year (2013-2014)

Expenditure of the project: Tk. 500000.00

Introduction

Tomato (*Solanum lycopersicon*) is sown mainly from October to November and becomes available for consumption from February to April. From March through September, tomatoes are practically not grown in Bangladesh due to the adverse weather conditions of summer including high temperature, high rainfall and high light intensity. But, in this period, the country has to import tomatoes every year from India. High temperature and heavy rainfall (humid condition) are the major problems of unfruitfulness for summer tomato production in Bangladesh. High temperature is responsible to limit fruit set due to an impaired complex of physiological process in the pistil, which results in floral or fruit abscission.

Nowadays, some anti-transpirants are using to minimize transpiration as well as to reduce leaf temperature. Using antitranspirants such as kaolin may reduce transpiration rates from the plant; consequently reduce the amount of used water and improved the water use efficiency while it did not reduce carbon assimilation. Kaolin is a non-toxic aluminosilicate ($\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$) clay mineral. It reflects back a portion of the incident radiation falling on the upper surface of the leaves and cooling down the surrounding temperature. The use of kaolin-based particle film technology would be an effective tool to alleviate heat stress and to reduce water stress in tomato production.

Foliar feeding is the best way for summer tomato production resulting higher yield as well as higher income from per unit area of land. Growers in some countries are commercially producing tomatoes at higher temperature through exogenous application of synthetic hormones. In recent-past, a large number of investigators have studied the effect of various micronutrients on vegetative and reproductive parameters including deficiency symptom and fruit setting. In this circumstance, our target was to take initiatives to make the cultivation of tomato possible and profitable even under adverse climate of high temperature, heavy rain and high light intensity of summer season in Bangladesh. We were planning to overcome the problem of summer tomato cultivation particularly associated with the flower abscission, lower pollination, lower fruit setting and fruit dropping due to the adverse climate.

Our initiative was to use polythene shed as a treatment to control the adverse affects from rain and to maintain stigmatic respectability. We planned to apply some elements such as plant growth regulator 4-CPA (4-Chloro Phenoxy Acetic Acid); micronutrients Zn, B, Mn, Se; and Kaolin as an antitranspirant by which we could improve the yield and quality of tomato by regulating or reducing the adverse effect of high temperature and increase fruit setting of the tomato plant.

Objectives

- Findout the efficiency of heat reducing chemical, polythene shed, suitable plant growth regulators and micronutrients to overcome the adverse climatic effect and to maximize the yield of tomato in the summer season of Bangladesh;
- Cultivation of tomato under adverse climatic conditions, including high temperature, high light intensity and rainfall;
- To assess the effect of polythene shed, anti transpirant, micronutrients and growth hormone on the quality of summer tomato;
- Efforts to improve the summer tomato production in Bangladesh by searching problem oriented findings to the tomato production technology.

Methodology

The pot experiment was conducted at Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka - 1207, Bangladesh to determine the productive potentiality of summer tomato (BARI-4) by the influence of foliar application of plant growth regulator 4-CPA (4-Chloro Phenoxy Acetic Acid) and micronutrients Zn as Zinc Sulphate ($ZnSO_4$), B as Boric Acid (H_3BO_3), Mn as Manganese Sulphate ($MnSO_4$), Se as Sodium Selenate and Kaolin as an antitranspirant which is non-toxic clay particle "aluminosilicate" ($Al_4Si_4O_{10}(OH)_8$). This chapter includes a brief description of materials used, treatments, location of the experiment, characteristics of soil, weather & climate and process of experimentations etc.

Treatments of the experiment:

The experiment consisted of two factors:

Factor A: Polythene shed

- i. P0: Without Polythene Shed
- ii. P1: With Polythene Shed

Factor B: Foliar application of yield and quality contributing elements

- i. F0: Control (Spraying of tap water only).
- ii. F1: Spraying of Plant growth regulator 4-CPA (4-Chloro Phenoxy Acetic Acid) as per manufactures recommendation (5 ml per liter of water).
- iii. F2: Spraying of Zn as Zinc Sulphate ($ZnSO_4$), B as Boric Acid, Mn as Manganese Sulphate ($MnSO_4$), Se as Sodium Selenate @ 100 ppm for each of the nutrient solution.
- iv. F3: Spraying of Kaolin as an antitranspirant which is non-toxic “aluminosilicate” ($Al_4Si_4O_{10}(OH)_8$) @2% solution.
- v. F4: Spraying of Plant growth regulator 4-CPA (4-Chloro Phenoxy Acetic Acid) as per manufactures recommendation (5 ml in per liter of water); Zn as Zinc Sulphate ($ZnSO_4$), B as Boric Acid, Mn as Manganese Sulphate ($MnSO_4$), Se as Sodium Selenate @100 ppm for each of the nutrient solution and Kaolin as an antitranspirant @ 2% solution.

There were 10 (2×5) treatment combinations such as P0F0, P0F1, P0F2, P0F3, P0F4, P1F0, P1F1, P1F2, P1F3, and P1F4.

Design and layout of the experiment

The experiment was carried out in a Complete Randomized Design (CRD). The total plants were divided into two groups (Inside polythene shed to protect rain and without shed in field condition) with 3 replications. Four plants were exposed to each treatment.

Application of the treatments

All the treatments were applied considering the design of the experiment.

Data collection

Data was collected on the following parameters, plant height, foliar coverage, length of internodes, number of effective fruiting branches, number of leaves, length of petiole, number of buds, days to first flower initiation, number of fruits clusters, number of fruit sets and fruit sets percentage, average fruit sets per cluster, number of fruits harvested per plants, fruit weight of individual plant, measurement of leaf temperature, measurement of chlorophyll, measurement of total soluble solids (TSS), measurement of PH, measurement of Vitamin-C, measurement of fruit inner temperature, measurement of β -Carotene and micronutrients, measurement of fruit firmness, etc.

Statistical Analysis

The collected data were statistically analyzed using MSTAT-C software.

Results

The experiment under the project “Approaches of optimizing the production and quality of tomato in the adverse climatic condition of summer season” was conducted at the Horticultural Farm of Sher-e-Bangla Agricultural University (SAU), Dhaka. The experiment consisted of two factors: Factor A: polythene shed condition and Factor B: different treatments of the foliar application of growth and yield contributing elements. The treatments were F0: spraying tap water only, F1: spraying 4-CPA (4-Chloro Phenoxy Acetic Acid) as per commercial formulation, F2: spraying micronutrients solution (Zn, B, Mn and Se) @ 100 ppm, F3: spraying kaolin @ 2% solution and F4: combined application of 4-CPA, Zn, B, Mn, Se and kaolin.

Polythene shed and application of micronutrients and kaolin (antitranspirant) had a positive effect on tomato production and quality. Polythene shed (P1) provided two weeks earlier and higher yield of tomato. P1 provided the maximum yield (1145.07 g/plant) of tomato with the higher content of β -carotene, Vitamin-C and total soluble solid (TSS). In case of F4 (combined application of Zn, B, Mn, Se, 4-CPA and kaolin), significant variation was found in respect of most of the characters studied. F4 treatment performed best in respect of fruit setting, yield (1472.45 g/plant) and quality (rich in Vitamin-C, β -carotene and TSS) of tomato. Considering the treatment combination, the maximum yield (1973.37g/plant) and improved quality tomato was found from the treatment combination P1F4.

Table 1: Effect of polythene shed on fruits yield contributing parameters

Treatments	Number of fruits set per cluster	Total fruit weight per plant (g)
P 0	3.11	668.65
P 1	3.64	1145.07
LSD	0.196	75.882
CV (%)	4.25	3.25

P0: Without Polythene shed and P1: With Polythene shed.

Table 2: Effect of foliar application of yield contributing elements on fruits yield contributing parameters

Treatments	Number of fruits set per cluster	Total fruit weight per plant (g)
F 0	2.63c	495.88d
F 1	3.38ab	910.07b
F 2	3.53ab	950.22b
F 3	2.99b	705.69c
F 4	4.36a	1472.45a
LSD	0.309	119.981
CV (%)	4.25	3.25

[F 0: Control; F 1: 4-CPA; F 2: Zn, B, Mn, Se; F 3: Kaolin and F 4: 4-CPA, Zn, B, Mn, Se, Kaolin]

Table 3: Effect of polythene shed on growth and quality parameters

Treatments	Chlorophyll content	Leaf temperature (°C)	pH	TSS
P0	50.66	34.75	3.70	3.59
P1	51.54	34.93	3.72	4.39
LSD	0.759	0.672	0.310	0.136
CV (%)	4.27	3.56	5.25	6.32

P0: Without Polythene shed and P1 : With Polythene shed.

Table 4: Effect of foliar application of yield contributing elements on growth and quality parameters

Treatments	Chlorophyll content	Leaf temperature (°C)	pH	TSS
F0	47.24c	35.60a	3.65	3.43c
F1	49.55b	35.67a	3.65	4.38a
F2	52.78ab	35.76a	3.87	4.07b
F3	52.60ab	33.67b	3.72	3.48c
F4	53.32a	33.53b	3.65	4.57a
LSD	1.200	1.063	0.491	0.215
CV (%)	4.27	3.56	5.25	6.32

[F0: Control; F1: 4-CPA ; F2: Zn, B, Mn, Se; F3 : Kaolin and F4: 4-CPA, Zn, B, Mn, Se, Kaolin]

Table 5: Effect of polythene shed on yield and quality parameters

Polythene shed	Inner fruit temperature (°C)	Fruit firmness (Neuton)	Vitamin -C (mg per 100 g)	β-carotene (μ gram per 100 g)
P0	35.21	8.84	18.94	3190.55
P1	35.84	9.21	21.49	3895.25
LSD	0.259	0.191	0.430	79.511
CV (%)	1.98	2.47	10.24	9.45

P0: Without Polythene shed and P1: With Polythene shed

Table 6: Effect of foliar application of yield contributing elements on yield and quality parameters

Treatments	Inner fruit temperature ($^{\circ}\text{C}$)	Fruit firmness (Neuton)	Vitamin-C (mg per 100 g)	β -carotene (μ gram per 100 g)
F ₀	36.22a	8.10b	17.25c	3134.41b
F ₁	35.82ab	9.07ab	20.68b	3620.82a
F ₂	35.85ab	9.06ab	20.77b	3649.35a
F ₃	34.80b	9.39a	20.89b	3663.38a
F ₄	34.94b	9.51a	21.47a	3646.55a
LSD	0.410	0.302	0.679	125.717
CV (%)	1.98	2.47	10.24	9.45

[F₀: Control; F₁: 4-CPA; F₂: Zn, B, Mn, Se; F₃: Kaolin and F₄: 4-CPA, Zn, B, Mn, Se, Kaolin]

The present study revealed that the combined foliar application of 4-CPA (4-chloro Phenoxy Acetic Acid as per commercial formulation: 5ml per liter of water), micro nutrients Zn as Zinc Sulphate (ZnSO_4), B as Boric Acid (H_3BO_3), Mn as Manganese Sulphate (MnSO_4), Se as Sodium Selenate @100 ppm, separately, and 2% solution of Kaolin as an antitranspirant (non-toxic clay particle: aluminosilicate: $\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$) under polythene shed condition performed the best compared with all other treatments.

Conclusion

Farmers can grow summer tomato under polythene shed with foliar application of antitranspirant like kaolin 2%, micronutrients like Zn (Zinc Sulphate), B (Boric Acid), Mn (Manganese Sulphate), Se (Sodium Selenate) @ 100 ppm each and growth regulator like 4-CPA (4-chloro Phenoxy Acetic Acid) @ 5ml/L. Hopefully, the findings of the study will help the growers to solve the problems of flower abscission, fruit setting and fruit dropping as well as will improve the yield and quality of tomato in the summer season of Bangladesh. Further trial of this research work in different locations of the country is needed to justify the results for precise recommendation.

Reactions of First Row Transition Metal Ions with Proton Pump Inhibitors

Md Aftab Ali Shaikh and Pradip K Bakshi

Location: Department of Chemistry, University of Dhaka, Bangladesh

Duration: Two years (2013-2015)

Expenditure of the project: Tk. 1700000.00

Introduction

Proton pump inhibitors (PPIs) are a group of drugs that decreases the amount of acid in the stomach and intestines. PPIs act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H⁺/K⁺ ATPase, or more commonly gastric proton pump) of the gastric parietal cells. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H⁺ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion [Liu, W., *et al.*]. Targeting the terminal step in acid production, as well as the irreversible nature of the inhibition, results in a class of drugs that are significantly more effective than H₂ antagonists and reduce gastric acid secretion by up to 99%. The lack of the acid in the stomach will aid in the healing of duodenal ulcers, and reduces the pain from indigestion and heartburn, which can be exacerbated by stomach acid.

The PPIs are given in an inactive form. The inactive form is neutrally charged (lipophilic) and readily crosses cell membranes into intracellular compartments (like the parietal cell canaliculus) that have acidic environments. In an acid environment, the inactive drug is protonated and rearranges into its active form. The active form will covalently and irreversibly bind to the gastric proton pump, deactivating it.

Doctors prescribe PPIs to treat people with GERD, ulcers in the stomach or intestine, or other digestive disorders that may cause excess stomach acid. The vast majority of these drugs are benzimidazole derivatives; however, promising new research indicates that imidazopyridine derivatives may be a more effective means of treatment [Preetha, C.R., *et al.*]. Clinically used proton pump inhibitors are omeprazole (Ome), pantoprazole (Pan), esomeprazole (Eso), rabeprazole (Rab), lansoprazole (Lan) and dexlansoprazole (Dlp).

Another group of pharmaceuticals with similar effects, but different mode-of-action, called H₂-receptor antagonists (H₂RAs). All four FDA-approved members of the group are cimetidine, ranitidine, famotidine, and nizatidine.

Objectives

Both PPIs and H₂-receptor antagonists heal gastric and duodenal ulcers by reducing gastric acid output as a result of H₂-receptor blockade. The drugs are taken orally and reach the H₂-receptors via the blood stream. It is also noticeable that two metal containing drugs such as tri-potassium dicitratobismuthate and sucralfate have long been used in ulcer therapy. The former is bismuth chelate and found to be effective in healing gastric and duodenal ulcers. Sucralfate is a complex of aluminium hydroxide and sulphated sucrose but has minimal antacid properties. Recently it has been reported that certain metal complexes of benzimidazole derivatives have in vitro antibacterial activities [Proceeding, IAEA]. Some H₂-receptor antagonists such as cimetidine [Gosser, D.K. *et al.*], famotidine [Proceeding, IAEA] which, in combination with metal ions, has antitumor effect. Thus the interaction of metal ions with gastrointestinal drugs and the possible effect on their conformation and biological activity are thus very important but research efforts in this area appear to be rare and there remains a vacuum of knowledge in this field.

Since in the blood serum, micromolar levels of loosely bound metal ions, e.g. Cu(II), Co(II), Zn(II) etc. are present, it is likely that these metal ions will interact with the drug molecules under in-vivo conditions. There is little doubt that these metal ions although present in the body system in extremely small quantities, play different roles that are absolutely essential for proper functioning of many large organic molecules. Thus this area of study and research has already emerged as a separate discipline in science drawing attention of many research groups. Here we propose to initiate a new research project on interactions of some biologically important transition metals (Cu, Mn, Fe, Co, Ni, Zn) with a selection of PPIs. This would be studied in a systematic way to probe the nature of their interactions and any implications that these may have in medicinal chemistry. The main objectives of our research plan are: (i) a systematic electrochemical study of the reactions of first transition metal ions with the PPIs (omeprazole, lansoprazole, pantoprazole, rabeprazole and esomeprazole). (ii) investigate the effect of pH on the electrochemical reactions of such transition metal ions with PPIs.

Methodology

Electrochemical Cell

A three electrode electrochemical cell consisted of a working electrode (Glassy carbon electrode (GCE)), a reference electrode (Ag/AgCl (satd. KCl)) and a counter electrode (Pt wire) was used in this cyclic voltammetric study. Besides the working, reference, and auxiliary electrodes the cell also includes a N₂ purge line for removing dissolved O₂ and an optional stir bar. A computerized electrochemical system is capable of performing a series of dynamic electrochemical techniques. All the experimental data were obtained by using CHI 620D Electrochemical Workstation by CHI Inc., USA. A software is available with this instrument for doing various types of electrochemical experiments and for data analysis.

Preparation of Stock Solutions

Acetate buffer solution was used. It was prepared by mixing requisite volume 0.1 M $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ and 0.1 M CH_3COOH solution. 0.1 M $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ and 0.1 M CH_3COOH solutions were prepared using 50% ethanol-water mixer individually.

Stock solution of 1.0 mM Cu(II) and 1.0 mM Mn(II), 1.0 mM Fe(III) and 1.0 mM Ni(II) were prepared with analytical grade of Cu(II) perchlorate hexahydrate, Mn(II) perchlorate, Fe(III) perchlorate hexahydrate and Ni(II) perchlorate respectively with acetate buffer solution. Solution of 0.8 mM Co(II) and 0.8 mM Zn(II) perchlorate were also prepared with analar grade of Co(II) perchlorate hexahydrate and Zn(II) perchlorate salt respectively with acetate buffer solution. 1.0 mM, 2.0 mM, 3.0 mM, and 4.0 mM solutions of omeprazole, pantoprazole sodium, rabeprazole sodium, esomeprazole magnesium were prepared with acetate buffer solution. The metal-ligand solutions for complexation reaction were prepared by adding equi-volume of metal and ligand solution of different concentration to get 1:1, 1:2, 1:3 and 1:4 ratios at desired pH.

Preparation of Working Electrode

This electrode preparation includes the polishing and conditioning of the electrode. At the beginning of each experiment, the working electrode was polished with alumina slurry (a few amount of alumina polishing powder of particle size 0.3 μm and a few drops of water) on the surface of water resistant polishing cloth. Then it was rinsed with plenty of de-ionized water and the whitish alumina was wiped off with a clean tissue paper.

Experimental Procedure for CV

Electrochemical interaction of Cu(II), Mn(II), Fe(III), Co(II), Ni(II) and Zn(II) with omeprazole, pantoprazole sodium, esomeprazole magnesium and rabeprazole sodium in four ratios (1:1, 1:2, 1:3 and 1:4), at various pH values (3.50, 4.00 and 4.50 for Cu(II), Mn(II), Fe(III) and Ni(II)) and 3.50, 4.00, 4.50, 5.00, 5.50, 6.00 and 6.50 for Co(II) and 3.50, 4.00, 4.50 and 5.00 for Zn(II)) were carried out using cyclic voltammetry. The chosen potential ranges for Cu(II) is +1000 to -800 mV, for Mn(II) is +1200 to -1200 mV, for Fe(III) is +1000 to -800 mV, for Co(II) is +1000 to -1100 mV, for Ni(II) is +1200 to -1000 mV and Zn(II) is -400 to -1400 mV.

Results

Electrochemical study of 1.0 mM Cu(II) in acetate buffer solution at different pH values (3.50, 4.00 and 4.50) was carried out at GCE. Fig.1(a) shows a cyclic voltammogram (CV) of 1.0 mM Cu(II) in acetate buffer solution with scan rate of 100 mVs⁻¹ at pH 3.50. In forward scan two cathodic peaks ipc1 and ipc2 at about -231.2 and -420.9 mV respectively and in reverse scan an intense anodic peak ipa2 at about 445.5 mV are observed.

The effect of the scan rate on the electrochemical response of Cu(II) under the identical condition was examined by taking the CVs of Cu(II) with scan rate of 25, 50, 75, 100, 125 mVs⁻¹. The voltammograms are analyzed and various parameters such as anodic and cathodic peak current (i_p), peak potential separation (ΔE_p), peak current ratio (i_{pa}/i_{pc}) are found and the ΔE_p and i_{pa}/i_{pc} reveals that the redox process Cu(II)/Cu(0) is quasi-reversible [Wang, J.] in nature.

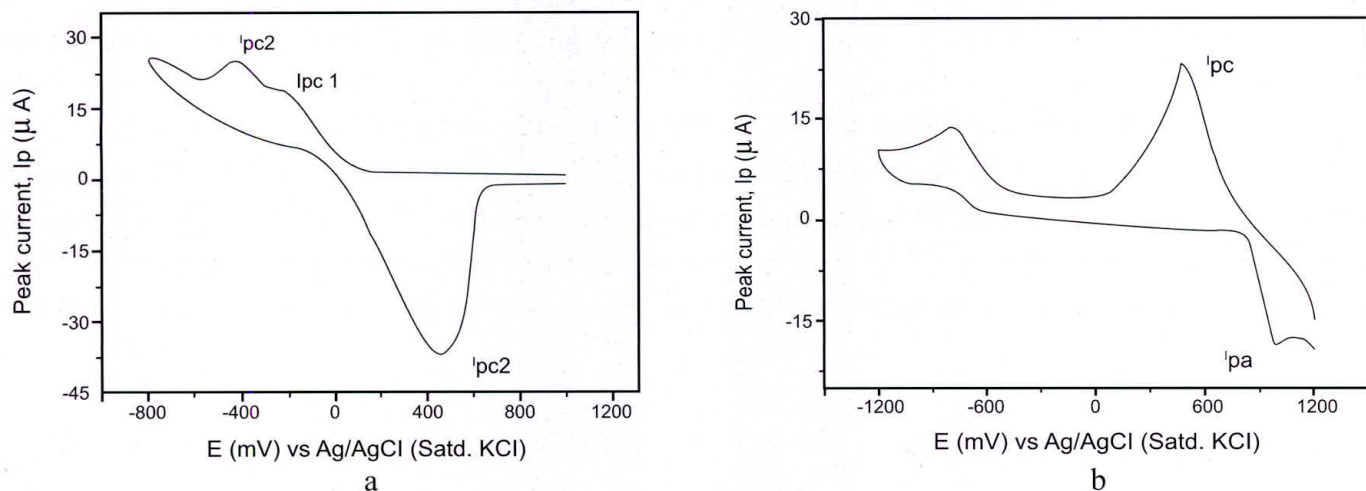


Fig. 1. Cyclic voltammograms of (a) 1.0 mM Cu(II) and (b) 1.0 mM Mn(II) in acetate buffer solution at pH 3.50 with a scan rate of 100 mVs⁻¹.

Therefore, it is a two steps one electron redox process. The effect of pH on the CVs of Cu(II) has also been investigated. With the increase of pH a distinguishable cathodic peak and an intense anodic peak are found. The first cathodic peak is almost disappeared at higher pH.

Table 1: Data for the cyclic voltammograms from Cu(II) in presence of Ome at pH 3.50.

Ratio of Cu(II)/Ome	Peak current, i_p (μA)		Peak potential, E_p (mV)		Peak current ratio, i_{pa}/i_{pc}	Peak potential separation, ΔE_p (mV)
	$i_{pa}(-)$	i_{pc}	E_{pa}	$E_{pc}(-)$		
Cu(II)/Ome	31.56	21.26	439.3	399.4	1.48	838.7
1:1	8.45	-	711.5	-	-	-
1:2	8.22	-	795.0	-	-	-
1:3	9.44	-	795.5	-	-	-
1:4	10.55	-	799.5	-	-	-

Electrochemical studies of the interaction of Cu(II) with Ome at pH 3.50 has been carried out. CVs recorded for Cu(II), and Cu(II) in presence of various molar concentration of Ome (Cu(II)/Ome = 1:1, 1:2, 1:3 and 1:4) and for each ratio of Cu(II) and Ome, the peak current for both anodic and cathodic peak decreases (Table 1) than those for free Cu(II). Moreover, it is observed that the cathodic peaks shifted significantly towards negative direction; while the anodic peaks are almost disappeared. It is suggesting that there is an interaction between Cu(II) and Ome and the strong interaction occurs at 1:2 molar ratio of Cu(II) and Ome.

CVs recorded for 1.0 mM Cu(II) in presence of Ome at pH 4.0 and 4.5 in an identical conditions. Similar results were found and a strong interaction was occurred at 1:2 molar ratio of Cu(II) and Ome. Electrochemical studies of the interaction of Cu(II) with Pan, Eso and Rab in acetate buffer solution at various pH (3.50, 4.00 and 4.50) has also been performed. Similar behavior was observed for the mentioned cases and in each case strong interactions were found at 1:2 molar ratio of Cu(II) and Pan, Eso or Rab.

Voltammetric study of 1.0 mM Mn(II) (Fig. 1(b)) in acetate buffer solution at different pH (3.50, 4.00 and 4.50) was carried out and it is found that the electrode process is quasi-reversible. With the increase of pH cathodic and anodic peak heights are decreased. The electrochemical interaction of Mn(II) with Ome in acetate buffer solution at various pH and different molar concentrations of Mn(II) and Ome, Pan, Eso and Rab (1:1, 1:2, 1:3 and 1:4) has been carried out. The CVs revealed that the strong interactions are found for 1:2 molar ratio of Mn(II) and Ome, Pan, Eso or Rab at every pH.

Cyclic voltammetric behavior of 1.0 mM Fe(III) in acetate buffer solution at different pH were investigated. A cathodic and an anodic peak were observed and the electrode process was quasi-reversible in nature. Effect of the pH on CVs was also investigated and it is found that with the increase of pH a distinguishable cathodic peak and an anodic peak with larger peak height were found. The electrochemical study of the interaction of Fe(III) with Ome, Pan, Eso and Rab in acetate buffer solution at various pH has also been carried out. From the results it is found that a strong reaction occurred at 1:2 molar ratio of Fe(III) and PPIs at mentioned pH.

Electrochemical study of 0.8 mM Co(II) in acetate buffer solution at different pH (3.50, 4.00, 4.50, 5.00, 5.50, 6.00 and 6.50) was also done and the redox reaction followed diffusion controlled quasi-reversible in nature. A strong effect of pH on the electrochemical behavior of Co(II) was also found. The electrochemical interaction of Co(II) with Ome, Pan, Eso and Rab in acetate buffer at different pH has also been observed. The CVs recorded for Co(II), and Co(II) in presence of various molar concentration of PPIs (Co(II)/ PPIs = 1:1, 1:2, 1:3 and 1:4) showed that a strong interaction was happened for 1:2 molar ratio of Co(II) and PPIs at every pH.

Cyclic voltammetric study of 1.0 mM Ni(II) in acetate buffer solution at different pH (3.5, 4.0 and 4.5) was carried out. In the forward scan one cathodic peak while in the reverse scan no peak was observed. The solution pH was also had an effect on the CVs of Ni(II). A strong interaction was observed for 1:2 molar ratio of Ni(II) and PPIs in all cases.

Voltammetric study of 1.0 mM Zn(II) in acetate buffer solution at different pH value (3.50, 4.00, 4.50 and 5.00) was carried out at GCE. The ΔE_p and i_{pa}/i_{pc} reveals that the redox process Zn(II)/Zn(0) is quasi-reversible. Effect of pH on the CVs of Zn(II) in acetate buffer solution has been investigated. With the increase of pH a distinguishable cathodic peak and an intense anodic peak are found. The electrochemical interaction of Zn(II) with Ome, Pan, Eso and Rab in acetate buffer at various molar concentrations and different pH has been carried out. Interaction between the metal ions and PPIs were found and a strong interaction was observed for 1:2 molar ratio of Zn(II) and PPIs at every pH.

Conclusion

Electrochemical studies of the redox behavior of first row transition metal ions such as Cu(II), Mn(II), Fe(III), Co(II), Ni(II) and Zn(II) in acetate buffer solution at various pH were carried out at GCE. Effect of pH on the redox reactions of the metal ions was also investigated. Electrochemical interactions of different metal ions with omeprazole, pantoprazole, esomeprazole and rabeprazole at different molar ratio and various pH were also investigated. Metal ions interacted with PPIs in every mentioned pH while strong interaction was occurred for 1:2 molar ratio of metal ions and PPIs.

Publications

- Biswas, R. Das, M. C., Islam, A., Haque, M. A. and Shaikh, A. A. 2015. Voltammetric study of the interaction of Cu(II) with proton pump inhibitor at different pH. *Bangladesh J. Sci. Ind. Res.*, 50(3), 219-226.
- Das., M.C., Biswas, R., Akter, H., Haque, M.A. and Shaikh, A. A. 2015. Electrochemical Study of The Interaction of Ni(II) Ion With Proton Pump Inhibitors. *Bangladesh J. Sci. Res.*, 28(1) 85-89.
- Das, M.C., Biswas, R., Akter, H., Haque, M. A., Bakshi, P. K. and Shaikh, A. A. 2016. Cyclic Voltammetric Study of the Interaction of Biologically Important Metal Ion with Proton Pump Inhibitors. *Dhaka Univ. J. Sci.*, 64(1), 25-30.
- Biswas, R., Islam, S., Rahman, M. S., Haque, M. A., Hasan, A., Bakshi P. K. and Shaikh, A. A. 2016. Interaction of Manganese(II) with Proton Pump Inhibitor at Different pH: A Cyclic Voltammetric Study. *Dhaka Uni. J. Sci.*, 64(2), 115-120.

- Kabir, A., Yeasmin, H., Hasan, M. A., Rahman, M. S., Hoque, M. A., Bakshi P. K. and Shaikh, A. A., 2017 Effect of pH on the Electrochemical Redox Behavior of Co^{2+} in Acetate Buffer Solution. *Dhaka Univ. J. Sci.*, 65(2), 107-112.
- Begum, S., Yeasmin, H., Shaikh A. A. and Bakshi, P. K., Cu(II), Zn(II), Cd(II) and Hg(II) Complexes Pantoprazole (PPZH): Synthesis and Characterization. *Dhaka Univ. J. Sci.*, (Submitted).

References

- Gosser, D.K. 1993. Cyclic Voltammetry (Simulation and analysis) Willey-VCH, Inc.
- Liu, W., Baker, S.S., Trinidad, J., Burlingame, A. L., Baker, R. D., Forte, J. G., Virtuoso, L. P., Egilmez, N. K., Zhu, L., 2013. Inhibition of lysosomal enzyme activities by proton pump inhibitors. *J. Gastroenterol*, 48:1343–1352.
- Preetha, C. R., Gladis, J. M. and Rao, P. T. 2002. Solid phase extractive preconcentration of thorium onto 5, 7-dichloroquinoline-8-ol modified benzophenone , *Talanta*, 58(4):701-709.
- Proceeding of the International Atomic Energy (IAEA), Vienna, Research Coordination meeting on, Nuclear Analytical Technique for Sustainable Development, Kualalampur, (1993).
- Wang, J. 1976. Analytical Electrochemistry, 2nd Ed., A Wiley-VCH publication, 1.

Chapter 2

Funding Year

2014-2015

Synthesis Characterization and Antimicrobial Activities of Some New Ligands and Their Metal Complexes

Saroj Kanti Singh Hazari and Joysree Das

Location: Department of Pharmacy, BGC Trust University

Duration: Three years (2014-2017)

Expenditure of the project: Tk.1000000.00

Introduction

It is well known that sulphur-containing compounds have potential biological activities. The characteristics and biological activities of dithiocarbazate ($\text{NH}_2\text{NHCS}_2^-$), its substituted derivatives and their metal complexes have been investigated for long time. These type of compounds have received much attention because (i) they provide an interesting series of ligands whose properties can be greatly modified by introducing different organic substituents, thereby causing a variation of ultimate donor properties, (ii) the interaction of these donor atoms to metal ions give complexes of various geometries and properties and (iii) these types of complexes have potential biological activities.

Moreover, molybdenum chemistry has received considerable interest in the recent years in view of its biochemical importance. The discovery of molybdenum in number of enzymes has led to the continued growth of the coordination chemistry of molybdenum. The potential for biological and physicochemical activities and industrial uses of the molybdenum complexes have stimulated the discovery of a variety of molybdenum complexes in various oxidation states. Molybdenum complexes have also played significant role in domains of analytical chemistry, catalysis, stabilizers, polymers, pigments and dyes, photography, electro-optical display devices and agriculture.

Objectives

Keeping the above facts in mind, it was planned

- To synthesise some ligands (bidentate, tridentate and tetradentate) by condensation of different carbonyl compounds and S-benzylidithiocarbazate;
- To synthesise some molybdenum complexes of these ligands by the reaction of molybdenum salts and these ligands;
- To characterise these ligands and complexes by by ir, ^1H nmr and electronic spectral data, molar conductance, magnetic moment measurement and metal analysis (in cases of complexes);

ii) One tetradentate ligand namely N-(1-Methyl-2-oxopropylidene) hydrazinecarbodithioic acid benzyl ester was synthesized from the reaction of $\text{CH}_3\text{-CO-CO-CH}_3$ with S-benzylthiocarbamate.

iii) Seven bidentate ligands (namely Schiff base ligands of Benzylacetone, 4-Hydroxy-benzaldehyde, Acetophenone, 2-Amino-acetophenone, 2-Amino-benzophenone, 4-Bromo-benzophenone and 2-Acetyl-naphthalin with S-benzylthiocarbamate) were synthesised from the reactions of different carbonyl compounds with S-benzylthiocarbamate.

iv) All these 15 ligands were allowed to react with different molybdenum salts and 15 new molybdenum complexes were isolated.

v) All the ligands and complexes were characterised by IR, ^1H NMR and electronic spectral data, molar conductance, magnetic moment measurement and metal analysis (in cases of complexes),

vi) Antifungal activities of all ligands and their molybdenum complexes against some fungi were studied. For antifungal activities study Griseofulvin has been used as a standard fungicide.

vii) Antibacterial activities studies against four selective Gram positive bacteria of all ligands and their molybdenum complexes reveals that almost all test compounds can inhibit the growth of the bacteria to some extent, except some ligands remain totally ineffective towards some bacteria. The ligands and all of their metal complexes showed higher zone of inhibition against *Bacillus cereus* than Ampicillin does, which have been used as a standard antibiotic. In other cases the zone of inhibition were lower than Ampicillin.

Conclusion

The study reveals that the investigated Schiff-base ligands undergo facile complexation with molybdenum to produce a variety of complexes. All tetradentate and bidentate ligands were found to produce octahedral complexes but all tridentate ligands were found to produce square pyramidal complexes. This may be due to the stereochemical differences of the ligands. The ligands and some of its complexes show good antibacterial activity against different types of gram-positive and gram-negative bacteria. The ligands and the complexes show remarkable activity against different types of fungi. So they may be commercially used as fungicides and as antibiotic. However further studies are needed for that use.

Publication from this Research

Hazari, S.K. S., Dey, B.K., Ganguly, B. and Das, T. 2015. "Synthesis and Characterisation of some molybdenum complexes with some polydentate S-N ligands" – *BGC Trust Univ. Journal* (ISSN 2073-5448), Volume 2, page -1-16,

References

- Barua, S., Rabi, S., Datta, A. K., Debanath, E., Shil, R. K., Roy, T. G., 2016. Palladium complexes with hexamethyl tetraazacyclotetradecadiene (L) and isomers of its reduced form ('tet-a' & 'tet-b'): synthesis, characterization and antimicrobial studies. *J. Incl. Phenom. Macro. Chem.*, 86, 291.
- Hazari, S. K. S., Dey, B. K., Palit, D., Roy, T.G., Ali, M.A. and Sen, K., 1999. Synthesis, characterisation and antifungal activities of some metal complexes of an NS-chelating ligand with ferrocene backbone. *J. Bangl. Chem. Soc.*, 12(1):83 – 91.
- Hazari, S. K. S., Dey, B. K., Palit, D., Ganguli, B. and Sen, K. 2001. Synthesis, characterisation and antifungal activities of some metal complexes of an NS-chelating ligand with ferrocene backbone. *Ceylon J. Sc. (Physical Science)*, 9(2): 23 – 30.
- Hazari, S. K. S., Dey, B. K., Palit, D., Roy, T. G. and Alam, K.M.D. 2006. Synthesis, characterisation and antimicrobial activities of a NS-chelating ligand with ferrocene backbone and some of its metal complexes, *Ceylon J. Sc. (Physical science)* 11:23-31.
- Hazari, S. K. S., Kopf, J., Palit, D., Rakshit, S. and Rehder, D. 2009. Oxidovanadium(IV) complexes containing ligands derived from dithiocarbazates - Models for the interaction of VO₂⁺ with thiofunctional ligands. *Inorgan. Chim. Acta* 362:1343 – 1347.
- Rakshit, S., Palit, D., Hazari, S. K.S., Rabi, S., Roy, T. G., Olbrich, F. and Rehder, D. 2016. Synthesis, characterization and biomedical activities of molybdenum complexes of tridentate Schiff base ligands. Crystal and molecular structure of [MoO₂(L10)(DMSO)] and [MoO₂(L11)(DMSO)], *Polyhedron*, 117: 224–230 .
- Wang, D., Ebel, M., Schulzke, C., Grüning, C., Hazari, S. K S. and Rehder, D. 2001. Vanadium(IV and V) Complexes Containing SNO (Dithiocarbonylhydrazone; Thiosemicarbozone) Donor Sets. *Eur. J. Inorg. Chem.*, 935-942.

Phytophthora in Bangladesh: Classification of Phytophthora Species Based on Ribosomal DNA Sequence

Abul Faiz and Md Jamal Uddin

Location: Department of Horticulture, Sher-e-Bangla Agricultural University

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 3293000.00

Introduction

Their high virulence and ability to spread rapidly throughout the world establishes Phytophthora as one of the most important groups of plant pathogens especially for Horticultural crops. Phytophthoradiseases are very important in ornamental horticulture (in vegetable crops and tree as well). A series of phytophthora species are available in Bangladesh, but the taxonomy of Phytophthoraspecies is not clear. The ability to accurately and rapidly identify the causal agent is essential for implementing disease management and regulatory measures to prevent major crop disease epidemics and mitigate long-term impacts that could result from such events. Accurate identification of new isolates is a challenge, even to experts. Given the expected magnitude of pathogen diversity in nature, the importance of systematically cataloging and sharing taxonomic and phylogenetic information for agricultural security cannot be overemphasized. Furthermore, the observation that fungal/oomycete species easily recognized using phylogenetic are not necessarily morphologically distinguishable (O'Donnell *et al.*, 2004) indicates that molecular identification is critical for recognizing new and emerging threats. In addition, because crop loss is caused by populations of strains that vary within species in traits such as virulence, host range, mating type, fungicide resistance, and/or toxin production, determining species identity is often insufficient. We need to generate a comprehensive picture of the genetic and phenotypic diversity within phytophthora species.

Objectives

Specifically, we aim to accomplish the following purposes:

- To establish a comprehensive phylogenetic framework for Phytophthoras spp.
- To develop and optimize molecular diagnostic tools.

Methodology

Lab establishment: with this project we have established a lab facilities for the advanced research. Demand related specific equipment were confirmed in this laboratory. Related chemicals were also ensured in this laboratory. Brief description of methodology (about one page) required to publish the paper

Results

A total, 19 isolates of Phytophthoraspp were studied in this experiment and strains were collected from varieties of host plants. Collected phytophthora isolates were cultured in the test tubes. DNA extracted and amplification were done with PCR techniques. DNA were tested with Gel electrophoresis.

Isolates and culture

Isolates originated from a wide range of host plants were isolated. All isolates were cultured on V8-agar (20% clarified V8 juice, 0.4% CaCO₃, and 1.5% agar in distilled water) and maintained at 25°C.

DNA extraction: DNA was extracted using the commercial DNA extraction solution (PrepMan Ultra Reagent, Applied Biosystems, Norwalk, CN, USA) according to the manufacture's instruction. About 2cm² of mycelium was scraped from about 1 week-old culture and transferred to 1.5ml micro tube containing 100 µl of half strength of the solution. The solution was incubated at 100°C for 10 min. After centrifugation at 15000g for 3 min., the supernatant was used immediately or stored at -20°C until further use.

Complete ITS region of rDNA was amplified with universal primers ITS1 (5'-TCCGTAGGTGAACCT GCG-3') as forward primer and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') as reverse primer (White *et al.*, 1990). Twenty five microlitres of the PCR reaction mixture contained 1 µm of each primer, 1.25 units of rTaq DNA polymerase (Takara Bio, Shiga), 0.2 mM dNTPs mixture, PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 1.5 mM MgCl₂), and 200 ng DNA template.

PCR was programmed with a 2700 DNA Thermal Cycler (Applied Biosystem, Norwalk, CN) with an initial denaturing at 94°C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30s, and 72°C for 1 min, with a final extension at 72°C for 10min. PCR products were electrophoresed in 2% agarose LO3 (TaKaRa Bio, Siga, Japan) gels was conducted in TAE

Buffer (40mM tris-HCL, pH 7.5, 19mM glacial acetic acid, and 2mM EDTA), stained with Ethidium bromide and visualized with uv light (Fig. 1).

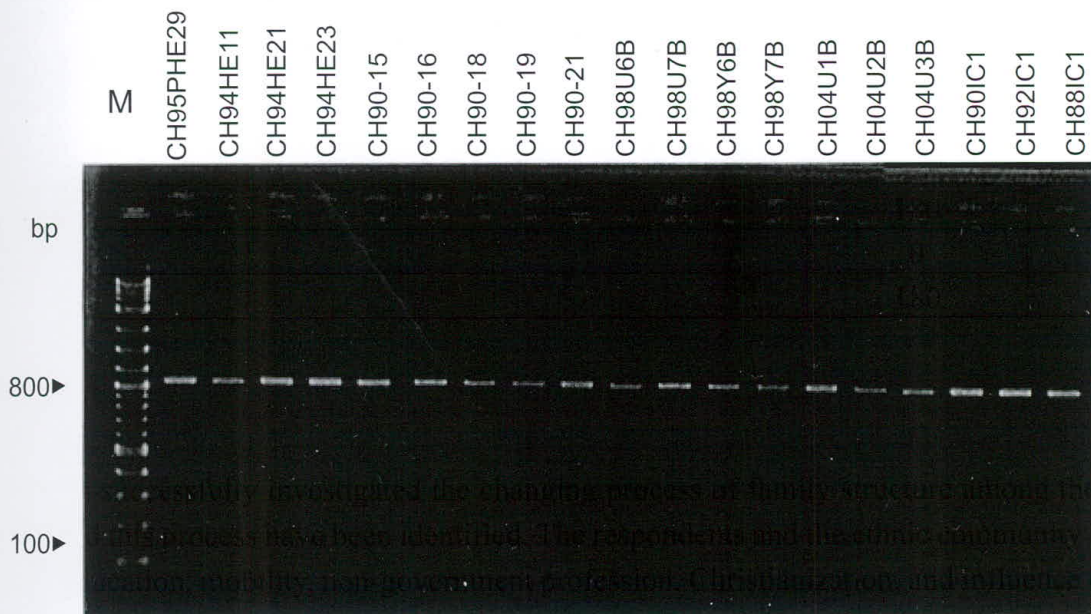


Fig. 1: Agarose gel electrophoresis of PCR products of *Phytophthora* isolates amplified with ITS1 and ITS4 primers, M, 100-bp DNA ladder marker

Conclusion

It was a preliminary work. Further research work should be conducted with other species of *Phytophthora* for conclusion

References

- O' Donnell, K. 1997. Two divergent intra-genomics rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Biology* 5: 93–107.
- White, T. J. 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*, eds. Innis.

Culture and Production of House Fly Larvae and Spirulina Using Poultry Waste and Their Use as Food for Catfish Post-Larvae

Md Ahsan Bin Habib and A H M Kohinoor

Location: Bangladesh Agricultural University, Mymensingh

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 3464000.00

Introduction

Walking catfish is very important table fish in Bangladesh due to its delicateness, good taste and rich in nutrient (Habib *et al.* 2001) and it can easily grow in ditches and small pond and even in polluted water (Habib *et al.* 1994; Habib *et al.* 2001). However, the growth performances of post-larvae showed good results when fed on frozen fresh house fly (*Lucilia sericata*) larvae (maggot) (Experiment 2), and maggot meal (MM) replacing fish meal (FM) and a control diet (Experiment 3). The fry gave better growth when fed control diet (T5) significantly ($p < 0.05$) higher than those fed the diets prepared with different levels of cassava tuber powder (CTV) (Experiment 4). The growth performances of walking catfish (*Clarias batrachus*) fed diets prepared with spirulina meal (SM) were observed better than fed diets with SM and FM, and the control diet prepared with FM (Experiment 5). So, these two feed ingredients such as MM and SM as partially or completely replacing FM and CTP replacing wheat flour up to 10% in diets.

Objectives

To assess the growth performances of post-larvae of catfish fed diets made with partial and complete replacement of FM with SM and MM, and CTP as replacer of 10% wheat flour.

Materials And Methods

1) Growth, production and collection of house fly larvae grown in poultry waste

The house fly (*Lucilia sericata*) usually lays eggs on raw poultry waste (PW) and their larvae grow. The flies were allowed to lay eggs in one day old PW in trays of 10 kg capacity just nearby a poultry farm. The larvae were hatched out at the morning of 2nd day and collected on 4th day morning. First the larvae grown in PW were taken on mosquito net, placed in pond water and allowed to washout all the dirt materials from inside the net. Then the net contained the remaining materials was taken out and the solid materials were carefully picked up, then the larvae was picked up using spoon and forceps, and kept in plastic bottles. The larvae was cleaned using tap water, dried in oven at 50°C overnight and the dried larvae was ground, sieved and powder of larvae was packed in polythene bags and kept in deep freeze for future use as ingredient of feed and chemical analysis.

2) Culture, production and collection of spirulina grown in supernatant of digested poultry waste

Spirulina (Spirulina platensis) was grown in the wet laboratory of Department of Aquaculture, BAU, Mymensingh. Collected poultry waste was air dried, ground and weighed. Ten gram per litre dry poultry waste was taken in 5 L glass jar and mixed with distilled water and allowed to digest under aeration for 12 days. The physico-chemical properties of poultry waste and supernatant of digested poultry waste were analysed. Finally spirulina was grown in 30 L aquaria in supernatant of digested 4.0 g/L poultry waste which gave the highest production. Best optical density of *Spirulina (S. platensis)* was found on 10th day of culture. In that day, spirulina was filtered, collected in petridish and kept in an oven at 40°C for overnight for drying. The proximate composition of meal of maggot, spirulina and CTP was analyzed in the laboratory, BAU, Mymensingh by standard methods (Horwitz, 1984).

3) Preparation of diets replacing FM with MM, SM and CTM and feeding trial of catfish post-larvae

The experiment was conducted to feed walking catfish (*Clarias batrachus*) post-larvae with five isonitrogenous diets including a control diet in the wet laboratory for 28 days in aquaria under recirculatory system. The diets were made using SM, MM and FM (contained high crude protein) and cassava tuber powder as source of carbohydrate (starch). The diets such as Diet 1 (T1) contained 25% (7.50% CP) fish meal, 50% (15% CP) maggot meal and 25% (7.50% CP) spirulina, Diet 2 (T2) contained 25% fish meal (7.50% CP), 25% (7.50% CP) maggot meal and 50% (15% CP) spirulina meal, Diet 3 (T3) prepared with 25% (7.50% CP) maggot meal, Diet 4 (T4) made with 100% (30% CP) spirulina and control Diet 5 (T5) contained 100% (30% CP) fish meal (Table 1). The growth performances of stinging catfish fry fed these five different diets were shown in Table 3). The growth performances of fry such as weight gain, percent weight gain, specific growth rate (SGR), food conversion rate (FCR), protein efficiency rate (PER), apparent protein digestibility (APD) and survival rate were analyzed following standard methods. The proximate composition of prepared diets (Table 2) and carcass composition of fish fry (Table 4) were analyzed following the methods (Horwitz, 1984).

Results

Crude protein content of the ingredients such as SM, MM and FM were high and more than 54% (Table 2). Crude protein around 30% where crude lipids around 10% and ash content around 12% were recorded (Table 3). The percent weight gain of catfish fry fed diet 2 (T2) contained 25% FM, 25% MM and 50% SM was found to be significantly ($p < 0.05$) higher than that of fry fed diet 1 (T1), diet 4 (T4), control diet 5 (T5) and diet 3 (T3) (Table 4). Whereas specific growth rate (SGR) of fry fed diet 2 (T2) had significant ($p < 0.05$) variation from that of fry fed other diets. Food conversion ratio (FCR) of fry fed control diet was significantly ($p < 0.05$) higher than that of fry fed other diets. There was variation among the FCR of fry fed four different diets but not significant. Protein efficiency ratio (PER) of fry fed four different diets prepared with different inclusion levels of MM and SM replacing FM was significantly ($p < 0.05$) higher than that of fry fed control diet prepared with FM as sole source of crude protein. Apparent protein digestibility (APD) fry fed four different diets was higher than that of fry fed control diet but variation was not significant. Survival rate (%) of fry fed diet 1 (T1), diet 2 (T2), diet 3 (T3) and diet 4 (T2) was significantly ($p < 0.05$) higher than that of fry fed control diet (T5). But there was variation observed among the survival rate (%) of fry fed four different diets prepared with different inclusion levels of MM and SM replacing with FM but not significant ($p > 0.05$). Carcass composition of fry fed different diets was analyzed and shown in Table 4.

Conclusion

The walking catfish (*Clarias batrachus*) post-larvae were allowed to feed five different diets including control prepared from three different ingredients as sole sources of protein. The fish meal (FM) was replaced by certain percentage of maggot meal (MM) and spirulina meal (SM) up to 100%. It was recorded that diet 2 (T2) prepared with 25% FM, 25% MM and 50% SM gave significantly ($p < 0.05$) higher growth performances than those fed diet 1 (T1), diet 4 (T4), diet 3 (T3) and control diet (T5) which indicate that fry gave better growth and development when FM was replaced by MM and SM (Table 1). Again, it showed higher growth when fed diet prepared with higher percentage spirulina up to 50% replacement (Diet 2, T2). When FM was replaced with 75% SM in Diet 3 (T3) and 100% SM in Diet 4 (T4), then fry also showed significantly ($p < 0.05$) higher growth performances than fry fed control diet (T5). The carcass composition of fry showed that the crude protein and ash content were higher in fry when fed four diets (T1, T2, T3 and T4) than that of fry fed control diet (T5). It is assumed that FM replacement with SM and then MM in diet enhanced the biosynthesis of protein in the body of catfish fry more than that of fry fed control diet (Ross and Dominy 1990; Nuov 1995; Toyomizu *et al.* 2001; Habib *et al.* 2001; Oluwole *et al.* 2004).

Publication from this research

A PhD and MS theses researches already completed using the fund of this project. The PhD thesis is completed and submitted for the degree recently.

Table 1. Formulation of diets of 30% crude protein for catfish post-larvae containing different levels of protein sources and control.

Ingredients (%)	Diet No.				
	1 (T1)	2 (T2)	3 (T3)	4 (T4)	5 (T5) (Control)
Fish Meal	13.39	13.39	-	-	53.57
Maggot Meal	26.50	13.25	13.25	-	-
Spirulina meal	13.72	27.44	41.16	54.88	-
Cassava tuber powder (CTP)	10	10	10	10	10
Wheat flour	15	15	15	15	15
Soybean oil	5	5	5	5	5
Vitamin premix	2	2	2	2	2
Mineral premix	3	3	3	3	3
Chromic oxide	0.50	0.50	0.50	0.50	0.50
α -Cellulose	10.89	10.42	10.09	9.62	10.93
Total	100	100	100	100	100
Crude protein					

Diet 1, T1 (7.50% CP of FM, 15% CP of MM & 7.50% CP of SM), Diet 2, T2 (7.50% CP of FM, 7.50% CP of MM & 15% CP of SM), Diet 3, T3 (7.50% CP of MM & 22.50% CP of SM), Diet 4, T4 (30% CP of SM) and control diet, T5 (30% CP of FM). 0.56g CP/1.0g FM, 0.566g CP/1.0g MM and 0.5467g CP/1.0g SM

Table 2. Proximate composition of five isonitrogenous formulation of diets (Treatments).

Components (%)	Diet No.				
	1 (T1)	2 (T2)	3 (T3)	4 (T4)	5 (T5) (Control)
Moisture	9.42 ± 0.03	9.48 ± 0.03	9.43 ± 0.02	9.46 ± 0.03	9.44 ± 0.02
Crude protein	30.15 ± 0.09	30.02 ± 0.08	30.01 ± 0.10	29.99 ± 0.08	29.96 ± 0.08
Crude lipids	10.32 ± 0.03	10.20 ± 0.03	10.15 ± 0.02	10.14 ± 0.03	10.16 ± 0.03
Ash	12.44 ± 0.04	12.32 ± 0.03	12.37 ± 0.03	12.40 ± 0.04	12.27 ± 0.03
Crude fibre	9.73 ± 0.03	8.80 ± 0.02	7.45 ± 0.02	6.66 ± 0.02	9.80 ± 0.03
NFE*	27.90 ± 0.07	29.13 ± 0.06	30.54 ± 0.07	31.30 ± 0.07	28.32 ± 0.05

*NFE = 100 – (Crude protein + Crude lipids + Ash + Crude fibre)

Table 3. Growth performances of catfish (*Clarias batrachus*) fry fed five different diets (Treatments).

Growth parameters	Diet No.				
	1 (T1)	2 (T2)	3 (T3)	4 (T4)	5 (T5) (Control)
Initial wt. (g)	0.203 ± 0.03	0.202 ± 0.03	0.204 ± 0.03	0.203 ± 0.03	0.202 ± 0.03
Final wt. (g)	1.552 ± 0.04	1.594 ± 0.04	1.520 ± 0.04	1.525 ± 0.04	1.452 ± 0.04
Weight gain (g)	1.349 ± 0.04	1.392 ± 0.03	1.316 ± 0.03	1.322 ± 0.03	1.250 ± 0.03
Weight gain (%)	665 ± 2.46 ^b	689 ± 2.25 ^a	645 ± 2.16 ^b	651 ± 2.05 ^b	619 ± 2.07 ^c
Specific growth rate	1.68 ± 0.03 ^b	1.82 ± 0.03 ^a	1.66 ± 0.03 ^b	1.62 ± 0.03 ^b	1.47 ± 0.03 ^c
Food conversion ratio	1.81 ± 0.03 ^b	1.79 ± 0.03 ^b	1.85 ± 0.03 ^b	1.90 ± 0.03 ^b	2.19 ± 0.02 ^a
Protein efficiency ratio	1.90 ± 0.03 ^a	1.93 ± 0.03 ^a	1.90 ± 0.03 ^a	1.84 ± 0.03 ^a	1.72 ± 0.02 ^b
Apparent protein digestibility (%)	84 ± 0.30 ^a	86 ± 0.40 ^a	85 ± 0.40 ^a	86 ± 0.40 ^a	82 ± 0.50 ^a
Survival rate (%)	87 ± 4 ^a	91 ± 3 ^a	89 ± 4 ^{ab}	86 ± 4 ^{ab}	83 ± 4 ^b

Figures with common letters in superscript of each row don't differ significantly at 5% level of probability.

Table.4. Carcass composition of catfish fry fed different diets (Treatments) including control.

Components (%)	Diet No.					
	Initial sample	1 (T1)	2 (T2)	3 (T3)	4 (T4)	5 (T5) (Control)
Moisture	9.80 ± 0.03	9.82 ± 0.03	9.83 ± 0.03	9.80 ± 0.03	9.82 ± 0.03	9.83 ± 0.03
Crude protein	45.34 ± 0.11	61.66 ± 0.10	61.85 ± 0.11	60.78 ± 0.10	60.88 ± 0.11	59.95 ± 0.10
Crude lipids	8.45 ± 0.03	13.33 ± 0.04	13.45 ± 0.04	14.38 ± 0.04	13.89 ± 0.04	15.46 ± 0.04
Ash	26.20 ± 0.05	13.60 ± 0.03	13.25 ± 0.03	13.35 ± 0.03	13.45 ± 0.03	13.15 ± 0.03
NFE*	10.24 ± 0.03	1.54 ± 0.03	1.57 ± 0.03	1.64 ± 0.03	1.90 ± 0.03	1.55 ± 0.02

* NFE (Nitrogen Free Extract) = 100 – (Crude protein + Crude lipids + Ash). Variations in treatment means don't differ significantly at 5% level of probability.

References

- Habib, M.A.B., Hasan, M.R and Akand, A.M. 1994. Evaluation of silkworm pupae poultry offal and blood meal as dietary protein sources for *Clarias batrachus* (L.). The Third Asian Fisheries Society, Manila, Phillipines. pp. 641-644.
- Habib, M.A.B. 1998. Culture of selected microalgae in rubber and palm oil effluents and their use in the production of enriched rotifers. Ph. D Thesis, Faculty of Biology and Environmental Studies, University Putra Malaysia, Malaysia. 530 pp.
- Habib, M.A.B., Ullah, Hasan, M.R. and Hossain, M.S. 2001. Use of silkworm pupae as partial replacement of fish meal in the diets with rotifer as feed additives of Asian catfish, *Clarias batrachus* (Linn.) fry. *Bangladesh Journal of Fisheries* 24(1-2) 133-144.
- Horwitz, W. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th Edition. Association of Official Analytical Chemists, Washington D.C., USA. 1018 pp.
- Nuov, S. 1995. The use of live maggot (*Lucilia sericata*) derived from pig manure in the cage culture of African catfish (*Clarias gariepinus*). MS Thesis, Asian Institute of Technology, Bangkok, Thailand.
- Oluwole, O. B., Olatunj, I. O. O. and Odunfa, S.F. 2004. A process technology for conversion of dried cassava chips into gari. *Nigerian Food Journal* p. 65-73.
- Ross, E. and Dominy, W. 1990. The effect of dehydrated *Spirulina platensis* on poultry. *Poultry Science* p. 794-800.

Economic Extraction and Utilization of Pectin from Under-Utilized Fruits and Industrial by-Products

M Burhan Uddin and Mohammad Gulzarul Aziz

Location: Department of Food Technology and Rural Industries, BAU, Mymensingh

Duration: Three years (2014-2017)

Expenditure of the project: Tk 3000000.00

Introduction

Food processing sector is an emerging sector in Bangladesh in terms of both creating employments and earning foreign currencies. For developing various value added products, food industries need to use secondary food ingredients like pectin, acid, and so on. In general, pectin is located in the peel, rind and skin not in the juice. In citrus fruits, the pectin is chiefly in the white part of the rind. Other sources are sunflower seeds, guava, peel of mango and orange. Commercial pectin is usually extracted from fruit and vegetable by-products like apple, lemon, carrot, mango, tomato, beets, banana etc. Most of these by-products are the waste of every fruit and vegetable industries. The project was concerned with the optimization of pectin extraction from different fruits and fruit waste. Pectin was extracted from Chalta, Jamrul, Starfruit and by-products of fruits like Mango Peel, Banana Peel, Jackfruit Waste, watermelon and so on. Comparison in terms of efficiency and economical feasibility of pectin extraction by hot water extraction (HWE), acid extraction (AE) and organic solvent extraction were made. The physical and chemical properties of extracted pectin and their application in commercial products were studied. The study generated new knowledge on different pectin extraction technology, that will help broaden our present state of knowledge and skill on fruit waste management and their economic use. Output of the project will facilitate establishing relevant industries for the production of secondary food ingredients and thus generate new jobs.

Objectives

- To identify and extract potential pectin content in fruits and by-products generated in food industries;
- To standardize and optimize economic pectin extraction method(s) from these fruits and byproducts;
- To characterize the extracted pectin; and
- To assess the diversified use of extracted pectin and train the beneficiaries.

Methodology

Principle: Pectin is extracted from plant material and saponified, precipitated as calcium pectate by the addition of calcium chloride to an acid solution. After thoroughly washing to eliminate chlorides ions, the precipitate is dried and weighed.

Materials:

Peels of jackfruit , mango , pineapple, longan, hog plum (amra) , elephant apple (chailta), pameló (chokair), banana and papaya peel were collected and pectin was extracted. The pectin content of these samples, as calcium pectate, was determined by a gravimetric method (Rangana,2004).

Raw materials preparation: The peels/skins/rinds were first washed many times with water to remove all adhering substances. Small pieces of sample was made using knife and these will be then dried in a cabinet drier at 60 0C. The dried peels was made to powder using a mechanical grinder. The ground powder was then suspended in 85% (v/v) ethanol at 700C for 30 min in a shaking water-bath. The resulting alcohol-insoluble-residue (AIR) will be collected and air-dried at 500C. The AIR will now used to extract pectin.

Standardization of methods of pectin extraction

i) Hot water extraction: Alcohol-insoluble-residue was mixed well with deionized water at a ratio of 1:30 (w/v). The solution was heated at 750C for 60min and cooled down, filtered and wash with hot water. The filtrates was collected and the liquid was concentrated in water bath to 1/4th of its original volume. Finally the concentrate was dispersed in an equal volume of 95% ethyl alcohol. The precipitate was collected, centrifuged, dispersed in 70% ethyl alcohol, stirred for 30 min and centrifuged. The washing was repeated with 70% ethyl alcohol and then with 100% ethyl alcohol. The product was dried at 400C in an air conventional drier to constant weight.

Yield of pectin will be calculated by the following formula:

$$\text{Yield of pectin} = \frac{\text{Amount of pectin obtained} \times 100}{\text{Total amount of peel powder}}$$

ii) Acid extraction: 5 gm AIR was transferred into a beaker (1000 mL) containing 150 mL of water, 0.1 N H₂SO₄ was added to give a pH of 2.5. Sample was heated at 80°C for 60 min with frequent stirring. filter the hot extract through two fold nylon cloth. The filtrate cool to room temperature and dispersed in an equal volume of 95% ethanol. and allowed to settle for 1 h. The precipitate was collected, washed in 70% ethanol 3-4 times, and then with 96% ethanol. The product was dried at 400C in an air oven to constant weight. Method was developed from Rehman et al. (2004).

iii) Extraction using Sodium-hexa-met-phosphate: 5 gm of AIR weighed into a tarred 1000 mL beaker add 150 mL distilled water. 1.2 grams of freshly ground sodium hexametaphosphate (BDH) was then added and the initial pH adjusted with 3 N HCl to 2.2 ± 0.1 , heated with constant stirring at $80 \pm 5^\circ\text{C}$ for 1 hour and water loss was replaced at intervals except in the last 20 minute of extraction. The extract was filtered through muslin cloth and residue washed with 200 mL of warm water. the washings will be added to the filtrate, which was concentrated by evaporation on a hot plate to approximately one fifth of its initial volume. The concentrated pectin solution was cooled to 50°C and poured into a volume of ethanol in a ratio of 1:3, the ethanol contained 0.5 M HCl. The mixture was stirred for 15 min and allowed to stand for one hour. The precipitate was filtered through cloth. Wash the precipitate at same pH with more ethanol-HCL solution and filtered. Finally the precipitate will washed with 95% alcohol. The precipitate will be dried at 40°C to constant weight in an air oven.

iv) Chelating agent extraction (ammonium oxalate): 5 gm AIR will be transferred into a beaker (1000 mL) containing 150 mL of 0.25% ammonium oxalate ($\text{pH } 4.6 \pm 0.1$ with oxalic acid). Sample was heated at 85°C for 60 min with frequent stirring. Filter the hot extract through two fold nylon cloth. The filtrate cool to room temperature and dispersed in an equal volume of 95% ethanol. and allowed to settle for 1 h. The precipitate was collected, washed in 70% ethanol 3-4 times, and then with 96% ethanol. The product was dried at 40°C in an air oven to constant weight. Method will be developed from Mollea *et al.* (2008).

Results

Effect of methods on extraction rate

Table 1. Pectin extracted from jack-frit (Gala) by different methods

Extraction Condition				Precipitation condition		% pectin
Acidify with/media	pH	Heating time (min)	Heating temp ($^\circ\text{C}$)	Alcohol Ration	Time (min)	
HCl	2.0	35	65	1:1	60	7.80
H ₂ SO ₄	2.5	60	80	1:1	60	9.80
Deionized water	7.0	60	75	1:1	60	4.10
Sodium hexameta phosphate & HCl	2.2	60	80	1:1	60	16.30

The extraction of pectin basically involved the aqueous extraction of pectin from the raw material (plant), the isolation of the extracted pectin and purification (Joye & Luzio 2000), followed by drying process. The pectin extraction process should use a suitable method to obtain the maximum yield and quality of pectin. The yield of pectin usually depends on the extraction conditions, such as temperature, extraction time, pH, type of extraction solvents (Yeoh *et al.* 2008), and drying method (Mohammed *et al.* 2005).

Before extraction begins, an alcohol-insoluble residue is prepared to remove low-molecular weight compounds, including any traces of free galacto-uronic acid (Happi et al. 2008). We plan to extract from jack fruit wastage by different methods to find the maximum yield. Initially pectin was extracted by hot water extraction, by adding sodium hexa-meta-phosphate, by acid extraction by H_2SO_4 at different pH, time and temperature. Experiments are going on. We could extract highest pectin by hexa-meta-phosphate (16.30%) and lowest in hot water extraction (4.10%).

The pectin content of different fruit waste

Table 2. Pectin content (as Calcium pectate) indifferent fruits wastage

Sample		% Moisture content	% Calcium pectate (Dry basis)
Jack fruit (Khaja)	Rind	83.60	9.57
Jack fruit (Gala)	Rind	86.27	16.61
Jack fruit (Durasha)	Rind	87.27	13.51
Longan	Peel	81.00	2.28
Pineapple	Wastage	86.14	4.95
Hog plum	Peel	75.00	2.64
Pomelo	Peel	7.40	9.00
Banana	Peel	92.22	19.44
Papaya	Peel	90.49	13.24
Mango	Peel	79.42	15.26
Rosela	Whole	9.10	9.00
Elephant apple	Rind	9.10	5.00

Pectin content of different fruit wastage was measured as calcium pectate by gravimetric method. From the data we may conclude that highest calcium pectate in Jackfruit rind (16.611) and lowest in pineapple wastage. It has been observed that pectin content of jackfruit varies with variety. Within three varieties gala jack fruit contain higher than other two varieties (Khaja, durasha). In case of three part of a jack fruit bulb (edible portion contain lowest and rind contain highest pectin).

Evaluation of pectin

Extracted jackfruit waste pectin were evaluated for their physicochemical properties such as moisture content, ash content, anhydrouronic acid (AUA), degree of esterification (DE), solubility and viscosity by using standard methods (Fig. 1). Moisture contents of all the samples were in the acceptable range (below 12%) except the one extracted with sodium hexametaphosphate (14.73%). The results showed that sodium hexametaphosphate extracted pectin contained higher moisture content compared to ammonium oxalate extracted pectin (9.78%), acid extracted pectin (7.72%) and commercial pectin (10.94%).

The inorganic impurities in pectin are indicated by the ash content. Pectin extracted by sodium hexametaphosphate had the highest (8.15%) and sulfuric acid extracted pectin had the lowest (3.71%) ash content. The structure pectin has been shown in Fig. 2. Commercial pectin contained significantly lower ($p < 0.05$) ash than that of extracted pectin from jackfruit waste. Acid insoluble ash of all extracted pectins were found to be between 0.06%-0.27% and did not show significant differences ($p < 0.05$) with commercial pectin except for sodium hexametaphosphate extracted pectin. Ranganna (2004) stipulated that the ash content in pectin could vary from the 0.76 to 10.69%. Since low ash content is more favorable for gel formation, it could be reduced by washing with acidified alcohol. Solubility in cold and hot water, and the effect of alkali on different types of extracted pectin were evaluated. The ammonium oxalate extracted pectin showed the highest solubility whereas the lowest solubility was exhibited by the pectin extracted with sodium hexametaphosphate dispersed both in cold and hot water. The viscosity of the different pectin extracted under different extraction condition from jackfruit waste and pure pectin were compared. Ammonium oxalate extracted pectin demonstrated significantly ($p < 0.05$) highest viscosity (0.884 Pa.S) and sodium hexametaphosphate extracted pectin was found to have significantly ($p < 0.05$) the lowest viscosity among all pectin samples. Sulfuric acid extracted pectin also showed higher viscosity (0.347 Pa.S) but was not significantly different from the commercial pectin (0.191 Pa.S). Degree of esterification has a great impact on viscosity. Higher degree of esterification shows higher viscosity Sodium hexametaphosphate extracted pectin showed lower degree of esterification, a higher ash content, and a very low viscosity.

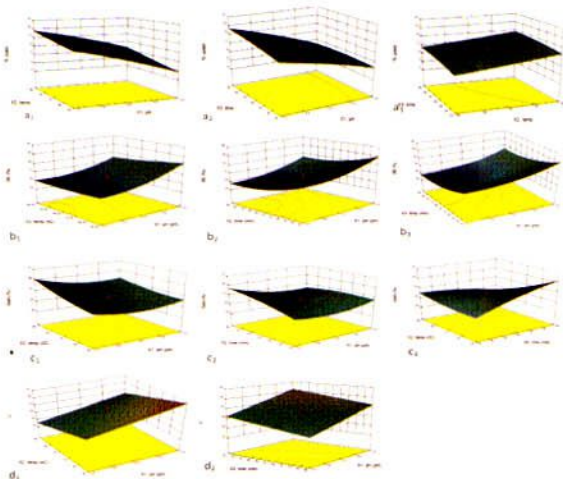


Fig. 1: Response surfaces plot showing the combination of pH, temperature and time on pectin yield (a1, a2, a3), DE (b1, b2, b3), GalA (c1, c2, c3) and l* (d1, d2) value respectively while extraction time (60 min), pH (7.5 oC) and pH (2.5)

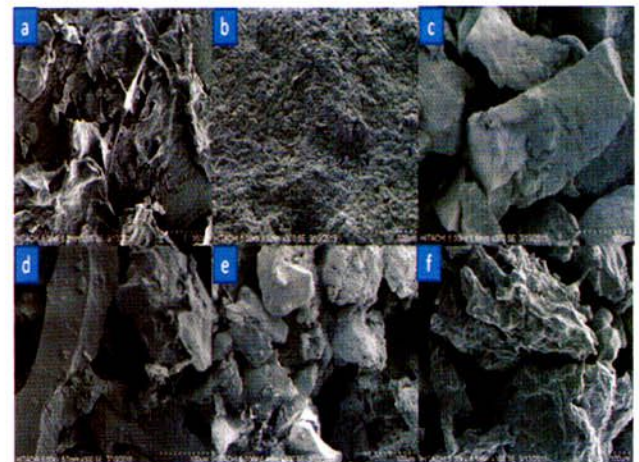


Fig. 2: SEM image of pectin powder: (a) Freeze dry, (b) Spray dry © Vacuum dry (d) oven dry (e) Commercial grade (f) analytical grade pectin

Conclusion

- The methods for extraction of pectin from 12 selected fruits waste standardized
- Quantities determination and qualitative assessment of pectin extracted from 12 fruits waste indicated there is prospect for commercial extraction of pectin from fruits waste available in Bangladesh.

Publications from this research

- Karim, R., Burhan, Uddin, M., and Jubayer, MF., 2014. Optimization of pectin isolation method from pineapple (*Ananas comosus l.*) waste. *Carpathian Journal of Food Science and Technology* ISSN-L 2066 -6845 pp:116-122
- Begum, R., Aziz, M. G., Uddin, M. B., and Yusof, Y. A. 2014. Characterization of Jackfruit (*Artocarpus heterophyllus*) Waste Pectin as Influenced by Various Extraction Conditions. Elsevier: *Agriculture and Agricultural Science Procedia* 2 : 244 – 251
- Begum, R., Yus, Y. A., Aziz, M. G., and Uddin, M. B. 2017. Structural and functional properties of pectin extracted from jackfruit (*Artocarpus heterophyllus*) waste: Effect of drying. *International Journal of Food Properties*. ISSN: 1094-2912.
- Begum, R., Aziz, M. G., Uddin, M. B., and Yusof, Y.A. 2014. Characterization of Jackfruit (*Artocarpus heterophyllus*) Waste Pectin as Influenced by Various Extraction Conditions. *International Conference in Agricultural and Food Engineering*, UPM, Malaysia, pp 35 -37.
- Begum, R., Aziz, M.G., Uddin, M.B., and Yusof, Y. A. 2016. Optimization of pectin extraction conditions from jackfruit waste using response surface methodology. OWSD Fifth General Assembly and International Conference, Kuwait, pp. 57-58.
- Begum, R., Aziz, M.G., Uddin, M.B., and Yusof, Y.A. 2016. Functional properties of jackfruit waste pectin as influenced by various drying methods. International conference on food properties (iCFP 2016), Bangkok, Thailand, pp 23-25
- Faruque, O. M. 2014. Standardizing Methods for Extraction and Characterization of Pectin From Mango Peel (*Mangifera indica*). M.S Thesis, Department of Food Technology and Rural Industries, BAU, Mymensingh.
- Karim, R. 2014. Optimization of Pectin Isolation Method from Pineapple (*Ananas comosus L.*) Waste. M.S, Thesis, Department of Food Technology and Rural Industries, BAU, Mymensingh.
- Begum, R. 2017. Extraction and utilization of pectin from selected fruit wastes. Ph.D Thesis, Department of Food Technology and Rural Industries, BAU, Mymensingh.

References

- Happi, E.T., Ronkart, S.N., Robert, C., Wathelet, B., Paquot, M. 2008. Characterisation of Pectins Extracted from Banana Peels (Musa AAA) under Different Conditions using an Experimental Design. *Food Chemistry* 108, 463-471.
- Joye, D. D., Luzio, G.A. 2000. Process for Selective Extraction of Pectins from Plant Material by Differential pH. *Carbohydrate Polymers* 43, 337-342.
- Mohamed, S. and Hasan, Z. 1995. Extraction and Characterization of Pectin from Various Tropical Agro-wastes. *ASEAN Food Journal* 10(2), 43-50.
- Mollea, C., Chiampo, F., Conti, R. 2008. Extraction and Characterization of Pectins from Cocoa Husks: A Preliminary Study. *Food Chemistry* 107, 1353-1356.
- Ranganna, S. 2004. Manual of Analysis of Fruits and Vegetable Products. Tata McGraw Hill publishing Company Ltd. New Delhi, pp. 40-42.
- Rehman, Z. U., Salaria, A. M., Habib, F., Shah, W. H. 2004. Utilization of Mango Peels as a Source of Pectin. *Journal of Chemical Society of Pakistan* 26(1), 73-76
- Yeoh, S., Shi, J., Langrish, T. A. G. 2008. Comparisons between Different Techniques for Water- Based Extraction of Pectin from Orange Peels. *Desalination* 218, 229-237.

Optimization of in Vitro Maturation and Fertilization of Oocytes in the Bovine in Bangladesh

Mohammad Musharraf Uddin Bhuiyan

Institution: Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 2500000.00

Introduction

Tropically adapted nondescript local zebu (*Bos indicus*) cows are low yielding ones with comparison to exotic (*Bos taurus*) cows (Cunningham and Syrstad, 1987). In Bangladesh, to upgrade our local zebu cattle by cross breeding, the Department of Livestock Services, Bangladesh introduced artificial insemination (AI) using semen of exotic breed since sixties. However, indiscriminate use of semen from exotic breeds without adapting any breeding policy in Bangladesh resulted in many cross bred cows without any known genetics although cross breeding (F1) possess both good adaptation and production (Cunningham and Syrstad, 1987). This F1 cattle can be produced by planned AI, MOET and IVF of in vitro matured (IVM) oocytes using 100% exotic bull semen followed by ET. Applying IVM-IVF-ET is more advantageous than that of AI and MOET as IVF-ET can use slaughterhouse derived oocytes for generation of F1 embryos within short duration. The IVF-ET technique has already been established in animals and humans in developed countries. However, these techniques have not yet been adapted in Bangladesh although the preliminary results are encouraging (Das *et al.*, 2006; Islam *et al.*, 2007; Talukder *et al.*, 2009). However, this technique needs to further optimize for routine embryo production followed by transfer in Bangladesh.

Objective

Using existing facilities of the Department of Surgery and Obstetrics, BAU, Mymensingh, and additional facilities made available through this research fund, the objective of the project was to optimize the IVM protocol for oocytes of local zebu cows in Bangladesh.

Methodology

The ovaries of indigenous zebu cows were collected from local slaughter house after slaughtering within 2 hrs and the follicular fluid of follicles was aspirated. The retrieved follicular aspirate was diluted with HEPES-buffered TCM 199 supplemented with BSA and oocytes were selected under a stereo-microscope. Four 50 μ l drops of maturation medium were prepared in 35mm petridish and covered with embryo tested mineral oil. For in vitro maturation, 7-10 COCs were cultured in each drop of medium in incubator at 39°C with 5% CO₂ in humidified air for 24 hrs. Presumptive maturation was confirmed by the presence of first polar body extrusion, the COCs were denuded by using denuding agent and pipetting. After pipetting, the denuded oocytes were kept in 10 μ l drops of HEPES buffered TCM 199 and examined for presence of polar body under inverted microscope with the help of a mouth controlled pipette.

Results

A total of 496 oocytes were collected from 148 ovaries and the mean number of oocytes collection from each ovary was 3.35. The overall maturation rate in the present investigation was 74.6% (206/276). In Experiment I, an effective basic medium for oocytes maturation was determined. The maturation rates of oocytes were 75.5 ± 3.9 and $62.2 \pm 20.2\%$ in TCM 199 and SOF medium, respectively. However the maturation rate did not vary between two media ($P > 0.05$) (Table 1).

Table 1. Effect of basic maturation media on IVM rate of Zebu oocytes

Basic media used	Number of oocytes cultured	Number of oocytes matured	Maturation rate (%)
	53	40	75.5 ± 3.9
SOF	45	27	62.2 ± 20.2

Number of replicates is 5. Proportion values are mean \pm SD. The maturation rate was not significantly different from each other ($P > 0.05$).

In Experiment II, an effective hormone supplementation in basic medium for oocytes maturation was determined. The maturation rate of oocytes was higher ($76.6 \pm 13.2\%$) in FSH supplemented TCM 199 than that of gonadotrophin supplemented counterpart ($69.7 \pm 10.8\%$). The difference in maturation rate was statistically significant between two hormone supplementations ($P < 0.05$) (Table 2).

Table 2. Effect of hormone supplementation on IVM rate of Zebu oocytes

Hormone supplementation	Number of oocytes cultured	Number of oocytes matured	Maturation rate (%)
FSH	49	38	76.6 ± 13.2^a
Gonadotrophin	39	27	69.7 ± 10.8^b

Number of replicates is 5. Proportion values are mean \pm SD. other ($P < 0.05$). a,b The values with superscripts within same column was significantly different from each

In Experiment III, an effective protein supplementation in basic medium for oocytes maturation was determined. The maturation rates of oocytes were $81.7 \pm 12.9\%$ in medium supplemented with FBS and OCS, respectively. However the maturation rate did not vary between two protein supplementations ($P > 0.05$) (Table 3).

Table 3. Effect of protein supplementation on IVM rate of Zebu oocytes

Protein supplementation	Number of oocytes cultured	Number of oocytes matured	Maturation rate (%)
FBS	45	36	81.7 ± 12.9
OCS	45	38	85.7 ± 12.7

Number of replicates is 6. Proportion values are mean ± SD. The maturation rate was not significantly different from each other ($P > 0.05$).

Conclusions

- i. Protocol for IVM of oocytes of zebu cows of Bangladesh is optimized and optimization of IVF and IVC is going on.
- ii. Two MS in Theriogenology thesis have already been produced by 2 students.
- iii. Preparation of dissertation by one PhD student is going on.

Publication from this research

- i) Doctor of Philosophy (PhD) Thesis:

One PhD student did research under this process and the dissertation is under preparation.

- ii) Master of Science (MS) Thesis:

Singh, J.K. 2014. Comparison of culture media for in vitro maturation of oocytes of indigenous zebu cows. Master of Science (MS) in Theriogenology, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Morshed, S.M.N. 2013. Optimization of culture conditions for in vitro maturation of oocytes of zebu cows. Master of Science (MS) in Theriogenology, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

- iii) Articles published in Journals:

Singh, J.K., Bhuiyan, M.M.U., Moshir, M.M. and Bari, F.Y. 2015. Comparison of culture media for in vitro maturation of oocytes of indigenous zebu cows in Bangladesh. *Journal of Embryo Transfer* 30(4): 327-333.

Morshed, S.M.N., Bhuiyan, M.M.U., Rahman, M.M., Singh, J.K. and Juyena, N.S. 2014. Culture conditions for in vitro maturation of abattoir derived oocytes of native zebu cows of Bangladesh. *Journal of Embryo Transfer* 29(3): 201-206.

iv) Abstracts published in Proceedings:

- Rahman, M.M., Bhuiyan, M.M.U., Rahman, M.M. and Juyena, N.S. 2017. Effects of protein and hormone supplementation in basic media on in vitro maturation of oocytes of indigenous zebu cows. 23rd Bangladesh Society for Veterinary Education and Research (BSVER) Annual Scientific Conference, 28-29 January, Mymensingh, Bangladesh, 42 : 28.
- Singh, J.K., Rahman, M.M., Bari, F.Y. and Bhuiyan, M.M.U. 2015. Comparison of culture media for in vitro maturation of oocytes of indigenous zebu cows. 21th Bangladesh Society for Veterinary Education and Research (BSVER) Annual Scientific Conference, 7-8 March, Mymensingh, Bangladesh, 40 : 80.
- Bhuiyan, M.M.U., Singh, J.K. and Rahman, M.M. 2015. Optimization of in vitro maturation and fertilization of oocytes in the bovine in Bangladesh. Bangladesh Agricultural University Research Progress, 25 : 18-19.

References

- Cunningham, E.P. and Syrstad, O. 1987. Crossbreeding *Bos indicus* and *Bos taurus* for milk production in the tropics. FAO Animal Production and Health paper 68, Rome, Italy.
- Das, Z.C., Siddiqui, M.A.R., Bhattacharjee, J., Bhuiyan, M.M.U. and Shamsuddin, M. 2006. In vitro fertilization using zebu and crossbred bull semen. In: Annual Scientific Conference of Bangladesh Society for Veterinary Education and Research (BSVER) Publication 31: 31.
- Islam, M.F., Bhuiyan, M.M.U., Banu, T.A., Bhattacharjee, J. and Shamsuddin, M. 2007. In vitro maturation and fertilization of oocytes in zebu cattle. *Bangladesh Veterinary Journal*, 41: 58-76.
- Talukder, A.K., Shamsuddin, M., Rahman, M.B., Bari, F.Y. and Parish, J.J. 2009. Normal and abnormal fertilization of zebu cattle oocytes in vitro. *Journal of Embryo Transfer*, 24 (2): 89-95.

Management of Climate Change with GIS Application to Ensure Food Security in Coastal Areas of Bangladesh

Md Ashraful Alam and Md Ashiq-ur-Rahman

Location: Urban and Rural Planning Discipline, Khulna University

Duration: Three Years (2014-2017)

Expenditure of the project: Tk. 2000000.00

Introduction

Global warming is great threat for development all over the world. The southwestern region of Bangladesh is facing different types of calamities such as sea level rise, river siltation, salinity intrusion, drought, excessive rainfall, seasonal variation and river erosion etc. However, low-income developing countries e.g. Bangladesh bear the highest burden in terms of the human lives and crop loss as a result of different disaster all over the year. The destructive impacts of recent natural disasters on southwestern coastal regions of Bangladesh have brought into focus the need for proper risk assessment, planning, preparedness and the implementation of sustainable disaster risk management strategies to ensure food security. Unfortunately, these vulnerable zones are often neglected and still there is hardly any adequate plan to resolve food insecurity that exists in the coastal community.

Objectives

- To identify the dynamics of disasters in response to different social groups in southwestern coastal areas of Bangladesh;
- To explore the main causes of food insecurity in southwestern coastal parts of Bangladesh; and
- To develop sustainable disaster risk management strategies to ensure food security for the southwestern coastal living communities of Bangladesh.

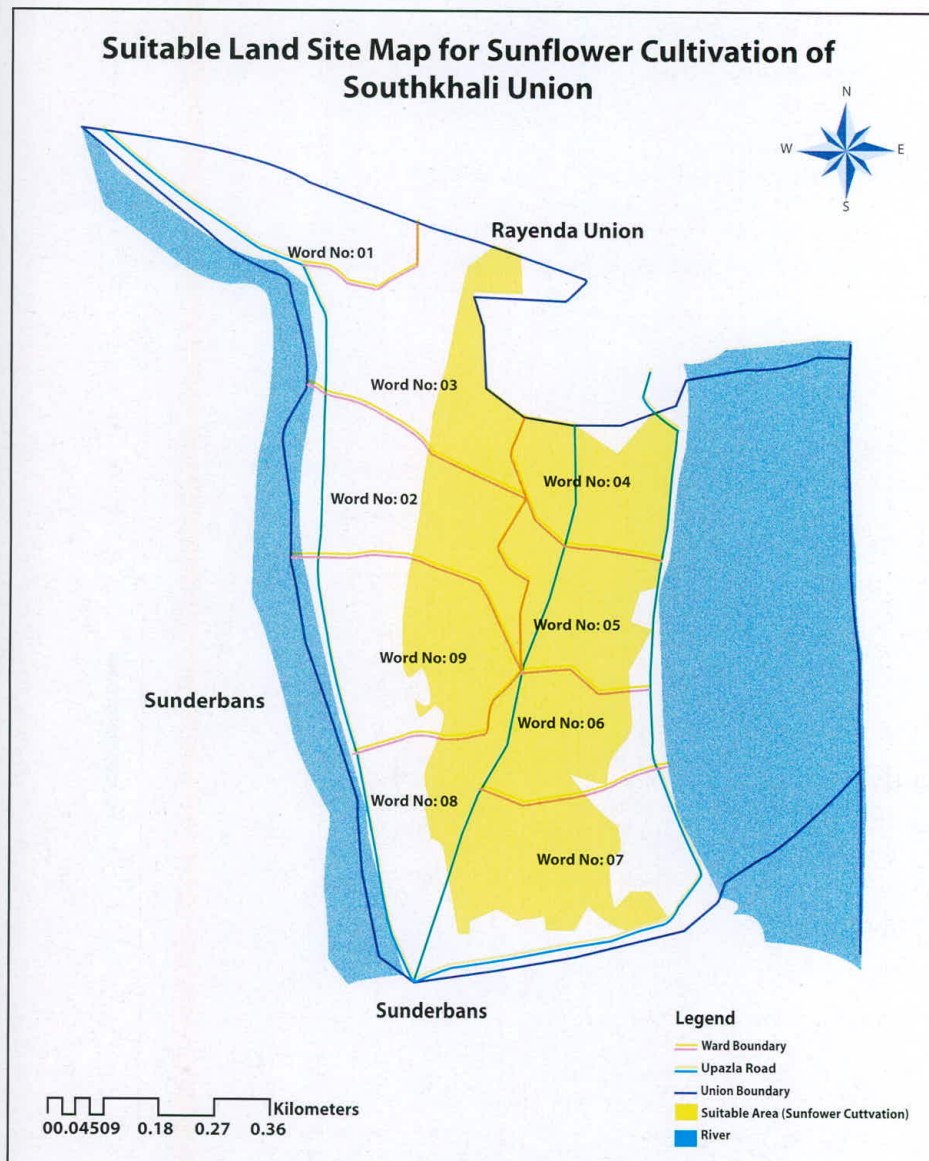
Methodology

Sarankhola Upazila, Bagerhat District has selected as study area. There are 9 wards for each union. From each wards 9 people are selected for questionnaire survey, 3 persons from civil society, 3 persons from NGO workers and 3 persons from farmers. So 81 (9x9) questionnaires surveys has conducted for each union. For 4 union of Sharankhoa Upazila, the sample size has been found as 324. One Focus Group Discussion was conducted comprising both men and women in every union of the upazila. Focus group helps to gather a wide range of information in a relatively short time. The participants of the FGD were selected haphazardly including subsistence farmer, small landholders, NGO worker, School teacher, UP members, student and other people o the area. This system helps to select individual or group from different places. Soil and water samples have collected to know the PH value from different location of the study area.



Results

Map-01 : Suitable land for Sunflower Cultivation of Southkhali Union

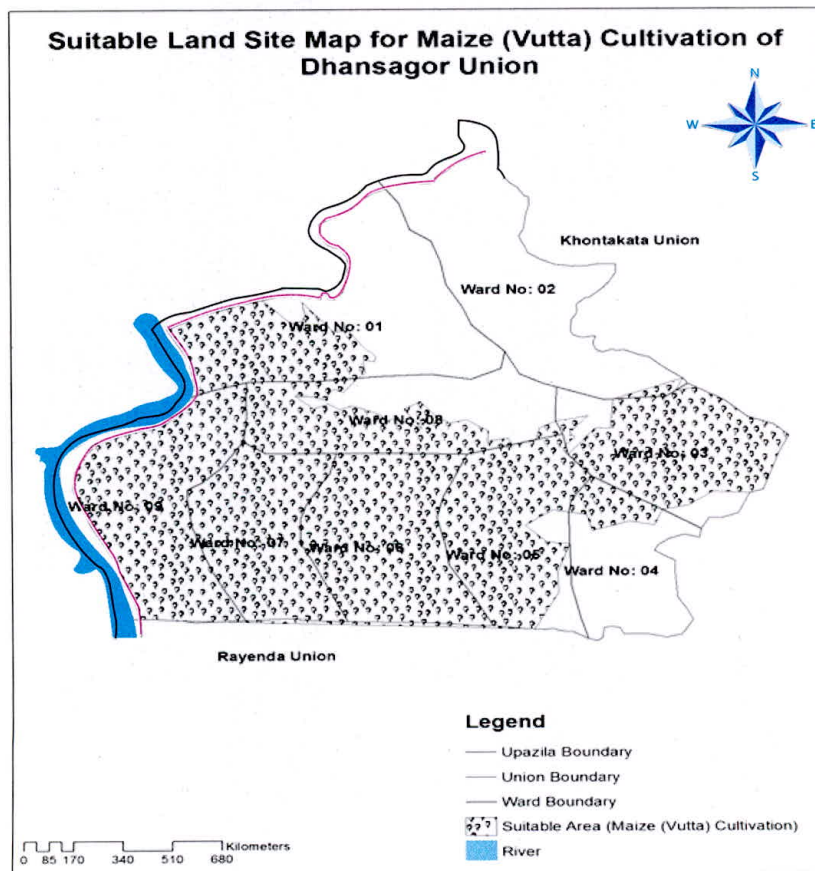


Source: Field Survey 2015, and LGED, 2015

According to Ph value and salinity level status of Southkhali union approximately 50% agricultural land is suitable for Sunflower cultivation. This great possibility of Sunflower cultivation is hindered for lacking of irrigation system and lacking of willingness of farmers. Proper irrigation system and awareness building programme for farmers on Sunflower cultivation can increase Sunflower oil production in coastal areas of Bangladesh. And this fellow land will be used as crop production purpose in dry season.

Water from Baleswar River is supplied for irrigation, a tremendous production will be occurred and it is expected by the author food security will be confirmed. Cyclone attacked Sarankhola upazila frequently and caused for huge damages of crop and people. Sidar and Aila caused for damages from past years. Every year it causes extensive damages to the agriculture sector and changes the cropping pattern in the dry season. It is the reality that rice production is decreasing due to the increasing rate of salinity in Sharankhola upazila. The increasing concentration of salinity is creating more pressure to the farmer by reducing yield on one hand and threating local livelihood and food security on the other hand. Government of Bangladesh and other national and international NGOs

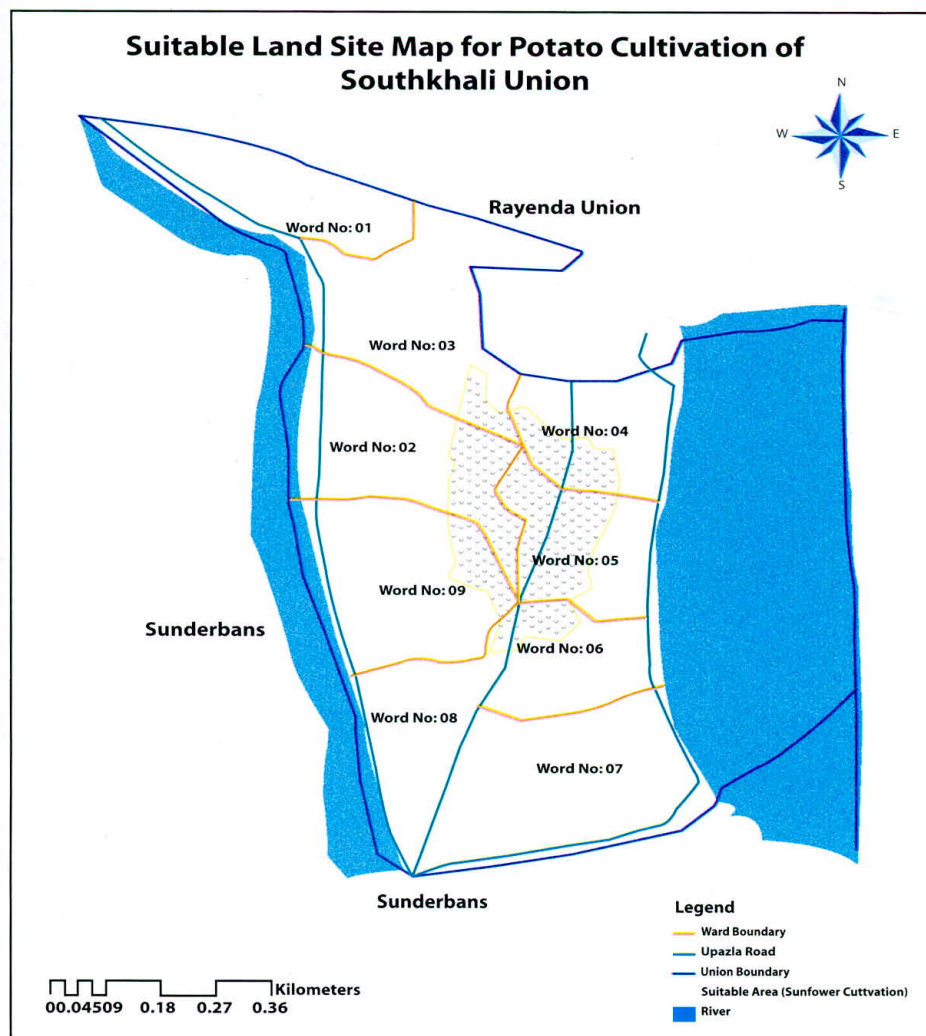
Map-02: Suitable land for Maize Cultivation of Dhansagor Union



Source: Field Survey 2015, and LGED, 2015.

According to Ph value and salinity level status of Dhansagor union approximately 55% agricultural land is suitable for Maize cultivation. But lacking of awareness of farmers and irrigation system this huge amount of land exists from outside of cultivation. And the great possibility is hindered for lacking of proper management. Whola River can be used as the source of water and internal canals can be used as irrigation channel.

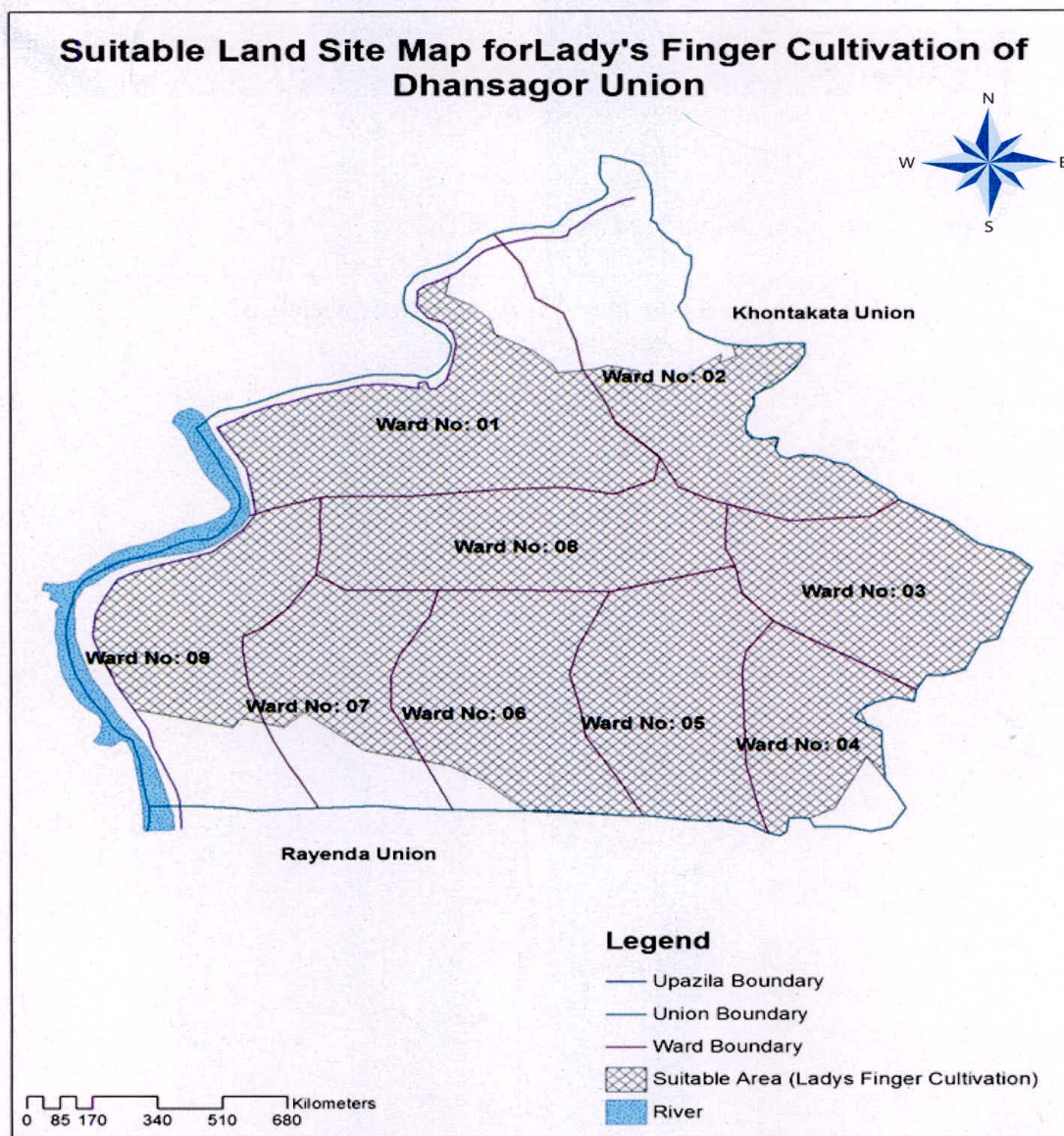
Map-03: Suitable land for Potato Cultivation of Southkhali Union



Source: Field Survey 2015, and LGED, 2015

According to Ph value and salinity level status of Southkhali union approximately 10% agricultural land is suitable for Potato cultivation. Some farmer in Southkhali union are agree to cultivate potato but they does not know which land is suitable for potato cultivation. This map shows the suitable agricultural land for potato cultivation. It helps farmer to increase potato production in this locality.

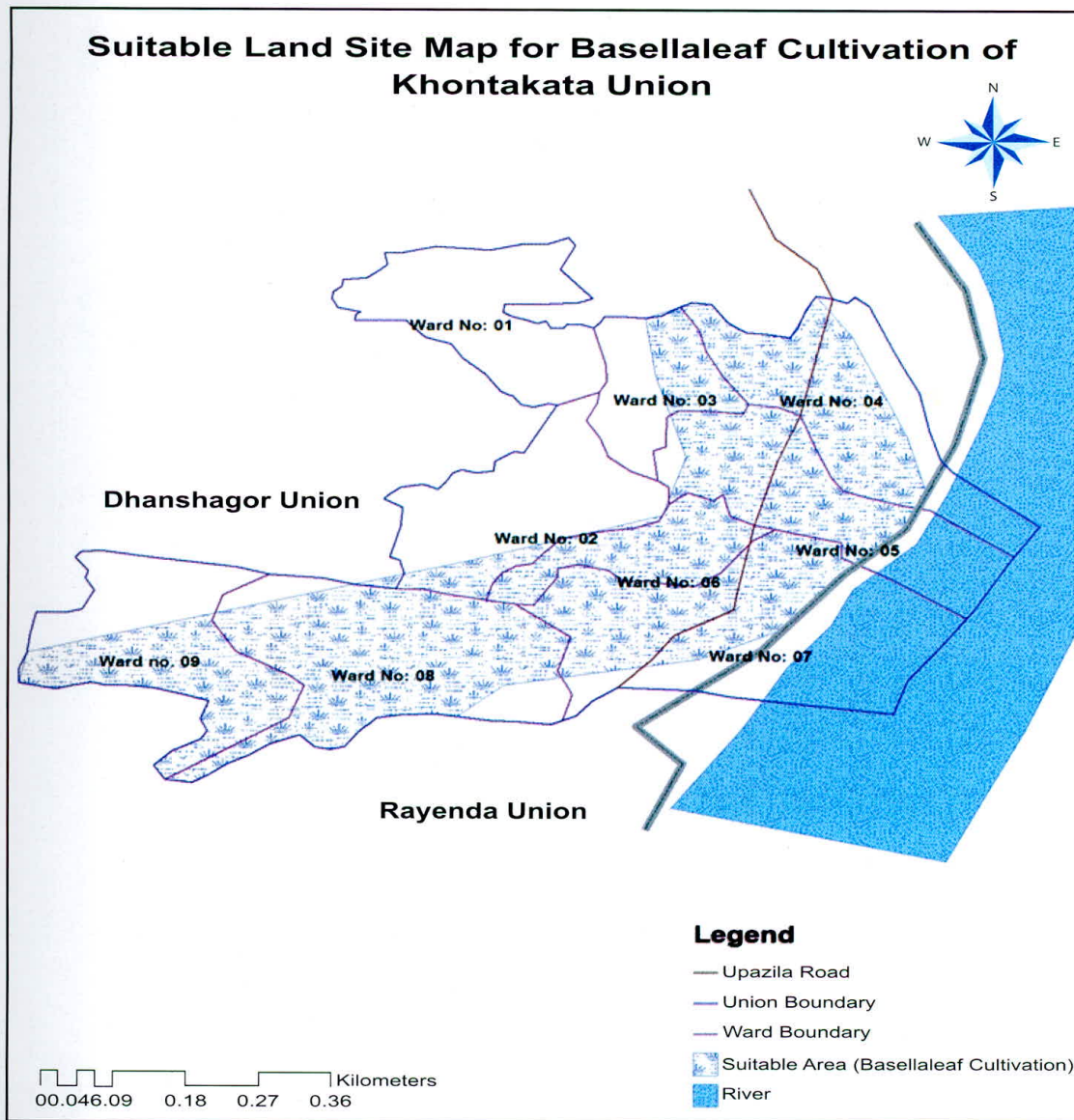
Map-04: Suitable land for lady's finger Cultivation of Dhansagor Union



Source: Field Survey 2015, and LGED, 2015

According to Ph value and salinity level status of Dhansagor union approximately 85% agricultural land is suitable for Lady's Finger cultivation. It is profitable to farmer because for Lady's Finger cultivation it needs less irrigation. There are many internal canal are exist beside the agricultural land. So proper use of this canal water make a huge profit to farmer of this union. The map shows that every ward of this union has a possibility to produce Lady's Finger and its production cost is lower than others.

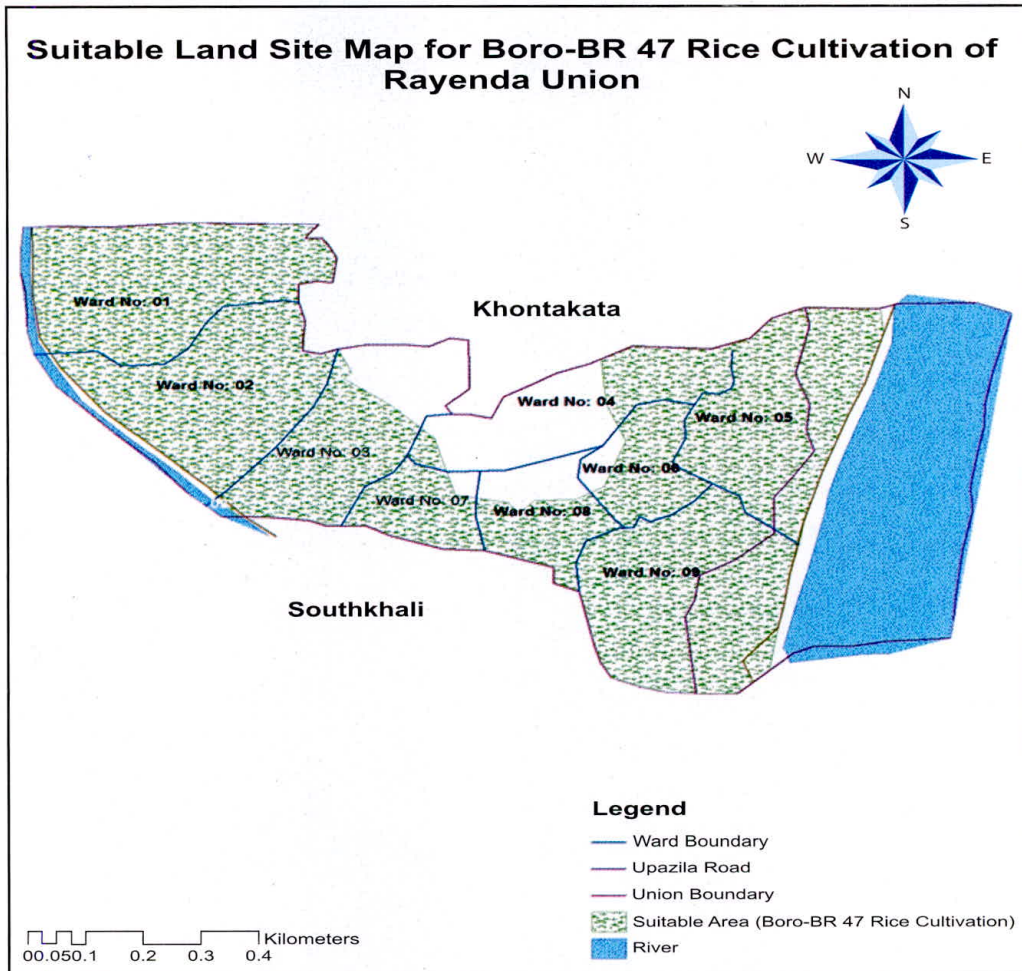
Map-05: Suitable land for basella leaf Cultivation of Khontakata Union



Source: Field Survey 2015, and LGED, 2015

According to Ph value and salinity level status of Khontakata union approximately 65% agricultural land is suitable for Basellaleaf cultivation. Most of the agricultural land is unused in dry season of this union for scarcity of water and lacking management of irrigation system. Small amount of water is needed for Basellaleaf cultivation thus farmer can be collected from canals or ponds. This research suggests to farmer that Basellaleaf cultivation is more profitable with low investment.

Map-06: Boro Rice Cultivation Map of Khontakata Union



Source: Field Survey 2015, and LGED, 2015

Rayenda union is situated in the north-east part of Sarankhola upazila. Salinity level of land is high in this union. Boro- BR 47 rice cultivation is profitable and suitable where salinity level is high. The land where rice cultivation is threatened for high salinity is also appropriate for Boro- BR 47 rice cultivation. Most of the agricultural land is not used in dry season for scarcity of water. With a great possibility there is no irrigation system in Rayenda union. According to Ph value and salinity level status of this union approximately 75% agricultural land is suitable for Boro- BR 47 rice cultivation, if Govt. or NGOs ensure irrigation system in this area. In dry season this 75% land is unused due to scarcity of water and lacking of knowledge of farmers. If farmers can be capable to cultivate this unused land in dry season, it will be a great task to eradicate food insecurity in Rayenda union as well as in coastal areas of Bangladesh.

Conclusion

The water of Baleswar River is comparatively less saline and may be used for irrigation. If a canal is constructed and water from Baleswar River is supplied for irrigation, a tremendous production will be occurred and it is expected by the author food security will be confirmed. Cyclone attacked Sarankhola upazila frequently and caused for huge damages of crop and people. Sidar and Aila caused for damages from past years. Every year it causes extensive damages to the agriculture sector and changes the cropping pattern in the dry season. It is the reality that rice production is decreasing due to the increasing rate of salinity in Sarankhola upazila. The increasing concentration of salinity is creating more pressure to the farmer by reducing yield on one hand and threatening local livelihood and food security on the other hand. Most of the community based disaster management initiatives that have been taken in Sarankhola are community driven initiatives. Muslim Aid and WFP, Ashroy Foundation and CODEC, PROSHAR Projects are working to make capacity building of local people. By different kind of programmes these organizations trying resilient people to cope with disaster but there is less initiatives to manage food security in coastal areas of Bangladesh. The researchers expect that if proper initiatives are taken by GOs and NGOs, crop production will be increased in the study area and ensure food security by managing climatic hazards. The well managed irrigation system can be ensured different crop cultivation in Sarankhola upazila. According to Ph value and salinity range, this study also finds out the suitable land which is perfect for specific crop cultivation. Basically this is the recommendations of this study how to increase crop cultivation in coastal areas of Bangladesh.

References

- Ali, A. 1999. Climate Change Impacts and Adaptation Assessment in Bangladesh. *Climate Research*, Vol.12, pp.109-116.
- FAO. 2007. Climate variability and change: Adaptation to Drought in Bangladesh. Pp.66, Bangladesh.
- Government of Bangladesh. 2010. National Plan for Disaster Management. Disaster Management Bureau, Dhaka, Bangladesh.
- McDonald, D. J. 1994. Temperature Rice Technology for the 21st century – the New South Wales Example. In: Proceedings of the first temperature rice conference, Yanco, Australia. Pp. 1-12.
- Munns, R., James, R.A. And Lauchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57:1025-1043.
- Rashid, M. H., Islam, M. and Islam, S. 2007. Adaptation to Climate Changes for Sustainable Development of Bangladesh Agriculture. 3rd session of Technical Committee of Asia and Pacific Centre for Agricultural Engineering and machinery (APCAEM), a China.
- USAID. 1992. Policy Determination: Definition of Food Security PD-19. Washington DC Pp. 4.

Study on Climate Variability and Its Impact on Major Food Crops in Bangladesh

Md Giashuddin Mia, Md Abiar Rahman and Satya Ranjan Saha

Location: Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 2000000.00

Introduction

Bangladesh is the most sensitive hotspots for the catastrophic events of climate variability due to juxtaposing geographical position of the funnel-shaped Bay of Bengal to the south and the biggest Himalayans mountains to the north (Rahman *et al.*, 2017a). It ranked fifth in the global climate risk index, which harbor 168.95 million peoples. Agriculture acts as a mainstay of the economy, which employs around 63% of the population and contributes over 18.3% of the country's GDP (BBS, 2015). Extreme climatic events for instances, increasing temperature and erratic rainfall along with austere and frequent floods, droughts and cyclones in the recent years, severely plagued the agricultural sector (Karim, 1993). The IPCC (2007) estimated that by 2050, there will be changing rainfall patterns with increasing temperatures, flooding, droughts and salinity that would cause decline in rice production in Bangladesh by 8% and wheat by 32%, against 1990 as the base year (Hossain and Silva, 2012). Despite technological advances (improved crop varieties, fertilization and irrigation); weather and climate are still key factors in agricultural productivity (Rahman *et al.*, 2017b). Unfortunately, there is no qualitative or quantitative information about the variability of climate change over time and space (ecosystem), and their impacts on different production systems especially food crop systems.

Objectives

- To analyze and understand the logics of climatic variability in different agro-ecosystems of Bangladesh using long time weather data;
- To monitor the current micro level climate data and production systems of major food crops in different agro-ecosystems; and
- To identify the critical stages of crop growth and performances due to climate change phenomenon.

Methodology

The study was carried out in Gazipur (rice), Comilla (rice and potato), Dinajpur (wheat and rice) and Jessore (wheat and potato) districts (Fig. 1). Both primary (field survey and monitoring) and secondary (climatic and crop productivity) data were used in this study. Long term weather (1960 to 2014) and crop productivity (2000 to 2014) data were collected from Bangladesh Meteorological Department (BMD) and Department of Agricultural Extension (DAE), respectively. The mean monthly maximum and minimum temperatures were derived by averaging the daily maximum and minimum temperatures.

To know the seasonal variability and changes, the monthly data were separated into three seasons namely hot summer (March to May), Monsoon (June to September) and dry winter (October to February). To understand climate change, lower confidence level (LCL) and upper confidence level (UCL) were calculated using standard method. Standardized Precipitation Index (SPI) was used to identify and monitor droughts, which was measured as suggested by McKee *et al.* (1993).

For primary data collection, fifty farmers from each site were selected randomly and surveyed to understand the farmers' understanding the climate change phenomenon and its impact on crop production. Field monitoring and discussion meetings with farmers were done to collect and validate the collected information. Climatic data were analyzed by MS Excel, while survey and monitoring information were analyzed by SPSS computer software.

Results

Climatic data (long-term maximum and minimum temperatures) showed increasing trends in most of the locations. The highest increment rates for maximum and minimum temperatures were found in Jessore and Dinajpur, respectively. The annual rainfall showed increasing pattern in Gazipur and Jessore and the increment rates were 3.24 mm and 1.68 mm per year, respectively; while it has been decreased slightly in Comilla (Fig. 2). The analyses of LCL and UCL clearly indicated climate change in recent years compared to 30 years ago. It was observed that temperatures during summer and winter months exceeded the UCL and LCL, respectively. In recent year, rainfall became more uncertain and has been shifting. Although the total annual rainfall, irrespective of region, did not vary significantly, the Monsoon rainfall showed increasing trends, while dry season rainfall decreased by 9.6, 2.8, 8.1 and 3.4 mm in Comilla, Dinajpur, Jessore and Gazipur in recent years compared to base year (1960-1969), respectively (Fig. 3). The SPI values indicated that drought is frequently occurring in Dinajpur and Jessore (Fig. 4).

It was recorded that the area for crop production has been decreased in most of the regions which might be due to unavailability of irrigation, uncertainty of rainfall and increasing temperatures. Although crop area has been decreasing in all locations, but the yields showed increasing trends due to technological development such as modern variety, management technique and protection options. However, crop yields could not maintain the potentiality because of various hazards under changing climate. Among the rices, boro rice did not affect much, while aus and aman rices were affected adversely as these are mostly rainfed. As a result, cropping pattern with aman rice has been decreased remarkably. Most of the farmers, irrespective of regions, opined that panicle initiation, tiller initiation, flowering and milky stages are critical for rice; whereas vegetative growth, tuber initiation and tuber bulking stages are critical for potato; and crown root initiation, flowering, joining, milky and tillering stages are critical stages for wheat (Table 1). Most of the farmers opined that climate has been changed in the recent years. Farmers have been experiencing irregular rainfall and temperature during critical stages of different crops that makes the farming more vulnerable. Due to uncertainty of rain, increasing of temperatures and fog rice, wheat and potato productions are being hampered in the recent years. Suitable techniques are needed to be developed to combat the adverse impacts of climate change for sustainable crop production.

Table 1. Critical stages of different crops to water and temperature based on opinion of farmers and agriculture extension officials of the study areas

Location	Rice	Potato	Wheat
Comilla	Tiller initiation, flowering and milky stage	Vegetative growth Tuber initiation	
Dinajpur	Tiller initiation, flowering and milky stage	Tuber initiation Tuber bulking	Crown root initiation, flowering, joining, milky and tillering
Jessore		Tuber initiation Tuber bulking	Crown root initiation, flowering, heading
Gazipur	Panicle initiation, tiller initiation, flowering and milky stage		

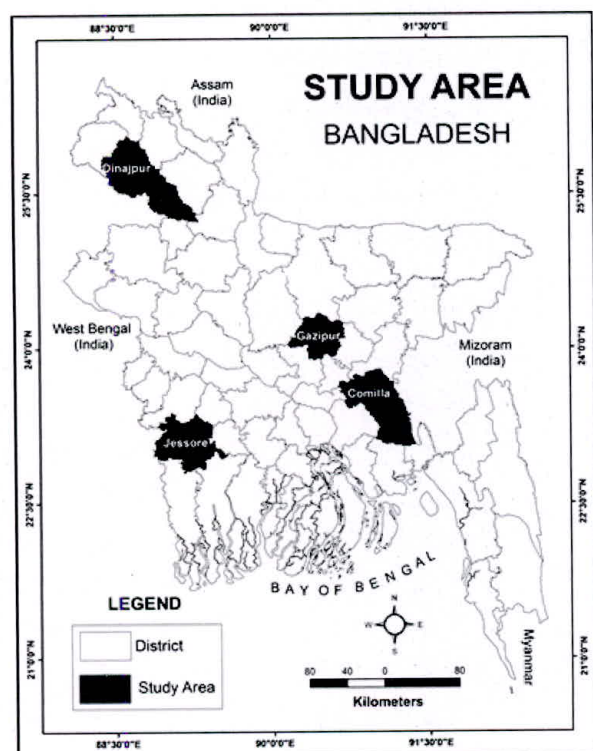
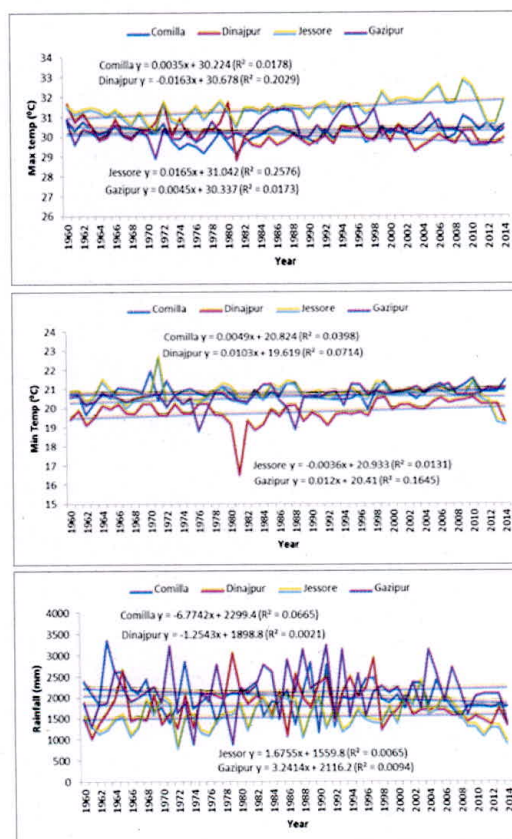
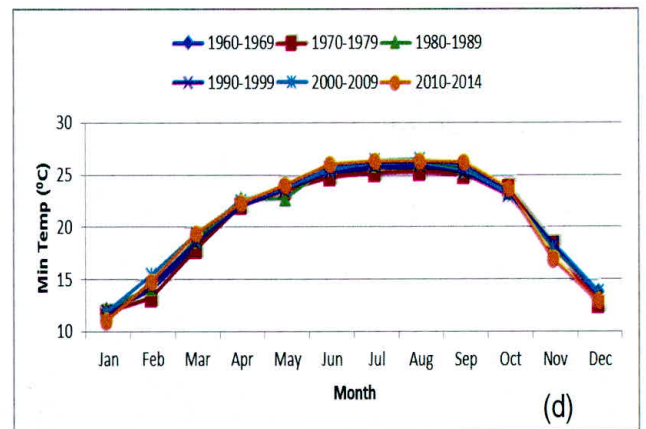
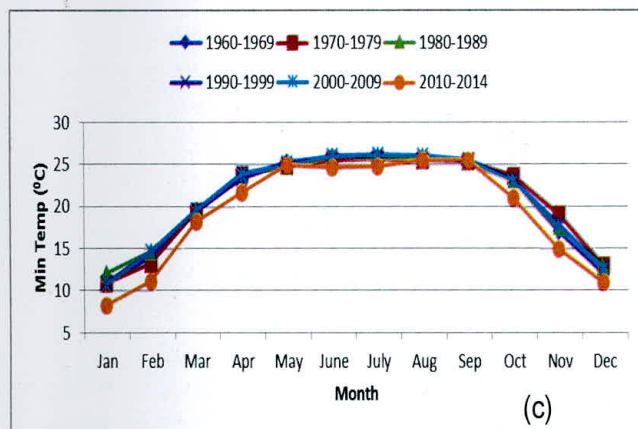
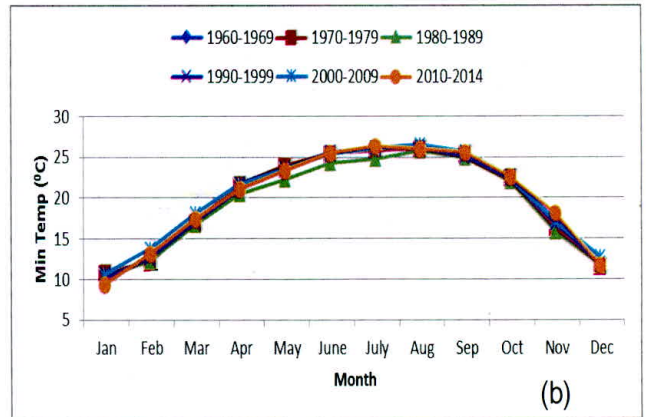
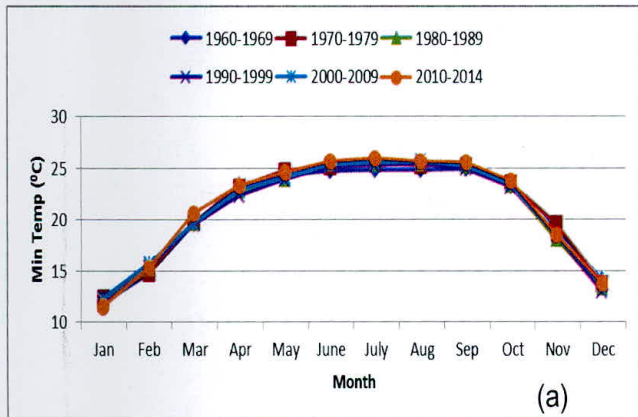
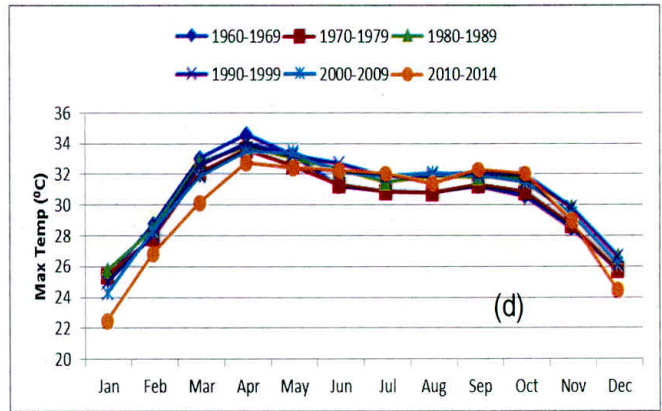
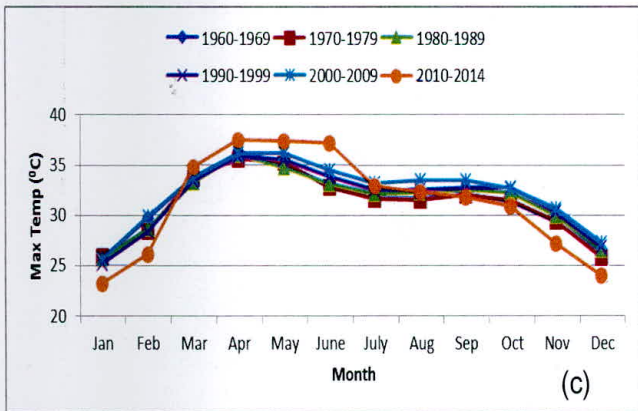
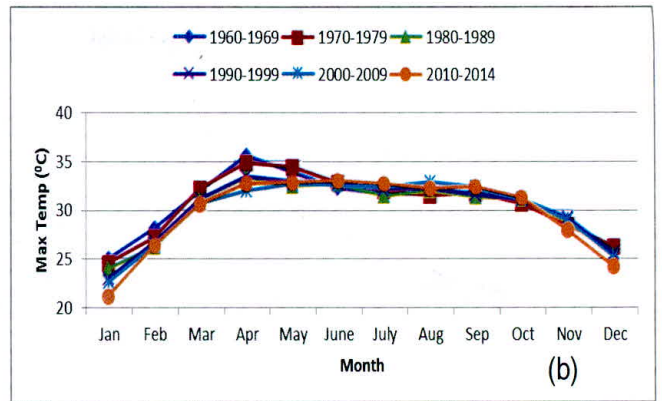
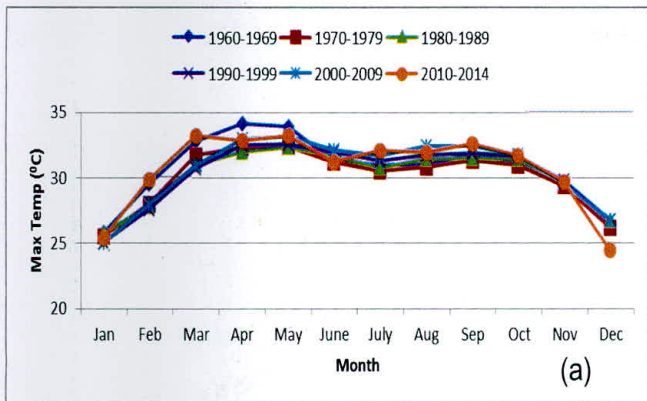


Fig. 1. Study locations





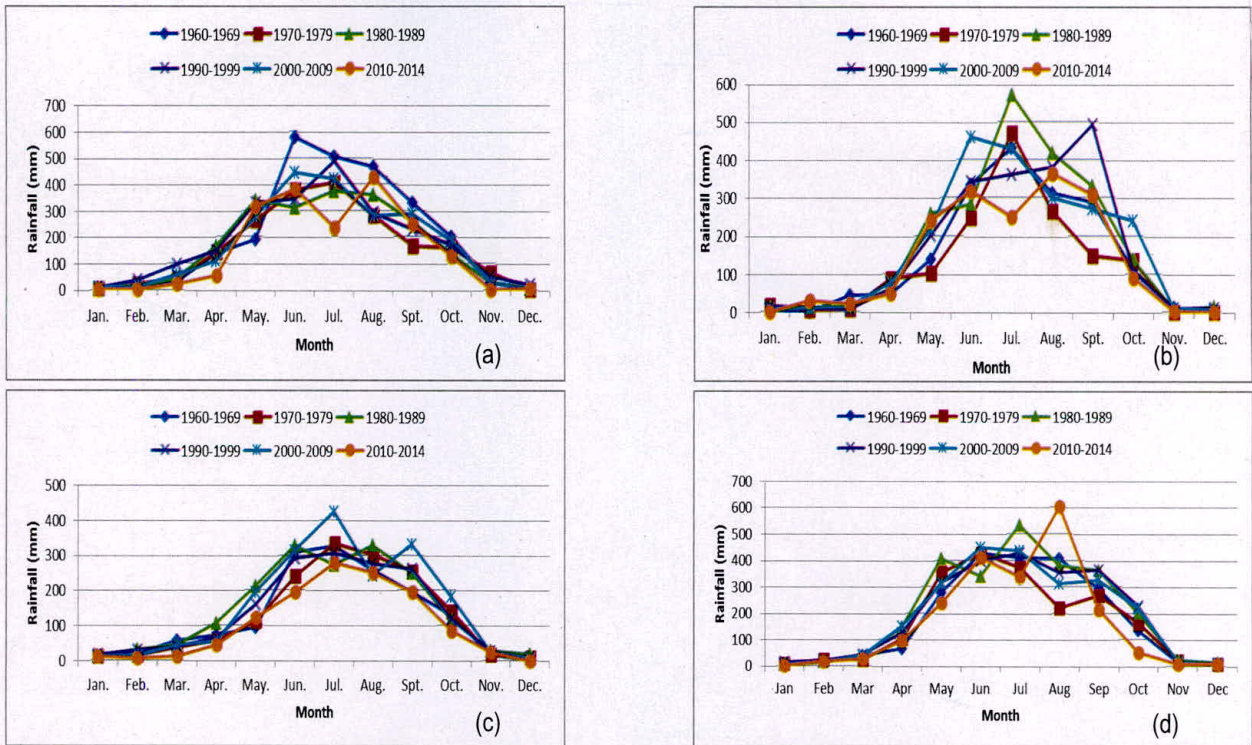


Fig. 3. Changes in decadal monthly maximum temperature, minimum temperature and rainfall in different locations (a) Comilla, (b) Dinajpur, (c) Jessore and (d) Gazipur over time.

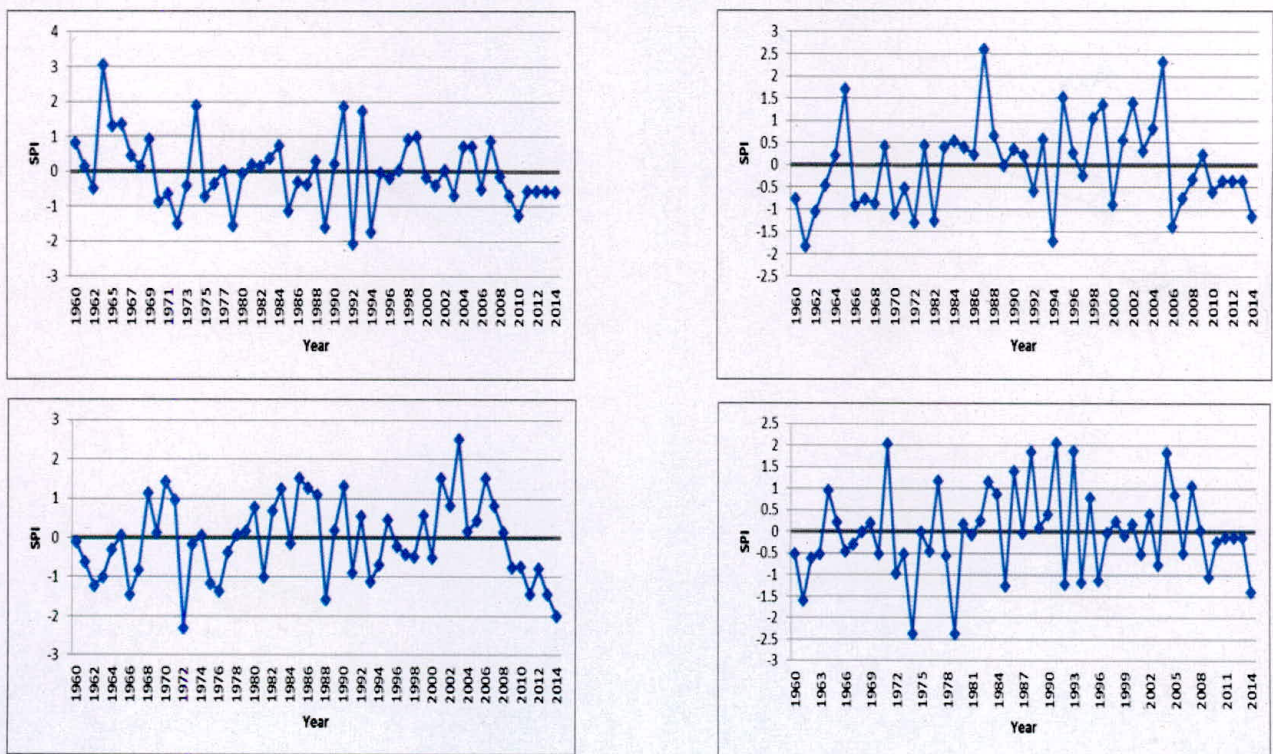


Fig. 4. Long-term (1960-2014) annual standardized precipitation index (SPI) in the different locations (a) Comilla, (b) Dinajpur, (c) Jessore and (d) Gazipur indicating frequency of dry and wet conditions.

Conclusion

Analyses of long-term data showed that maximum and minimum temperatures have been increasing in most of the regions. Increasing rate of temperatures was more in Jessore and Dinajpur regions. It was observed that temperatures during summer and winter months exceeded the critical levels. On an average, the rainfall showed increasing trends and the increment rate was 6.72 mm, 4.98 mm and 3.84 mm per year in Dinajpur, Jessore and Gazipur, respectively. Monsoon rainfall has been increased by 24.2, 7.5, 11.4 and 13.0%; whereas dry season rainfall decreased by 57.6, 19.7, 37.8 and 23.3% in Comilla, Dinajpur, Jessore and Gazipur in recent years compared to base year (1960-1969), respectively. Decrease in dry season rainfall increased drought frequency.

The data revealed that cropping area has been decreasing at an alarming rate. Along with various causes, unavailability of irrigation, uncertainty of rainfall and increasing temperatures are responsible for decreasing area of crop. Crop production, however, did not decrease due to technological development such as modern variety, management technique and protection options. In the recent years, the yield potentiality is decreasing due to various hazards under changing climate. As a result, cropping patterns in different locations have been changed. Farmers are not interested in rainfed crops and they are interested in vegetable cultivation. Irregular rainfall and temperatures are being observed particularly during the critical stages of different crops that making the farming more vulnerable. Increasing temperatures and fog are responsible for high incidence of diseases and pests. Under these circumstances wheat and potato production are being affected severely. To sustain crop production under changing climate, suitable variety and techniques are needed. Proper programs should be undertaken to build farmers resilience with good strategy.

Publication from this Research

The findings have been accepted in the *Annals of Bangladesh*, a peer review journal from the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU).

References

- BBS. 2015. Statistical Year Book, Bangladesh Bureau of Statistics. Government of the People's Republic of Bangladesh, Dhaka, Bangladesh.
- Hossain, A. and Silva, J. A. 2012. Phenology, growth and yield of three wheat (*Triticum aestivum* L.) varieties as affected by high temperature stress. *Notulae Scientia Biologicae*, 4: 97–106.
- IPCC. 2007. Climate change 2007. Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, Pachauri RK, Reisinger AJ, eds]. Geneva, Switzerland: IPCC, 104.

- Karim, Z. 1993. Preliminary agricultural vulnerability assessment: drought impacts due to climate change in Bangladesh. IPCC Eastern Hemisphere Workshop on Vulnerability Assessment to Sea-Level Rise and Coastal Zone Management, 3–6 August 1993, Tsukuba, Japan.
- McKee, T.B., Doesken, N.J. and Kleist, J. 1993. The relationship of drought frequency and duration to time scales. In: Proceeding of the 8th conference on applied climatology. Boston: *American Meteorological Society*, 179–84.
- Rahman, M.A., Kang, S., Nagabhatla, N. and Macnee, R. 2017b. Impacts of temperature and rainfall variation on rice productivity in major ecosystems of Bangladesh, *Agric & Food Secur*, 6:10 DOI 10.1186/s40066-017-0089-5.
- Rahman, M.M., Mostofa, M.G., Rahman, M.A., Miah, M.G., Saha, S.R., Karim, M.A. and Tran, L.S.P. 2017a. Mechanistic insight into salt tolerance of *Acacia auriculiformis*: the importance of ion selectivity, osmoprotection, tissue tolerance and Na⁺ exclusion, *Front. Plant Sci.*, 8:155. doi:10.3389/fpls.2017.00155.

Studies on the Reactions of Cyclopentadienylmolybdenum Tricarbonyl with Different Mercaptanes to Form New Molybdenum Metal Clusters

Md Manzurul Karim

Location: Jahangirnagar University

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 2500000.00

Introduction

Transition metal complexes containing sulfur and nitrogen donor ligands attracted a considerable attention on account of their relevance to organic, inorganic, biological and industrial processes (Stiefel *et al.* 1996, Raper 1994, Cruikshank *et al.* 1997, Drukeret *et al.* 1995, Rizaet *et al.* 1994 and DuBois 1989). In particular, thiolato derivatives of transition metals are of great importance in coordination chemistry and a large number of mono- and polynuclear compounds containing both simple and functionalized thiolato and dithiolato ligands have been synthesized and structurally characterized (Nguyen *et al.* 2005, Akrivos 2001, Torrens 2000, Raper 1996, Raper 1997, Stephan *et al.* 1989 and Stephan *et al.* 1996). Sulfide ligands e.g., RSH and RSSR are usually the source of the thiolate ligands SR in the synthesis of thiolato complexes with M-SR bonding (Stephan *et al.* 1996). In addition metal thiolate itself can transfer thiolate ligand to other metal centers and thus it has been proposed as a thiolate ligand source in the synthesis of thiolato complexes (Cruikshank 1997). The effects of polynuclear coordination to promote the cleavage of C-S bonds in organosulfur ligands in osmium and ruthenium carbonyl clusters have also been reported (Drukeret *et al.* 1995). Here we are discussing about the reactions of $[\text{CpMo}(\text{CO})_3]_2$ with different N and S donor ligands to make Mo-Complexes.

Objectives

- To extend our research vista to synthesize mixed metal clusters; and
- To synthesize molybdenum clusters with a view to synthesize some catalytically active metal clusters which may give catalytic activity relevant to polymerization processes.

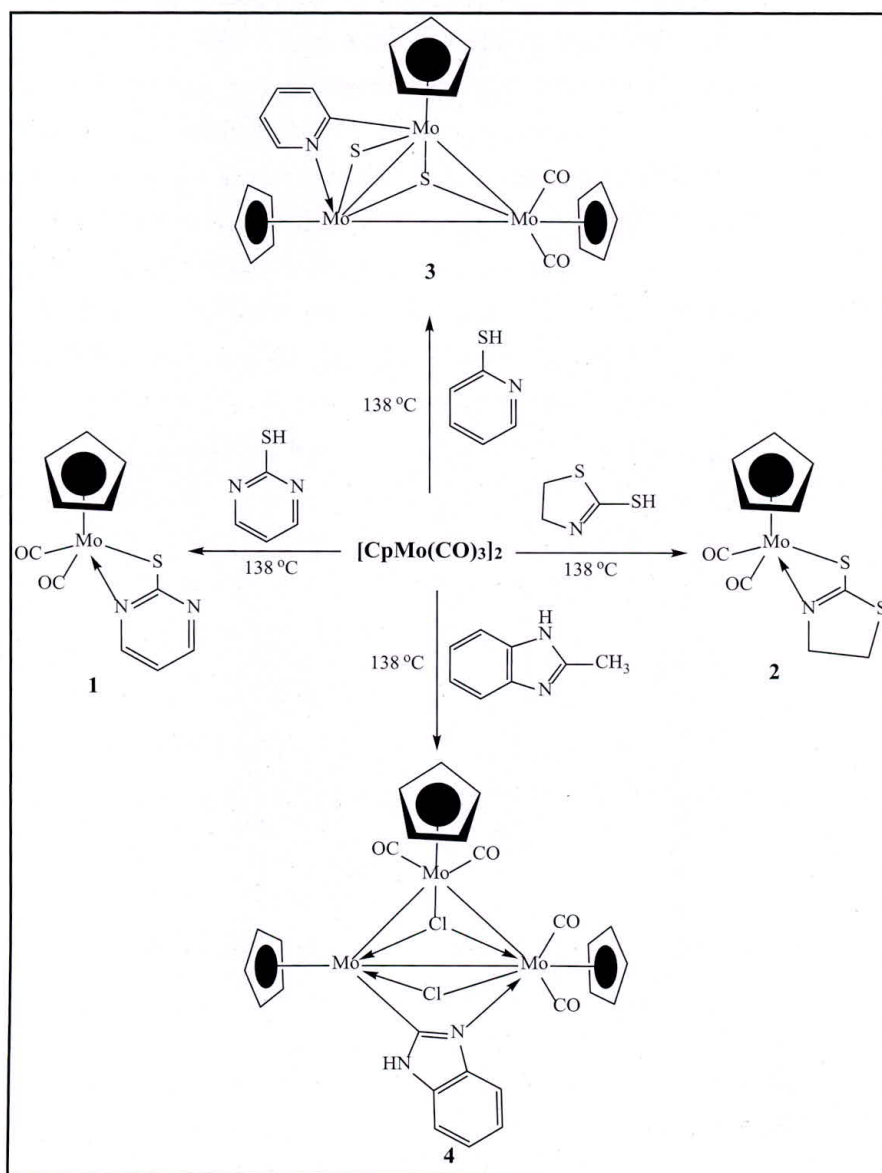
Methodology

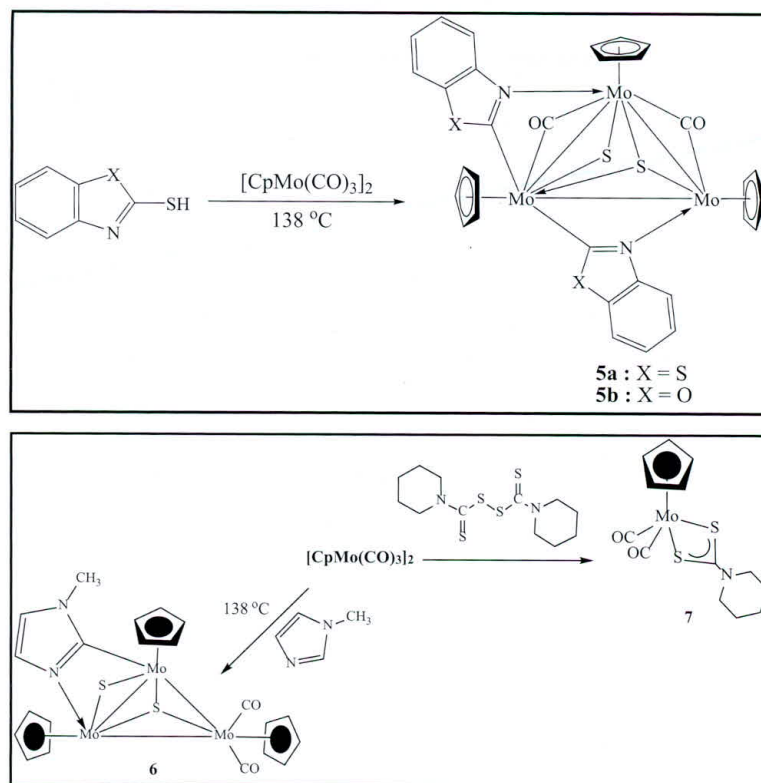
Metal clusters were synthesized by using appropriate metal carbonyls and ligands by thermal means. The compounds were separated by preparative thin layer chromatography. Chromatographic separation by TLC method followed by recrystallization from appropriate solvents was used for the purification of the compounds. Final characterization of the compounds was based on IR, NMR, mass spectroscopic data and single crystal X-ray diffraction method where possible.

Results

1. Syntheses of the compounds 1-7

The reactions of $[\text{CpMo}(\text{CO})_3]_2$ with 2-mercaptopyrimidine, 2-mercaptothiazoline 2-mercaptopyridine, 2-methylbenzimidazole, 2-mercaptobenzothiazole, 2-mercaptobenzoxazole, 2-mercapto-1-methylimidazole and dicyclopentamethylenethiuram disulfide in appropriate solvent resulted in the isolation of $[\text{CpMo}(\text{C}_4\text{H}_3\text{N}_2\text{S})(\text{CO})_2]$ (1), $[\text{CpMo}(\text{C}_3\text{H}_4\text{NS}_2)(\text{CO})_2]$ (2), $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_5\text{H}_4\text{N})(\mu\text{-S})(\mu_3\text{-S})(\text{CO})_2]$ (3), $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NNH})(\mu\text{-Cl})(\mu_3\text{-Cl})(\text{CO})_2\cdot\text{CH}_2\text{Cl}_2]$ (4), $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NS})_2(\mu_3\text{-S})(\mu\text{-S})(\mu\text{-CO})_2]$ (5a), $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NO})_2(\mu_3\text{-S})(\mu\text{-S})(\mu\text{-CO})_2]$ (5b), $[\text{Cp}_3\text{Mo}_3(2\text{-C}_4\text{H}_5\text{N}_2)(\mu\text{-S})(\mu_3\text{-S})\text{CO}]_2$ (6) and $\text{cis-}[(\text{CpMo}(\text{CO})_2\text{S}_2\text{-N-CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2)]$ (7) respectively.





Scheme- 1

Spectroscopic characterization of 1 - 7

The IR spectrum of 1-4,6 and 7 (in CH_2Cl_2) showed $\nu(\text{CO})$ absorption bands above 1850 cm^{-1} indicating the presence of terminal CO groups. IR spectrum of compounds 5a and 5b showed two $\nu(\text{CO})$ absorption bands below 1850 cm^{-1} supporting the presence of doubly bridging CO groups (Missleret. *et. al.* 2014).

The $^1\text{H-NMR}$ spectrum of 1-7 in CDCl_3 showed signals which were consistent with the structures of the compounds. The molecular ion peaks (in mass spectra of the compounds) supported the molecular weights of the compounds.

Spectroscopic data

Compound-1: IR (νCO ; in CH_2Cl_2): 1847 (s) and 1957 (s) cm^{-1} . $^1\text{H NMR}$ (CDCl_3): 5.52 (s, 5H) , $8.33\text{ (dd, 1H, } 3J=12\text{ Hz, appeared as triplet)}$, $\delta\ 8.23\text{ (dd, 1H, } 3J=12\text{ Hz, appeared as triplet)}$ and $6.69\text{ (dd, } 3J=8.0\text{ Hz, appeared as triplet)}$ ppm. MS (m/z) = 328, 300, 272, 165 etc.

Compound- 2: IR (νCO ; in CH_2Cl_2): 1942 (s) and 1849 (s) cm^{-1} . $^1\text{H-NMR}$ (CDCl_3): 5.51 (s, 5H) , 3.76 (m, 2H) and 3.30 (m, 2H) . MS (m/z): 335, 279, 164, 96 etc.

Compound- 3: IR (νCO ; in CH_2Cl_2): 2156 (s) , 2126 (vs) and 2055 (s) cm^{-1} . $^1\text{H NMR}$ (CDCl_3): $8.20\text{ (d, } 3J=8.0\text{ Hz, 1H)}$, $7.50\text{ (d, } 3J=8.0\text{ Hz, 1H)}$, $6.70\text{ (dd, } 3J=8.5\text{ Hz, } 3J=5.56\text{ Hz, 1H)}$, $6.30\text{ (dd, } 3J=8.4\text{ Hz, } 3J=5.5\text{ Hz, 1H)}$ $6.00, 6.09, 7.25\text{ (s, three Cp)}$ ppm. MS (m/z): 681, 653, 625, 594, 560, 519, 324, 267, 164, 96 etc.

Compound- 4: IR (ν_{CO} ; in CH_2Cl_2): 2056 (s), 2127 (vs) and 2054 (s) cm^{-1} . ^1H NMR (CDCl_3): 7.20 (d, $J = 7.5$ Hz, 1H), 7.10 (d, $J = 7.5$ Hz, 1H), 6.90-7.00 (m, 2H), 6.23, 6.08, 5.07 (s, 3Cp), 9.10 (s, NH, br), 5.53 (s, CH_2Cl_2). MS (m/z): 812 (very weak), 727, 699, 671, 635, 600, 574 etc.

Compound- 5a: IR (ν_{CO} ; in CH_2Cl_2): 1711 (m) and 1656 (w) cm^{-1} . ^1H NMR (CDCl_3): 8.03 (1H, d, $3J = 8.0$ Hz), 7.86 (1H, d, $3J = 8.0$ Hz), 7.73 (1H, d, $3J = 8.0$ Hz), 7.66 (1H, d, $3J = 8.0$ Hz), 7.18 – 7.43 (4H, m), and three singlets at 6.61, 6.16 and 6.10 ppm. MS (m/z): 871, 839, 811, 783, 751, 782, 762, 720, 701, 648, 565, 509, 239 etc.

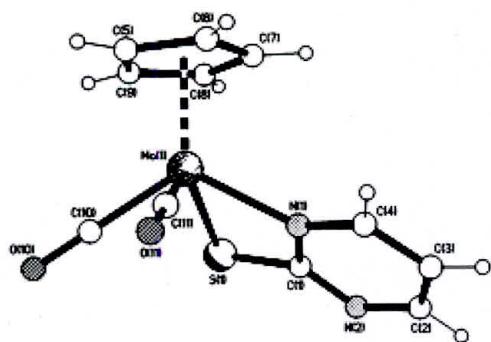
Compound- 5b: IR (ν_{CO} ; in CH_2Cl_2): 1834 (s) and 1778 (m) cm^{-1} . ^1H NMR (CDCl_3): 8.33 (1H, d, $3J = 7.5$ Hz), 8.23 (1H, d, $3J = 7.5$ Hz), 8.03 (1H, d, $3J = 8.0$ Hz), 7.86 (1H, d, $3J = 8.0$ Hz), 7.25 – 7.43 (4H, m), and three singlets at 6.61, 6.45 and 6.10 ppm. MS (m/z): 839, 779, 732, 783, 700, 669, 645, 598, 439, etc.

Compound- 6: IR (ν_{CO} ; in CH_2Cl_2): 1925 (w), 1819 (vs) and 1760 cm^{-1} . ^1H NMR (CDCl_3): 6.86 (d, 2.0, H), 6.30(d, 2.0, H), 6.11, 6.02 and 5.07 ppm. Mass (m/z): 684.

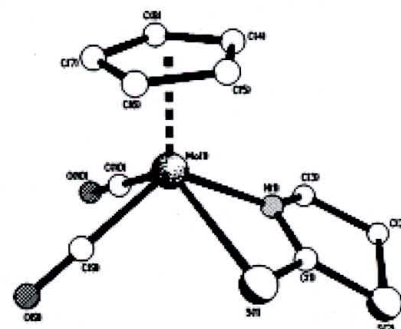
Compound- 7: IR (ν_{CO} ; in CH_2Cl_2): 1857 (s) and 1949 (s) cm^{-1} . ^1H NMR (CDCl_3): 5.41(5H, Cp), 1.54 (m, 4H), 1.63 (m, 2H), 3.56 (q, 2H), 3.79 (m, 2H) ppm.

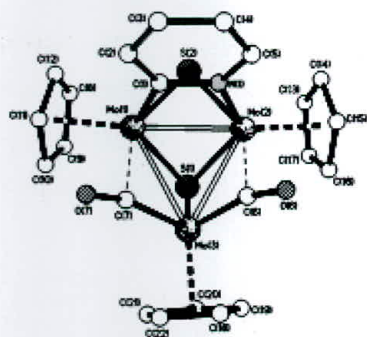
2. Solid state molecular structure of compounds 1-4, 6 and 7

The X-ray crystal structural analysis of 1-4,6 and 7 has been carried out for crystals selected from those grown by slow diffusion of solvents from dichloromethane and hexane mixture. The ORTEP diagrams along with selected bond lengths and bond angles of compounds 1-4, 6 and 7 are shown in figure-1.

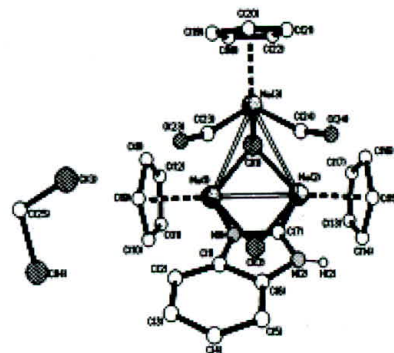


ORTEP diagram of 1; Selected bond lengths (\AA): Mo(1)–C(10) 1.960(2), Mo(1)–C(11) 1.968(2), Mo(1)–N(1) 2.1716(18), Mo(1)–S(1) 2.5218(6), Av. Mo – C (Cp) 2.3306(2); Selected bond angles ($^\circ$): C(10)–Mo(1)–C(11) 75.05(9), C(10)–Mo(1)–N(1) 119.33(8), C(11)–Mo(1)–N(1) 83.80(8), C(10)–Mo(1)–S(1) 80.43(6), N(1)–Mo(1)–S(1) 64.48(5).

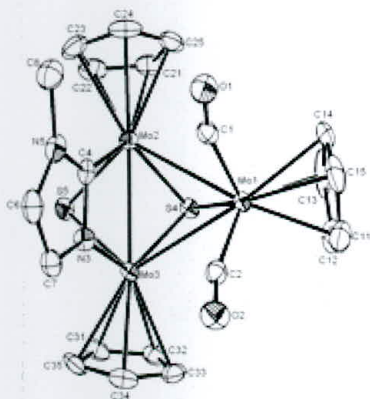




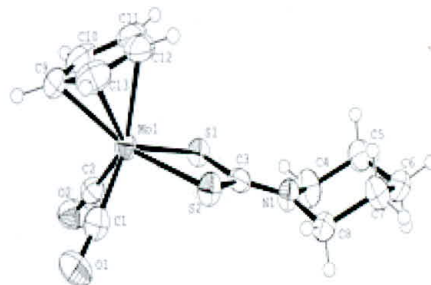
ORTEP diagram of 3; Selected bond lengths (Å): Mo(1)–Mo(2) 2.7508(2), Mo(1)–Mo(3) 3.0115(2), Mo(2)–Mo(3) 2.9760(3), Mo(1)–S(1) 2.3839(5), Mo(1)–N(1) 2.1463(2); Selected bond angles (°): Mo(2) – Mo(1) – Mo(3) 61.996(6), Mo(1) – Mo(2) – Mo(3) 63.308(7), Mo(2) – Mo(1) – Mo(3) 54.696(5).



ORTEP diagram of 4; Selected bond lengths (Å): Mo(1)–Mo(2) 2.7547(2), Mo(1)–Mo(3) 2.9803(3), Mo(2)–Mo(3) 3.0110(8), Mo(1)–Cl(1) 2.3674(14), Mo(1)–N(1) 2.144(4); Selected bond angles (°): Mo(2) – Mo(1) – Mo(3) 61.195(19), Mo(1) – Mo(2) – Mo(3) 62.062(19), Mo(2) – Mo(1) – Mo(3) 54.743(14).



ORTEP diagram of 6; Selected bond lengths (Å): Mo(1)–Mo(2) 2.9822(4), Mo(1)–Mo(3) 3.0105(5), Mo(2)–Mo(3) 2.7605(4), Mo(1)–S(4) 2.3740(10), Mo(3)–N(3) 2.119(3); Selected bond angles (°): C(2) – Mo(1) – C(15) 99.1(2), S(4) – Mo(1) – Mo(2) 51.42(2), N(3) – Mo(3) – C(33) 119.05(16).



ORTEP diagram of 7; Selected bond lengths (Å): Mo(1)–C(2) 1.966(6), Mo(1)–C(1) 1.958(6), Mo(1)–S(1) 2.505(2), Mo(1)–S(2) 2.495(3), Av. Mo – C (Cp) 2.3306(2); Selected bond angles (°): C(2)–Mo(1)–C(1) 75.7(2), C(2)–Mo(1)–C(9) 98.3(3), S(2)–Mo(1)–S(1) 68.55(7), C(3)–S(1)–Mo(1) 90.05(6).

Fig. 1: Solid state structure of 1-4, 6 and 7

The molecular structures of 1, 2 and 7 show a mononuclear core stabilized by a cyclopentadienyl ring and a bidentate ligand. The shapes of these complexes are square pyramidal. The cyclopentadienyl ring forms the zenith of the pyramid whose basal unit is a distorted square defined by two CO groups and two heteroatoms of the ligands. The most common feature of compounds 1, 2 and 7 is the chelation of Mo atom by the ligands. The compounds 1, 2 and 7 are electron precise and the core Mo atom follows 18-electron rule.

Compound 3,4 and 6 consists of a trinuclear framework of isosceles triangle having Mo atoms joined by bridging sulfur or chlorine (in 4) atom inside the triangular framework. The other parts of the ligands are also acting as bridging ligand joining two Mo atoms. The compounds have one longer and two remarkably shorter Mo-Mo bonds thus defining the isosceles structures. The TVEC for the compounds are 48 and all Mo cores satisfy 18 electron rule.

Conclusion

The reaction of $[\text{CpMo}(\text{CO})_3]_2$ with 2-mercaptopyrimidine and 2-mercaptothiazoline in refluxing m-xylene at 138°C gave two novel mononuclear compounds $[\text{CpMo}(\text{C}_4\text{H}_3\text{N}_2\text{S})(\text{CO})_2]$ (1) $[\text{CpMo}(\text{C}_3\text{H}_4\text{NS}_2)(\text{CO})_2]$ (2) respectively. Treatment of $[\text{CpMo}(\text{CO})_3]_2$ with 2-mercaptopyridine, 2-methyl benzimidazole, 2-mercaptobenzothiazole, 2-mercaptobenzoxazole and 2-mercapto-1-methyl imidazole produced the trinuclear clusters $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_5\text{H}_4\text{N})(\mu\text{-S})(\mu\text{3-S})(\text{CO})_2]$ (3) $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NNH})(\mu\text{-Cl})(\mu\text{3-Cl})(\text{CO})_2\cdot\text{CH}_2\text{Cl}_2]$ (4) $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NS})_2(\mu\text{3-S})(\mu\text{-S})(\mu\text{-CO})_2]$ (5a) $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_7\text{H}_4\text{NO})_2(\mu\text{3-S})(\mu\text{-S})(\mu\text{-CO})_2]$ (5b) and $[\text{Cp}_3\text{Mo}_3(\mu\text{-C}_4\text{H}_5\text{N}_2)(\mu\text{-S})(\mu\text{3-S})(\text{CO})_2]$ (6) respectively. The reaction of $[\text{CpMo}(\text{CO})_3]_2$ with dicyclopentamethylenethiuram disulfide in refluxing xylene afforded a mono nuclear compound, cis- $[(\text{CpMo}(\text{CO})_2\text{S}_2\text{N-cyclo}(\text{CH}_2)_5)]$ (7) in moderate yield. All compounds have been characterized by IR, ^1H NMR and mass spectroscopic data. Structure of compounds 1-4, 6 and 7 was unambiguously determined by single crystal X-ray diffraction studies.

Publications from this research

- Chowdhury, M. M. H., Rajbangshi, S., M. Karim, M., Ghosh, S., Kabir, S. E., Siddique, T. A., Nesterov, V. N. and Richmond, M. G., 2015, Reactivity of $[\text{CpMo}(\text{CO})_3]_2$ towards heterocyclic thiols: Synthesis, structure and bonding in the sulfide-ligated cluster $\text{Cp}_3\text{Mo}_3(\mu\text{CO})_2(\mu,\kappa^2\text{-C}_7\text{H}_4\text{NS})(\mu\text{-S})(\mu\text{3-S})$, *Inorg. Chim. Acta*, 434, 97-103.
- Karim, M. M., Ahmed, L., Islam S. and Rahman, M. M., Metal, J., 2015, sulfur nitrogen bonded complexes of molybdenum and their x-ray crystal structures, *Bangladesh Chem.Soc*, 27, 92.
- Karim, M. M., Islam, S., Islam, M. R., Karim, M. R. and Siddiquee. T. A., Dicyclopentamethylenethiuram Disulfide as Precursor of Mononuclear Complexes: Oxidative Cleavage of Metal-Metal Bond in $[\text{CpMo}(\text{CO})_3]_2$ and Molecular Structure of cis- $[\text{CpMo}(\text{CO})_2\{\text{S}_2\text{C-N}(\text{CH}_2)_5\}]$, *J. Cluster Science* (submitted).

References

- Akrivos, P. D. 2001. Recent studies in the coordination chemistry of heterocyclic thiones and thionates, *Coord. Chem. Rev.*, 213:181.
- Cruirs, M. D. and Dungey, K. E. 1997. Homolytic C-S Bond Cleavage on a Heterogeneous Co/Mo/S Hydrodesulfurization Catalyst, *J. Amer. Chem. Soc.*, 119:842.
- Cruirs, M. D. and Druker, S. H. 1997. Homolytic C-S Bond Scission in the Desulfurization of Aromatic and Aliphatic Thiols Mediated by a Mo/Co/S Cluster: Mechanistic Aspects Relevant to HDS Catalysis, *J. Amer. Chem. Soc.*, 119:1027.
- Druker H. and Cruirs, M. D. 1995. Activation of C-S Bond Homolysis by Coordination to a Mo/Co/S-Containing Cluster, *J. Amer. Chem. Soc.*, 117:6366.
- DuBois, M. R. 1989. Catalytic applications of transition-metal complexes with sulfide ligands, *Chem. Rev.*, 89:1.
- Nguyen, T., Panda, A., Olmstead, M. M., Richards, A. F., Stender, M., Brynda, M., Power, P. P. 2005. Synthesis and Characterization of Quasi-Two-Coordinate Transition Metal Dithiolates $M(SAr^*)_2$ ($M = Cr, Mn, Fe, Co, Ni, Zn$; $Ar^* = C_6H_3-2,6(C_6H_2-2,4,6-Pri_3)_2$), *J. Am. Chem. Soc.*, 127:8545.
- Raper, E. S. 1994. Copper complexes of heterocyclic thioamides and related ligands, *Coord. Chem. Rev.*, 129:91.
- Raper, E. S. 1996. Complexes of heterocyclic thionates. Part 1. Complexes of monodentate and chelating ligands, *Coord. Chem. Rev.*, 153:199.
- Raper, E. S. 1997. Complexes of heterocyclic thionates Part 2: complexes of bridging ligands, *Coord. Chem. Rev.*, 165:475.
- Riza, U., Curnow O. J. and Cruirs, M. D. 1994. *J. Amer. Chem. Soc.*, 116:4357.
- Stephan, D. G. 1989. Early-late heterobimetallics, *Coord. Chem. Rev.*, 95:41.
- Stephan, D. G., Nadasdi, T. T., 1996. Early transition metal thiolates, *Coord. Chem. Rev.*, 147:147.
- Stiefel E. I. and Matsumoto, K. 1996. Transition Metal Sulfur Chemistry: Biological and Industrial Significance Eds.; ACS: Washington DC.
- Torrens, H., 2000. Pentafluorobenzenethiolato derivatives of the platinum group metals, *Coord. Chem. Rev.*, 196:331.

Production of Bioethanol by Thermotolerant Microbes

Ali Azam Talukder and Anowar Khasru Parvez

Location: Jahangirnagar University, Savar, Dhaka, Bangladesh.

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 3300000.00

Introduction

An eco-friendly biofuel like ethanol is one of the alternate fuels that can be used in unmodified petrol engines with current fueling infrastructure. It is desirable fuel additive because it allows fuel to burn more cleanly and lowers greenhouse gas emissions. The emission and toxicity of ethanol is carbon neutral are lower than those of fossil fuels such as petroleum, diesel etc. Despite the recent advances, developments of eco-friendly inexpensive bioethanol production still become a challenge. In the proposed project we have isolated, identified and characterized thermo tolerant microorganisms mainly yeasts from the natural fermented products of Bangladesh for high temperature bioethanol production. High temperature ethanol fermentation technology is anticipated to become one of the economical next-generation fermentation technologies (1). High temperature fermentation technology permits to execute economical fermentation, which cuts down cooling cost, contamination risk and operation cost in addition to advantages specific for bioethanol production including higher saccharification and fermentation rates and continuous ethanol removal.

Objectives

In the proposed project we have identified yeast strains able to produce higher ethanol yields during fermentation. On the above background the proposed project has undertaken to achieve the following objectives in three phases in years:

- Development of efficient low cost screening method for isolating thermotolerant microorganisms from the natural sources of Bangladesh.
- Identification of useful microorganisms and standardization fermentation process for laboratory scale bioethanol production.
- Apply genetic engineering technology for the production of high yielding variety from the wild isolates.
- Establishment of suitable conditions for microbial growth, inexpensive procedure using low cost manpower and cheap readily available raw materials for mass production of bioethanol industrially.
- Application of bioethanol to recover the shortage of our national demands including fuel, electricity and other sectors, and 6) Earns foreign revenue through export excess bioethanol.

Methodology

Microbial cells growth was carried out in Yeast Extract Peptone Dextrose (YPD) liquid medium and fresh growing culture were diluted 100-fold and then further incubated at various growth temperatures upto 96 hours. The fermented sample was used for various experiment purposes as shown below. Cells were harvested by mild centrifugation (5000 rpm for 5 min) and then prepared for microscopic study. Cell growth curve was estimated by measuring optical density at 600 nm. Bioethanol content was measured after centrifuged fermented sample. The cell supernatant was collected and then filtered using syringe filter (0.22-0.45µm). Bioethanol concentration was estimated either by solvent extraction and dichromate oxidation method or by Gas Chromatography (GC) method as described (2). Optical density (OD) for standard ethanol solution and samples were measured at 595nm in a UV spectrophotometer (Thermo Scientific). Bioethanol content of unknown sample was estimated from the ethanol standard curve. Bioethanol content was further confirmed by GC (standard ethanol solutions from 0.0-1.0 % (v/v) were prepared with water and 1-2l was injected into the injection sport of the GC and then subjected to quantitative analysis of ethanol on a GC apparatus (SRI Model 8610A GC 5890 GC, Shimadzu, Japan).

Results

Research Results are summarized below

- Potential natural thermotolerant strains have been identified for bioethanol production.
- Laboratory scale bioethanol production rate is optimized.
- Set-up industrial microbiology laboratory in Jahangirnagar University.
- Twelve Masters and ten undergraduate students completed their dissertation from 2012 to till date.
- Eight-research reports have been published in international peer reviewed journals.
- Research findings are yet to be announced nationwide through mass communication and electronic media.

Brief results are shown in 4 Figures below

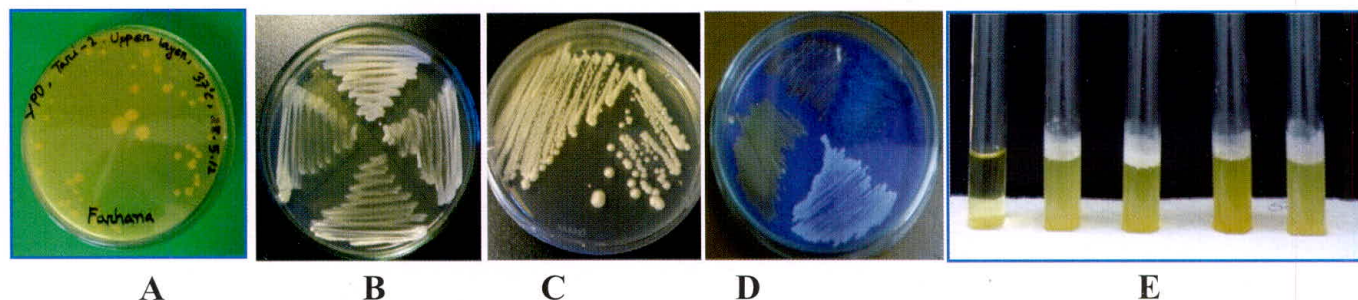


Fig. 1: Screening thermotolerant microorganisms collected from various natural fermented product of Bangladesh. Various microorganisms were screened on either YPD agar plates or YPD broth. Here, A) YPD agar plate used as a parental plate where you could see various types of colonies; B) various strains were screened (Wm-1, Tari-3, Sc-4, and pv-1) on YPD plate; C) isolation of single colony from the strain Sc-4 (the colonies were round, cream colored, smooth and elevated surface); D) growth on YPG agar plate which containing various acetic acid producing bacteria and E) image represents the growth of yeast cells in YPD broth, where you could observed fermentation. All yeast cells were grown in YPD liquid medium (pH 4.5) at 37 °C for 4 days. Each test tube contains 5 ml YPD broth. Top left image represents as control (without sample), which was used to compare the growth of other yeasts. After incubation the YPD broth became turbid and formed ethanol and CO₂ gas are the by-product of fermentation process.

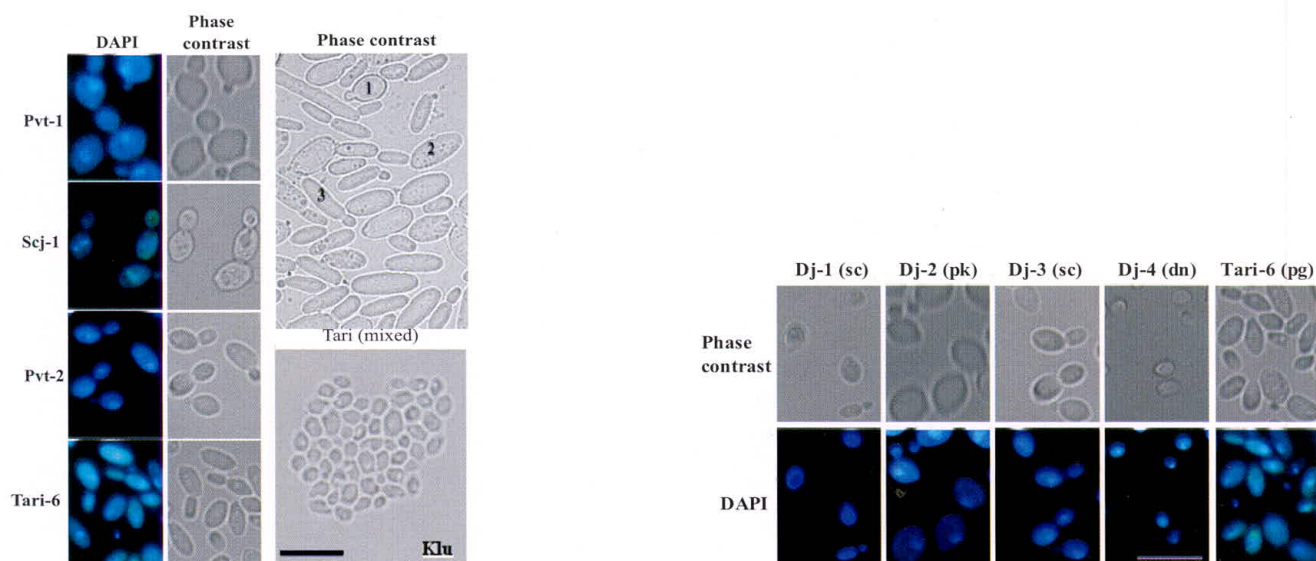


Fig. 2: Immunofluorescence microscopic observation of thermo tolerant yeasts isolated from the various natural fermented sources of Bangladesh. Various yeast cells (Pvt-1, Scj-1, Pvt-2, Tari-6, Tari-mixed sample (Left panel) and Dj-1, Dj-2, Dj-3, Dj-4 and Tari-6 (Right panel) were grown in YPD liquid medium at 30 °C for 24 hours at 100 rpm, collected, fixed and stained with DAPI and examined by Fluorescence microscope (3). DAPI images (blue color) represented the location of nucleoid onto the cell, while phase contrast images showed the cell shapes and sizes. Scale bar represents 15 μ.

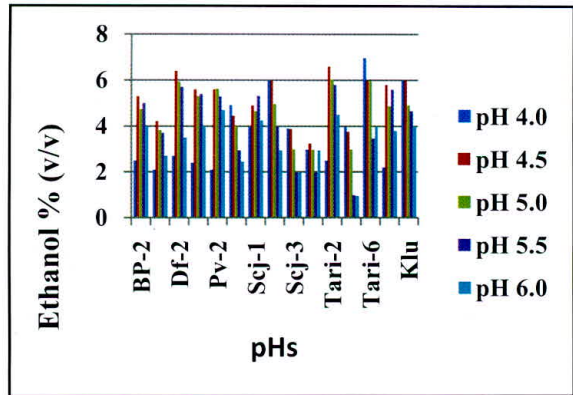


Fig. 3: Effect of medium pHs on bioethanol production. Various cells were grown in different medium pHs are shown in a basal medium composed of total sugar (20%), H₂O and 0.05% (NH₄)₂SO₄ and incubated at 37 °C for 72 h. The highest amount of bioethanol was also produced at pH 4.5 by Tari-2 strain.

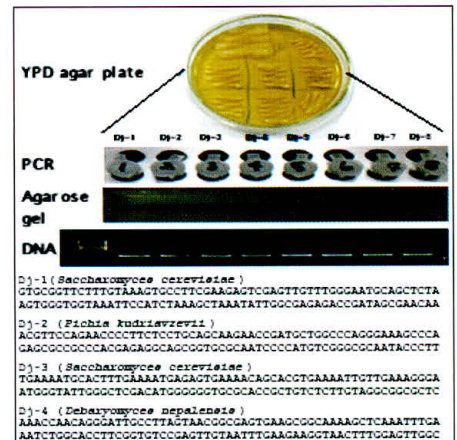


Fig. 4: PCR amplification, purification and sequencing of yeasts D1/D2 domain isolated from various natural fermented sources of Bangladesh. Left and right panel show the samples collected from the sugar-cane juice and date palm juice (left) and date juice (right), respectively.

Conclusion

After screening 51 microorganisms morphologically, physiologically, biochemically and genetically (DNA sequencing), we concluded that the isolates from the Tari (Tari-6, *Pichiaga leiformis*), Pantavat (Pvt-1, *Pichiaguilliermondii*), Boiled Potato (Bp-2, *Candida tropicalis*) are potential thermotolerant microorganisms produced higher amount of bioethanol under the condition we employed here. Because, both strains have broad substrate spectrum, thermotolerance and high growth rate we have performed. Another thermosensitive strain isolated from the Date palm juice, Dj-3, which encoded *Saccharomyces cerevisiae* which utilizes Xylose as a sole carbon source might be used for Beer producing industry under low temperature fermentation process. Further research is required to establish suitable conditions and various parameters for optimum growth and production of bioethanol in laboratory as well as industrial scale.

Publication from this Research

- Talukder, A.A. and Ishihama, A., 2014. Dps is a stationary phase-specific protein of *Escherichia coli* Nucleoid. *Advances in Microbiology*, USA, 4, 1095–1104.
- Diba, F., Alam, F. and Talukder, A.A., 2015. Screening of acetic acid producing microorganisms from decomposed fruits for vinegar production. *Advances in Microbiology*, USA, 5, 291–297.
- Talukder, A.A, Sujon, S.I., Hossain, M.M, Gomes, D.J. and Yamada, M., 2015. Production of bioethanol at high temperature from Tari. *Advances in Microbiology*, USA, 5, 325–335.
- Talukder, A.A and Ishihama, A., 2015. Growth phase dependent changes in the structure and protein composition of nucleoid in *Escherichia coli*. *Science China Life Sciences*, 58: 902–911.
- Talukder, A.A., Islam, F., Mahmud, S.A. and Yamada, M., 2016. Thermotolerant yeasts capable of producing bioethanol: isolation from natural fermented sources, identification and characterization. *Biotechnology & Biotechnological Equipment*. 30. 1106–1114.
- Talukder, A.A, Siddiqa, Miah, R., Tuli, J.F., Barman, N.K. and Yamada, M., 2017. Inexpensive procedure for measurement of ethanol: Application to bioethanol production process (In Press, *Advances in Microbiology*, USA).
- Talukder, A.A., Islam, T., Diba, F., Alam, F. and Matshushita, K., 2017. Optimization of high temperature vinegar production by *Acetobacter* and *Bacillus* sp. from decomposed fruit materials in Bangladesh (In Press, *Advances in Microbiology*, USA).

Biological Nitrification Inhibition – A Novel Genetic Strategy Can Increase Nitrogen Use Efficiency and Combat N₂O Emissions in Cereal Production System

A K M Zakir Hossain and Md Abul Khair Chowdhury

Location: Department of Crop Botany, Bangladesh Agricultural University, Mymensingh

Duration: Three Years (2014-2017)

Expenditure of the project : Tk. 2500000.00

Introduction

Plants that are efficient in absorption and utilization of nutrients, greatly enhance the efficiency of applied fertilizers, reducing cost of inputs, and preventing losses of nutrients to ecosystems. Inter- and intra-specific variation for plant growth and mineral nutrient use efficiency (NUE) are known to be under genetic and physiological control and are modified by plant interactions with environmental variables. Some of the major concerns of modern production agriculture are the high level of N-pollution and low efficiency of N utilization. Nitrification of NH₄⁺ to NO₃⁻ results in the transformation of the relatively immobile NH₄⁺ to highly mobile NO₃⁻, that can result in large N losses from denitrification and nitrate leaching (Subbarao *et al.*, 2006). This results in environmental pollution and inefficient use of both soil N and applied N, as shown by the lower fertilizer-N recovery and N-utilization during crop production (Raun & Johnson 1999). Suppression of nitrification and keeping N in the NH₄⁺ form can be a key strategy in extending N residence time in the soil, thus increasing N recovery and improving nitrogen use efficiency.

Among cereals, rice is the staple food of the people of Bangladesh. It contributes Bangladeshi economy and agriculture accounting 18% of the gross domestic product. The rice area totals about 10 million ha and accounts for 75% of the total area of agricultural crops, and 93% of the total planted to cereals. Rice is a high nitrogenous fertilizer consumptive crop and total annual demand of urea in Bangladesh is 2.7 million tons. Recently it has been confirmed that *Brachiaria humidicola* a tropical pasture grass, *Leymus racemosus*-a wild relative of wheat and sorghum release significant amounts of biologically active compounds that suppress soil nitrification (Subbarao *et al.*, 2006b; Hossain *et al.*, 2008) while other important cultivated cereals such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) did not show BNI capacity (Subbarao *et al.*, 2007a). One novel active constituent which acts as a strong natural biological nitrification inhibitor, has been identified from sorghum root exudates (Hossain *et al.*, 2008). The characterization of this trait in cereals to inhibit soil nitrification process is not fully characterized yet. Among the identified species *Brachiaria* grass and wild relative of wheat, *Leymus racemosus* are very slow growing cereals. So the research was designed to focus the detailed characterization of BNI release mechanisms of sorghum and the effect of released natural nitrification inhibitors from sorghum root exudates on rice production system.

Objectives

The present study was aimed at evaluating the following objectives:

- Collection and evaluation of sorghum germplasms for BNI function;
- To check the stability and ability of BNI from sorghum in the soil;
- Evaluation of the nitrification inhibitory compound/s from sorghum on rice growth environment; and
- Physiological studies on the mechanisms effecting BNI-compounds release; mode of action of BNI release from roots.

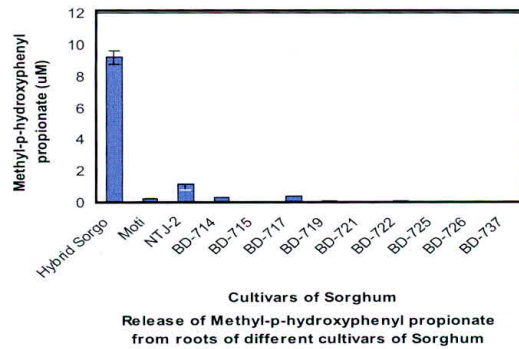
Methodology

- Soil (dry soil 2 gms;/ 10 ml glass bottle with 60% WHC)
- 0.72 ml of 1M(NH₄)₂SO₄ (182 mg N Kg⁻¹) control
- (NH₄)₂SO₄ +BNI(MHPP) I.e.0 10, 20, 50, 100 μM /g soil Replication-4
- Placed in temperature (200 C) and humidity (95%) controlled incubator for 45 and 60 days
- Soil extraction was done with 2M KCl for 30 min and filtered
- HPLC analysis was done for the stability of BNI compound/s
- The filtrate was also analyzed for NH₄⁺ with in indophenols colorimetric method and NO₃⁻ was determined with sulfanilamide-a-naphthylene method.

Results

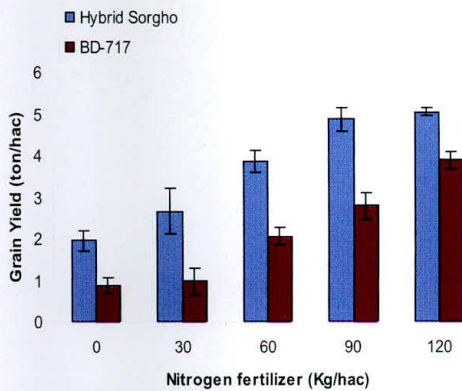
Activity 1: Collection and evaluation of sorghum germplasms for BNI function

Experiment-1: Seedlings were grown in sand for 5 days. Seedlings were transferred to hydroponic culture for 21 days with full nutrient solutions at pH 6.25 with continuous aeration. Root exudates were collected with 1 mm NH₄Cl solution (4 plants). Root exudates was concentrated and extracted with methanol and then dissolved in acetonitrile for HPLC analysis. Samples were chromatographed by gradient elution on a reverse phase C18 column. Detector measured at 280 nm.



Several sorghum species from Bangladesh and abroad were surveyed for BNI capacity in roots using hydroponics. Among the tested sorghum varieties, none of the varieties showed detectable BNI capacity except hybrid sorgho from Japan.

Experiment 2 : Field Experiment: Methodology: Place: Crop Botany Field Laboratory; **Varieties:** 1. Hybrid Sorgho, 2. BD-717, Doses of nitrogen: 0,30,60,90,120 Kg/hac; Replication: Four (4); RCBD



Different doses of nitrogen on grain yield

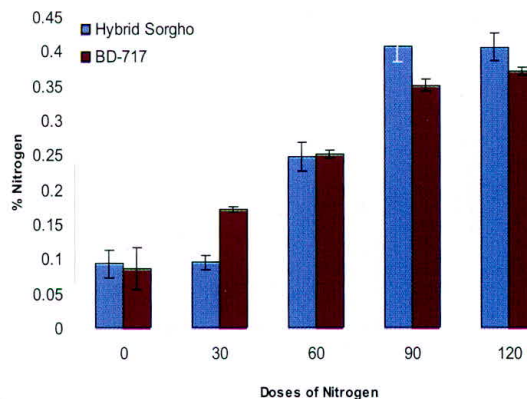
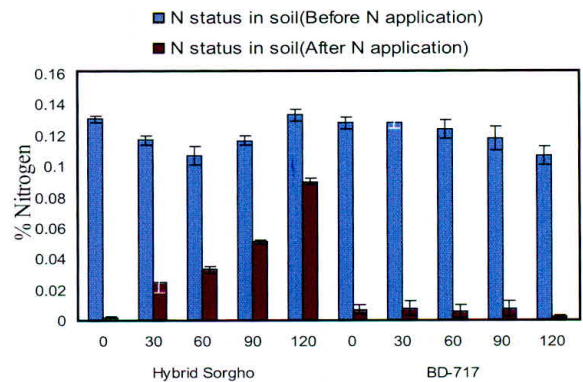


Fig: Nitrogen status in Grain

Activity 2: To check the stability and ability of BNI from sorghum in the soil

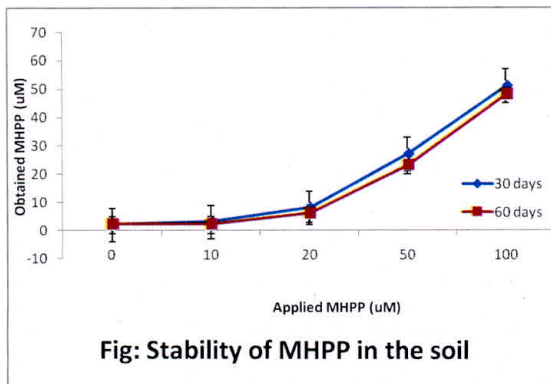


Fig: Stability of MHPP in the soil

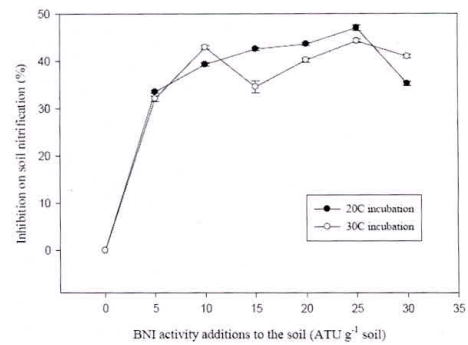


Fig: Ability of MHPP on inhibition of nitrification

It has been confirmed that the identified compound has the ability to stay in the soil for longer period of time and significantly inhibited the soil nitrification process.

Activity 3

Evaluation of the nitrification inhibitory compound/s from sorghum on rice growth environment

Preliminary results of two experiments have indicated that the BNI has significant contribution in increasing nitrogen use efficiency in rice production.

Experiment 1 (Treatments) T0 (Control) = 200 Kg urea ha⁻¹ (Urea alone) (recommended dose); T1 = 150 kg urea ha⁻¹ plus 250 µM MHPP; T2 = 100 kg urea ha⁻¹ plus 250 µM MHPP;

T3 = 50 kg urea ha⁻¹ plus 250 µM MHPP (250µM MHPP propionate was applied @1litre/plot except control).

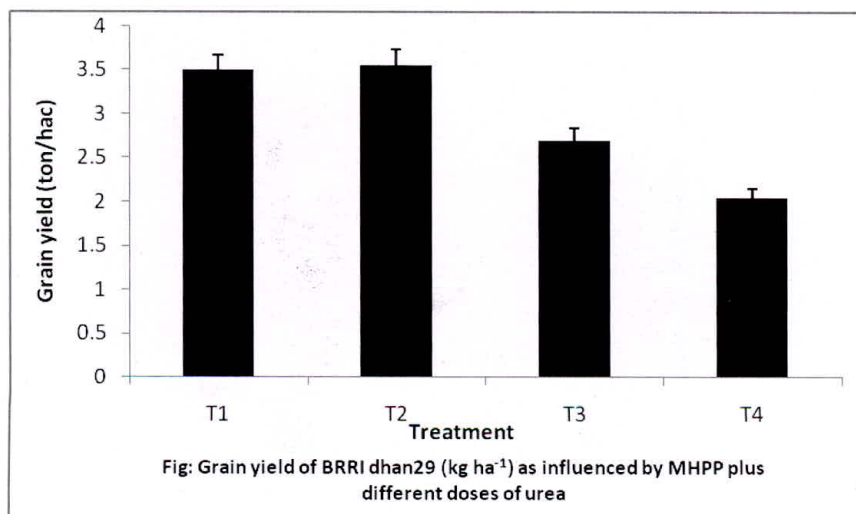


Fig: Grain yield of BRR1 dhan29 (kg ha⁻¹) as influenced by MHPP plus different doses of urea

Overall results of this experiment indicate that the inhibitor from sorghum (i.e. MHPP) effectively works on nitrification process and improves the nitrogen use efficiency by improving yield parameters of rice.

Activity 4: Physiological studies on the mechanisms effecting BNI-compounds release; mode of action of BNI release from roots

Experiment 1: Effect of different forms of nitrogen (NH_4^+ vs NO_3^-) on the release of methyl-p-hydroxy-phenyl propionate.

Seedlings were grown in hydroponics as described in the above expt. The samples were processed as described in the activity-1

Plants of Sorghum grew well with both sources of N (i.e. NH_4^+ or NO_3^-). The growth was similar and the plants looked healthy in both sources of N. Specific BNI activity was detected higher in root exudates in NH_4^+ treated plants in compare to NO_3^- treated plants when solution pH was continuously controlled. The HPLC spectra of NH_4^+ treated exudates showed 2.5-3 times higher concentration (considering peak area) of active compounds than NO_3^- treated exudates.

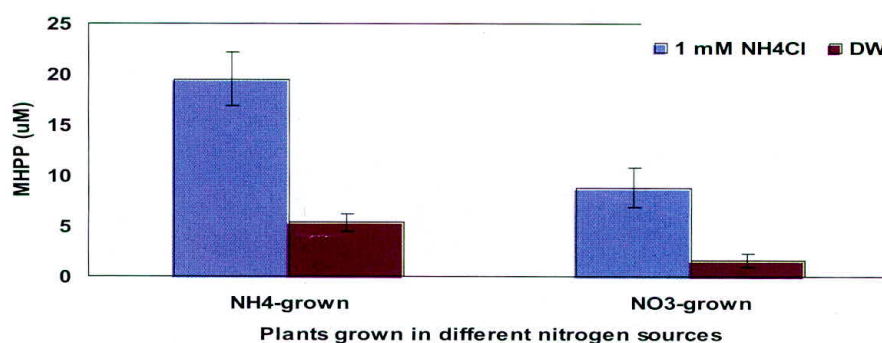
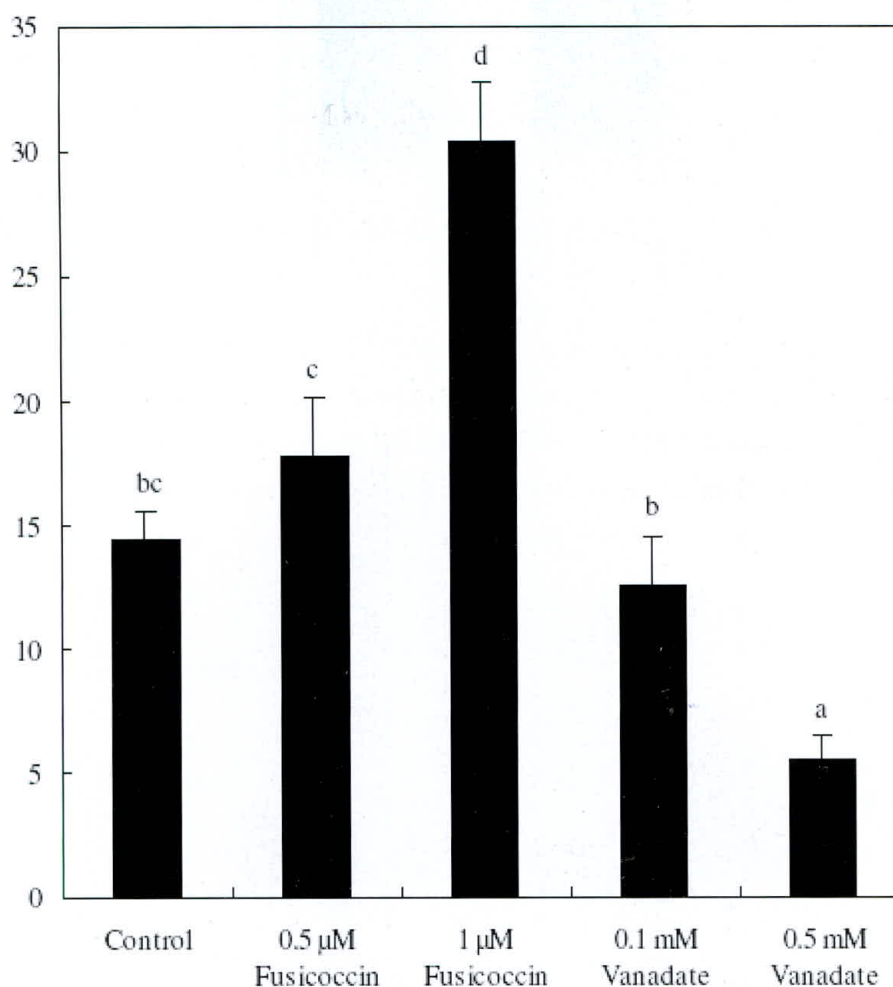


Fig: BNI activity from root exudates of NH_4^+ or NO_3^- grown plants (Plants grown in hydroponics with 1 mM N added as $(\text{NH}_4)_2\text{SO}_4$ and KNO_3 . Exudates collected with 1 mM NH_4Cl and distilled water for 24h)

Experiment 2: Effect of the modification of plasma membrane H^+ -ATP-ase on BNI release from roots

Root plasma membrane from sorghum was isolated according to Yan *et al* (2002). To understand further the role of PM H^+ -ATPase on BNI release we modified the root PM H^+ -ATPase activity by keeping the intact plant roots in solutions of fusicoccin and vanadate before the collection of root exudates. Fusicoccin, a phytotoxin produced by *fusicoccum amygdali*, is a known stimulator of PM H^+ ATPase in plant cells (Reid *et al* 1985). Vanadate is an inhibitor of PM H^+ ATPase, an analog of phosphate.

Results revealed that fusicoccin at 1 μM significantly increase MHPP release (fig). In contrast MHPP release was significantly suppressed by 0.5 μM vanadate. The results indicated that modification of PM H^+ ATPase had a direct impact on the release of BNI.



Conclusion

From the present results we could not detect significant release of BNI compounds from collected sorghum varieties except Hybrid Sorgho from Japan and MTJ-2 from ICRISAT, India. None of the cultivars from Bangladesh showed detectable BNI capacity. Still the experiments are being conducted in screening cultivars for high BNI capacity using BARI accessions. It has also been proved that the identified compound from sorghum is highly stable in the soil for longer period of time and effectively inhibits the nitrification process. Overall results of the field and pot experiments indicate that the inhibitor (i.e. MHPP) may effectively inhibits nitrification process and improves the nitrogen use efficiency by improving yield parameters of rice. The field experiment also reveals that sorghum can also be cultivated as an alternate crop with rice based cropping system for minimizing the uses of nitrogen fertilizer. Physiological studies on the mechanisms of BNI-compounds release revealed that NH_4^+ has the trigger (stimulatory) effect on BNI compound release from sorghum; and NH_4^+ uptake, Plasma membrane H^+ -ATP-ase activity and rhizosphere acidification are functionally inter connected with BNI release in sorghum

Publications

- Razzak, M. A., Hossain, A. K. M. Z., Rahman, M. M., Debsharma, S. K. and Roy, C. 2013. Effect of nitrification Inhibitors on yield and yield attributes of T. Aman rice varieties. *The Planter*. 88 (1041):865-8732.
- Rahman, A. M. S., Hossain, A. K. M. Z., Hossain, M. A., Saha, B. K. and Anowarul, H. M. 2014. Variation among grain sorghum varieties in response to nitrogen fertilizer. *Bangladesh Journal of Seed Science & Technology*. 17(1-2): 7-14.

References

- Hossain, A.K.M.Z, Subbarao G.V., Pearse S.J., Gopalakrishnan, S., Ito O., Ishikawa T., Kawano N., Nakahara K., Yoshihashi T., Ono, H., and Yoshida M. 2008. Detection, isolation and characterisation of a root exuded compound, methyl 3-(4-hydroxyphenyl)propionate responsible for Biological Nitrification Inhibition by sorghum (*Sorghum bicolor* L.) *New Phytologist*. 180: 442–451
- Raun, W.R., Johnson, G.V. 1999. Improving nitrogen use efficiency for cereal production. *Agronomy Journal* 91: 357-363
- Subbarao, G.V., Ban, T., Kishi, M., Ito, O., Samejima, H., Wang, H.Y., Pearse, S.J., Gopalakrishnan, S., Nakahara, K., Hossain. A.K.M.Z., Tsujimoto, H., Berry, W.L. 2007. Can biological nitrification inhibition (BNI) genes from perennial *Leymus racemosus* (Triticeae) combat nitrification in wheat farming? *Plant and Soil* 299: 55-64.
- Subbarao, G.V., Ito, O., Sahrawat, K.L., Berry, W.L., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., Rao, I.M. 2006. Scope and strategies for regulation of nitrification in agricultural systems—challenges and opportunities. *Critical Reviews in Plant Sciences* 25: 303–335.

Exploration of The Diversity in the Boro Rice Cultivars from the Sundarbans and Adjacent Areas and Determination of the Variability in the Tolerance of Sodium Chloride Salinity within the Varieties

Md Sarwar Jahan

Location: Agrotechnology Discipline, Khulna University, Khulna

Duration: Three years (2014- 2017)

Expenditure of the project: Tk. 2000000.00

Introduction

Salinity is a major constraint on crop production in the coastal areas in Bangladesh where rice is the predominant crop. Although there are extensive studies of salinity effects on rice, our understanding of the quantitative effects of salinity on rice, especially with respect to commonly used cultivars, is still limited. Rice cultivation in the saline soils are restricted due to unavailability of suitable salt tolerant planting materials. Bangladesh Rice Research Institute (BRRI) released a few modern varieties for cultivation in aman season only. BRRI dhan47 (HYV) was recommended for saline soils of Khulna and farmers usually grow local varieties during boro season. Therefore, emphasis must be given to the identification of salinity tolerance in existing local rice cultivars as compared to the few improved varieties. The project was designed to study the effects salinity on germination and seedling growth and on yield attributes of boro rice.. After seed collection, germination test for rice varieties was carried under various levels of salinity. The growth and yield performance of selected rice cultivars under saline conditions were also examined. The outcome of the project will help to build up our scientific knowledge of rice cultivation in coastal areas.

Objectives

- To collect the indigenous boro rice cultivars from the Sundarbans and adjacent areas;
- To study the influence of salinity on germination and seedling growth of boro rice cultivars; and
- To examine the effects of salt concentration on yield of boro rice cultivars.

Methodology

Study 1 - Germination and seedling growth of boro rice varieties under different levels of sodium chloride salinity

Design of experiment: Completely Randomized Design (CRD) with 2 factors and 5 replications.

Factor A: 16 boro rice varieties viz. Chandramoni, Jea, Nayantara, Lataibalam, Tero bele, Nayan moni, Mala, Bapi aus, Kalo sate, Sylhet boro and Koijor.

Factor B: 6 salinity levels viz. 0, 4, 8, 12, 16 and 20 dS m⁻¹.

Data on following parameters was collected:

- i) Germination (%)
- ii) Shoot length (cm) and root length (cm)
- iii) Shoot dry weight (g) and dry weight (g)

Study 2 : Yield performance of boro rice varieties under various levels of salt stress

Eight rice varieties were used. The following parameters were studied:

- i) Plant height
- ii) Number of effective tillers
- iii) Panicle length (cm)
- iv) Grains/hill
- v) Seed weight plant-1(g)
- vi) Straw weight plant-1(g)

Results

Table 1. Effect of NaCl salinity on germination percentage of boro rice varieties

Variety	Germination (%) at different salinity levels (dS m ⁻¹)					
	0	4	8	12	16	20
Chandramoni	100.00	96.67	91.67	68.33	45.00	36.67
Jea	100.00	95.00	91.67	70.00	48.33	36.67
Nayantara	100.00	93.33	86.67	76.67	70.00	48.33
Lataibalam	100.00	90.00	86.67	51.67	45.00	31.67
Tero bele	81.67	70.00	56.67	46.67	41.67	31.67
Nayan moni	75.00	40.00	35.00	26.67	18.33	16.67
Mala	98.33	93.33	91.67	90.00	55.00	41.67
Bapi aus	95.00	90.00	83.33	78.33	58.33	48.33
Kalo sate	71.67	56.67	46.67	33.33	30.00	23.33
Sylhet boro	85.00	65.00	50.00	41.67	35.00	18.33
Koijor	83.33	78.33	65.00	60.00	48.33	38.33
BINADHAN -8	93.33	76.67	66.67	65.00	48.33	38.33
BRRi dhan47	80.00	71.67	61.67	53.33	43.33	30.00
Khatomala	76.67	65.00	53.33	41.67	33.33	28.33
BRRi dhan55	83.33	71.67	61.67	46.67	31.67	26.67
Assam boro	90.00	85.00	80.00	73.33	50.00	45.00

Germination of boro rice varieties varied widely across salinity levels and as salinity level increased seed germination reduced (Table 1). The higher germination percentage was observed in controlled condition while it was below 40% at 20 dS m⁻¹. Varieties Chandramoni, Jea, Nayantara, Lataibalam, Mala, Bapi aus and Assam boro responded well but Kalo sate, Khatomala, Nayan moni and Koijor were found sensitive to salinity. The variability in salt tolerance among rice varieties for germination has been reported by Hakim et al. (2010), Narale et al. (1969) and Reddy and Vaid (1983). The osmotic effect due to salinity is the main inhibitory factor that reduced final germination percentages as indicated by Akbar and Ponnampereuma (1982).

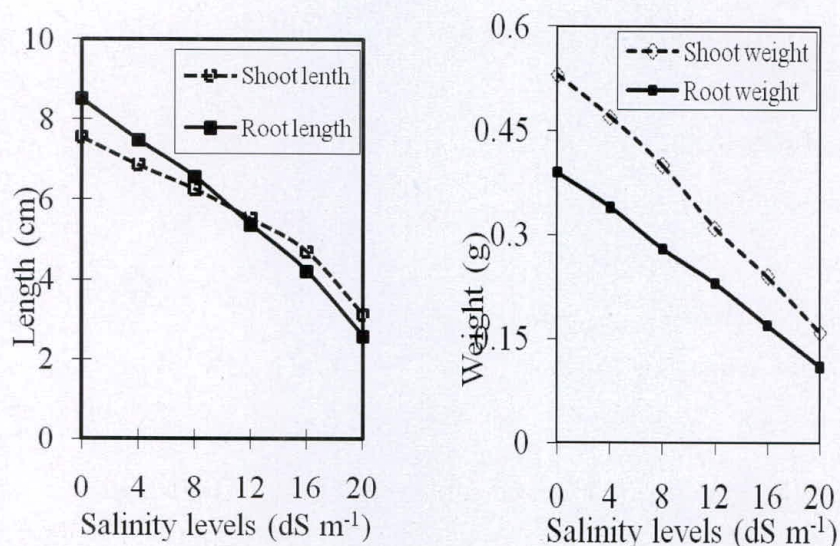


Fig. 1: Effect of NaCl salinity on root and shoot length (left) and on root and shoot weight (right) of boro rice varieties.

Shoot and root length differed greatly due to increase in NaCl salinity. Shoot length ranged from 7.55 cm to 3.13 cm while root length varied from 8.50 to 2.59 cm (Fig.1). Average over cultivars, salinity caused a decrease in shoot length of 42.5% and root length of 30.5% over control. Shoot and root weight also differed due NaCl salinity. Shoot weight was from 0.53 g to 0.16 g while root weight differed from 0.39 to 0.11 g (Fig.1). Accordingly, salinity reduced shoot weight of 30.2% and root weight of 28.2% compared to control. Amin et al. (1996), Jamil and Rha (2007) and Hakim et al. (2010) observed that shoot lengths, root lengths and dry weight were decreased with increasing salt stress.

All the yield contributing characters of local boro rice varieties differed significantly (Table 2) except plant height. Grain weight of rice varieties covered a wide range from 1.15 g to 4.74 g. The maximum grain weight was recorded in Noyanmoni (4.74 g) followed by Kajollota (4.12 g).

The minimum grain weight was recorded in Bareyratna (1.58 g). The highest grain weight in Noyanmoni was attributed due mainly to more number of effective tillers/hill relatively and elevated number of grains/hill (Table 2).

Table 2. Effect of NaCl salinity on yield and yield attributes of boro rice varieties

Variety	Plant height (cm)	Effective tiller/hill	Panicle length (cm)	Grains /hill (no.)	Grain weight (g)	Straw weight (g)
No yanmoni	34.33	16.00 a	6.22 ab	103.00a	4.74 a	22.78b
Abdulhai	25.78	9.22 ab	3.39 bc	61.22bc	2.28 b	21.78b
Kaliboro	22.67	12.44 ab	5.39 ab	48.67bc	2.04 b	15.79d
BRRRI dhan 55	28.89	7.11 b	5.78 ab	29.11bc	1.65b	14.22d
Noyantara	22.44	6.67 b	5.97 ab	46.67bc	2.20 b	15.67 d
Kajollota	28.44	10.22 ab	7.00 ab	99.44b	4.12 ab	17.67 c
Bareyratna	30.22	10.11 ab	7.94 a	29.33bc	1.58 b	19.56 c
BINA dhan -8	38.67	14.44 a	3.00 bc	41.22bc	1.79 b	26.67 a
LS	NS	*	**	**	*	**

LS= Level of significance

Conclusion

- Salinity reduced germination, shoot length and root length as well as shoots weight and root weight in local boro rice varieties.
- Varieties Noyanmoni and Kajollota exhibited superior performance in terms of yield.

Publication from this Research

Sk. R.U. Rashid, U. Paul, M.B. Ahmed and M.S. Jahan. 2015. Present Scenario and Problem Confrontation of Local Boro Rice Cultivation in Two South Western Districts of Bangladesh. *South Asian Journal of Agriculture* 6: 199-208.

References

- Akbar, M. and Ponnampereuma, F.M, 1982. Saline soils of South and Southeast Asia as potential rice land. In: *Rice Research Strategies for the Future*. International Rice Research Institute (IRRI), pp: 265-281.
- Amin, M., Hamid, A, Islam, M.T. and M.A. Karim, M.A,1996. Root and shoot growth of rice cultivars in response to salinity. *Bangladesh Journal of Agronomy*, 6: 41-46.
- Hakim, M.A., Juraimi, A.S., Begum, M., Hanafi, M.M., Ismail M.R. and Selamat, A. 2010. Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, 9: 1911-1918.
- Jamil, M. and Rha, E.S, 2007. Response of transgenic rice at germination and early seedling growth under salt stress. *Pakistan Journal of Biological Sciences*, 10: 4303-4306.
- Narale, R.P., Subramangam T.K. and Mukherjee, R.K,1969. Influence of salinity on germination, vegetative growth, and grain yield of rice (*Oryza sativa* var. Dular). *Agronomy Journal*, 61: 341-344.
- Reddy, P.J. and Vaid, Y, 1983. Note on the salt tolerance of some rice varieties of Andra Pradesh during germination and early seedling growth. *Indian Journal of Agricultural Sciences*, 52: 278-285.

Potential and Prospect of True Potato Seed (TPS) Technology for Sustaining Food Security in Bangladesh

Tuhin Suvra Roy and Parimal Kanti Biswas

Location: Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 2000000.00

Introduction

Shortage of good quality seed is recognized as the most important factor limiting potato production in Bangladesh. The availability of quality planting material in adequate quantities, therefore, is the major issue that needs to be carried for. The national average yield of potato in Bangladesh is 19.03 t/ha, which is lower than that of many other potato growing countries like New Zealand (50.3 t/ha) (FAOSTAT, 2014). Farmer received only 15-20% of certified seed potato.. As a result very year potato farmers have been facing serious Problem due to paucity of quality seed. To overcome this, an alternative technology of True Potato Seed (TPS) (botanical seed of potato produced through artificial pollination between male and female parents) for commercial potato production has shown a great promise for producing both disease-free and cheaper seed and thereby, reducing the cost of cultivation.



Fig. 1: Flowers of potato plant



Fig. 2: Berries of potato plant

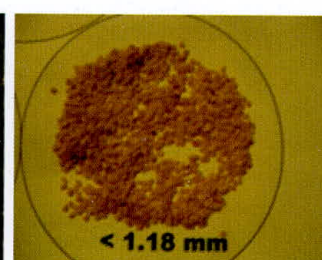


Fig. 3: True potato seed (TPS)



Fig. 4: Seedling tubers of potato

By adopting this technology farmers can produce certified grade potato seed in their own fields. Only 60-100g of TPS or 500- 550 kg seedling tuber is sufficient for planting per hectare instead of using 2.0-2.5t of seed tubers that is required in the conventional system. Thus, TPS is cost effective. The demand of potato in Bangladesh as vegetable along with acceptable processed chips and french fry is increasing day by day. However, the productivity and processing characteristics of potato derived from TPS are largely unknown. This study will compare the productivity and examine the chips and french fry processing attributes of some TPS progenies against the conventional method of potato production.

Objectives

- To find out the optimum level of N, P and K for the production of hybrid TPS;
- To study the effect of plant growth regulators on the quality of TPS; and
- To screen the potato varieties for processing purpose.

Methodology

Experiment # 1	True Seed Production from Potato Mother Plant as Influenced by N, P and K Fertilization
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Methodology

For production of hybrid TPS, parental lines were MF-II (*Solanum tuberosum* L.) as female and TPS-67 (*Solanum andigena* L.) as male, Twenty seven combinations of fertilizers from 3 different levels of N, P and K i.e., N1 = 175 kg ha⁻¹, N2 = 200 kg ha⁻¹, N3 = 225 kg ha⁻¹; P1 = 50 kg ha⁻¹, P2 = 75 kg ha⁻¹, P3 = 100 kg ha⁻¹; K1 = 125 kg ha⁻¹, K2 = 150 kg ha⁻¹ and K3 = 175 kg ha⁻¹ were applied in a three factors split-plot design with 3 replications each. N was assigned to main plot, P to sub plot, K to sub-sub plot.

Production of TPS: Well sprouted whole tubers for both parental lines were planted by maintaining 75 cm line to line and 25 cm plant to plant distance. Being the prevailing climatic condition of Bangladesh is not favored to flower the potato plant, so the natural photoperiod was extended up to 16 hrs with the use of 400 watt high pressure sodium light bulbs placed 8 m above the crop field from 25 DAP up to completion of harvesting of berries. Artificial pollination (9:00-11:00 am) was done.

Extraction of TPS: Well ripened soft berries were crushed mechanically, allowed to ferment for 24 hr at room temperature and then washed under running tap water through a 0.5 mm mesh strainer. The collected seeds were treated with 10% HCl for 20 min with continuous stirring and then washed well with tap water. The seeds were then treated with 0.5% sodium hypochlorite solution for 10 min and finally washed 3 to 4 times with distilled water. The seeds were dried at room temperature until the moisture content was reduced to about 7% and then stored in desiccators for 15 days for further reduction of the moisture content to 4.0-4.5%.

Experiment # 2	Efficacy of Plant Growth Regulators (PGRs) on Potato mother Plant for the production of Hybrid True Potato Seed
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125 kg Murate of Potash (MOP), 100 kg gypsum, 15 kg zinc sulphate, 100 kg magnesium sulphate and 6.5 kg borax per hectare were applied at the time of planting in the furrows made on both sides of the rows. Cowdung and mustard oil cake were applied @10 tons and 500 kg per hectare, respectively, during final land preparation. After 30 DAP, 62.5 kg urea and 62.5 kg MOP per hectare were applied on both sides of the plant rows. After side dressing, the second earthing up was done. The rest 62.5 kg and 62.5 kg urea hectare⁻¹ were applied at 50 DAP just after 3rd earthing up. The procedure of production and extraction of TPS wer done as per our previous study. The collected data were analyzed statistically and means were adjusted by using the Least Significant Difference (LSD) test.

125 kg Murate of Potash (MOP), 100 kg gypsum, 15 kg zinc sulphate, 100 kg magnesium sulphate and 6.5 kg borax per hectare were applied at the time of planting in the furrows made on both sides of the rows. Cowdung and mustard oil cake were applied @10 tons and 500 kg per hectare, respectively, during final land preparation. After 30 DAP, 62.5 kg urea and 62.5 kg MOP per hectare were applied on both sides of the plant rows. After side dressing, the second earthing up was done. The rest 62.5 kg and 62.5 kg urea hectare-1 were applied at 50 DAP just after 3rd earthing up. The procedure of production and extraction of TPS wer done as per our previous study. The collected data were analyzed statistically and means were adjusted by using the Least Significant Difference (LSD) test.

Experiment 3	Screening potato varieties derived from true potato seed for chips and french fry quality for industrial purposes in Bangladesh
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Methodology:

Forty potato varieties (shown in Table 1) were collected from different potato growing areas of Bangladesh Thirty tuber samples from each variety were used to determine dry matter content, specific gravity, color, crispness firmness anthocyanin, antioxidant of chips and french fry. Potato slices for chips were prepared (Lulai and Orr, 1980) and fried in 1900C heated sunflower oil for 3 minutes On the other hand, after being peel and trim, tuber samples of french fries were cut in to 6 mm strips and then fry in 1700C heat sunflower oil for 6 minutes. Weight after frying (WFY) will be recorded for both chips and french fries. Fried samples of chips were evaluated using a colour meter ($L^* a^*b^*$) and crispness were measured by Force gauge

Results

Expt. 1 : The combination of 200 kg N ha 1, 75 kg P ha 1 and 175 kg K ha 1 produced the highest TPS yield but on the basis of 1000-seed weight 225 kg N ha 1, 75 kg P ha 1 and 150 kg K ha 1 produced better quality TPS compared to other combinations.

Table 1: Interaction effect of NPK fertilizer on weight of different size berry, total weight of seeds kg 1 berry and total seed yield of MF-II×TPS-67

Treatment combination	Weight of seeds plant ¹ obtained from berries of different sizes (mg)			Total weight of seeds plant ¹ (mg)	Total seed yield (kg ha ¹)
	<2 cm	2-3 cm	>3 cm		
N ₁ P ₁ K ₁	356.40 q	447.40 q	336.20 o	1140.00 u	99.48 l-n
N ₁ P ₁ K ₂	381.40 o	445.80 q	329.80 p	1157.00 t	98.15 mn
N ₁ P ₁ K ₃	398.00 kl	467.20 m	328.00 p	1193.20 r	96.83 n
N ₁ P ₂ K ₁	351.40 r	442.80 r	341.20 n	1135.40 u	102.20 k-n
N ₁ P ₂ K ₂	374.40 p	445.60 q	347.40m	1167.40 s	103.20 j-n
N ₁ P ₂ K ₃	405.40 i	472.00 kl	362.00 l	1239.40 o	105.50 i-m
N ₁ P ₃ K ₁	384.80 n	453.60 p	387.00 h	1225.40 p	118.20 b-e
N ₁ P ₃ K ₂	400.40 jk	469.80 l	387.60 h	1257.80 n	114.60 c-g
N ₁ P ₃ K ₃	421.80 f	488.20 h	405.60ef	1315.60 h	120.10 a-d
N ₂ P ₁ K ₁	389.40 m	484.20 i	414.40 b	1288.00 jk	104.60 i-m
N ₂ P ₁ K ₂	475.20 a	546.20 a	413.60 b	1435.00 a	109.20 g-k
N ₂ P ₁ K ₃	436.40 d	514.40 d	409.60 d	1360.40 d	105.80 i-m
N ₂ P ₂ K ₁	408.60 h	472.40 k	412.40bc	1293.40 j	120.10 a-d
N ₂ P ₂ K ₂	445.80 c	525.00 c	403.60 f	1374.40 c	124.00 ab
N ₂ P ₂ K ₃	426.40 e	501.60 f	418.00 a	1346.00 e	126.50 a
N ₂ P ₃ K ₁	397.60 kl	465.40 m	412.40 bc	1275.40 l	110.20 f-j
N ₂ P ₃ K ₂	470.60 b	507.60 e	406.00 e	1384.20 b	113.70 d-h
N ₂ P ₃ K ₃	413.00 g	480.80 j	410.60 cd	1304.40 i	111.70 e-i
N ₃ P ₁ K ₁	395.60 l	499.00 g	390.80 g	1285.40 k	111.20 e-i
N ₃ P ₁ K ₂	434.20 d	536.00 b	391.00 g	1361.20 d	105.70 i-m
N ₃ P ₁ K ₃	426.00 e	509.20 e	387.00 h	1322.20 fg	103.00 j-n
N ₃ P ₂ K ₁	373.20 p	456.80 o	383.40 i	1213.40 q	117.60 b-f
N ₃ P ₂ K ₂	420.20 f	502.60 f	405.00 ef	1327.80 f	124.60 ab
N ₃ P ₂ K ₃	400.40 jk	490.40 h	392.20 g	1283.00 k	121.80 a-c
N ₃ P ₃ K ₁	391.60 m	460.00 n	373.40 k	1225.00 p	106.70 h-l
N ₃ P ₃ K ₂	421.20 f	516.60 d	380.00 j	1317.80 gh	110.70 e-j
N ₃ P ₃ K ₃	401.60 j	483.60 i	381.40 ij	1266.60 m	110.20 f-j
Level of Significance	**	**	**	**	**
N×P	**	**	**	**	**
P×K	**	**	**	**	ns
N×K	**	**	**	**	**
LSD _(0.05) for (N×P), (P×K) and (N×K)	8.66	7.35	9.40	9.33	4.44
LSD _(0.05) for interactions	0.16	0.13	0.10	0.10	7.69

N₁ = 175 kg ha¹, N₂ = 200 kg ha¹, N₃ =225 kg ha¹, P₁ = 50 kg ha¹, P₂ = 75 kg ha¹, P₃ = 100 kg ha¹, K₁ = 125 kg ha¹, K₂ = 150 kg ha¹, K₃ = 175 kg ha¹. **: significant at p<0.01 and *: significant at p<0.05. Different lowercase letters beside the mean value indicate significant at p<0.05 or p<0.01

Expt. 2 : The application of GA3 @ 100 ppm in flower buds and inflorescences of female parent (MF II), resulted higher retention of flowers. The application of 200 ppm GA3 in the flower buds and inflorescences of female parent (MF II) demonstrate better performance in respect of quality TPS production.

Table 3: Effect of different doses of plant growth regulators on seed yield attributes of female parent (MF II)

Treatment	Av. yield of seed kg ⁻¹ berry (g)	Total yield of seed plant ⁻¹ (mg)	Total yield of seed plot ⁻¹ (g)	Total yield of seed (kg ha ⁻¹)
T ₁	19.15	1831	79.14	94.69
T ₂	20.02	2017	87.12	103.0
T ₃	22.08	2285	99.17	118.0
T ₄	19.83	1822	78.77	94.31
T ₅	20.16	1923	82.79	98.68
T ₆	20.88	1983	86.80	103.5
T ₇	22.05	2158	94.55	111.9
LSD(0.05)	3.166	340.4	11.96	17.89
CV(%)	8.64	9.55	7.74	9.72

T1 = Control, T2 = GA3-100 ppm, T3 = GA3-200 ppm, T4 = Benzyl Adenine (BA) -100 ppm , T5 = Benzyl Adenine (BA) - 200 ppm, T6 = Planofix (NAA) - 2.5 ppm and T7 = Planofix (NAA) - 5.0 ppm

Expt. 3: Among the varieties, Raja, Lady Rosetta, Dheera, Elgar, Cardinal, Ailsa, Rumba, Omega, Endeavour, Caruso, Forza, Belarossa, Amanda, Ludmila and Tomensa had optimum moisture, dry matter, starch, reducing sugar, non-reducing sugar and total sugar as well as better color score which were desirable for processing. However, this has to be investigated further conducting trials.

Table 1. Dry matter content, starch content, reducing sugars, non reducing sugars and total sugars content of different potato varieties

Variety	Dry matter content (%)	Starch (%)	Reducing sugars (%)	Non reducing sugars (%)	Total sugars (%)
Granola	16.72 pq	10.30 o-q	0.29 ef	0.17 fg	0.46 k-n
Diamant	21.36 g-i	15.40 gh	0.13 h	0.37 cd	0.50 h-k
Raja	21.45 f-i	15.43 gh	0.11 hi	0.37 cd	0.48 k-m
Lady Rosetta	23.07 c-e	17.17 c-e	0.05 ij	0.23 e	0.28 qr
Binella	17.46 o-q	11.17 n-p	0.32 de	0.16 gh	0.48 j-m
Jerla	16.75 pq	10.30 o-q	0.41 c	0.15 g-i	0.56 f-j
Dheera	22.05 fg	16.10 fg	0.06 ij	0.33 d	0.39 m-o
Elgar	20.17 jk	14.03 ij	0.16 h	0.35 cd	0.51 g-k
Agila	17.63 n-p	11.37 m-o	0.34 de	0.44 b	0.78 a
Dura	17.39 o-q	11.03 n-q	0.15 h	0.43 b	0.58 d-h
Sagitta	16.63 pq	10.20 pq	0.52 b	0.12 hi	0.64 c-f

Saikat	19.50 kl	13.33 jk	0.24 fg	0.33 d	0.57 f-i
Patrones	19.12 lm	12.97 kl	0.25 fg	0.36 cd	0.61 d-f
Provento	16.93 pq	10.57 o-q	0.34 de	0.32 d	0.66 c-e
Cardinal	21.55 f-h	15.53 f-h	0.06 ij	0.40 bc	0.46 k-n
Steffi	16.46 q	10.03 q	0.54 b	0.16 gh	0.70 a-c
Felsina	18.74 lm	12.53 kl	0.44 c	0.23 ef	0.67 cd
Laura	16.97 pq	10.60 o-q	0.34 de	0.26 e	0.60 d-f
Ailsa	23.33 b-d	17.50 b-d	0.03 j	0.36 cd	0.39 no
Multa	18.96 lm	12.80 kl	0.34 de	0.36 cd	0.70 a-c
Espirit	14.18 r	7.56 rs	0.54 b	0.22 ef	0.76 ab
Quincy	18.70 lm	12.50 kl	0.35 d	0.25 e	0.60 d-f
Meridian	19.33 k-m	13.20 jk	0.16 h	0.25 e	0.41 l-o
Asterix	18.28 m-o	12.03 l-n	0.15 h	0.36 cd	0.51 g-k
Almerah	13.56 r	6.80 s	0.61 a	0.09 i	0.70 a-c
Cumbica	17.48 o-q	11.17 n-p	0.25 fg	0.36 cd	0.61 d-f
Rumba	21.29 g-i	15.30 gh	0.14 h	0.35 cd	0.49 i-l
Omega	22.45 d-f	16.57 d-f	0.05 ij	0.31 d	0.36 op
Endeavour	20.52 ij	14.50 hi	0.13 h	0.45 b	0.58 d-h
Caruso	24.52 a	18.83 a	0.02 j	0.25 e	0.27 r
Forza	23.53 bc	17.80 bc	0.07 ij	0.36 cd	0.43 k-o
Belarossa	20.59 h-j	14.53 hi	0.14 h	0.44 b	0.58 e-h
Amanda	23.53 bc	17.73 bc	0.06 ij	0.53 a	0.59 d-g
Ludmila	24.60 a	18.93 a	0.02 j	0.33 d	0.35 o-q
Connect	14.41 r	7.83 r	0.44 c	0.13 g-i	0.57 e-i
Svenja	17.67 n-p	11.40 m-o	0.16 h	0.44 b	0.60 d-f
Tomensa	24.53 a	18.86 a	0.03 j	0.26 e	0.29 p-r
BARI TPS-1	18.54 l-n	12.30 k-m	0.34 de	0.35 cd	0.69 bc
Bat Pakri	18.73 lm	12.57 kl	0.22 g	0.35 cd	0.57 f-i
Jam Alu	22.25 e-g	16.33 e-g	0.13 h	0.24 e	0.37 o
SE _{Value}	0.32	0.34	0.01	0.01	0.02
CV (%)	2.85	4.43	14.89%	11.47%	8.84%

In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by DMRT at 0.01 level of probability

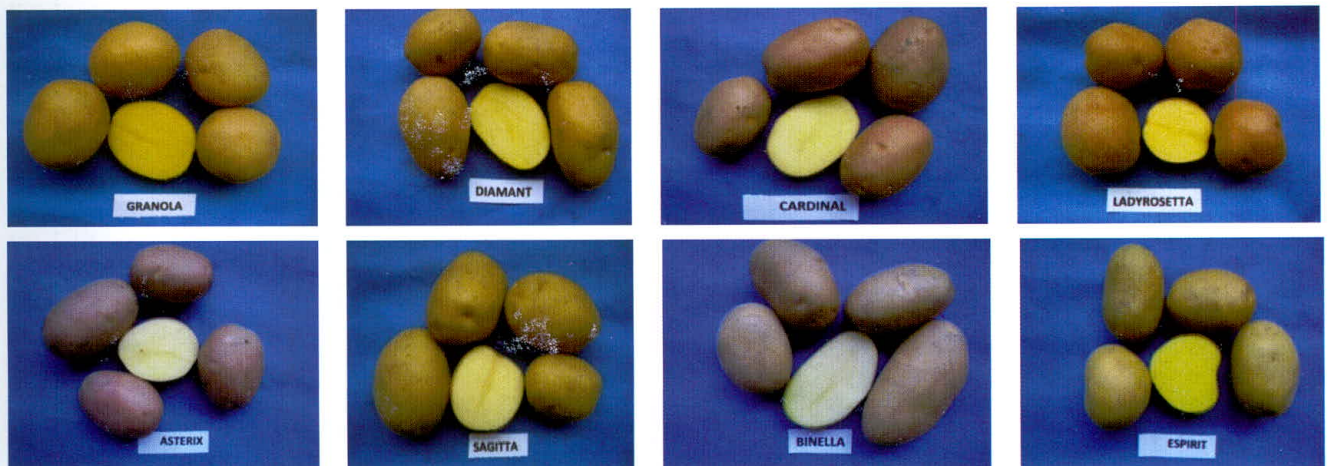


Fig. 5: Promising potato variety for processing industry

Conclusion

- Considering the results of the present experiment, it may be concluded that, quality TPS production is possible through optimum fertilizer management.
- From the point of economic production of quality true potato seed under present study it may be concluded that, by applying the plant growth regulators, it is possible to enhance the flowering and fruit setting in female parent (MF II).
- The potato variety 'Lady Rosetta', 'Ailsa', 'Caruso', 'Forza', 'Amanda', 'Ludmila', and 'Tomensa', had optimum dry matter, starch, reducing sugar, non reducing sugar and total sugar were demonstrated better performance in respect of most of the processing quality studied in this experiment. However, this has to be investigated more by using the other parameters which affect on the processing qualities of potato tubers.

Publications

- Bhattacharjee, A., Roy, T. S., Haque, M. N., Pulok, M. A, I. and Rahman, M. M. 2014. Changes of Sugar and Starch Levels in Ambient Stored Potato Derived from TPS. *International Journal of Scientific Research and Publication*, 4(11): 1-5.
- Rahman, M.M, Roy, T.S., Chowdhury, I.F., Haque, M.N., Afroj, M. and Ahmed, S. 2016. Biochemical composition of different potato varieties for processing industry in Bangladesh. *Agriculture-Science and Practice*, 1/2(97- 98): 81.89.
- Roy, T.S., Haque, M.N., Chakraborty, R., Islam M.N. and Haque. M. A. 2016. True Potato Seed production from potato mother plant as influenced by N, P and K fertilization. *World Journal of Agricultural Sciences*, 12(1): 07-14.
- Roy, T.S., Chakraborty, R., Choudhury, T. E., Islam, M.J. and Quamruzzaman. M. 2015. Efficacy of plant growth regulators (PGRs) on potato mother plant for the production of hybrid true potato seed. *European Academic Research*, 11(12): 15940-15946.
- Chakraborty, R. and Roy, T.S. 2016. Splitting of Nitrogen and Boron: a new dimension for quality TPS. Lambert. *Academic Publisher, Germany*. 1-108pp.
- Rahman, M.M., Roy, T.S. and Mahfuza Afroj, M. 2016. Assessment of potato varieties for processing Industries in Bangladesh. *Lambert Academic Publisher, Germany*. 1-97 pp.

Disaster Risk Analysis Using GIS and Remote Sensing Techniques in South West Coastal Bangladesh

Md Mujibor Rahman and Dilip Kumar Datta

Location: Environmental Science Discipline, Khulna University

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 1000000.00

Introduction

Our country is highly vulnerable to cyclones, floods, droughts, tornados, river erosion and now earthquakes emerging as a hot issue. The risk of disaster is rising continues due to concentration of population low pattern settlement in disaster prone area. Disasters are a significant threat to the live hood of the poor people and also their lives. Its damage extent depends on the socio-economic, status of people, physical location of the area; available assets to cope with disaster etc. disaster risk analysis will help people to reduce the probable damage. It is a complex issue. It varies widely within the community and also changes over time. Vulnerability of poor people can be reduced by analyzing the risk and build an asset base and capacity to respond. Understanding vulnerability of an early stage is essentials to enhances preparedness and reduce disaster risk. Vulnerability of the people can either be due to lack of resources or lack of knowledge or lack of access to both. Access to information, different resources is determined by the rights of people in that country .significant persons of the study area belongs to the profession of low income and depend on the Sundarbans reserve forest and catching fish to the river side. Access to reserve forest is restricted by different forces. It is more severe for marginalized people. So vulnerability analyses will identify the reasons of vulnerability and denial of rights of poor people in that community. It should help to delineate the strategies to cope with disaster. Most of the people living in Sarankhola upazila are poor. Different disaster in this area also increase the poverty of people .it is seen that poor people are poor vulnerable to disaster due to lack of resources to settle down again from disaster damages. Moreover development initiatives in this area is also hindering by different disaster like river erosion, water logging etc. Several studies have been taken in the coastal area of Bangladesh but in the Sarankhola Upazila none of the research has been undertaken. GIS will be used in the study for the representation of the hazard, vulnerability and capacity assessment for disaster risk analysis. Particularly cyclone has been taken under consideration because that area has faced some devastating cyclone and storm surges in very recent decades. The people of study area are also support the cyclone as number one disaster and flood due to high tide and dam collapse as second disaster.

Objectives

- To delineate the nature, extent and characteristics of the common natural disaster of Sarankhola for hazard assessment;
- To determine the socio-economic and spatial vulnerability factors and their intensity over the population of Sarankhola;
- To measuring existing resources and management efficiency for coping with disaster;
- To identify the disaster risk index (DRI) of Sarankhola upazila; and
- To prepare hazard, vulnerability and disaster risk maps of the study area using GIS.

Methodology

Sarankhola Upazila of Bagherhat district is selected for this research because of its geo-spatial characteristics e.g., location and physical characteristics. The study area is situated in the south- western coastal region of Bangladesh which is highly disaster prone in nature. Almost every year natural disasters pay visit to this region. In November 2007, devastating SIDR caused a serious damage for human life and livestock and in may 25,2009 AILA caused a huge devastating in this area and caused dam collapse and flood due to high tide. History reveals that it is the most vulnerable area for the disaster. There is a wide scope of implementation of GIS and RS based approach against the disaster to identify risk index. Questionnaire, participatory risk assessment (PRA) checklist is the prime element for collecting the primary data. These were delineated considering the focal issue of the study and guideline to conduct that. A semi-structured questionnaire is prepared for survey of household. It has been prepared both open and close ended questionnaire to conduct the interview of the household member in the study area. Data is the primary and obligatory element for any analysis absence or inadequacy of data often affects the results of the study. In this study mainly secondary data collection method were applied to gather information. Primary data and information are collected through Questionnaire survey. Disaster Risk indexing is one of the major objectives of this research. Cyclone risk index identifies the cyclone risk of different area and flood risk index identify the flood risk of different area. In this research disaster risk index is prepared in order to identify the vulnerable group whom need easy access to cyclone shelter in a short time for cyclone risk assessment and easy access to unions Parishad office in a short time for flood risk assessment. Disaster risk assessment has done according to available information of different hazards, vulnerabilities and capacities of the local people of cope with disasters over time. Different criteria have been identified according of the views of the community people in order to assess the risk of severe disasters in the study area. There are several tools to measure risk but in this study Participatory Disaster Risk Analysis (PDRA) method is selected. Participatory disaster risk analysis (PDRA) is not a new method new method but a modified version and a coordination of some prevailing method to analyze people's vulnerability and risk. In PDR analysis it is believed that 'Risk' is an outcome of probable magnitude of any particular hazard and its impact upon any population group or other physical or non-physical features after considering the capacity to reduce the impact of the cumulative total of the hazard and the vulnerability. To calculate the disaster risk index in the study area, hazard and vulnerability scores are derived from the factors that are locally applicable and manageable in terms of data collection and time.

The value of hazard threat, vulnerability and capacity factors are categorized under high (4), medium (3), moderate (2) and low (1) and assign their respective score. Again, uniform values of Hazard, vulnerability and capacity factors are summed up at union level for better understanding and mapping. To evaluate risk for disaster each of these variables: hazard, vulnerability and capacity, is assessed by fixing some criteria. Furthermore each of these criteria can be assessed separately to make it more logical special for any participatory assessment which made peoples' opinion more analytical and judge mental. History analysis of disaster (cyclone, flood) identified the time duration of occurrence of different disaster (cyclone, flood). Vulnerability of people is discussed here considering the social and economic status of people. Extent, magnitude, duration of disaster (cyclone, flood) also include in support of vulnerability analysis. Capacity of the stakeholders of this region is also considered in this study to identify the likelihood to cope with disasters physical, organizational and motivational capacities that identified in respect of vulnerability of disaster (cyclone, flood). The risk index identified overall effects and the risk of people for disaster (cyclone, flood). It also finds the worst affected people of the study area and identify what is available at all levels to reduce the risk.

Disaster vulnerability is mainly depends on the socio-economic factor of the local communities as well as their geographic location. These factors are well known as vulnerability factors, which indicate what might be the devastation level if a hazard occurs. Though UN standards suggest a number socio-economic vulnerability factors for risk assessment but limitations of data and lack of sources are constrain to follow the prescribed guidelines. Capacities refer to the qualities that both help communities resist hazards and other shocks and recover from them. It is through an understanding of capacities that we can shift from merely providing goods and services towards supporting and strengthening existing community strategies. Understanding people's capacities and how they cope in times of disaster is a important step to assess the risk of disaster (cyclone, flood). In the next stage, the maps were reproduced for outlining the spatial factors using buffer function identified the river risk zone, accessibility map and administrative centres buffer zone. River risk zone defines three types of areas at fixed distance from the river. Obviously closest areas have higher risk than that of farthest areas. Accessibility map shows the areas having higher accessibility and areas which are less or far from the transportation facilities. Finally union centres buffer zone illustrates the areas close or far from Upazila/Union headquarters. Considering the zone characteristics, the buffers were assigned weight score and reclassified.

Results

This research reveals that the actual situation of coastal region in terms of different disasters facing over time. Vulnerability of this coastal area varies due to diversified socio-economic settings. Perception of the local people of the study area is that they are not at so severe risk due to presence of the Sundarbans, but the Sundarbans itself is at a risk due to change of sweet water flow, and increasing salinity. Moreover illegal intervention on embankment or "Bheri Bundh (BB)" by shrimp farmers of this area augmented the risk of people to floods, water surge etc. Therefore, a sustainable approach to disaster management policy and appropriate mitigation plan are crucial for this area. And it cannot be achieved unless participation of local communities is involved in the course of action from the outset. It is not enough to just involve communities

but it is significant to disaggregate the community and view them as distinct individuals with different rules and responsibilities, capacities and interest. More important, the time has come now to support them in having a voice to change and choice. This study made a little effort to involve the coastal region to disaster analysis directly. "Disaster Risk Assessment (DRA)" will be completed on the basis of their particular information. The theme involve in this research; is preparation of "Disaster Risk Index (DRI)" which mainly provides information about hazard, vulnerability and capacity of the study area and the combination of hazard, vulnerability and capacity identifies the total risk of disaster. By improving or modifying the necessary factors whether it is hazard, vulnerability or capacity, it is easy to reduce the disaster risk of any area. Flood due to high tide and dam collapse and cyclone are two severe disasters which risk have assessed. The study enriched and feed backed by the local people with valuable information. It is essential to take better disaster mitigation and preparedness efforts involving local people according to them mainstreaming disaster management activities with mainstreaming of development issue of is essential to reduce the disaster damage over time. The cyclone shelter facilities improvement, river bank rising, understandable early warning systems etc. are their primary demand to the government and respective organization.

Conclusion

The aim of disaster risk analysis (DRA) is to determine the nature and dimensions of disaster risk within a defined geographical area. A review of the literature indicates that Disaster risk assessment practice lacks a single spatial-analytical framework that is both conceptually holistic and supports diverse methodologies. In the southeast coastal region in particular the development of a regionally applicable method for assessing the risk in the region is particularly warranted and for this reason the area Sarankhola upazila, Bagherhat district was taken as my study area. Disaster risk analysis are made with the objective of measuring hazard assessment for disaster, indentifying the disaster risk index (DRI) and preparing hazard, vulnerability and disaster risk map using GIS. The GIS Adaptive participatory disaster Risk analysis method that is to be developed, tested, validated and implemented consists of three modules: hazard assessment, vulnerability assessment, and capacity assessment and over all disaster risk assessment. In an effort to achieve the aim of adequately and equally studying the human and physical dimensions of disaster risk, a mixed-methods methodology will be used. The risk assessment method will consist of mapping and compiling hazard and vulnerability data onto a GIS platform and weighting and combining each component (layers) into a single representation of risk. In this study, it is reveals that the total disaster risks score of disaster in different unions of Sarankhola Upazilla. It has been identified that Southkhali and Royenda are more risk prone for flood due to high tide and dam collapse and the risk score is as follow 1.43 and1.16 where as Southkhali and Khontakata are more risk prone for cyclone and the risk score is 1.52 and1.31.but overall most disaster risk area is Southkhali union. Then Royenda, Khontakata and less disaster risk area is Dhansagar union. As we know, GIS is a decision making tool that helps to make prudent decision, and is a lesser amount of time consuming, economical and reliable. Then selected criteria were fixed to identify which area is severe in risk, moderate in risk slight in risk and finally recognize the suitable location to save local people from the natural disaster on the basis of the distinctiveness of the study area.

Synthesis and Characterization of Polynuclear Transition Metal Clusters: Precursor for Supported Heterogeneous Catalysts and Viable Hydrogen Storage Medium

Shariff Enamul Kabir, Shishir Ghosh and Md Kamal Hossain

Location: Department of Chemistry, Jahangirnagar University, Dhaka, Bangladesh

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 3000000.00

Introduction

The chemistry of low-valent transition metal clusters has observed a tremendous resurgence from the beginning of 21st Century predominantly due to the explosion of nanotechnology and the realization that large molecular clusters are on the cusp of the nano-domain 1. It also stems from the development of small clusters as nanoparticle precursors that, especially when supported on metal-oxide frameworks, can act as extremely active catalysts for a range of chemical transformations. Coupled with this is the slow but constant development of cluster synthesis and an increased understanding of their often unique physical properties allow them to be incorporated into a range of chemical systems.

Reversible uptake and storage of hydrogen presents a major challenge towards a hydrogen-based economy. Very recently, it has been found that low-valent clusters may have potential as hydride sponge. Although the use of clusters as hydrogen uptake/storage devices is currently primarily of academic interest, the rapid developments made recently suggest that this is an area which could be further exploited and such clusters might also provide useful models for the binding of hydrogen to nanomaterials – an area which is currently much in the news. In view of this, we have decided to synthesize new polynuclear transition metal clusters as precursors for supported heterogeneous catalysts and viable hydrogen storage medium.

Objectives

The specific objective of the study are:

- To develop new high-yielding synthetic routes for the preparation of novel transition metal clusters at ambient temperatures.
- To prepare nanoparticles using the transition metal clusters synthesized at the first phase as precursors by decomposing them into appropriate surface (mainly oxide-based) following suitable techniques.
- To study the physical and chemical properties of both the newly synthesized polynuclear transition metal clusters and the resultant nanoclusters to explore their ability as viable hydrogen storage medium.

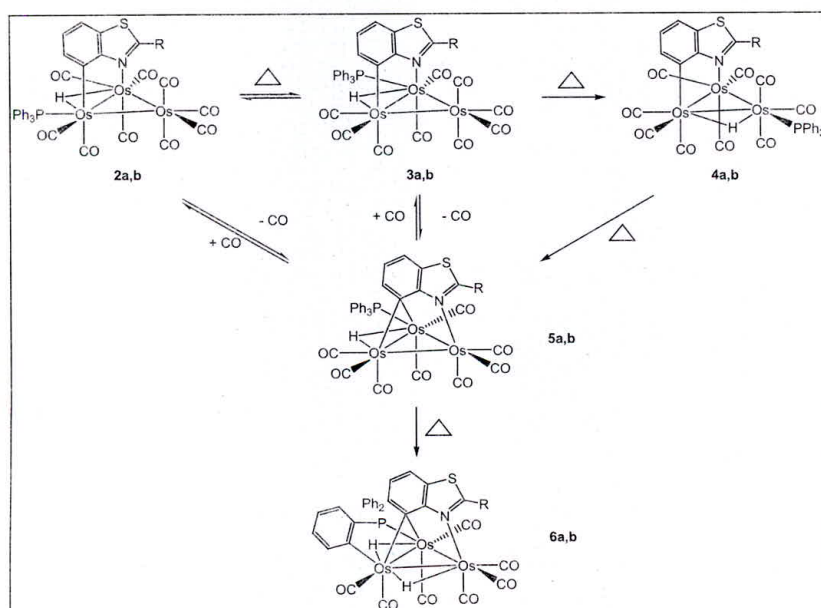
Methodology

The low-valent $M_3(CO)_{12}$ ($M = Fe, Ru, Os$) type clusters are commercially available which will be used as starting point for the synthesis of new precursor cluster molecules for this project. The heavier congeners of $Fe_3(CO)_{12}$ are unreactive at ambient temperatures, thus they will be converted either into labile clusters such as $Os_3(CO)_{10-n}(NCMe)_n$ ($n = 1, 2$) before the desired reactions or their reactions with appropriate substrates will be carried out in the presence of a carbonyl removing reagent e.g., Me_3NO or in the presence of catalysts e.g., $Na[Ph_2CO]$ which removes carbonyl(s) from these clusters at room temperature.

The preparation of cluster nanoparticles using these newly synthesized clusters as precursors will be carried out by decomposing them into appropriate surface (mainly oxide-based) following suitable techniques with the help of our foreign collaborators if necessary. The catalysis will be performed by treating the substrate(s) in the presence of catalysts at ambient temperatures and the process will be monitored by TLC, GC/MS and 1H NMR as necessary. The hydrogen storage capability of these clusters will be tested by treating them with molecular hydrogen to see whether they can form hydrogen rich clusters that can release hydrogen under desired conditions.

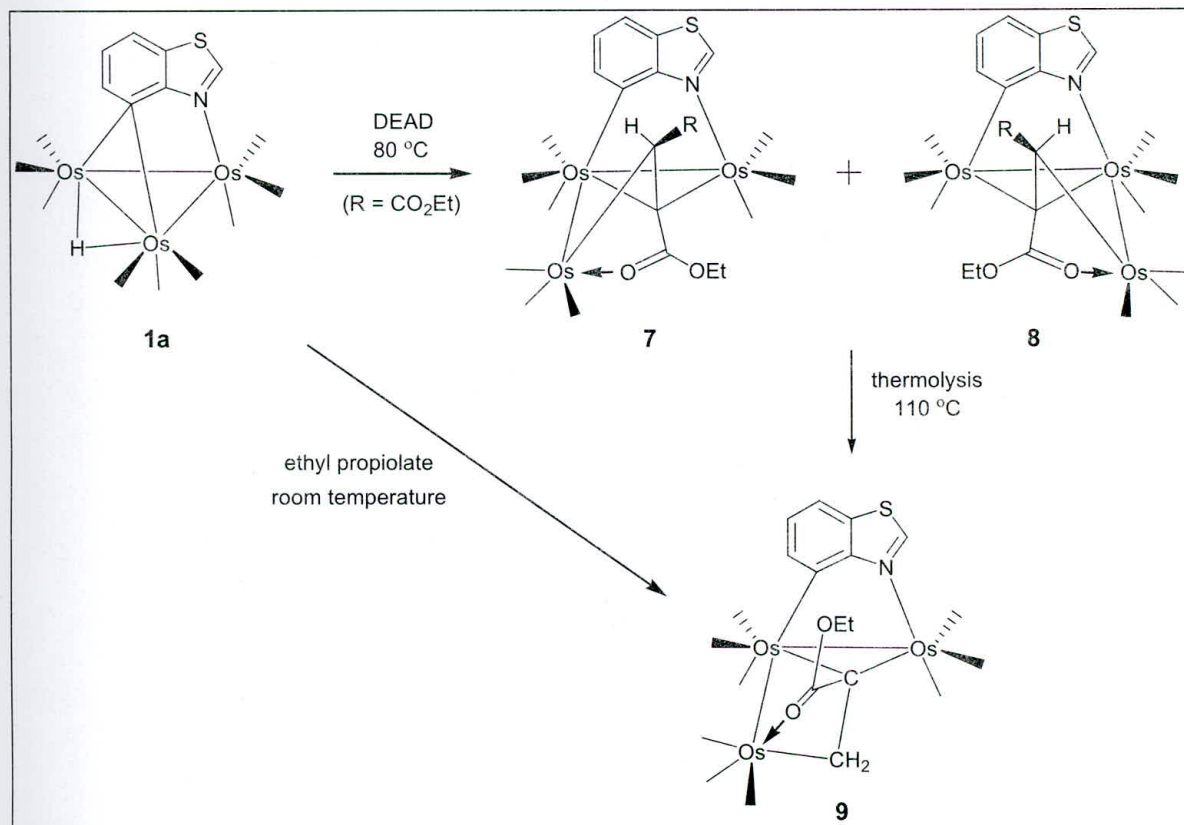
Results

A number of new precursor clusters have been synthesized and appropriately characterized from this project. For example, the reaction of the benzothiazolate-capped triosmium clusters $Os_3(CO)_9(\mu_3-2-C_7H_3NSR)(\mu-H)$ (1a, $R = H$; 1b, $R = Me$) with PPh_3 furnish $Os_3(CO)_9(PPh_3)(\mu_3-2-C_7H_3NSR)(\mu-H)$ (2a, $R = H$; 2b, $R = Me$) in high yields. Subsequent thermolysis reactions of these clusters have also led to the isolation of a series new clusters as shown in Scheme 1.



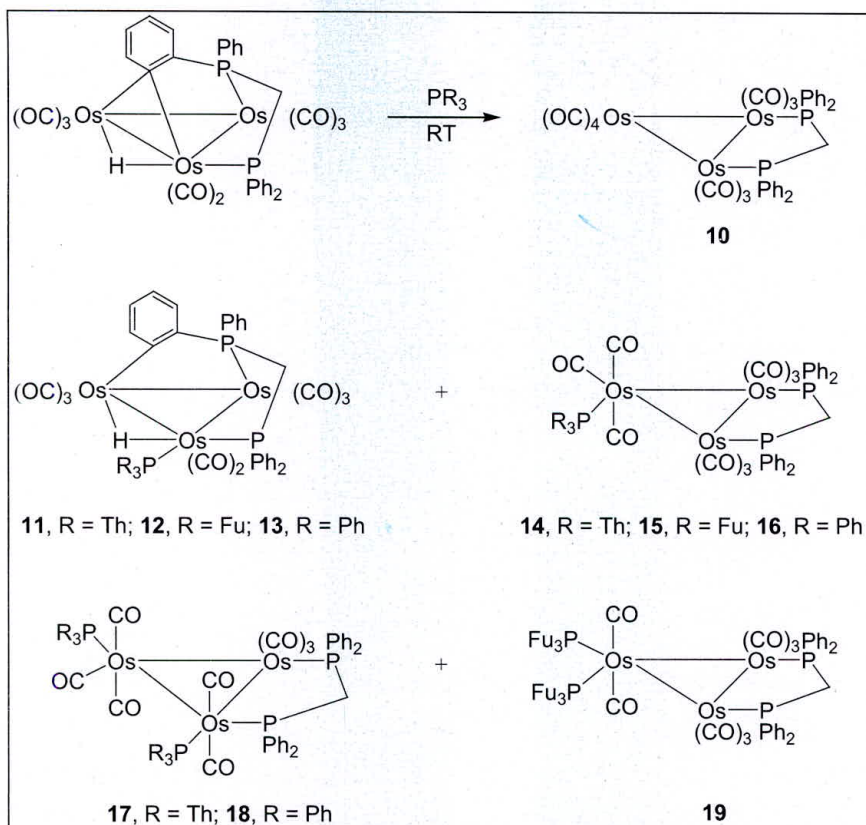
Scheme 1. Thermolysis behavior for clusters 2a,b through 6a,b.

Cluster 1a reacts with DEAD at 67°C to furnish the diastereomeric alkenyl complexes $\text{Os}_3(\text{CO})_9(\mu\text{-C}_7\text{H}_4\text{NS})(\mu\text{3-EtO}_2\text{CCCHCO}_2\text{Et})$ (7 and 8) both of which convert into $\text{Os}_3(\text{CO})_9(\mu\text{-C}_7\text{H}_4\text{NS})(\mu\text{3-EtO}_2\text{CCCH}_2)$ (9) upon heating in refluxing toluene through activation of the alkenyl ligand. Cluster 9 can be independently synthesized from 1a and ethyl propiolate at room temperature as shown in scheme 2.

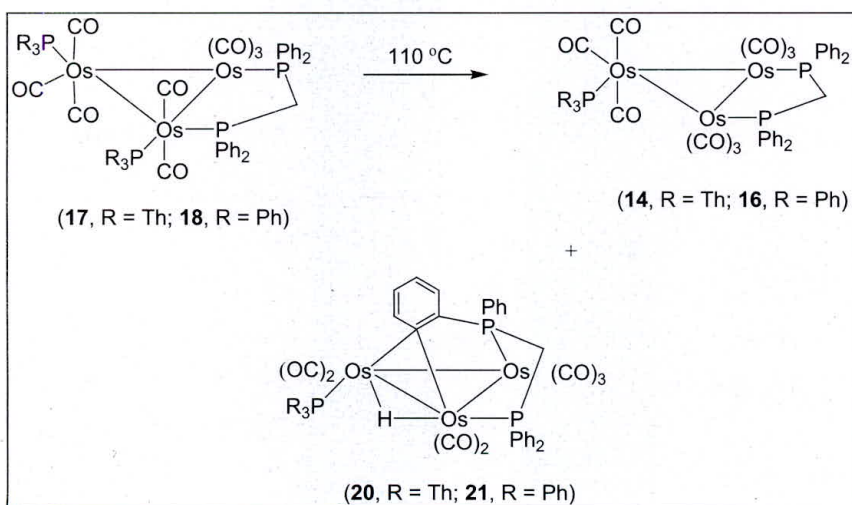


Scheme 2. Reaction of $\text{HOs}_3(\text{CO})_9(\mu\text{3-C}_7\text{H}_4\text{NS})$ (1a) with DEAD and ethyl propiolate.

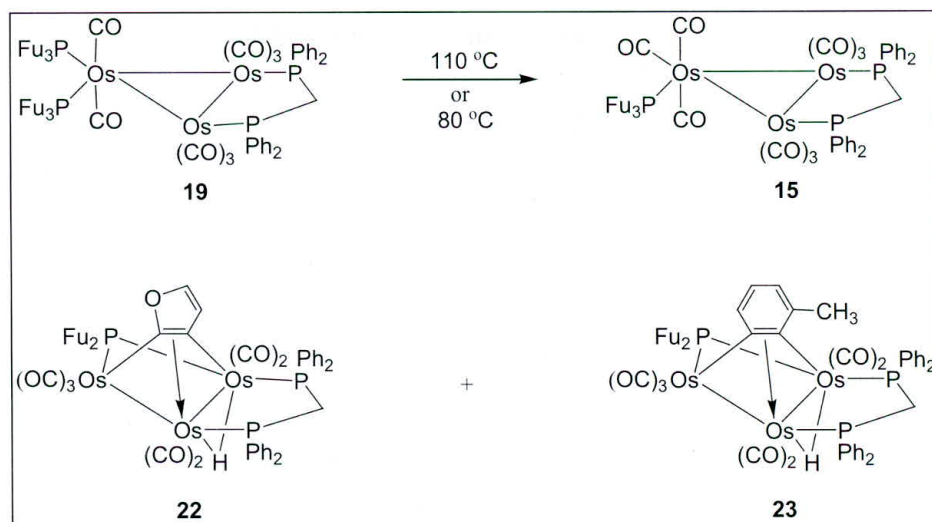
A number of new triosmium clusters have also been synthesized from the reactions of unsaturated $[\text{Os}_3(\text{CO})_8\{\mu\text{3-Ph}_2\text{PCH}_2\text{P(Ph)C}_6\text{H}_4\}\mu\text{-H}]$ with tri(2-thienyl)phosphine (PTh₃), tri(2-furyl)phosphine (PFu₃) and triphenylphosphine (PPh₃) as shown in Schemes 3-5. At room temperature four different complexes were obtained; $[\text{Os}_3(\text{CO})_{10}(\mu\text{-dppm})]$, $[\text{Os}_3(\text{CO})_8(\text{PR}_3)\{\mu\text{3-Ph}_2\text{PCH}_2\text{P(Ph)C}_6\text{H}_4\}\mu\text{-H}]$, $[\text{Os}_3(\text{CO})_9(\text{PR}_3)(\mu\text{-dppm})]$ and $[\text{Os}_3(\text{CO})_8(\text{PR}_3)_2(\mu\text{-dppm})]$ (Scheme 3). Heating $[\text{Os}_3(\text{CO})_8(\text{PR}_3)_2(\mu\text{-dppm})]$ (R = Th, Ph) at 110°C afforded $[\text{Os}_3(\text{CO})_9(\text{PR}_3)(\mu\text{-dppm})]$ and unsaturated $[\text{Os}_3(\text{CO})_7(\text{PR}_3)\{\mu\text{3-Ph}_2\text{PCH}_2\text{P(Ph)C}_6\text{H}_4\}\mu\text{-H}]$, the latter being formed by loss of phosphine and CO with concurrent metalation of a phenyl ring (Scheme 4). Heating $[\text{Os}_3(\text{CO})_8(\text{PFu}_3)_2(\mu\text{-dppm})]$ at 110°C gave $[\text{Os}_3(\text{CO})_9(\text{PFu}_3)(\mu\text{-dppm})]$ and the carbon-phosphorus bond cleavage products $[\text{Os}_3(\text{CO})_7(\mu\text{-PFu}_2)(\mu\text{3-}\eta\text{-2-C}_6\text{H}_3\text{O})(\mu\text{-H})(\mu\text{-dppm})]$ and $[\text{Os}_3(\text{CO})_7(\mu\text{-PFu}_2)(\mu\text{3-}\eta\text{-2-C}_6\text{H}_3\text{CH}_3)(\mu\text{-H})(\mu\text{-dppm})]$ (Scheme 5).



Scheme 3. Reactions of $[Os_3(CO)_8\{\mu_3\text{-Ph}_2\text{PCH}_2\text{P(Ph)C}_6\text{H}_4\}(\mu\text{-H})]$ with PR_3 ($\text{R} = \text{Th, Fu, Ph}$).

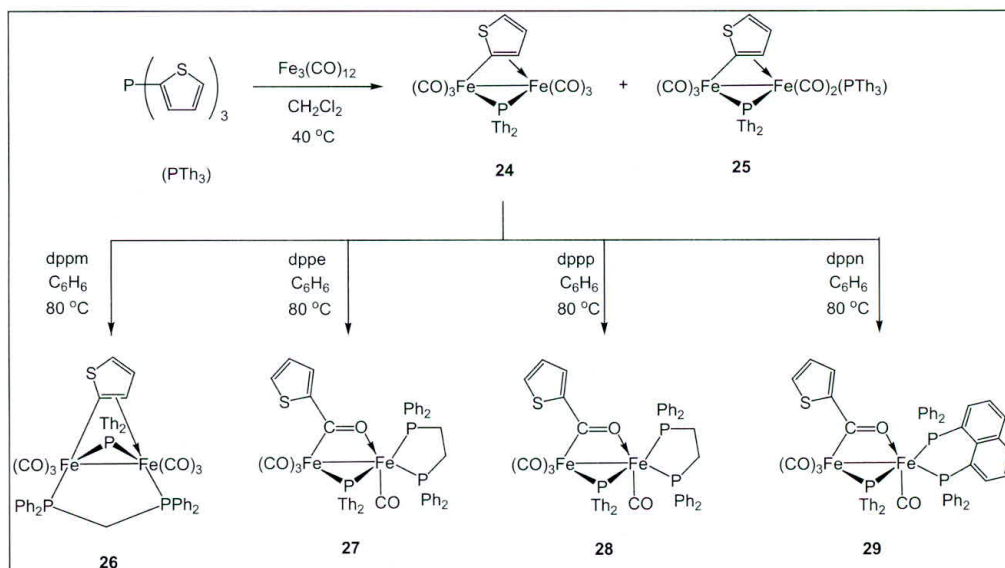


Scheme 4. Thermal stability of $[Os_3(CO)_8(\text{PR}_3)_2(\mu\text{-dppm})]$ ($\text{R} = \text{Th, Ph}$).

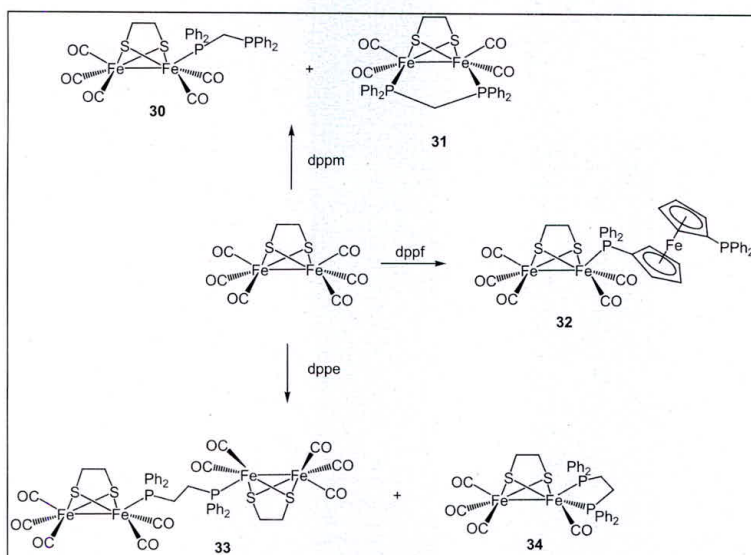


Scheme 5. Thermal stability of $[\text{Os}_3(\text{CO})_8(\text{PFu}_3)_2(\mu\text{-dppm})]$.

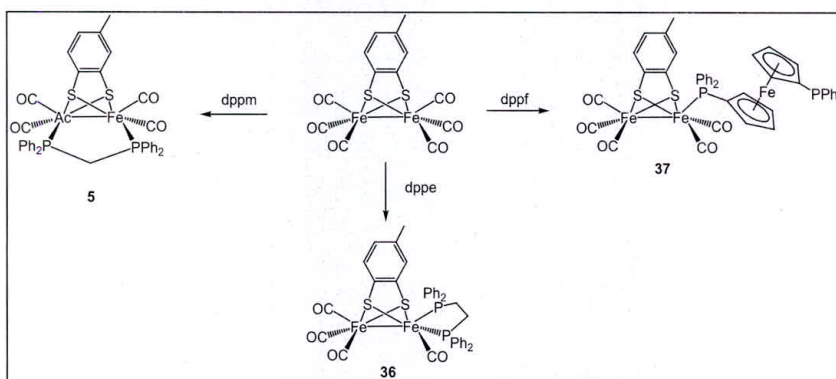
A number of trinuclear clusters containing various mono- and bi-dentate phosphine(s) have also been prepared during this study (please see the list of publications from this research). Besides the synthesis of clusters, we have also prepared a good number of binuclear low-valent transition metal complexes as precursor molecule for nanoclusters during the project as shown in Scheme 6. Several diiron complexes have also been prepared and characterized as electrocatalysts for proton reduction during the study as shown in Schemes 7 and 8.



Scheme 6. Preparation of $\text{Fe}_2(\text{CO})_6(\mu\text{-Th})(\mu\text{-PTh}_2)$ and its reactions with various diphosphines.



Scheme 7. Reactions of $\text{Fe}_2(\text{CO})_6(\mu\text{-edt})$ with various diphosphines.



Scheme 8. Reactions of $\text{Fe}_2(\text{CO})_6(\mu\text{-edt})$ with various diphosphines.

Conclusion

Some of the triosmium clusters synthesized during the study contains hydride ligands. We are testing their reactivity towards hydrogen now for the development of new molecular systems for hydrogen storage. Some of these clusters indeed react with molecular hydrogen at elevated temperatures, but more investigation needs to be done in order to make these molecules a potential medium for the storage of hydrogen which are ongoing in our laboratory.

Publications

Begum, S.A., Hossain, M.K., Ghosh, S., Tocher, D.A., Richmond, M.G. and Kabir, S.E. 2017. Alkyne activation and polyhedral reorganization in benzothiazolate-capped osmium clusters on reaction with diethyl acetylenedicarboxylate (DEAD) and ethyl propiolate. Dalton Transactions, in press (DOI: 10.1039/c7dt02933k).

- Begum, S.A., Chowdhury, M.A.H., Ghosh, S., Tocher, D.A., Richmond, M.G., Rosenberg, E. and Kabir, S.E. 2017. Reactions of the face-capped benzothiazolate- substituted clusters $\text{Os}_3(\text{CO})_9(\mu_3\text{-h}2\text{-C}_7\text{H}_3\text{NSR})(\mu\text{-H})$ ($\text{R} = \text{H}, \text{Me}$) with PPh_3 : Kinetic formation of $\text{Os}_3(\text{CO})_9(\text{PPh}_3)(\mu_3\text{-h}2\text{-C}_7\text{H}_3\text{NSR})(\mu\text{-H})$ and thermally induced ligand isomerisation. *Journal of Organometallic Chemistry*, in press (DOI: 10.1016/j.jorganchem.2016.10.024).
- Miah, A.R., Rajbagnshi, S., Hossain, K., Siddiquee, T.A. and Kabir, S.E. 2015. Synthesis, structure and reactivity of triosmium cluster derived from the reaction of $[\text{Os}_3(\text{CO})_{10}(\mu\text{-dppm})]$ and $[(\mu\text{-H})\text{Os}_3(\text{CO})_8\{\mu_3\text{-Ph}_2\text{PCH}_2\text{P}(\text{Ph})\text{C}_6\text{H}_4\}]$ with tris (4-flourophenyl) phosphine and tris (cyanoethyl) phosphine. *Indian Journal of Chemistry*, 54A: 581-587.
- Miah, A.R., Rajbagnshi, S., Rahaman, A., Hossain, M.K., Siddiquee, T.A. and Kabir, S.E. 2015. New tertiary phosphine derivatives of $\text{Os}_3(\text{CO})_{12}$: X-ray structure of 1,2- $[\text{Os}_3(\text{CO})_{10}\{\text{PhP}(\text{o-Tol})_2\}]$, 1,2,3- $[\text{s}_3(\text{CO})_9\{4\text{-FC}_6\text{H}_4\}_3]$, 1,2,3- $[\text{Os}_3(\text{CO})_9\{\text{PhP}(\text{Cy})_2\}_3]$ and $[\text{Os}_3(\mu\text{-OH})_2(\text{CO})_8\{4\text{-FC}_6\text{H}_4\}_3\text{P}\}_3]$. *Indian Journal of chemistry*, 54A: 161-169.
- Rana, S., Ghosh, S., Hossain, M.K., Rahaman, A., Hogarth, G., Kabir, S.E. 2017. Hydrogenase biomimetics: structural and spectroscopic studies on diphosphine-substituted derivatives of $\text{Fe}_2(\text{CO})_6(\mu\text{-edt})$ (edt = ethanedithiolate) and $\text{Fe}_2(\text{CO})_6(\mu\text{-tdt})$ (tdt = 1,3-toluene dithiolate). *Transition Metal Chemistry*, 41:933-942.
- Raha, A.K., Uddin, M.N., Ghosh, S., Miah, A.R., Richmond, M.G., Tocher, D.A., Nordlander, E., Hogarth G. and Kabir, S.E, 2014. A comparative study of the reactivity of the lightly stabilized cluster $[\text{Os}_3(\text{CO})_8\{\mu_3\text{-Ph}_2\text{PCH}_2\text{P}(\text{Ph})\text{C}_6\text{H}_4(\mu\text{-H})\}]$ towards tri(2-thienyl)-, tri(2-furyl)- and triphenylphosphine. *Journal of Organometallic Chemistry*, 751: 399-411.
- Rahaman, A., Alam, F.R., Hossain, M.K., Magied, A.F.A., Ghosh, S., Tocher, D.A., Haukka, M., Kabir, S.E., Nordlander, E. and Hogarth, G, 2015. Phosphine addition to the σ,π thienyl complex $[\text{Fe}_2(\text{CO})_6(\mu\text{-Th})(\mu\text{-PTh}_2)]$ ($\text{Th}=\text{C}_4\text{H}_3\text{S}$): Carbonyl substitution and migratory carbonyl insertion to give the thienyl-acyl complexes $[\text{Fe}_2(\text{CO})_4(\text{diphosphine})\text{-}(\mu\text{-O}=\text{C-Th})(\mu\text{-PTh}_2)]$. *Inorganica Chimica Acta*, 430:208-215.
- Sarker, J.C., Raha, A.K., Ghosh, S., Hogarth, G., Kabir, S.E. and Richmond, M.G, 2014. Backbone modified small bite-angle diphosphines 2014. Synthesis, structure, and DFT evaluation of the thermal activation products based on $[\text{Os}_3(\text{CO})_{10}\{\mu\text{-Ph}_2\text{PC}(\text{Me})_2\text{PPh}_2\}]$. *Journal of Organometallic Chemistry*, 750: 49-58.
- Uddin, M.M., Rajbangshi, S., Siddiquee, T.A., Ghosh, S. and Kabir, S.E, 2015. Reaction of electron-deficient triosmium cluster $\text{Os}_3(\text{CO})_8\{\mu_3\text{-Ph}_2\text{PCH}(\text{CH}_3)\text{P}(\text{Ph})\text{C}_6\text{H}_4\}(\mu\text{-H})$ with HCl : X-ray structure of two isomers of $\text{Os}_3(\text{CO})_8\{\mu_3\text{-Ph}_2\text{PCH}(\text{CH}_3)\text{PPh}_2\}(\mu\text{-Cl})(\mu\text{-H})$. *Indian Journal of Chemistry*, 54A: 1104-1108.

Establishment of Cost Effective Protocols for Mass Propagation and Conservation of Indigenous Orchids of Bangladesh

Mohammad Musharof Hossain and Mohammad Mizanur Rahman

Location: Department of Botany, University of Chittagong, Chittagong, Bangladesh

Duration: Three years (2014-2017)

Expenditure of the project: Tk. 1500000.00

Introduction

Orchids, the doyens among ornamentals, attributed outstanding royalty in the world floricultural market are one of the most important global cut flower and potted floricultural crop (Hossain *et al.*, 2013a). The global orchid trade exceeded billion dollar and countries of Asia-Pacific regions mainly Thailand, Singapore and Malaysia dominated in the world floriculture market. In 2012, the global orchid trade was estimated US\$504 million. This figure undoubtedly indicates the necessity of production, improvement and conservation of orchids (Cheamuangphan, 2013). Apart from their ornamental value many orchids are enriched with wide range of bioactive phytochemicals (Hossain, 2011). Because of high floricultural & medicinal importance, makes orchids as an important candidate for research. Unfortunately, no extensive programs were taken in Bangladesh for mass propagation and conservation of our indigenous orchids.

Orchids are extremely peculiar group of plants in the plant kingdom for their highly specialized pollination system, smallest and non-endospermic seeds; obligate requirement of mycorrhizal association for natural seed germination, diverse habit, cosmopolitan habitats, extraordinary adaptation mechanism and persistency in adverse environmental conditions (Hossain *et al.*, 2013b). Unfortunately, ruthless collection by increasing orchid lovers, over-exploitation for medicinal purposes, deforestation for urbanization, destruction of habitats by reclamation, shifting cultivation, killing of pollinators and unauthorized trade has led to reduction in natural populations of many orchids. Taking into consideration the present status of orchids, the family Orchidaceae as a whole was included in the CITES Appendix II (Hossain, 2015). Therefore, conservation of orchids is utmost necessary for meet up our future demand.

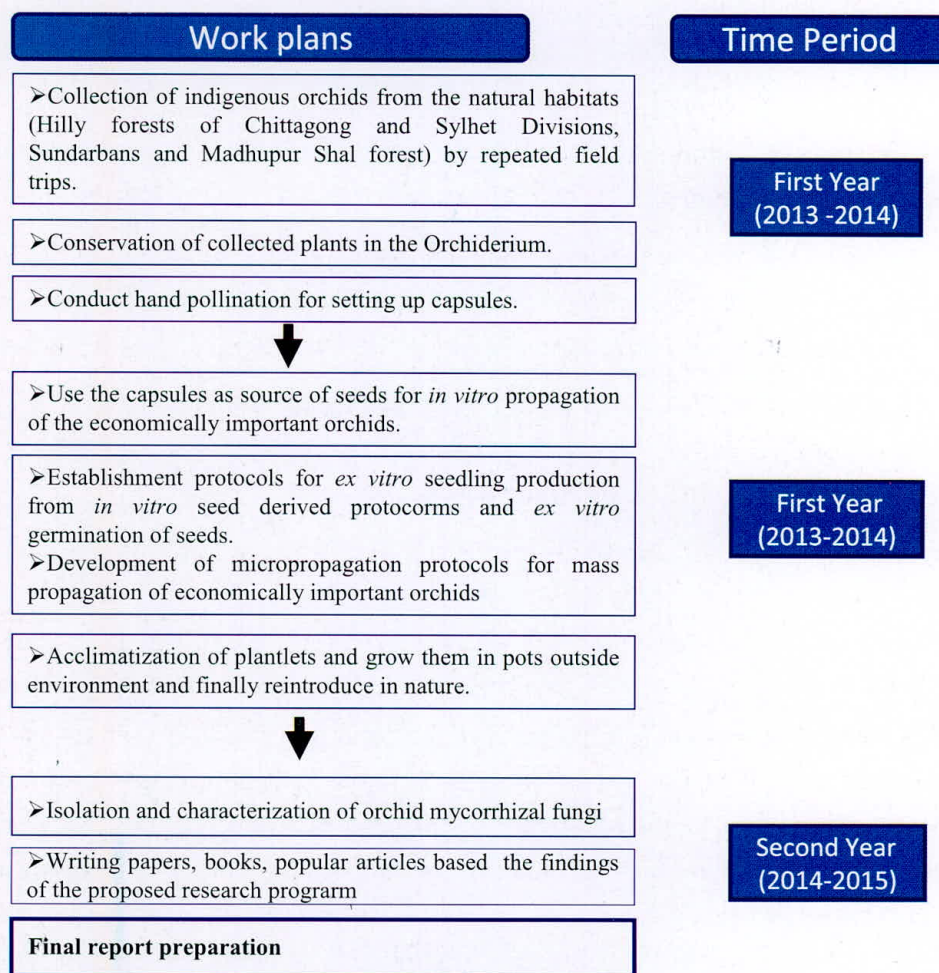
Objectives

The present research project was proposed with following objectives:

- Collection of indigenous orchids from natural habitats and grown in orchiderium for ex situ conservation;
- Development of efficient and cost effective protocols for mass propagation of economically important orchids;

- Production of synthetic/artificial seeds using protocorms or PLBs;
- Characterization of orchid mycorrhizal fungi and use for biological hardening of in vitro raised seedlings; and
- Adjustment of slow growth system for in vitro conservation.

Methodology



Results

- Forty one indigenous orchid species were collected from the natural habitats (Hilly forests of Chittagong and Sylhet Divisions, Sundarbans and Madhupur Shal forest).
- Collected orchid samples were maintained in the Orchidarium of the Chittagong University for their ex situ conservation.
- Successful hand pollination was made in some orchid species for setting up capsules (Fig.1).

- Capsules collected from nature as well as artificial pollinated plants were used as source seeds for in vitro propagation of the economically important indigenous orchids.
- Germination of seeds was successful in nine economically important indigenous orchid species namely, *Aerides odoratum*, *Arundina graminifolia*, *Cymbidium aloifolium*, *Dendrobium aphyllum*, *D. aggregatum*, *Eulophia promensis*, *Geodorum densiflorum*, *Rhynchostylis retusa* and *Spathoglottis plicata* (Fig. 2).
- Ex vitro seedling production from in vitro seed derived protocorms in *Eulophia promensis* and ex vitro seed germination in *Spathoglottis plicata* were noticeable achievement of the project (Fig. 3).
- Characterization of two mycorrhizal fungi namely, *Ceratobasidium* sp. strain RR and *Ceratobasidium* sp. strain AM was done on the basis of their cultural morphology as well as microscopic features (Fig. 4).
- Micropropagation was completed in four orchid species namely, *Cymbidium aloifolium*, *Arundina graminifolia*, *Eulophia promensis* and *Dendrobium aggregatum* and three research papers published and more two papers is under review in reputed peer reviewed journals.
- In vitro grown seedlings of *Cymbidium aloifolium*, *Dendrobium aphyllum*, *Spathoglottis plicata*, *Geodorum densiflorum* and *Eulophia promensis* were reintroduced in nature according their growth habitat for ex situ conservation (Fig.5).
- A research oriented book on orchids entitled “Orchid: Biology and Biotechnology” was published from the project.

Conclusions

The orchid species collected from the different parts of the country and conserved in the *Orchiderium* of the Botany Department of the Chittagong University may be used as a germplasm centre of our indigenous orchids. Protocols developed for in vitro seed germination and micropropagation of our indigenous orchid species may be applied for their commercial seedling production and conservation. A protocol for ex vitro seedling production from in vitro protocorms in *Eulophia promensis* is a new technique for orchid propagation. Besides, the protocol developed for ex vitro seed germination in *Spathoglottis plicata* is to be a reliable technique for hobbyist, amateur growers and Horticulturists. Artificial seeds were also produced in *C. aloifolium* using protocorms. These protocols can be extended to other economically important, rare and endangered orchids for mass propagation and conservation. Characterization of two mycorrhizal fungi from orchid roots is another achievement of the project. A research oriented book entitled “Orchid: Biology and Biotechnology” published from the project which is the first book in Bangladesh devoted exclusively to orchid biology and biotechnology. It is exceptionally informative as it is addressed many aspects of orchid biology and biotechnology including Introduction to orchids, History, Morphology, Biology and Taxonomy of orchids, Application of biotechnology to orchids, Therapeutic uses, Modern breeding, *Orchid mycorrhiza*, Pests and diseases management and Propagation & conservation of orchids.

Special information on present status of indigenous orchids of Bangladesh with some pictorial evidence was also included in this book. It will be a valuable guide for readers and researcher especially interested in orchid biology and biotechnology and will be a signpost for the next level of orchid research.

Publication from this study

No	Title of the Book/ research paper	Name of Journal Vol. & page No.	Publisher's name and address	Year of Publication
1	Orchid: Biology and Biotechnology ISBN: 978 -984-33-9330 -2	Page: I -XIII+264	Protiva Prokash, Dhaka, Bangladesh	2015
2	<i>Ex Vitro</i> seedling development from <i>in vitro</i> rhizome-like bodies in <i>Eulophia promensis</i> Lindl.: A new technique for orchid propagation	Journal of Botany, Article ID 207694, pages 1 -7	Hindawi Publishing Corp., Amsterdam, Netherlands	2014
3	A classroom exercise for propagation of Bamboo orchid - <i>Arundina graminifolia</i> (D. Don) Hochr.	The Journal of the Orchid Society of India 27(1&2):83 -88	The Orchid Society of India, Chandigarh, India	2013
4	<i>In vitro</i> embryo morphogenesis and micropropagation of <i>Dendrobium aggregatum</i> Roxb.	Plant Tissue Culture & Biotechnology, 23(2): 241 -249	BAPTC&B , Dhaka, Bangladesh	2013
5	Dual phase regeneration system for mass propagation of <i>Cymbidium aloifolium</i> (L.) Sw. - an economically important orchid	Indian Journal of Experimental Biology (Under review)	CSIR, New Delhi, India	-
6	Orchid Mycorrhiza: Past, Present and Future	Turkish Journal of Botany (Submitted)	The Scientific and Technological Research Council of Turkey, Ankara, Turkey	-

References

- Cheamuangphan, A., Panmanee, C., Tansuchat, R. 2013. Value Chain Analysis for Orchid Cut Flower Business in Chiang Mai. *Business and Information* pp 712-721.
- Hossain, M. M., Kant, R., Van, P.T., Winarto, B., Zeng, S., Teixeira da Silva, J.A. 2013. The application of biotechnology to orchids. *Critical Reviews in Plant Sciences* 32: 69–139.
- Hossain, M. M. 2011. Therapeutic orchids: Traditional uses and recent advances - *An overview. Fitoterapia* 82:102–140.
- Hossain, M. M., Rahi, P., Gulati, A., Sharma, M. 2013. Improved ex vitro survival of asymbiotically raised seedlings of *Cymbidium* using mycorrhizal fungi isolated from distant orchid taxa. *Scientia Horticulturae* 159:109–112.
- Hossain, M. M. 2015. *Orchid: Biology and Biotechnology*, Protiva Prokash, Dhaka, Bangladesh.

Development of Database on the Fisheries Resources; Emphasis on Food and Feeding Habits, Breeding Biology of the Selected Indigenous Species in the Greater Jessore Area

Md Sherazul Islam, Md Anisur Rahman, Md Mer Mosharraf Hossain and Md Kamrul Islam

Location: Jessore University of Science & Technology, Jessore

Duration: Two years (2014-2016)

Expenditure of the project: Tk. 1500000.00

Introduction

Bangladesh is rich with diverse freshwater fishes and reported 237 inland fishes (Fish Base, 2013). Fisheries sector contributing 63% national animal protein consumption (FAO, 2005). Not so long back, we the people are denoted as “Mache vathe Bangali” means our meal should be with fish. But at present, middle or poor class people have merely chance to take fish in their diet once in a week. Where the fishes gone? The more important matter is that we don't know exactly how many fishes remain in our natural system. We do not put our attention to save our existing resources at all. So, the general objectives of this project were to identify the native freshwater fish species available in the region. The project also covered to find out the breeding technique which is not practicing yet in our country for the culture potentiality of native species.

Objectives

- To identify the available freshwater fish species and their rational availability in the greater Jessore region;
- To notify food and feeding habit of the selective fishes and their breeding biology such as ovarian development, size at maturity, and seasonality; and
- To develop breeding technique of the selective indigenous fishes.

Methodology

- **Study area:** The study was conducted in the greater Jessore area which covers all the districts. Samples were collected from three fish market in each of the districts (Fig. 1).

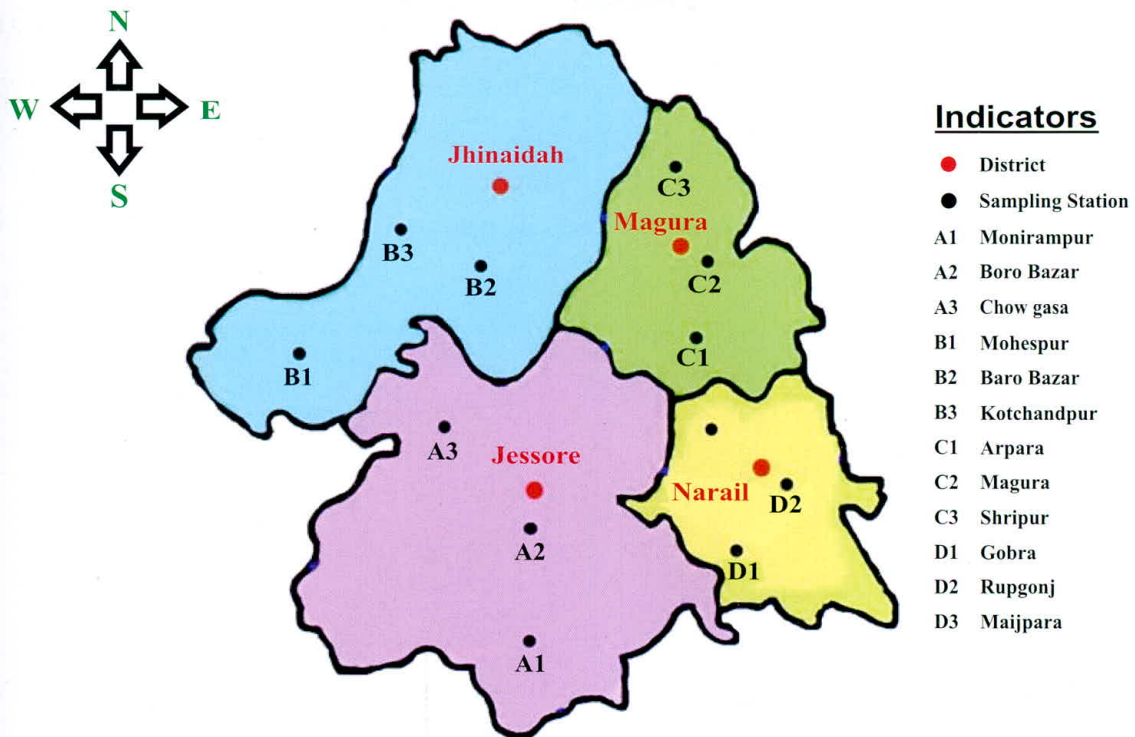


Fig. 1: Map of greater Jessore area with mentioning all districts and sampling stations.

- **Sampling and Identification of the species:** Fishes were collected year round from the greater Jessore region year round. Fishes was identified according to the available books and materials.
- **Estimation of food & Feeding habit:** In every month, gut of the major of the fishes was dissected to know their feeding habit. The samples were categorized into different life stages and their food types were identified.
- **Breeding technique:** Details investigation and trial on the induce breeding technique of the selective species were done to establish hatchery for the native species. For this, four species such as kholisha, bele, guchi and tara baim were selected to induce breeding in the laboratory by different hormones.
- **Development of Database:** All the related data of a specific fish was published as a Handbook. At the same time a web-base database was developed with the collaboration of the Department of Computer Science & Engineering of the same university.

Results

Available fish species: In the greater Jessore area during study period, 88 freshwater fishes and 10 crustaceans have been identified. Among the freshwater fishes, 77 species are indigenous and 11 species are exotic.

Food and feeding habits

Some selected fishes such as guchi baim, tara baim, taki, tengra, puti, bele, mola, khoilsa, kakila and chapila were further investigated to know the food and feeding habit, feeding intensity, and gut status. It was showed that different species taken different food and even the same species have different feeding habit and intensity with accordance to season, sex and maturity. Some of the food and feeding habit mention in the following figures 2 and 3.

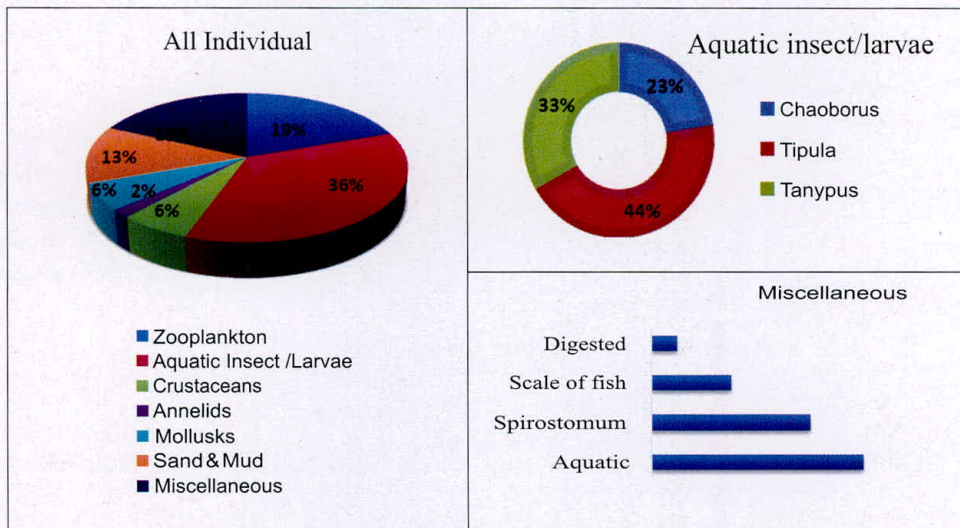


Fig. 2: The different feed and their availability in the gut of Tara baim.

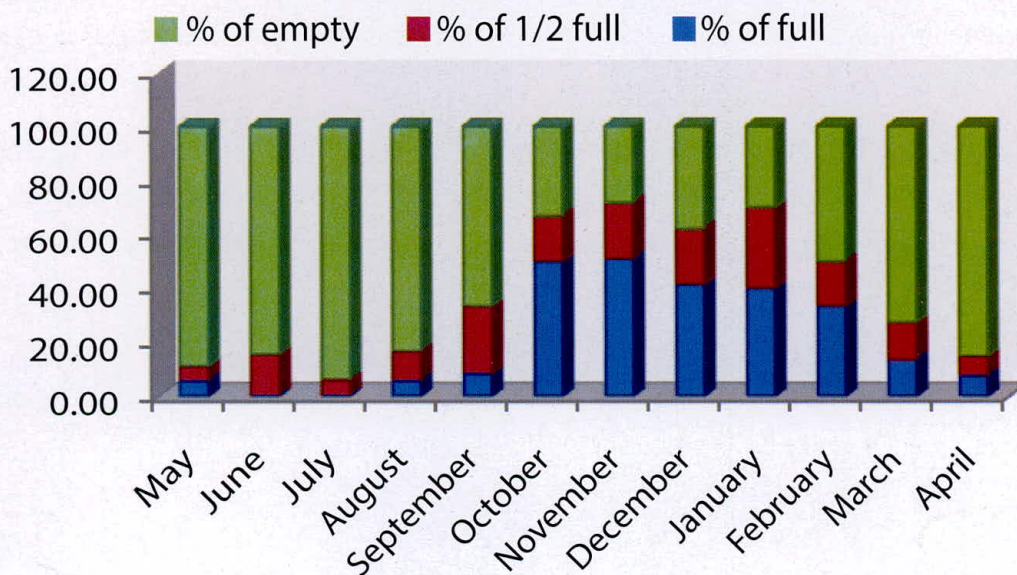


Fig. 3: The gut index of Khoilsa fish in differnt months.

Induce breeding technique

The induced natural breeding was performed on the selected species such as Khoilsa, bele, tara and ghuchi baim. The species breed successfully in the aquarium condition by inducing with different hormones. The embryonic and larval development stages were also observed for the better understanding of the species early life stages. The induce breeding performance, ovarian and larval development stages are mentioned in the following table 2 and figures 4, 5 and 6.

Table-2: The different hormones, doses and the spawning, fertilization and hatching performance of khoilsa with different hormones.

Treatment	Hormone	Fish	Doses		Time interval (hrs)	Spawning (%)	Fertilization (%)	Hatching (%)
			1st	2nd				
T ₁	PG (mg/kg)	Female	4	6	6	100	60	47
		Male	-	4				
T ₂	HCG (IU/kg)	Female	400	800	6	67	75	51
		Male	-	400				
T ₃	Ovaprim (ml/kg)	Female	0.5	1	6	100	46	40
		Male	-	0.5				



Fig. 4: The male-female khoilsa and their deposited eggs at the bubble nest.



Fig. 5: The egg laying mechanism and deposited eggs on the aquarium wall of befe fish.

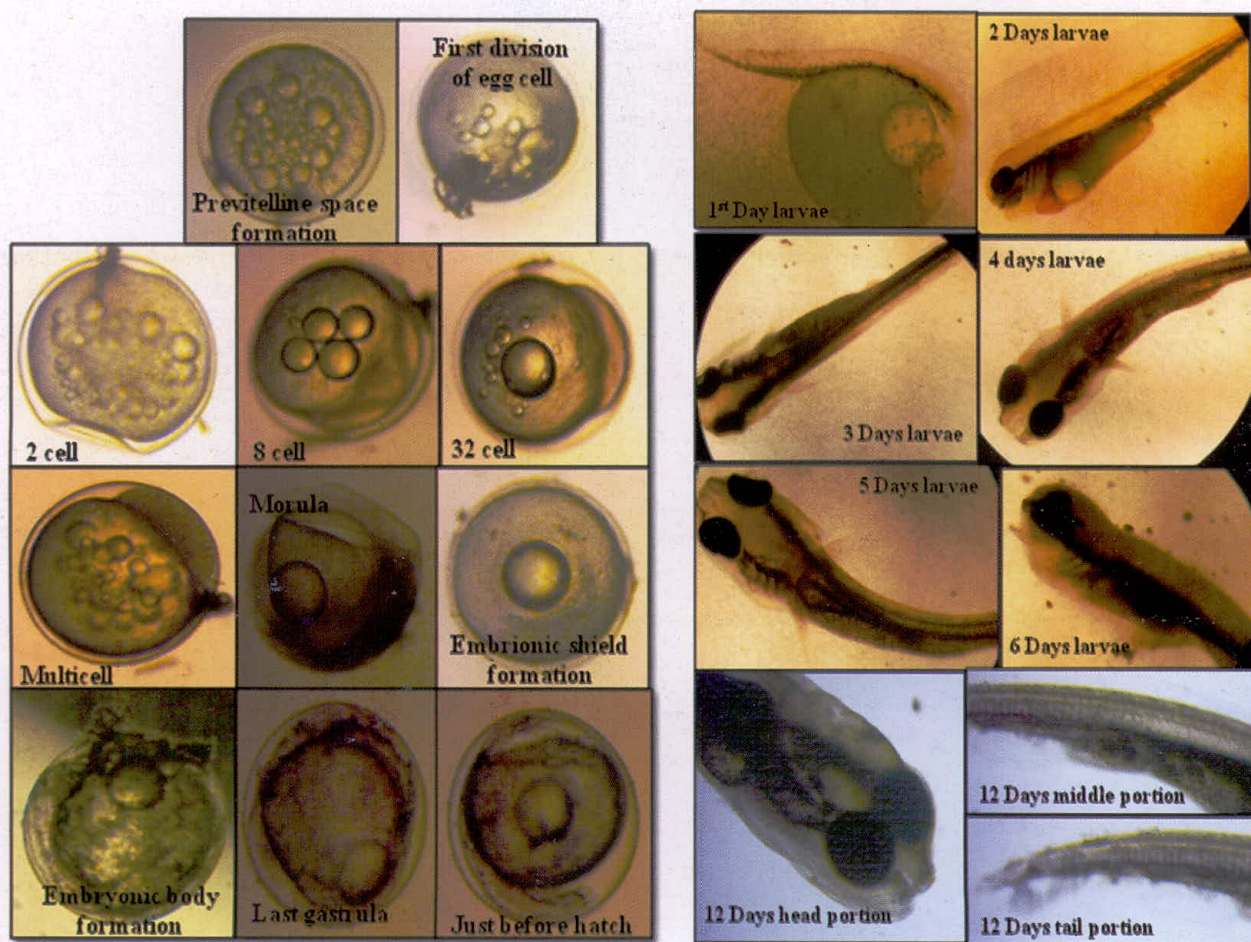


Fig. 6: The embryonic development stages of guchi baim (left) and the larval development of Tara Baim fish (right)

Conclusion

The present work was successfully completed and recorded 88 freshwater fish species in the area. Among them 77 species are indigenous and 11 species are exotic fish species. In addition, 12 crustaceans were recorded in which eight of them were freshwater shrimp. The identified species were published in a Book and web-base database was developed (www.fishinjessore.com). It was noticed that the different species showed different food and feeding habit. Even, the same species showed different feeding habit and feeding intensity regarding their sex, size and maturity of the species. The induced breeding was done successfully of the tara baim, guchi baim, khoilsa and bele fish in the aquarium condition. All the species were breed successfully and their embryonic and larval development stages were recognized and stated. We can say that the indigenous fish could be propagated through inducing with different hormones. However, different fish needed different types of hormones and off course different doses. There were several limitation recorded during the study like parasitic diseases (like Nematode and Tricodina) attract during breeding and larval rearing. Thus further study required to confirmation of the breeding hormones, their applicable doses and better management for better success to produce large number of indigenous fish seed in the locality.

Publications

বৃহত্তর যশোর অঞ্চলের মৎস বৈচিত্র্যঃ খাদ্যাভ্যাস ও প্রজনন পদ্ধতি (FISH BIODIVERSITY IN GREATER JESSORE AREA FOOD HABIT AND INDUCED BREEDING TECHNIQUE. Nutondhra printing press, Dhaka-1205; June 2015.

Islam, M.S., Akter, S., Hasan, M.R., Sheba, S.S. 2016. Some aspects of biology of banded gourami, *Colisa fasciatus* (Bloch and schneider 1801) in Jessore, Bangladesh *Int. J. of Bioscience*, 9 (4): 72-80.

Hasan, M.R., Islam, M.S., Afroze, A., Bahadur, P. and Akhter, S. 2016. Captive breeding of Striped Spiny Eel, *Mastacembelus pancalus* (Hamilton, 1822) considering the various hormonal responses. *Int. J. of Fisheries & Aquatic Studies*, 4 (3): 07-11.

Development of DNA Fingerprinting Yased Patient-Specific Breast Cancer Marker Through Alu Sequence

Gazi Nurun Nahar Sultana and Mizanur Rahman

Location: University of Dhaka, Bnagladesh

Duration: Two years (2014-2016)

Expenditure of the projcet: Tk. 2500000.00

Introduction

Breast cancer is the most common non-cutaneous malignancy among female and ranked number one cancer in Bangladesh (Bergstein *et. al.* 1999). In India, it is the second most common cancer in females (Heim *et. al.* 1985). The etiology of the highest breast cancer in Bangladesh remains unclear, although epidemiologic investigation have identified numerous risk factors in the origin of breast cancer (Taback *et. al.* 2001). Genetic study reveals fact that breast cancer is associated with multiple genetic disorders, including defects in DNA mismatch repair genes, epigenetics, mitochondrial defects (Gazi *et.al.* 2011) and BRC1 and BRC₂ mutations in hereditary breast cancer (Piri *et. al.* 2001). With progression of tumors, early malignant molecular changes converts into large genomic instability reflected as chromosomes lose and gain. Some of these rearrangements of chromosomes occur at higher rate than expected (Grover *et. al.* 2003). Prognosis and survival rates for breast cancer vary greatly depending on the cancer type, stage, grading, treatment, and geographical location of the patient. Survival rates in the Western world are high (85%) (World cancer report, 2008), in developing countries, however, survival rates are much poorer due to lack of early diagnosis method.

'Alu' are the most abundant repetitive elements in the human genome; which altogether represent 10.7% of the whole genome mass (Lander *et.al.* 2001). They occur semi randomly in genome and are located in between or within genes; any increase in 'Alu' amplification, might contribute to further destabilization of human genome and inactivation of tumor suppressors genes that could contribute to the progression of breast cancer (Deininger *et. al.* 1999). Therefore this study attempted to develop a unique genetic marker that would not rely on specific gene expression rather would depend on specific polymorphic changes in genomic DNA by using 'Alu' specific primers.

Objectives

Detection of systemic breast cancer recurrence is limited by lack of universally expressed tumor cell markers. We hypothesized that a test that detects genetic alterations specific to breast cancer cells of an individual patient would provide a superior breast cancer marker for the women of Bangladesh.

Methodology

Sample collection

All approval by the local ethical committee of Bangladesh Medical Research Council (BMRC) and University of Dhaka has been taken. Sixty four breast cancer samples (Stage I to IV) from National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh were included for the study in 2014-2015 (Table1). A healthy cohort of 26 female individuals from mainstream population was also included. Approximately 3 to 5 mL of blood samples were collected in EDTA coated tubes from breast cancer patients and approx 2.3 cm² tissues samples were collected from patients during surgery.

DNA isolation, Alu-PCR and Electrophoresis

Cancer and normal blood DNA was isolated by standard proteinase K treatment followed by phenol/chloroform/ isoamyl alcohol extraction. DNA quantification was performed by taking absorbance at 260 nm and visualized by 0.8% agarose gel electrophoresis (Campbell *et.al*, 2007). PCR amplification of DNA from each of the blood, tumor tissue and normal tissue were performed for each patient. Finally one primer R14B/264 was selected for the results to be concluded. The PCR amplification with following validated thermal condition; Samples were amplified between 400bp and 2000bp until the DNA fingerprint was firmly validated. The resulting DNA fragments were visualized as a series of bands on nondenaturing 1.5% agarose. Genetic changes between patient's normal blood DNA and other samples were identifiable as the presence or absence of a band or by intensity and specificity of bands.

Statistical Analysis: Two tailed non-directional Fisher-Irwin (Fisher's exact test) has been used to verify the Alu change probability difference in cancer and healthy (control) populations (Sheskin, 2007).

Results

When control blood samples compared, twenty four samples of 26 showed no band changes between bloods DNA of control themselves (Fig.1). Two samples C26 and C29 showed polymorphisms (loss of bands) when compared with other control samples in respect to band number and intensity 19% and 28%.

The blood and tissue samples from cancer patients showed less intense or complete deletion of bands at 1700bp and 2000bp compared to control blood with the exception of two samples (Figure 2). The loss of intensities in 92% breast cancer tissues and blood samples was observed which statistically significant ($P < 0.001$) except for two samples (C26, C29). Loss of another band at 870bp is found in approx. 89% breast cancer blood and 86% in cancer tissues samples when compare to control blood samples (Table 2) (Figure 3.) which is also statistically ($P < 0.02$) significant. This 870bp band was present in 92% control blood samples. Again we compared the number of bands in control blood; varies from 9-12 but in cancer samples the numbers of band varies only 7-9 in total and were reliable and reproducible.

Conclusion

There are over 500,000 Alu elements in human genome, which have clearly played a major role in sculpting and damaging the genome. Alu elements have contributed to genetic disease like; breast cancer both by the disruption of genome through the insertion of newly retroposed elements and by deletion/loss of elements between genes (Deininger and Batzer 1999). In this study, genomic DNA from tumor tissue, peripheral normal tissue, and blood from 64 patients were collected during surgery and 26 healthy control samples was analyzed with 'Alu' specific-PCR. The most frequently observed alteration in tumor tissues, cancer blood and peripheral tissue was the loss of fragments. The results show less intense or loss of two 'Alu' specific bands at 1700bp and 2000bp in tumor tissue, normal tissue, and cancer blood samples compare to control blood samples where these two bands are more intense. This may be due to breast cancer shifts the genomic position of 'Alu' sequences (Ana *et.al*, 2009), which results in complete or partial loss of bands. The results of this study showed that breast cancer 'Alu' polymorphism, which is reflection of genomic rearrangement, is distinct from that found in control healthy individuals (Gu *et.al*, 2008). Another band at 870bp was lost in >86% cancer samples but intense 870bp band was observed in all healthy control blood. Multiple Alu polymorphic loci specifically two loci observed less intense in 92% breast cancer patients, compared to control patients are significant and can be useful maker for diagnosis of breast cancer. These specific changes observed in breast cancer patients need further investigation for the characterization of 'Alu' polymorphic loci at 1700 bp and 2000bp and 870bp with substantial number of blood samples.

Table 1. Staging of total 64 breast cancer samples included for the study

Cancer stage	Patient number
T0	0
T1	24
T2	18
T3	12
T4	4
Unknown stage	6

Table 2. Case-control comparison of 'Alu' mediated insertion and deletion

Band size (bp)	Control Blood (%) n = 26	Cancer blood (%) n = 61	Cancer tissues (%) n = 64
2000bp deletion	8% (N = 2)	92% (n = 56)	92% (N = 59)
Low intensity of 1700bp	26% (N = 6)	85% (n = 52)	75% (N = 48)
870bp -deletion	8 % (N=2)	89% (n = 54)	86% (N = 58)

* Out of 64 cancer samples only 61 blood samples were approved for gel check, three bloods DNA (03) Samples were degraded.

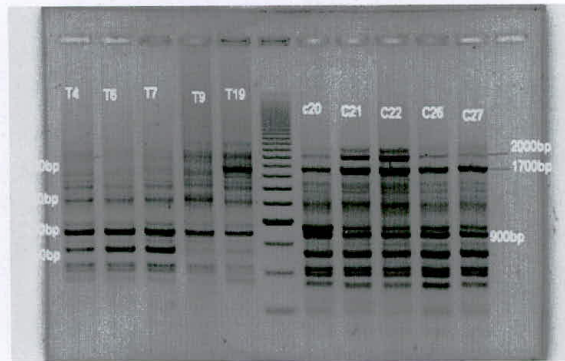


Fig. 1: Shows Alu-PCR profile of cancer tissue (T) and control blood (C) DNA samples. The arrows indicate that bands at 1700bp and 2000bp are present in the control blood samples that are less visible or absent in cancer tissue.



Fig. 2: Shows Loss of 2000bp and 1700bp band in breast cancer (bc) patients compared with Control Female (CF) which is present as less intense. Total band changes and band numbers in control and breast cancer patients are reproducible and specific.



Fig. 3: Electrophoretic profile of all cancer blood (T) samples show less intense or complete loss of band at 1700 base pair and complete loss of band at 2000 base pair.

References

- Fazza, A.C., Sabino, F.C., Newton, N.S., Bordin, N.A., Silva, E.H. and Carareto, C.M.A. 2009. *Genetics and Molecular Biology*, 32(1): 25-31.
- Bergstein, I. 1999. Molecular alterations in breast cancer. In: Bowcock, AM, eds. *Breast Cancer*. Totowa, Humana Press.
- Campbell, I. 2007. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. *Stat. Med.*, 26: 3661-3675.
- Gazi, N.N.S., Rahman, A., Karim, M.M. Shahinuzzaman, A.D.A, Begum, R., Begum, R.A. 2011. Breast cancer risk associated mitochondrial N.A.D.H-dehydrogenase subunit-3 (ND3) polymorphisms (G10398A and T10400C) in Bangladeshi women. *J Med Genet Genomics*; 3(8): 131-135.
- Grover, D., Majumder, P.P., Rao, C.B., Brahmachari, S.K. and Mukerji, M. 2003. Non-random distribution of Alu elements in genes of various functional categories: Insight from analysis of human chromosomes 21 and 22. *Mol. Biol. Evol.*, 20: 1420-1424.
- Heim, S., Mitelman, F., Heim S, Mitelman, F. 1985. Tumors of the breast. *Cancer Cytogenetics*, 2nd ed. New York: *Wiley-Liss*, 369 – 88.
- Piri, L., Welch and King, M.C. 2001. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Human Molecular Genetics*, 10(7): 705-713.
- Sheskin, D.J. 2007. Handbook of parametric and nonparametric statistical procedures Chapman & Hall, Boca Raton, 229 pp.
- Shurjo, K., Sen, Kyudong, H.a.n, Jianixin, Wang, Jungnam Lee, Hui Wang, Pauline A, Callinan, Matthew Dyer, Richard Cordaux, Ping Liang, and Mark A. Batzer. 2006. Human genomic deletion mediated by recombination between Alu elements. *Am J Hum Genet*, 79 (1): 41-53.
- Taback, B., Chan, A., Kuo, C. 2001. Detection of occult metastatic breast cancer cells in blood by a multi molecular marker assay: correlation with clinical stage of disease. *Cancer Res.*, 61: 8845–50.
- Wenli, Gu., Zhang, F., and James, R. Lupski. 2008. Mechanisms for human genomic rearrangement. *Patho Genetics*, 1(4): 1-17.
- International Agency for Research on Cancer. 2008. *World Cancer Report*. 2011-02-26.

p53 Gene Mutation in *H. pylori* Infected Gastric Cancer Patients

Md Mizanur Rahman and Gazi Nurun Nahar Sultana

Location: National Institute of Cancer Research and Hospital, Mohakhali, Dhaka

Duration: Two years 2014 – 2016

Expenditure of the project: Tk. 1800000.00

Introduction

Gastric Cancer (GC) is now leading cause of cancer-related morbidity globally, constituting 9.7% of all cancer-related mortality (Ferro A, *et. al.* 2014). Almost one million new cases of stomach cancer were estimated to have occurred in 2012 (952,000 cases, 6.8% of the total), making it the fifth most common malignancy in the world, after cancers of the lung, breast, colorectum and prostate (Globocan 2012). More than 70% of cases (677,000 cases) occur in developing countries (456,000 in men, 221,000 in women), and half the world total occurs in Eastern Asia (mainly in China). (Globocan 2012). Though Bangladesh is lacking a population based statistics for cancers, there are very few hospital based statistics. According to the reports from the national guideline on gastric cancer management it is ranking as the fifth most common cancer and third most common among the males. From the unpublished data across the country from different medical institutions it has been estimated that GC possesses second position after lung cancer in males (Rahman MM 2104).

Gastric cancer may be caused by environmental or lifestyle risks, host genetic polymorphisms, as well as aging (WCRF 1997). Many laboratory studies have pointed to roles for carcinogenic substances, including amine pyrolysate products and nitrosamines. A probable risk factor for stomach cancer is salt or salty foods, which act synergistically with *Helicobacter pylori* (*H. pylori*) infection in the development of stomach cancer in experimental animals and humans. (Nozaki K 2002). The International Agency for Research on Cancer (IARC 2004) has concluded that gastric cancer is a smoke related malignancy. *H. pylori* is a definite carcinogen, (Uemura N, *et. al.* 2001) which is a precursor lesion for stomach cancer. (Brenner H. *et. al.* 2014), (Tokudome S 2009). Interaction among environmental factors, host genetic polymorphism and bacterial virulence attributes collectively influence the clinical outcome of *H. pylori* infections (Blaser M.J, *et. al.* 2001), (Khaton J 2016).

Numerous cytogenetic and molecular genetic studies concluded a common cancer hypotheses, such as oncogene overexpression, suppressor, mutator, and methylator pathway hypotheses, and mismatched DNA repair, but exact molecular mechanisms of gastric cancer development remain elusive. (Resende, C. 2010), (Panani, A. D *et. al.* 2008).

The TP53 a tumour suppressor gene encodes a main regulator of cell growth and division. Studies showed that mutations in TP53 are present in a range of 40%-70% of early and advanced gastric cancers, and inactivation of TP53 resulting from Loss of heterozygosity (LOH) is found in 60%-70% of intestinal-type gastric cancers, thus making this gene among the most frequently mutated genes in cancers (Hamilton, J. P *et. al.* 2006). However, there are conflicting results regarding the prevalence and of TP53 mutations and its expression and their relationship to clinicopathological features of gastric cancer (Hudlet P *et. al.*). Frequent p53 mutation has been shown in many human cancers; thus, this gene has been associated with carcinogenesis in humans. Gastric mucosal integrity is maintained by apoptosis balanced with cell proliferation. This balance of epithelial cell turnover is altered in the gastric mucosa of patients with gastric cancer. With regard to apoptosis, many studies have shown an increased expression and mutation of p53 in *H. pylori* infected gastric mucosa. Therefore, the alteration of the p53 gene is recognized to play a role in gastric carcinogenesis in subjects infected with *H. pylori*. (Nardone G *et. al.* 1999), (Sawaya M *et. al.* 2008).

In Bangladesh though stomach cancer is a leading cancer and also an important cause for cancer related death we have yet to find both environmental and molecular the important risk factors. Like in other developing countries *H. pylori* infection is also prevalent in Bangladesh. In a recent study on dyspeptic patients 67% of the subjects showed evidences for *H. pylori* infection done in gastric biopsy samples taken by endoscopy(Habib AM *et. al.* 2008).This study was aimed to identify the status of p53 mutation, its association with H pylori infection and other clinicopathological and environmental factors in gastric cancer patients.

Objectives

General: To find out association of P53 gene mutation with H Pylori infection in gastric cancer patients

Specific:

- To investigate the status of p53 mutation in gastric cancer patients
- To investigate the status of H Pylori infection in gastric cancer patients.
- To find correlation of the patient factor, tumour factor and environmental factors like smoking, extra salt intake in the daily diet with gene mutation.

Methodology

This was a cross sectional study carried out from January 2014 to December 2015 in the department of Surgical Oncology, National Institute of Cancer Research and Hospital, Mohakhali, Dhaka, Bangladesh.

Patient selection of cases

After endoscopic diagnosis and histopathologically proven was adenocarcinoma, patient was admitted for surgical intervention. Diagnostic work up was completed by routine haematological tests, tests for general anaesthesia, ultrasonography abdomen and in selected cases CT scan of abdomen. Patients were randomly assigned as every alternative patients were taken into the study when they were finally selected for surgery. Surgeons were unaware of the subjects. Informed consent for surgery and study were taken from each patients. Approval was taken from institutions' ethical committee. For *H. pylori* antibody laboratory facilities were taken from the department of Biochemistry Bangabandhu Sk Mujib Medical University, Dhaka.

For *H. pylori* antibody study started a bit earlier. A control group of healthy patients were selected in the endoscopy room in Dhaka, Khulna and Sylhet matching with age and socioeconomic status of the patients.

Results

In this study, 114 GC patients and 520 control subjects were studied. Mean age of the control patients was 46.01 (± 13.56) years and that of the case patients was 51.11 (± 12.65) years. Patients were significantly less educated than control patients ($p < 0.05$) (Table 1). In case group 99 patients (86.8%) exhibited seropositivity against *H. Pylori* infection while in control group this percentage was 67.5%. This difference of seropositivity was statistically significant (Table 2). Finally tissue samples from 71 patients could be examined for genetic tests. The mean age of the patients was 52.96 with SD 13.26, (Fig 1) younger group was categorized as 40 years or below were 18 in number, male: female ratio was 48:23. Location of the tumour- Tumour staging T1-T3 were. and grading G1 G2 G3. Morphology Ulcerative, proloferative and ulceroproliferative - *H. pylori* antibody analysis show that among the 51 positive cases 41(80%) had mutated p53 gene. Rest 11 mutation was found among the 20 *H. pylori* non infected cases. Chi square test and Fisher's exact test show that *H. pylori* infection has got strong association with the mutation($p = .033$). Analysing the tumour profile like location of the tumour (cardia, midbody and antral), majority(>75%) of the lesion found in antral part(found no significant co relation with mutation), morphology of the tumour was categorized as ulcerative, proliferative and ulceroproliferative- which was the majority didn't have any significant co relation with the mutation, but tumour size (T4) has significant corelation with the mutation ($p = .05$). Similarly lymph node involvement (N1-N3) has got co relation with mutation ($p = .05$)Table 3. Other factors like gender, smoking carried no impact on the mutation status. But smoking had strong corelation with the mutation. Considering the age group, 18 patients were at or below 40 years of age, 17 of them had p53 mutation, it showed very strong association with age ($p = .015$).The logistic regression was performed to ascertain the effects of age, gender Tumour status (T), grading, node status, smoking, and extra salt intake in their daily dietary habits. The model explained 66.0% (Nagelkerke R²) of the variance gene mutation of gastric cancer and correctly classified 87.0% of cases. *H. pylori* infected cases, younger patients, T4 diseases, body tumours have more chances of association with p53 mutation. Table 4,5.

Conclusion

Like that of international studies, in Bangladesh *H. pylori* is also found in more than 80% of gastric cancer cases, again it is prevalent in the community also. To date, existing findings indicate that gastric cancer is the biologic translation of carrying an infectious disease, which is interestingly preventive with anti- *H. pylori* regimen.

On the other hand a substantial number of *H. pylori* infected patients expresses gene alteration which is also influenced by age, tumour stage, grading of the tumour and extra salt intake. Most of the younger patients suffer from gene mutation.

Therefore, as an inevitable consequence, identification of *H. pylori* colonized in people with high risk of gastric cancer is the main direction of the future research. It is postulated that If *H. pylori* can be removed from the population, it has been estimated that approximately 75% of gastric cancer would be eliminated. So it is the high time initiative to take ant *H. pylori* measures which will help to reduce the number of new cases.

References

- Blaser, M. J, Berg, D. E. 2001. Helicobacter pylori genetic diversity and risk of human disease. *J Clin Invest*; 107: 767-773 [PMID: 11285290 DOI: 10.1172/JCI12672]
- Brenner, H. Arndt, V. Stegmaler C. Ziegler, H. Rothenbacher D. 2004. Is Helicobacter pylori infection a necessary condition for noncardia gastric cancer? *Am J Epidemiol*; 159: 252–8.
- Ferro, A., Peleteiro B., Malvezzi, M., Bosetti, C., Bertuccio, P., Levi, F., Negri, E., L. a Vecchia, C., Lunet, N., 2014. World wide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. *Eur J Cancer*; 50: 1330-1344 [PMID: 24650579]
- Habib, A. M., Alam, M.J., Rudra, B., Quader, M.A. and Al-Forkan, M. 2016. Analysis of Helicobacter pylori Prevalence in Chittagong, Bangladesh, Based on PCR and CLO Test. *Microbiology Insights*:9 47–50 doi:10.4137/MBI.S39858.
- Hudler, P., Vogelsang, M. and Komel, R., 2017. Genetic Instability in Gastric Cancer. Available on www.intechopen.com

- IARC. 2004. Monographs on the Evaluation of Carcinogenic Risks to Humans. Tobacco Smoke and Involuntary Smoking, Vol. 83. Lyon: IARC.
- Khatoon, J., Rai, R.P., Prasad, K.N., 2016. Role of *Helicobacter pylori* in gastric cancer: Updates. *World J. Gastrointest Oncol*, 15; 8(2): 147-158
- Nardone, G., Staibano, S., Rocco, A., *et al.* 1999. Effect of *Helicobacter pylori* infection and its eradication on cell proliferation, DNA status, and oncogene expression in patients with chronic gastritis. *Gut*;44:789–799.
- Nozaki, K., Shimizu, N., Inada, K., *et al.* 2002. Synergistic promoting effects of *Helicobacter pylori* infection and high-salt diet on gastric carcinogenesis in Mongolian gerbils. *Jpn. J. Cancer Res*; 93: 1083–9.
- Panani, A., D. 2008. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett*, 266, (2), pp. 99-115, ISSN 0304-3835
- Rahman, M. M. 2014. Gastric Cancer Management. National Guideline of Gastric Cancer.
- Resende, C., Ristimaki, A. & Machado, J. C. 2010. Genetic and epigenetic alteration in gastric carcinogenesis. *Helicobacter*, 15 Suppl 1, pp. 34-39, ISSN 1523-5378.
- Sawaya M, Yoshimura T, Shimoyama, T., Munakata, A., and Fukuda, S. 2008. Difference of p53AIP1 mRNA Expression in Gastric Mucosa Between Patients With Gastric Cancer and Chronic Gastritis Infected With *Helicobacter pylori*. *J Clin Gastroenterol*; 42:351–355.
- Tokudome S, Witjitra D. Samsuria, Soeripto, F. X., Triningsih, E., Suzuki, S., Hosono, A. 2005. *Helicobacter pylori* infection appears essential for stomach carcinogenesis: Observations in Semarang, Indonesia. *Cancer Sci*; 96:873–875.
- Uemura, N., Okamoto, S., Yamamoto, S. *et al.* 2001. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med*; 345: 784–9.
- World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: a Global Perspective. Washington, DC: World Cancer Research Fund/American Institute for Cancer Research, 1997.

Simultaneous Electrochemical Detection and Estimation of Dihydroxybenzenes in Ternary Mixtures at a Poly Amino Acid Modified Electrode

Md Qamrul Ehsan and Md Elius Hossain

Location : Department of Chemistry, University of Dhaka, Dhaka, Bangladesh

Duration : Two years (2014-2016)

Expenditure of the project : Tk. 1900000.00

Introduction

The isomers of dihydroxybenzene catechol (1,2-dihydroxybenzene, CT), hydroquinone (1,4-dihydroxybenzene, HQ) and resorcinol (1,3-dihydroxybenzene, RS) are widely used in medicines, pesticides, cosmetics, tanning, flavoring agents, antioxidants, dyes and photography chemicals^{1,2}. Due to their high toxicity and low degradability in the ecological environment, they are considered as environmental pollutants^{3,4}. Moreover, these isomers are often coexisting in environmental samples^{5, 6} and interfere with each other during their identification⁷. Therefore, it is necessary to develop simple and rapid analytical method for the detection and determination of these isomers in mixture.

Many analytical methods have been established to determine dihydroxy benzene isomers, such as HPLC⁸, fluorescence⁹, chemiluminescence¹⁰, spectrophotometry¹¹, GC-MS¹² and electrochromatography¹³. Among them, electrochemical methods have attracted ever growing attention due to the advantages such as fast response, low cost, simple operation, fast analysis, high sensitivity, and excellent selectivity¹⁴. At ordinary bare electrodes these isomers show highly overlapping voltammograms where their redox peaks are not resolved¹⁵⁻²⁰. Detection and estimations of CT and HQ in binary mixture has been reported in literature²¹⁻²⁴. However, the simultaneous determination of CT, RS and HQ in a ternary mixture by electrochemical methods is insufficient. Recently, though the simultaneous determinations of CT, HQ and RS have been performed by single-wall carbon nanotube (SWCNT)²⁵, modified glassy carbon electrode (GCE), modified multielectrode array²⁶, and graphite doped carbon ionic liquid electrode²⁷, it is still worthwhile to investigate novel electrode material for the simultaneous determination of CT, HQ and RS in ternary mixture. In this article, an effective and practical method for the simultaneous detections and quantitative estimation of CT, HQ and RS using CV and DPV techniques at PGA modified GCE is presented.

Objectives

The objective of the research was to establish a method for the detection and estimation of the isomers of Dihydroxy Benzenes Catechol, Hydroquinone and Resorcinol in binary and ternary mixtures using CV and DPV techniques.

Methodology

The modified electrodes obtained by using different amino acids have significant changes in conductivity as well as selectivity. In the present project, the following methodology was adopted for the simultaneous detection and estimation of dihydroxy benzene isomers:

- (i) Selection of suitable amino acid to prepare a thin layer of poly amino acid on Glassy Carbon Electrode (GCE) by electro polymerization. It was done by using multiple scan of the electrode in a suitable amino acid solution containing supporting electrolyte.
- (ii) The target molecule was then immobilized onto the electrode. The characterization of the immobilized target molecule onto the electrode was done by using SEM, and/or IR spectroscopy.
- (iii) The prepared modified electrode was applied to detect the analytes. The prepared modified electrode monitored the recognition of the compounds.

Electrode modification: The bare glassy carbon electrode was first polished on polishing cloth with 0.3 alumina and then washed with distilled water and sonicated in ethanol. The cleaned and polished GCE was placed in 0.01 M glutamic acid solution in a pH 7.0 phosphate buffer, which was previously purged with high purity nitrogen for 10 minutes. The electrode was treated with 4 cycles of CV between -1.5 to 2.0 V at a scan rate of 100 mV/s. A uniform adherent blue polymeric layer was observed on the electrode surface. The electrode was ready for experiment after a rinse with deionized water. Voltammograms correspond to 1.0 mmol. L⁻¹ of analyte at 50 mV.s⁻¹ scan rate unless otherwise mentioned. By this way, we could design on low cost device with this modified electrode for easy use.

Results

Behavior of dihydroxybenzenes at Bare and modified GCE

At bare GCE in acetate buffer solution (ABS, pH4.5) CT and HQ showed highly overlapping character where their anodic and cathodic peaks merged together forming an overall voltammogram in a binary mixture (Fig 1a). This impeded the simultaneous detection using CV at bare GCE. On the other hand at PGA modified GCE, both CT and HQ showed highly reversible behavior (i_{pa}/i_{pc} close to unity) in CV with dramatic signal enhancement compared to that of at bare GCE (Fig 1b and 1c).

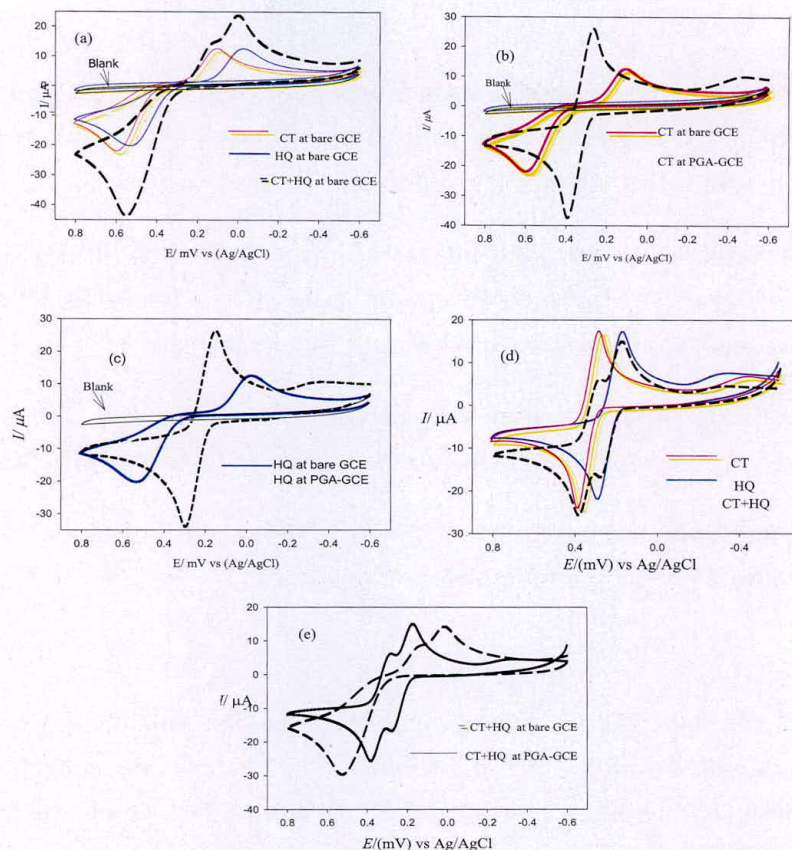


Fig. 1: CVs of (a)CT and HQ (solid lines), CT-HQ binary mixture (dashed line) at bare GCE (b) CT at bare GCE (solid line)and at PGA-GCE (dashed line) (c) HQ at bare GCE (solid line) and at PGA-GCE (dashed line) (d) overlay of individual CT and HQ (solid lines) and CT-HQ binary mixture (dashed line) at PGA-GCE (e) comparison between simultaneous CT and HQ at bare GCE (dashed) and at PGA-GCE(solid line) in ABS pH 4.5.

Fig 1d shows the CV of CT-HQ binary mixture at PGA-GCE overlaid with that of individual CT and HQ. It is clear that both CT and HQ retained their corresponding redox peaks in the mixture. A comparison between the CV responses of binary mixture of CT and HQ at bare and PGA modified GCE is depicted in Fig 1e. Similar behavior was observed in phosphate buffer solution (PBS, pH 7.0).

Electrochemical behavior of dihydroxybenzenes in binary mixtures at the modified GCE

A series of CV and DPV experiments were performed on binary mixtures of CT, HQ and RS in PBS pH 7.0 for their simultaneous detection in presence of another.

Detection of CT and HQ in mixture in PBS

At PGA modified GCE, CT and HQ showed well separated anodic peaks at +201mV and +94 mV and two cathodic peaks at +133 mV and +19 mV respectively. An overlay of CVs of individual CT and HQ with that of CT-HQ binary mixture (Fig 2a) shows that both CT and HQ retains their corresponding redox peaks in binary mixture at the modified GCE.

A DPV of the CT-HQ binary mixture in PBS is shown on Fig 2b, which clearly shows that CT and HQ responses with well separated anodic peaks while retaining their individual peak positions (at +296 mV and +193 mV respectively) at the modified GCE. The peak separation is measured to be 103 mV which is quiet good for the simultaneous detection of these isomers in the presence of another.

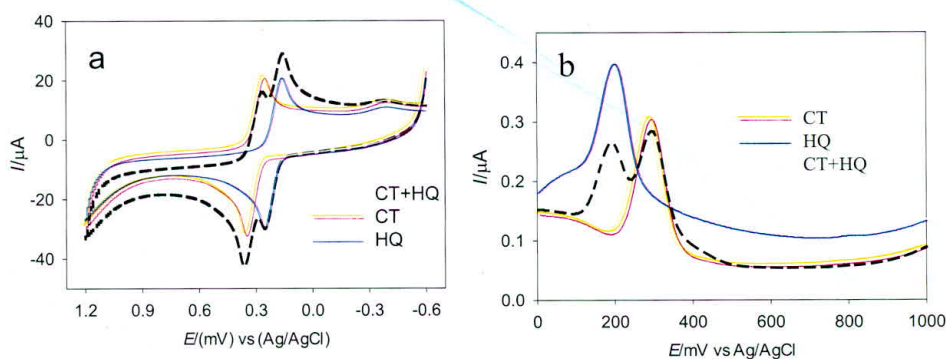


Fig. 2: Overlay of (a) CV and (b) DPV responses of individual (solid lines) and binary mixture (dashed line) of CT and HQ in PBS at PGA modified GCE.

Detection of CT and RS in mixture in PBS

Similar experiments were done on CT-RS binary mixture. In CV, CT and RS gave responses with very large peak separation which was retained in the binary mixture (Fig 3a). In DPV, CT and RS showed oxidation peaks (at +293mV and +702 mV) separated by 409 mV which is well suited for their simultaneous detection at PGA modified GCE (Fig 2b).

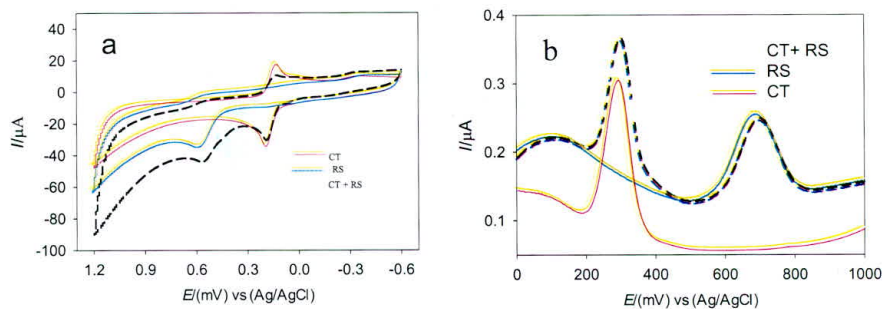


Fig. 3: Overlay of (a) CV and (b) DPV responses of individual (solid lines) and mixture (dashed line) of CT and RS in PBS at PGA modified GCE.

Detection of HQ and RS in mixture in PBS

Same experiments were performed on HQ-RS binary mixture which exhibited even better peak separation both in CV and DPV depicted in Fig 4a and Fig 4b respectively. In DPV the binary mixture shows two anodic peaks at +202 mV and +701 mV respectively resulting into peak separation of 499 mV.

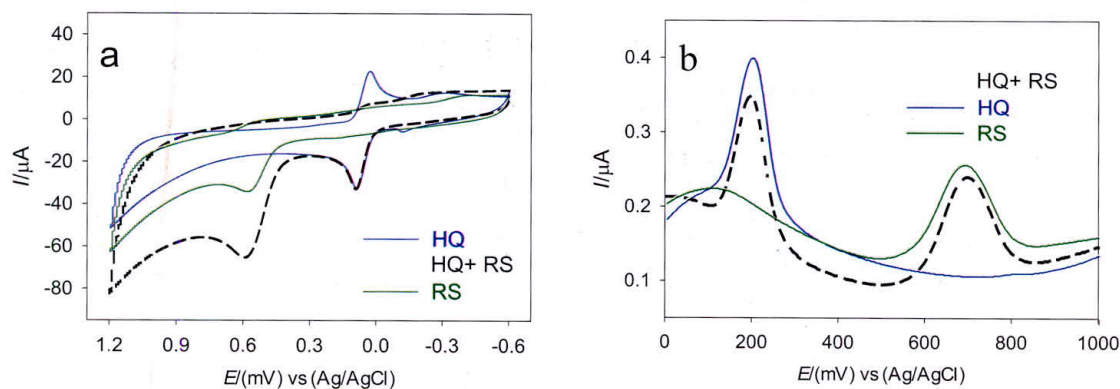


Fig. 4: Overlay of (a) CV and (b) DPV responses of individual (solid lines) and mixture (dashed line) of HQ and RS in PBS at PGA modified GCE.

Detection of CT, HQ and RS in ternary mixture

CV and DPV experiments were performed in order to detect CT, HQ and RS simultaneously from ternary mixtures in both acetate buffer (ABS) and phosphate buffer solution (PBS) at PGA modified GCE.

Detection of CT, HQ and RS in ternary mixture in PBS

Cyclic voltammograms of individual CT, HQ and RS and that of the mixture in PBS at PGA-GCE are overlaid on Fig 5a. Three anodic peaks at +94 mV, +201 mV and +605 mV and two cathodic peaks at +134 mV and +19 mV were observed. These peaks correspond to the anodic and cathodic peaks of CT, HQ and RS.

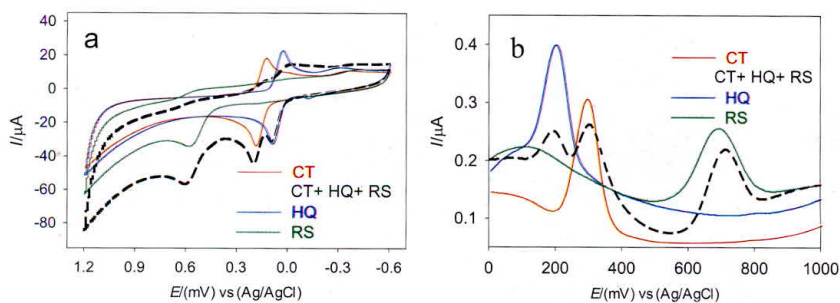


Fig. 5: Overlay of (a) CV and (b) DPV responses of individual (solid lines) and ternary mixture (dashed line) of CT, HQ and RS in PBS at PGA modified GCE.

The DPV study of CT, HQ and RS as individual isomer and as amixture are shown in Fig 5b, which shows that at PGA modified GCE, CT, HQ and RS retained their corresponding anodic peaks at +295 mV, + 202 mV and +715 mV respectively in ternary mixture.

Detection of CT, HQ and RS internary mixture in ABS

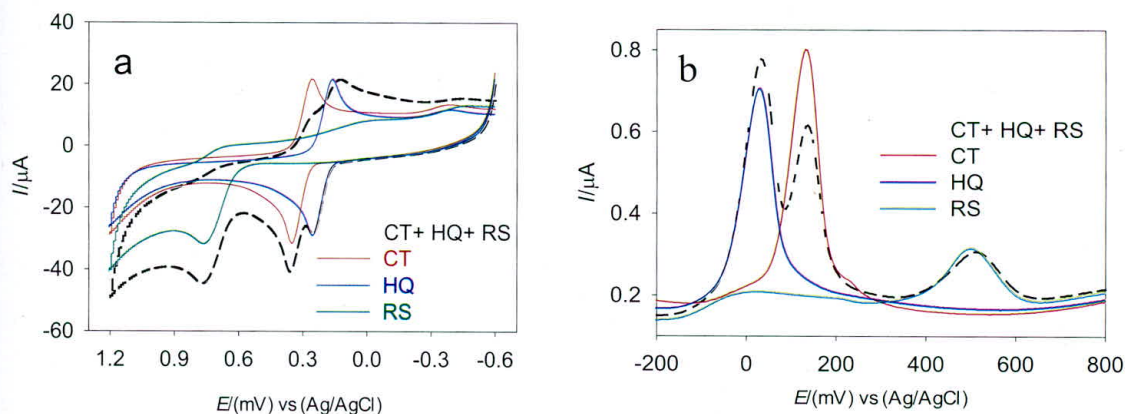


Fig. 6: (a) CVs of individual (solid lines) and ternary mixture (dashed line) of CT, HQ and RS (b) DPV responses of individual (solid lines) and ternary mixture (dashed line) of CT, HQ and RS in ABS at PGA modified GCE.

The CVs of ternary mixture and individual CT, HQ and RS in acetate buffer solution at PGA modified GCE were taken and are shown in Fig 6a. Three anodic peaks at +247 mV, +354 mV and +769 mV for the three isomers and two cathodic peaks at +224 mV and +135 mV for CT and HQ respectively were found.

Fig 6b shows the overlay of DPV responses of individual CT, HQ and RS (at +138 mV, + 33.6 mV and +513 mV respectively) and that of in ternary mixture in ABS.

Quantitative Estimation

Quantitative estimation of CT in the presence of HQ

DPV was performed on the binary mixture of catechol and hydroquinone at PGA modified GCE within the potential range of +100 mV to +400 mV. Catechol was added successively by a volume of 10.0 μL using a micro pipette. Concentration of hydroquinone was kept constant. The resulting DPVs are shown in Fig 7a.

A calibration curve (Fig 7 b) was drawn for different concentrations of catechol. This calibration curve can be used to quantify catechol (CT) in the presence of hydroquinone (HQ) in a binary mixture. Detection limit of catechol in presence of hydroquinone was found in micromolar range.

Quantitative estimation of HQ in the presence of CT and RS

DPV was performed on the binary mixture of catechol and hydroquinone at PGA modified GCE within the potential range of +100 mV to +400 mV. Catechol was added successively by a volume of 10.0 μL using a micro pipette. Concentration of hydroquinone was kept constant. The resulting DPVs are shown in Fig 7a.

A calibration curve (Fig 7 b) was drawn for different concentrations of catechol. This calibration curve can be used to quantify catechol (CT) in the presence of hydroquinone (HQ) in a binary mixture. Detection limit of catechol in presence of hydroquinone was found in micromolar range.

Quantitative estimation of HQ in the presence of CT and RS

DPV was performed on a ternary mixture of CT and HQ and RS at PGA modified GCE in the potential range of -200 mV to +800 mV. HQ was added successively by a volume of 10.0 μL using a micro pipette. Concentration of CT and RS were kept constant. The resulting DPVs are shown in Fig 8a. A calibration curve (Fig 8b) was drawn for different concentrations of HQ which can be used for its quantitative estimation in the presence of CT and RS. Detection limit of HQ in presence of other isomers was found in micromolar range.

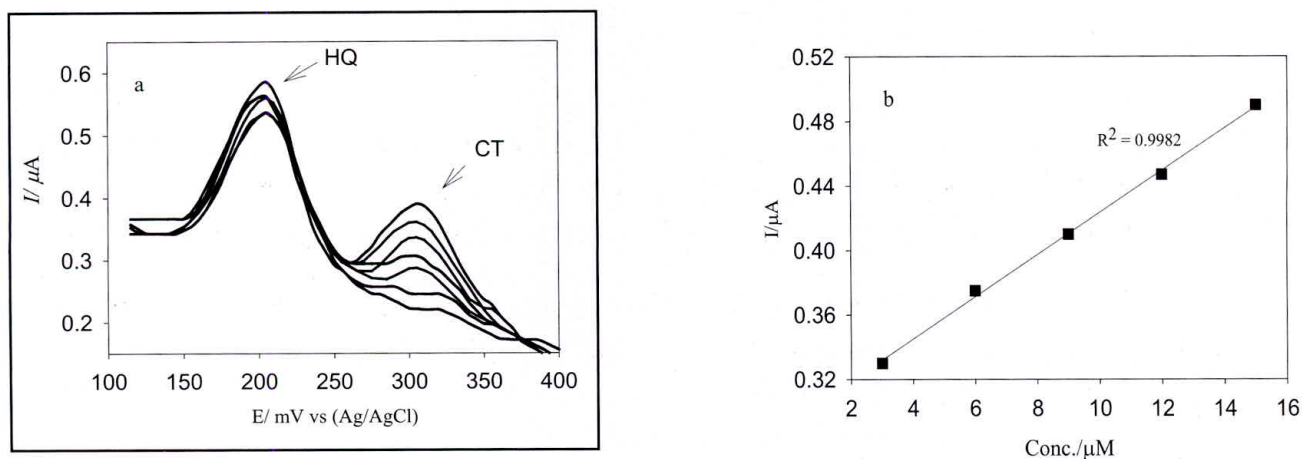


Fig. 7: (a) Differential pulse voltammogram (DPV) of CT in the presence of HQ at PGA modified GCE in PBS (b) Calibration curve for the quantitative determination of CT in the presence of HQ.

This separating ability of the poly glutamic acid modified glassy carbon electrode can be used to estimate catechol, hydroquinone and resorcinol quantitatively in presence of others.

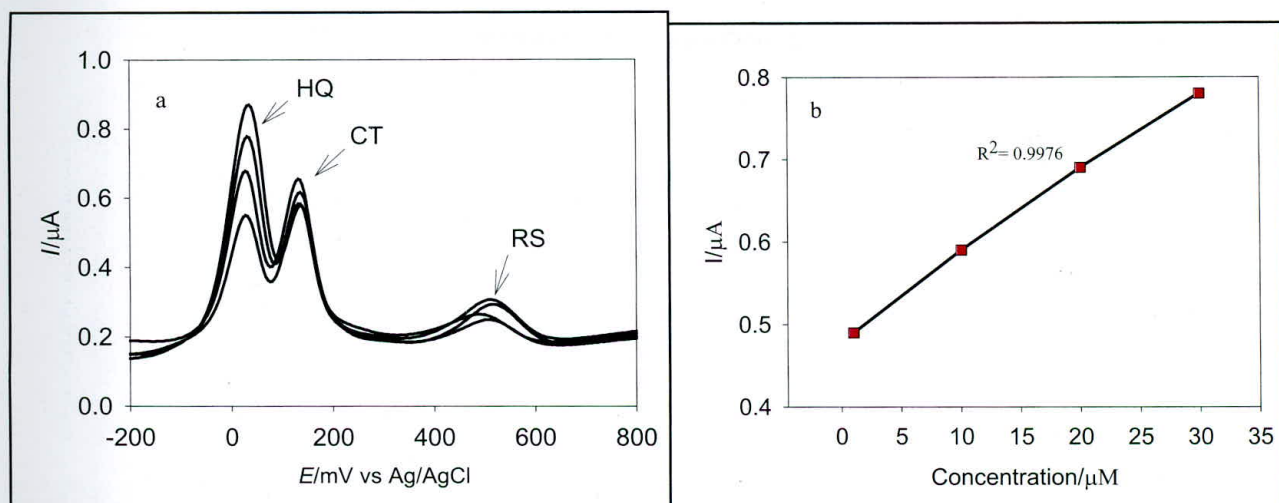


Fig. 8: (a) Differential pulse voltammogram (DPV) HQ in the presence of CT and RSPGA modified GCE in PBS. (b) Calibration curve for the quantitative determination of HQ in ternary mixture with CT and RS.

Conclusion

In summary, a simple yet effective method for the simultaneous detection and estimation of catechol, hydroquinone and resorcinol using electrochemical techniques has been presented. Catechol, hydroquinone and resorcinol has been detected from binary and ternary mixtures in ABS and PBS at PGA modified GCE. This method could be used to detect and quantify dihydroxybenzenes as pollutants and/or contaminants in real environmental samples.

Publication from this research

Md. Uzzal Hossain, Md. Toufiqur Rahman, and Md. Qamrul Ehsan. 2015. Simultaneous Detection and Estimation of Catechol, Hydroquinone, and Resorcinol in Binary and Ternary Mixtures Using Electrochemical Techniques. Hindawi Publishing Corporation. *International Journal of Analytical Chemistry*, Vol. 2015. Article ID 862979, 8 pages.

Development of Bio-Degradable Plastic Bags in Context of Bangladesh

Dil Afroza Begum and M Ruhul Amin

Location: Chemical Engineering Department, BUET, Dhaka Bangladesh

Duration : Three years (2014-2017)

Expenditure of the project: Tk. 1000000.00

Introduction

Owing to low production cost, good physical properties and light weight, plastic objects have slowly substituted glass, paper and metals in several fields of application. At the same time, the current huge global production of plastics has generated enormous environmental concerns.

In Bangladesh, plastic bags have been banned completely since early 2002. They were found to have been the main culprit during the 1988 and 1998 floods that submerged two-thirds of the country. The problem was that discarded bags were choking the drainage system.

Plastic wastes have already contributed to a great deal of environmental hazards in Bangladesh. Our project work has started its journey to make and develop biodegradable and environmentally friendly polymers. This venture aims to reduce the environmental impact that plastic wastes has on the country. We have started this project in May 2012. For the first time, a polymer laboratory has been set up in the Chemical Engineering Department in collaboration with Bangladesh Council of Scientific and Industrial Research (BCSIR), experiments have been carried out to test the viability of the product. Starch has been successfully used in preparation of blends with polyethylene (PE).

Objectives

The objectives of the project are

- Identify types of starch, master batches and additives that enhance environmental degradation (both biodegradation and photo degradation) of polyethylene plastic bags;
- Identify local plant waste products that can be utilized as biodegradable additives;
- Produce plastic films of Polyethylene containing the additives;
- Produce plastic films containing local plant waste materials; and
- Investigate photo degradation of the films and investigate compost ability of the films.

Methodology

In this experiment, we have prepared plastic films by first premixing the film grade polyethylene pellets with different amounts of additives. Additive loadings were 10, 20, 30, 40 and 50 percent mixed with pure polyethylene. Subsequently, the mixer was placed between the plates of a manually operated press to produce the film. The films were exposed in outdoor environment. The films were exposed for one year and samples were drawn at regular intervals and evaluated for degradation.

A portion of the specimens were exposed to sunlight and the rest buried underground. The deterioration of the starch-polyethylene composites following exposure was determined by measuring changes in tensile properties, flexural properties, SEM imaging and FT-IR analysis of the samples.

SEM analyses, coupled with elemental surface microanalysis, of polymer samples retrieved from degradation experiments were performed using a scanning electron microscope, and the collected images was compared with those recorded on the original untreated samples. Original and degraded polymeric materials, as well as the relevant extractable fractions were characterized by FTIR.

Results

The relationships between the compositions and mechanical properties, chemical properties and morphology of the samples have been shown and discussed below.

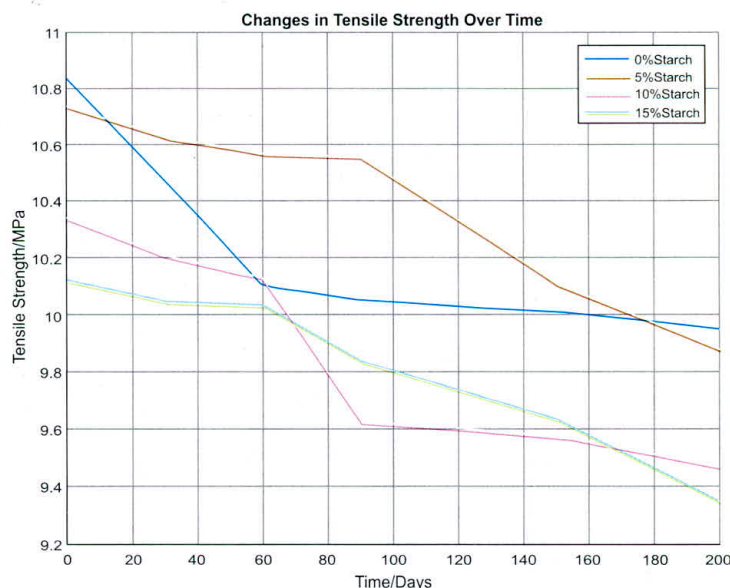


Fig. 1: Relationship between Tensile Strength and composition with time

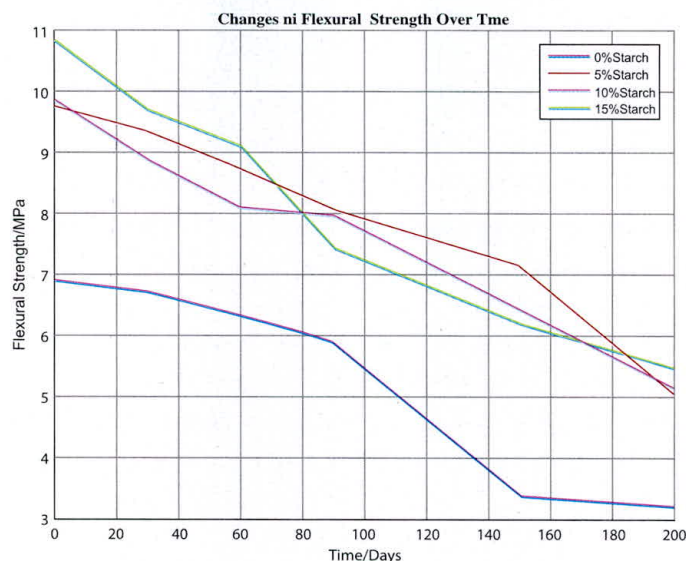


Fig. 2: Relationship between Flexural Strength and composition with time

The mechanical properties showed a progressive degradation with increase in starch content. Here, it can be seen that on Day 0, as the amount of starch increases from 0 to 15%, the tensile strength of the blend decreases from 10.83 MPa to 10.12 MPa.

More significant variation was observed for the blend having 15% Starch. This is because the additive used during the formulation has tensile properties much lower when compared to PE. Similar trends were observed for the LDPE/cassava starch blend by Nakamura et al. (Nakamura, 2005). Young's modulus for the specimens increased with the amount of starch. This can be explained by the crystallinity, hydrogen bonding and stiffening effect of the starch present in polystarch N (Chandra, Rustgi 1997).

Structural changes such as oxidation level of PE due to exposure to UV radiation (sunlight) can be accurately detected by FTIR. Spectra FTIR, for 0% starch, 5% starch and 10% starch at 0 day and after 30 days are given below:

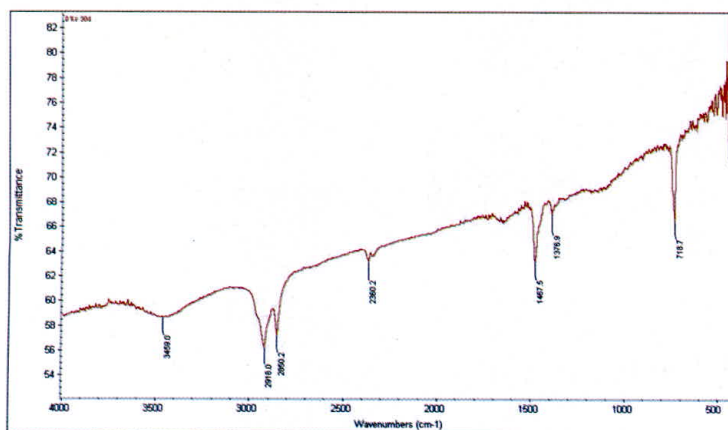


Fig. 3: FTIR spectra for 0% starch at 0 day.

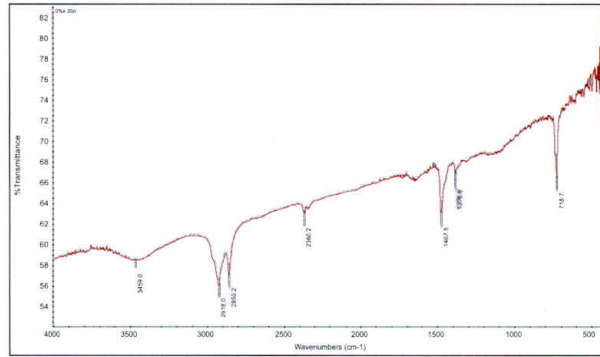


Figure 4: FTIR spectra for 0% starch after 30 days.

For 5% Starch-PE, peaks are observed at 2917 and 2843 cm^{-1} corresponding to the vibration mode of $-\text{CH}_2$ group. The peak at 1715 cm^{-1} shows the band vibration of the $\text{C}=\text{O}$ group which appears due to the photo-oxidation of the PE. This band increases more considerably with time. The peak at 1463 cm^{-1} corresponds to the deformation and elongation mode of CH_2 group (Gisjsman, 1997 and Allen, 2000). The FTIR spectra of the 5% starch-PE sample also shows more peaks at 30 days than at Day 0 which indicates the degradation of the sample. Similar trends are found in the spectra of the 10% Starch-PE blend. A significant amount of peaks are seen after 30 days.

The SEM images for the polyethylene - starch blend (0% starch , 5% starch, 10% starch, 15% starch) taken at different exposure time (0, 30 and 60 days) are presented in figures. Initially it's clear that there is no crack on the surface of the blend. When the time of exposure increases, degradation of the material starts and gradually the affected area increases. For the material after 30 days of exposure, initiation of cracks and degradation occurs; after 60 days of exposure, degradation propagates rapidly, bigger cracks are shown. This degradation is due to the degradation for the starch as well as for the oxidized the PE amorphous regions. Thereby, the surface area of polymer blends increases and this improves oxygen-based reactions that enhances and increases PE chain oxidation reaction.

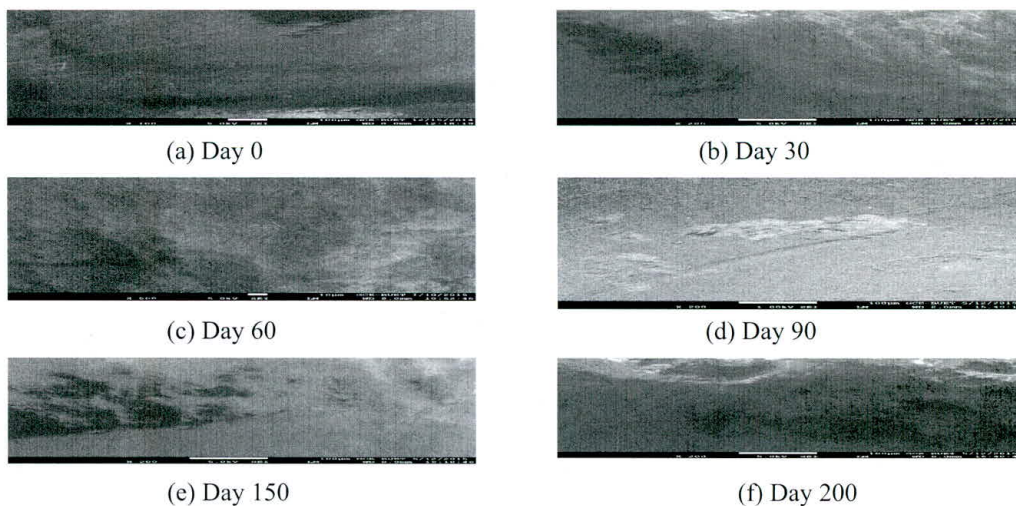


Fig. 5 (a-f): SEM images of 0% polyethylene-starch samples

From these figures, it can be interpreted that there is no crack on the surface of the sample at 0 day and same smooth surfaces are also shown after 30 and 60 days of exposure. For later days, cracks seem to have developed and the surface is no longer smooth as on Day 0.



(a) Day 0



(b) Day 30



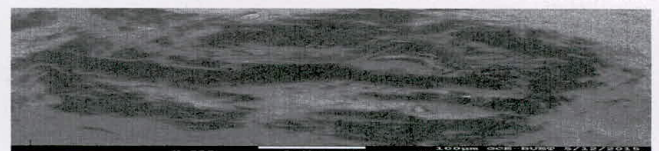
(c) Day 60



(d) Day 9/10



(e) Day 150



(f) Day 200

Fig. 6 (a-f): SEM images of 5% polyethylene-starch samples

These images clearly indicate that with the increase of exposure days, the surface cracking of the sample increases gradually due to photo-degradation. At 0 day, the surface of blend sample is smooth without any cracking. After 30 days of exposure, the image shows the initiation of cracking on the surface. The surface cracking becomes bigger after 60 days of exposure. At 0 day, the surface of blend sample is so smooth without any cracking. After 30 days of exposure, the image shows the initiation of cracking on the surface. The surface becomes rougher after 200 days of exposure.

Conclusion

Blending of food grade corn starch with PE was planned with the aim of making the waste PE films biodegradable. Various combinations of different starch concentrations ranging from 0 to 15% in PE were blended and converted into plates and films. The specimens were studied for various properties like physic-mechanical, morphological, chemical, etc., for packaging applications.

The mechanical properties show the drastic fall in tensile and flexural strength upon exposure. Brittleness and stiffness of the specimens was evident from the rise in the E-Modulus after exposure. FTIR of the samples revealed breakdown of the polyethylene to multiple compounds by breaking bonds. SEM study revealed that PE/starch systems showed corroded morphology. The extruded films show degradation of the structure indicating biodegradability in both UV radiation and under microbial action.

All of these results prove the successful synthesis of a starch-polyethylene composite suitable for making biodegradable plastics, in particular for making waste PE biodegradable as well as for packaging materials and shopping bags to reduce their environmental impact.

Publication from this Research

Ruhul, A., Afroza, D. B, Gafur, A. 2017. Study of Process Parameters for Making Biodegradable Linear Low Density Polyethylene in Natural Weathering Conditions. *Journal of Applied Chemistry* (IOSR-JAC) e-ISSN: 2278-5736. Volume 10, Issue 5 Ver. II (May. 2017), PP 79-90

Designing and Implementation of a Grid-Connected Solar Water Pumping Station for Bangladesh

Md Abul Hossain, Md Shafiqul Islam, M Shamim Kaiser and Shamim Al Mamun

Location: Dept. of Physics, Jahangirnagar University, Savar, Dhaka

Duration: Two years (2014-2016)

Expenditure of the project: Tk.1500000.00

Introduction

Bangladesh is a country of more than sixty-eight thousand villages where major profession is farming. Due to climate change and lack of river water, northern part of the country is dry and needs water pumping station not only to supply water to the field for irrigation but also to supply water for the household use (Chueco-Fernández, 2010; Ramos, 2009; Al-Karaghoul, 2000; Arab, 2009 and Bhave, 1994).

Power is one of the most important factors for a third world developing country like Bangladesh. In our country, the demand of energy is increasing day by day. Energy infrastructure of Bangladesh is quite small, insufficient and poorly managed. Low-income developing country like Bangladesh, the per capita energy consumption is one of the lowest (265kW) in the world. In 2012, around 53% of Bangladeshi population had access to electricity. Bangladesh's generation capacity is 8099MW (Rahman, 2013 and Kaiser 2006). Bangladesh largely depends on natural gas and hydro power stations to generate major portion of the power. The country lags behind than its expected production capacity. High demand and increasing need of power have created challenge for the power sector to meet the demand. To solve this energy crisis we can use different form of renewable energy to generate power. Renewable energy is the energy which comes from different form of renewable resources mainly from sunlight, wind, tides, biofuel and geo thermal heat. The country's energy demand is actually growing 12% annually and not as per government's estimated of 7.0%. Renewable energy as solar energy is a great source for solving power crises in Bangladesh. The total solar energy absorbed by earth's atmosphere, oceans and land masses in one hour is more than the world's demand for energy in a year. But it is untapped. There are studies that suggest- if solar energy is adopted as much as 10,000 MW daily of solar electricity can be generated in short and medium-runs - this is equivalent to almost twice the total amount of electricity produced and supplied on the national grid (Kaiser, 2006, and Siddique, 2013). From the geographical point of view, due to the high solar insolation, the solar energy has huge potential. A solar powered water pumping system can be served for ensuring the accessibility of water in remote areas (Kaldellis, 2011; Meah, 2008; Kordab, 2007; Mahmoud, 2004 and Mahmoud, 2003). For starters, a good amount of capital investment is required which may not be forthcoming. Furthermore, whenever the conditions are not suitable- cool, cloudy days- the efficiency of the system suffers (Hammad, 1999). Hence, a separate water storage tank is required to be filled in preparation for such days which is another source of expenditure (Amrouche, 2010; Sutthivirode, 2009 and Suleimani, 2000). Also, in remote, off-grid areas, the amount of skilled technicians capable of repairing a solar powered system may be very low, putting the feasibility of the whole system under scrutiny (Hasan, 2016).

Objectives

This research work is based on the design and performance analysis of a reliable grid-connected PV system for the irrigation in Bangladesh. The aim is to study the designing of connecting to the proposed PV system. The specific objectives of the research are to:

- Propose the best and reliable grid connected PV system for solar water pumping system;
- Deduce the mathematical model for the grid connected solar water pumping system; and
- Propose the financial feasibility of the proposed system and compare the performance with the diesel generator

Methodology

To accomplish the objectives in this research work the following methods are important;

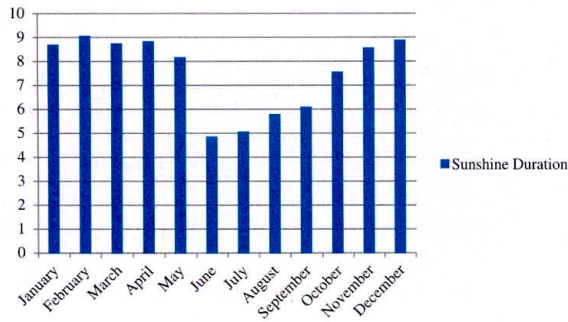
- a) Develop an optimal PV system which can meet the demand of the load by using proper sizing.
- b) Determine the Financial analysis using HOMER
- c) Analyze the performance of the grid tie inverter
- d) Determine the performance by comparing the proposed system with existing system. RET screen and HOMER can be used to do it.

Results

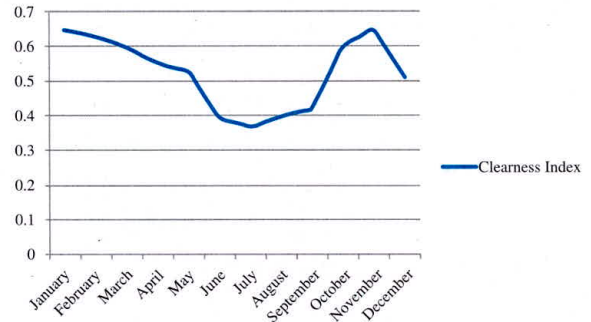
This section summarized the results obtained from this work. Fig. 1 (a) shows sunshine duration on different months of the year over Bangladesh. It has been found that the sunshine duration is lower in the rainy season. The cloud cover index is also high during the months of rainy season (Figure 1 (b)). Figure 1 (c) shows the daily energy generation for different months of the year by the proposed 5 kw solar water pumping system. The monthly average generation for different months of the year is shown in Figure 1 (d).

Figure 2 shows the effect of solar insolation on the flow rate of the motor. The flow rate is a linear function of the solar insolation received on the solar panel tilted on the angle of latitude of Dhaka.

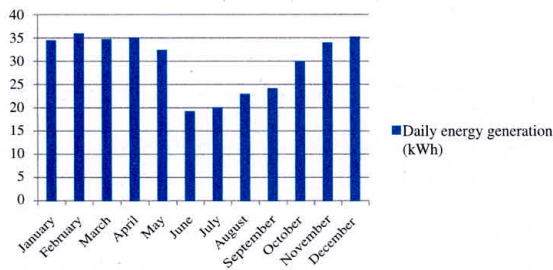
Table 1 shows summary of internal rate of return (IRR) and cost of energy (COE) of a 5 KW system for stand alone, grid tie and DEG. It has been found that COE for the proposed system is low although the payback is relatively high. Also, it has been found that the system is feasible technically and economically for the country like Bangladesh where the solar insolation is high.



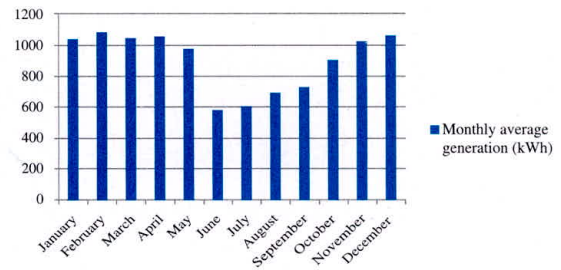
(a) Sunshine duration



(b) clearness index



(c) daily Energy from the system



(d) monthly Energy from the system

Fig. 1: Sunshine duration, clear sky index and energy generation from the system

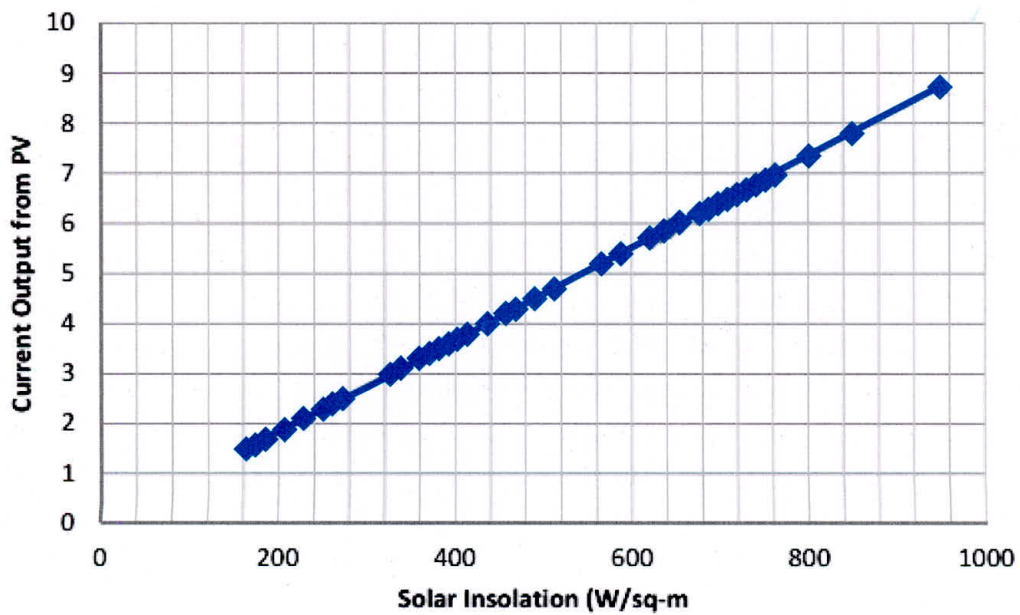


Fig. 2: Effect of Solar Radiation on PV output as well as on the Flow Rate

Table 1: Summary of internal rate of return (IRR) and cost of energy (COE) of a 5 KW system

Type of system	Pay back period (Years)	COE (USD/KWh)
Stand alone	11.1	19.58
Grid Tie	12	12.5
Diesel Engine (escalation rate 5%)	6	15.55
Diesel Engine (escalation rate 10%)	6	18.55

Conclusion

Irrigation is the basis of the economy and society, ranging from Asia to America. Similarly irrigated agriculture is playing a key role for the growth of agricultural crops in Bangladesh. Farmers uses shallow tubewells (STWs), deep tubewells (DTWs), hand tubewells (HTWs) and low-lift pumps (LLPs) as irrigation system. Energy is the major concern of the development of any country. The demand of energy in our country is increasing day by day and the demand is always greater than that of the generation. Bangladesh Government has taken a lot of steps to reduce the difference between generation and demand. It has been reported in different newspaper that government is purchasing grid electricity from different rental plant by paying unit charge of 21 BDT. On the other hand the Life cycle cost of generation of energy from grid connected PV system is found to be 3 BDT. In this project, we have designed and implemented a 5 kW grid connected solar submersible pumping station for Bangladesh which supplied water for irrigation and at the same time the system is connected to the local grid. The main components of the proposed systems are: solar array, charge controller, grid connected inverter, bi-directional meter, control circuit and wiring. If the generation is higher than that of the energy required by the pumping station, the surplus energy will be fed to the national grid for the others use. Contrary, if energy-demand of the pumping station is higher than that of the energy generation during the rainy days, the required energy will be taken from the local grid. The financial feasibility analysis shows that the internal rate of return of the proposed grid tie solar water pumping system is less than that of standalone and DEG based pumpingsystem. Moreover the COE of the proposed system is lower than its counterparts. Performance evaluation reveals that the proposed grid connected solar water pumping system is technically feasible. The key features needed to be processed for successful development of the proposed system. These are: firstly, energy planning at government level and NGOs are required. They should set up a goal for developing countries; secondly, government should help entrepreneur by establishing PPP to spur market development. Thirdly, government should offer the installation cost with very low interest rate. Fourthly, government may avoid offering subsidy to the conventional energy and may negotiate flexible loan facility and repaying plan to serve the goal. Australian and Japanese governments have initiated Rebate program for grid-tie system. A feed-in tariff is a rate paid for electricity fed back into the electricity grid from a designated renewable electricity generation source such as a rooftop solar panel system. If unit energy is fed to grid, government give two units energy for generating energy from grid-tie system. Thus the government should offer some rebate like the Australia and Japan.

Publication from this Research

Feasibility of Solar powered Grid tied pumping system- A case study for Bangladesh, *Journal of Solar Energy, A Journal of International Solar Energy Society*.

References

- Al-Karaghoul, A. and Al-Sabounchi, A. M. 2000. A PV pumping system. *Applied Energy*, 65(1), 145-151.
- Amrouche, S. O., Rekioua, D., and Hamidat, A. (2010). Modelling photovoltaic water pumping system and evaluation of their CO₂ emissions mitigation potential. *Applied Energy*, 87(11): 3451-3459.
- Arab, A. H., Chenlo, F., Mukadam, K. and Balenzategui, J. L. 1999. Performance of PV water pumping systems. *Renewable Energy*, 18(2):191-204.
- Bhave, A. G. (1994). Potential for solar water-pumping systems in India. *Applied Energy*, 48(3):197-200.
- Chowdhury, A. R. 2004. Evaluation of Occupational Health Problems of Cycle Rickshaw Pullers And Re design Of Cycle Rickshaw On Ergonomic Principles. *Indian Journal of Renewable Energy*, 23, 112-134.
- Chueco-Fernández, F. J. and Bayod-Rújula, A. A. 2010. Power supply for pumping systems in northern Chile: Photovoltaics as alternative to grid extension and diesel engines. *Energy*, 35(7) 2909-2921.
- Hammad, M. A. 1999. Characteristics of solar water pumping in Jordan. *Energy*, 24(2):8592, 1999.
- Hasan, M. R., Islam, M. S. Hossain, M. A., Sarjana, U., Hasan, M. R., 2016. Design and Development of a Cost Effective Standalone PV System for a Residential Building in Pabna, Bangladesh. *International Journal of Scientific & Engineering Research*. 7(9):1664-1669
- Kaiser, M. S., Anwar, A. Adyita, S. K. and Mazumder, R. K. 2006. Financial Analysis of a Roof-top grid-connected PV system in Dhaka, *Journal of Science, University of Dhaka*. 54(2): 157-162,.
- Kaldellis, J.K, Meidanis, E., and Zafirakis, D. 2011. Experimental energy analysis of a stand-alone photovoltaic-based water pumping installation. *Applied Energy*, 88(12):4556-4562
- Kordab, M. 2007. Priority option of photovoltaic systems for water pumping in rural areas in ESCWA member countries, *Desalination*, 209(1-3): 73-77
- Mahmoud, E. and Nather, H E. 2003. Renewable energy and sustainable developments in Egypt: photovoltaic water pumping in remote areas, *Applied Energy*, 74(1-2): 141-147.

- Manolakos, D., Papadakis, G., Papantonis, D., and Kyritsis, 2004. A stand-alone photovoltaic power system for remote villages using pumped water energy storage. *Energy*, 29(1):57-69
- Meah, K. Fletcher, S., and Ula. S. 2008. Solar photovoltaic water pumping for remote locations. *Renewable and Sustainable Energy Reviews*, 12(2):472-487.
- Rahman, M. 2013. Report on Energy Scenario of Bangladesh. Bangladesh Power Development Board (BPDB) Annual Report 2013.
- Ramos, J. S., and Ramos, H. M. 2009. Solar powered pumps to supply water for rural or isolated zones: A case study, *Energy for Sustainable Development*, vol. 13(3):151-158.
- Siddique, A.R., Khondokar, M.A., Patoary, M. N. H., Kaiser, M. S. and Imam, A. 2013. Financial feasibility analysis of a micro-controller based solar powered rickshaw. *International Conference on Electrical Information and Communication Technology (EICT)*, 1-5.
- Suleimani, Z. Al and Rao, N. R. 2000. Wind-powered electric water-pumping system installed in a remote location. *Applied Energy*. 65(1): 339-347.
- Sutthivirode, K., Namprakai, P., and Roonprasang, N. 2009. A new version of a solar water heating system coupled with a solar water pump. *Applied Energy*, 86(9):1423-1430.

Geochemical Vulnerabilities of Ground- and Surface Waters of the Southwestern Coastal Bangladesh under Stressed Geo-climatic Conditions

Dilip Kumar Datta, Md Mujibor Rahman and Prosun Kumar Ghosh

Location: Environmental Science Discipline, Khulna University, Khulna, Bangladesh

Duration: Two years (2014-2016)

Expenditure of the project: Tk. 1500000.00

Introduction

The coastal Bangladesh is one of the vulnerable regions globally and its water security shall have to face multiple stresses in the upcoming decades due to rapid changes in the climatic pattern. The water resources and its security in the southwestern coastal Bangladesh is already critical due to adverse changes in the physical and chemical characteristics of ground and surface waters of the region. The consequent stresses on the ecosystem is aggravating most because of the fact that this deltaic region undergoing a period of transition from being an active deltaic system with strong freshwater front pressing tidal waters downwards to a much weaker local freshwater front with strong tidal front pressing upstream due to human intervention to the natural system. The population pressure, landuse/land cover changes, deforestation, changes in chemical fluxes due to various activities upstream etc have contributed to the changes in the coastal zone. The threat of geo-climatic changes in the delta as well are imposing quantitative and qualitative impacts on ground- and surface waters in the region. However, quality-water is essential for sustainable livelihood activities that determines quality of life and social wellbeing and is integral to the issues of poverty, social (gender) security and development. Scientific literature on the coastal aquifers and surface water system of Bangladesh is very scarce and is most true in case of geochemical characterization of the waters. The variability and complexity in geochemical behavior of coastal ground- and surface waters introduces uncertainties in proving options during stresses environmental conditions. Therefore a detail study of water geo-chemistry considering both spatial and temporal attributes is essential for southwestern coastal Bangladesh is essential in sustainable water management plant as mentioned in Coastal Development Strategy (CDS) 2005. This research project therefore aims to address the geochemical vulnerabilities of ground- and surface waters of the southwestern coastal Bangladesh (Satkhira, Khulna and Bagerhat districts) and to assess these attributes of the water resources and water security in relation to poverty alleviation and gender security.

Objectives

The broad objective of the proposed research project was to decipher the geochemical vulnerabilities of ground and surface water of the south western coastal Bangladesh under stressed geo-climatic conditions. The specific objectives include-

- Construction of geochemical profile of major water bodies such rivers of the southwestern coastal

- Construction of geochemical profile of major groundwater bodies adjacent to the rivers from existing bore-holes (tube-wells) with both spatial and temporal considerations.
- Understanding pollution of water bodies and their geochemical link and its spatial and temporal variation between ground- and surface waters and
- relating changes in the land-use/land cover pattern with the geochemical parameters to assess the relationship with the geo-climatic changes.

Methodology

This research project was completed in two consecutive years following the steps mentioned below: (i) The land-use/land cover pattern of the region and the natural drainage system was identified from satellite imageries and the sampling stations for both surface-and ground water representative of the water bodies were selected avoiding instant sources of pollution. The water sampling representative of temporal variation were carried out during post-monsoon (Sept-Oct to Feb-March), pre-monsoon (March-April to May-June) and monsoon (june-July to Aug-Sept). (ii) Both surface- and groundwater samples were collected considering both spatial and temporal variations and the chemical parameters such as pH, EC, TDS, the major cations (Ca_2^+ , Mg_2^+ , Na^+ and K^+) and major anions (HCO_3^- , Cl^- , SO_4^{2-} and PO_4^{3-}), silica and trace elements such as F-, I- and Br- were estimated following standard procedures. (iii) The data were analyzed and interpreted by standard statistical and specially designed software to prepare the hydro-geochemical profiles, and to decipher the source and controlling mechanism of the nature of solute load, the route to pollutants/contaminants in water and the quality of water.

Results

This research project entitled Geochemical Vulnerabilities of Ground- and Surface waters of the Southwestern Coastal Bangladesh under Stressed Geo-climatic Conditions was intended to decipher the hydro geochemical characteristics of ground- and surface waters of southwestern coastal Bangladesh – the Satkhira, Khulna and the Bagerhat districts representing the core of the Bengal delta - where from the samples were collected during August 2014 (Monsoon), December 2014 (Winter) and April 2015 (Pre-monsoon) from 45 stations having uniform spatial distribution and were analyzed for the physicochemical attributes such as electrical conductivity (EC), Total Dissolved Solids (TDS), pH, major cations (such as Na^+ , K^+ , Ca_2^+ , Mg_2^+), major anions (such as Cl^- , HCO_3^- , SO_4^{2-} , PO_4^{3-}) and Br-, I-, F-, NO_3^- , and SiO_2 . The general trend of cationic abundance during monsoon and pre-monsoon for both ground- and surface waters is $\text{Na}^+ > \text{Ca}_2^+ > \text{Mg}_2^+ > \text{K}^+$; however during winter the trend of cationic bundance is $\text{Na}^+ > \text{Mg}_2^+ > \text{Ca}_2^+ > \text{K}^+$ both for ground- and surface waters. . Although the general trend of anionic abundance for surface water during all the seasons is $\text{Cl}^- > \text{HCO}_3^- > \text{SO}_4^{2-} > \text{Br}^- > \text{NO}_3^- > \text{NO}_3^- > \text{PO}_4^{3-}$ but the trend in ground water a bit different in that Br- is more during winter compared to that of NO_3^- during winter. Such abundance suggests that sea water contribution to the water chemistry is significant during winter although continental contribution governs the mechanism during monsoon and pre-monsoon ground water chemistry.

The temporal variation in water chemistry for both ground- and surface waters also statistically significant suggests that the surface and groundwater has a seasonal link and atmospheric contribution to the chemistry is quite evident. Furthermore more the correlation matrix among different estimated attributes of both ground- and surface waters indicates their source as co-original. The major source minerals contributed to the water chemistry has been identified as calcite, albite, dolomite, feldspars and ferromagnesian minerals. The major mechanism as revealed by Gibb's diagram influencing the groundwater chemistry is the rock weathering processes, however there is an equal contribution of rock weathering processes and evaporation and recrystallization processes representing sea water may be detected in surface water chemistry. The groundwater samples also shows a state of both super- and under saturation with respect to calcite and dolomite suggesting both CO_2 outgassing and both presence and absence of nucleation for calcite precipitation. The monsoon surface waters are under saturated while the pre-monsoon waters are super saturated. The groundwater are mostly of Na-Cl- HCO_3 type; however Na-Cl and Na- HCO_3 -Cl type water are also prevalent. The surface waters are mostly Na-Cl type and shows both permanent and temporary hardness. Generally the alkali metals exceeds alkaline earths and strong acidic anions exceeds weak acidic anions in the water chemistry and suggest their detrimental quality when considering their usage pattern. Thus both the ground- and surface water shows their poor suitability in USSL salinity hazard- and Wilcox diagram with respect to irrigation during all the seasonal cycles. The concentration of PO_4^- , SO_4^- and NO_3^- are mostly within the WHO allowable standard for both ground- and surface water but the other elemental concentration suggests their incompatibility for potable purposes.

The current state of water chemistry of the region is related to the changes of land cover and land-use pattern over the six decades as manifested by the integrated approach done through remote sensing, GIS and evaluation of water management in the region. Land cover pattern during 1980, 1990, 2000 and 2015 were analyzed by satellite where images were selected (LANDSAT 8 OLI, LANDSAT 4-5 TM and LANDSAT 1-3 MSS downloaded from <earthexplorer.usgs.gov>) both on their spatial and spectral attributes. The changes during the 35 year period (1980-2015) were identified with reference to five categories of land pattern such as forest land, agricultural land, fallow land, water bodies, settlements and gheer areas that have been processed by ArcGIS 10.3 and remote sensing software ENVI 5.3. The results suggest that major changes were located at the proximity of gheer areas through conversion of agricultural and fallow land to gheer areas. During 1980s the agricultural, fallow and gheer coverage was 14.13%, 19.59% and 3.48% respectively; however during 2015 the agricultural and fallow land has shrink down to 13.68% and 8.10% respectively of the total area while the gheer coverage increased to 10.19% of the total area – an annual increment of 23.43 km. The settlement coverage also decreased during 1980 to 2015 from 21.66% to 15.50% of the total area – a major consequence incremental coverage of waterlogged conditions. Such changes has been prompted by massive infrastructural interference to the natural systems of the southwestern coastal Bangladesh through construction and implementation of mega-structures such as polders, sluice gates, closures etc that hindered the natural flow of the river system and thus the tidal processes. The changes in the land cover pattern has changed the land use pattern that too contributed as a positive feedback input to salinity and waterlogged conditions in the southwestern coastal Bangladesh.

Conclusion

The water chemistry of the southwestern coastal Bangladesh – the core of the Bengal delta – represents the current state of chemical environmental of the region. The prevalence of alkali metals compared to that of alkaline earths and the prevalence of strong acidic anions compared to that of weak acidic anions in the water composition of both ground- and surface water suggests a projected oceanic influence that is hindering the continental processes as manifested by the major mechanism of rock weathering that contributing significantly to the water chemistry. Such situation indicates a waterlogged condition where natural processes of inflow and out of flow of tidal waters is deterred. Such situation occurs due to interference to the natural flow pattern by interference of mega-structures as is evident in the southwest coastal Bangladesh. Thus it may be concluded that the major geochemical vulnerability of ground- and surface waters of the southwestern coastal Bangladesh under stressed geo-climatic conditions lies with the consequences of mega-structures constructed in the region that have aggravated waterlogging and hence the salinity.

Development of an Intelligent Information Prediction System for Stock Exchange Using Different Data Mining Techniques

Kazi Shah Nawaz Ripon, Lasker Ershad Ali and Masudul Islam

Location : Khulna University, Bangladesh

Duration : Two years (2014-2016)

Expenditure of the project: Tk. 706639.00

Introduction

Stock market forecasting is the process to determine the price of an asset (stock) value in advance of a company available in the stock market. A stock market is a public market for companies or for people to invest money for trading shares of listed companies. But financial time series prediction is one of the most challenging applications in the recent days. Gaining high profit is the dream of every investor. However, a lot of risk factors including political events, general economic conditions and trader's expectations are involved in the stock market and the stock data is highly noisy, irregular, random, non-linear, non-seasonal and chaotic in nature. Therefore, predicting finance market price movements is quite difficult. So it has always remained as a challenge for the common investors and stock buyers/sellers to gain knowledge about the daily stock market price values. Because of this two-fold criterion, there is a great necessity for automated stock market forecasting system to provide an insight to the investors for better and safer investment. This work presents an automated stock market prediction system based on bio-inspired algorithm for Dhaka Stock Exchange (DSE), Bangladesh.

Objectives

The specific objectives of this research are:

- To build an accurate model by using computational intelligence (bio-inspired) techniques to facilitate the methods accessible and user-friendly;
- To use the developed algorithms for designing and building an intelligent knowledge-discovery system for stock exchange environment; and
- To propagate and endorse data mining technology in the financial and stock exchange industries.

Methodology

Statistical method can handle only linear data, they are unable to follow the non-linear pattern hidden within the stock data (Chauhan *et. al.* 2014; Kaur *et al.* 2014; Mohapatra *et. al.* 2013). In recent years, Bio- Inspired Algorithms have been applied to stock market forecasting, and produced better performance than competing approaches (Kaur *et. al.* 2014; Shetaet *et. al.* 2013). This is due their ability to find patterns and irregularities

as well as detecting multi-dimensional non-linear connections in data which is usual for stock market. Since all Bio-Inspired Algorithms have advantages and disadvantages, developing hybridized Bio- Inspired Algorithms for stock market forecasting is clearly a necessity. Motivated by this, a hybridized bioinspired scheme for stock prices forecasting based on Genetic Algorithm (GA) and Artificial Neural Network (ANN) with Neighborhood Replica-Window Scheme has been proposed in this research. The Proposed scheme is presented in Figure-1.

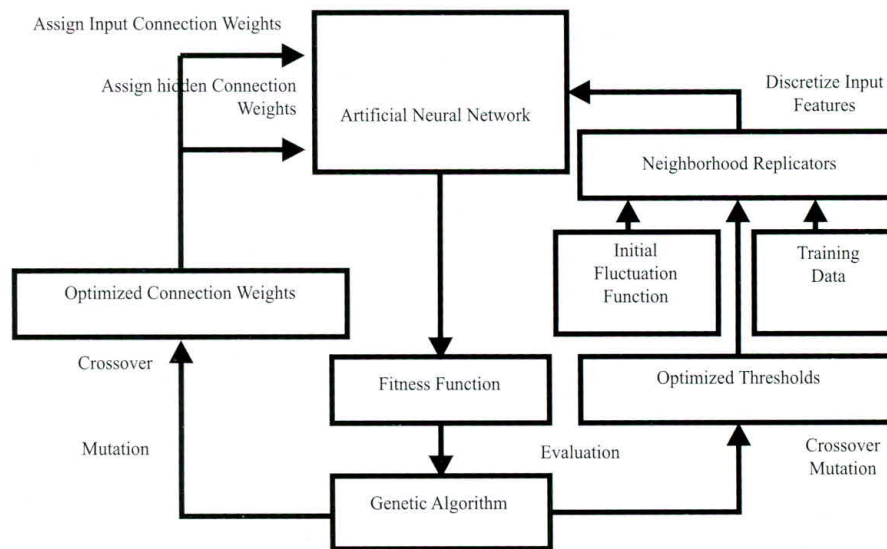


Fig. 1: Proposed Scheme for Stock Market forecasting based on Hybridized Bio-inspired Scheme

Along with the neighborhood replicator parameter, other parameters for connection weights of ANN were used as prescribed by Kaur *et. al.* 2014. For the GA, initial population size was randomly selected within the range of 10 to 40 Initial population was chosen by analyzing the best of average MSE for varying the population sizes and then taking the best of average. The crossover probability was 60 with mutation probability of 0.0015 Stopping Criteria was considered for 1000 generations. Maximum number of iterations during learning was considered 1000 and the learning coefficient used to change within the range of 0.1 and 0.8 Maximum number of iterations during neural network learning for fitness calculation in GA was considered 200.

Results

For the experiments, the data was collected from DSE from January 01, 2013 to August 15, 2015 and from the real-market prices, conjoint data was chosen to evaluate the effectiveness of the proposed scheme along with the data to train up the schema. Data procured from DSE has been used for the training and testing data. In the following figures (Fig. 2 to Fig. 5), the actual value and predicted value of last traded price for random selection of training data has been presented.

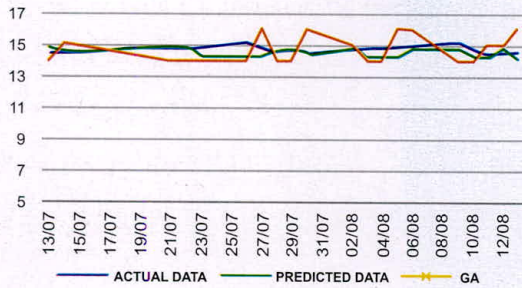


Fig. 2. Actual and Forecasted Last Traded Price Value for BANK ASIA

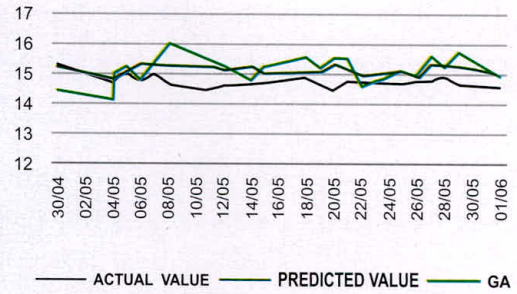


Fig. 3. Actual and Forecasted Last Traded Price Value for ALARAFAH BANK

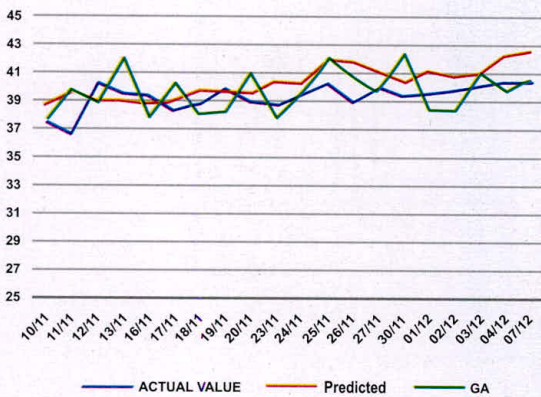


Fig. 4. Actual and Predicted Share Price for BEXIMCO

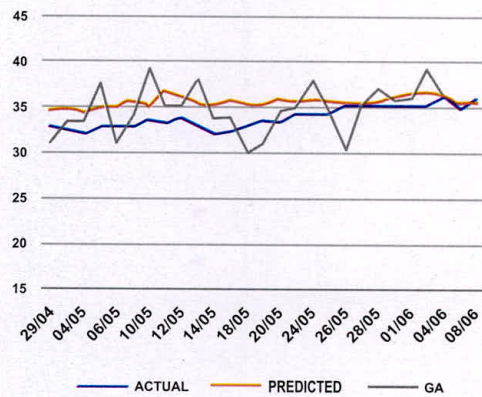


Fig. 5. Actual and Predicted Share Price for BARC Bank

The highest price has been predicted using the same approach. In the following figures (Figure-6 to Figure-10), some random executions has been presented. Along with the highest actual price and predicted highest price using proposed approach; we have also presented the predicted highest price by applying GA. Experiments show that, the proposed hybridized scheme performs better than the simple GA approach for the considered instruments for all cases.

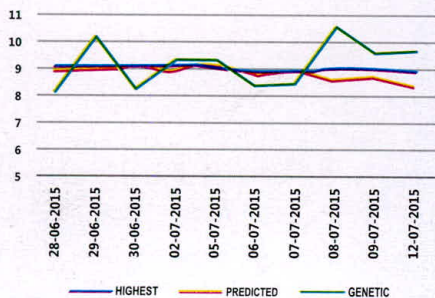


Fig. 6. Highest Price Forecasting for EXIM Bank

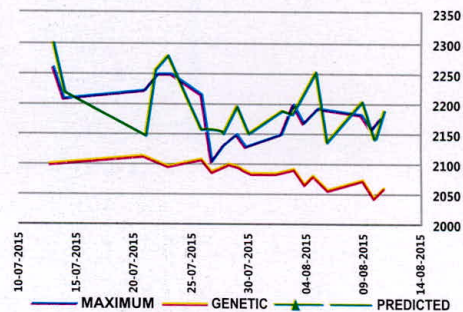


Fig. 7. Highest Price Prediction for Glaxo Smith instrument

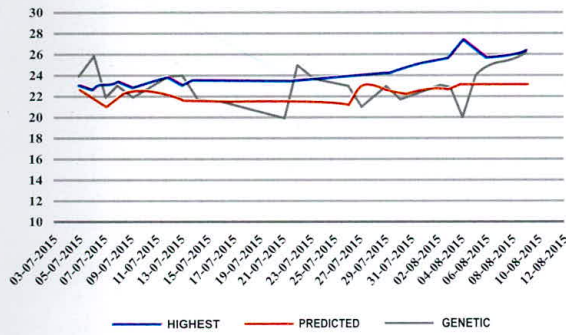


Fig. 8. Highest Price Prediction for Grammen Phone

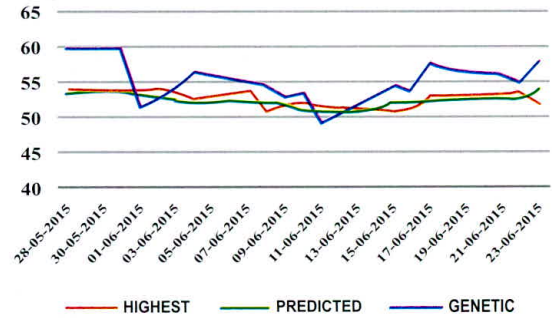


Fig. 9. Highest Price Forecasting for IDLC

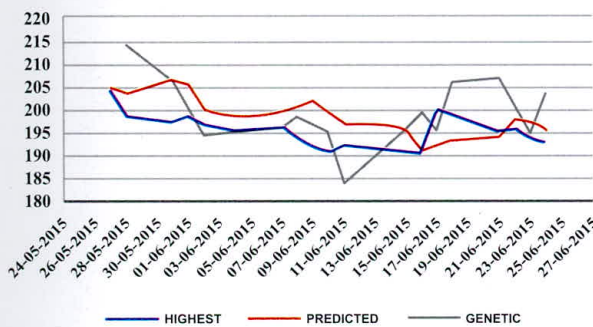


Fig. 10. Highest Price Forecasting for Jamuna Oil

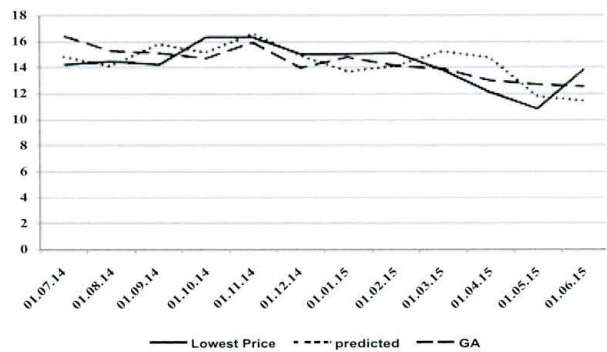


Fig. 11. Minimum Price Prediction of a Fiscal Year for Al-Arafah-Bank

Again the month-wise minimum traded price has also been predicted for a number of companies along the fiscal year 2014-2015 which are presented in Fig. 11 to Fig. 13. The performance of proposed scheme is better than GA because, the scheme gets the trend information (the upward sloping criteria towards the record date and the downward sloping criteria of the stock price from record date) from the training data and applying the neighborhood replica scheme proposed scheme assists a lot in achieving better performance.

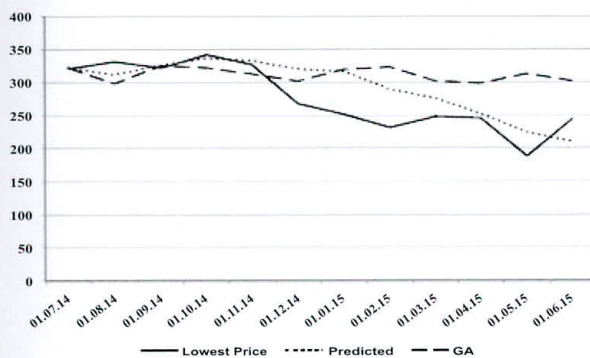


Fig. 12. Minimum Price Forecasting of a Fiscal Year for Padma Oil

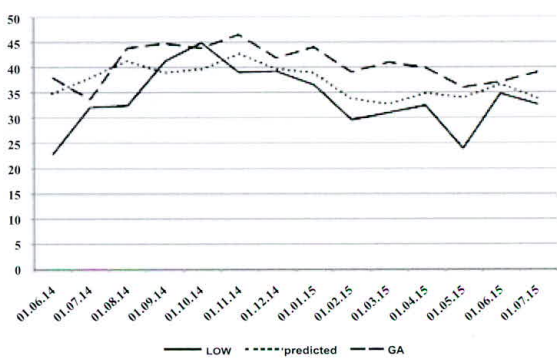


Fig. 13. Minimum Price Forecasting of a Fiscal Year for Beximco.

The last traded price has also been predicted on weekly basis for the period of January 01,2014 to December 31,2014 which has been presented in Figure-14. The weekly data also resembles better performance by dint of hybridized bio-inspired approach and neighborhood replica scheme. The average error rate is also lower than Genetic algorithm. Also the performance of the proposed scheme has been measured against MSE (Mean Square Error) as shown in Fig. 15.

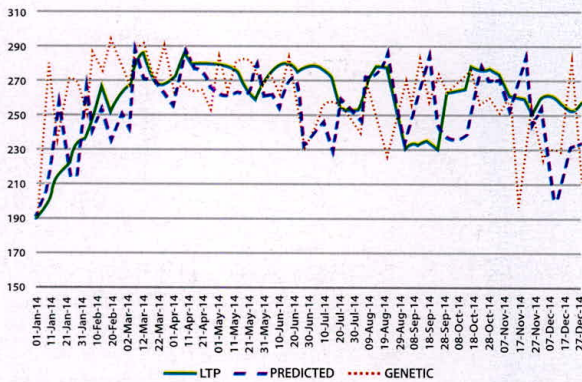


Fig. 14. Weekly Last Traded Forecasting for Squarepharma

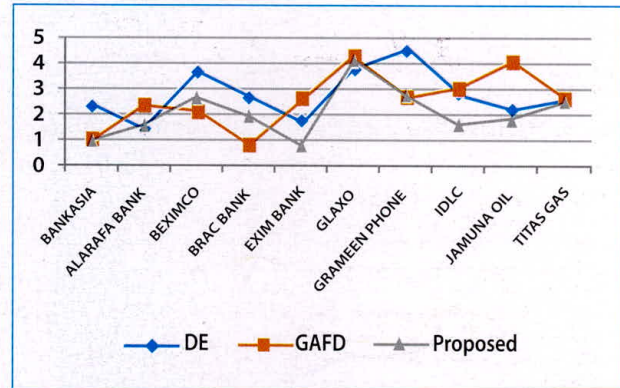


Fig. 15. Comparison of Various Schemes for Stock Market Forecasting

The performance in terms of MSE is presented in the following graph for DE, GAFD and the proposed scheme. Experimental results for the schemes on average of randomly selected data shows that the proposed GAFD focused Neighborhood Replica based scheme performs better in most cases. In comparison with the only paper that worked on the instruments from Dhaka Stock Exchange, average error rate of our scheme (1.038) is better than the Khan *et. al.* in 2011.

Conclusion

This research presents an effective approach for stock market forecasting using hybrid bio-inspired techniques for Dhaka Stock Exchange (DSE), Bangladesh. The proposed method is developed to facilitate accessible and user-friendly atmosphere for economists, analysts, and financial professionals. We analyzed the 24 months stock data for the DSE main index as well as ten of the companies listed therein. Experimental results show that the proposed scheme performs better than the existing schemes in terms of last traded price, close price, and maximum/minimum price with regard to various statistical measures.

Publication from this Research work

Ripon, K.S.N and Rajon, S.A.A. 2016. Stock market forecast using Bio-Inspired computing. In: IEEE 2016. International Conference on Control, Decision and Information Technologies (CoDIT), St. Julian's, Malta, 2016, pp. 523-529.

References

- Chauhan, B., Bidave, U., Gangathade, A. and Kale, S. 2014. Stock market prediction using Artificial Neural Networks. *International Journal of Computer Science and Information Technologies*, 5(1):904-907.
- Dase, R.K. and Pawar, D.D. 2010. Application of Artificial Neural Network for stock market predictions: A review of literature. *International Journal of Machine Intelligence*, 2(2):14-17.
- Isfan, M. Menezes, R. and Mendes, D.A. 2010. Forecasting the Portuguese stock market time series using Artificial Neural Networks. In: 7th International Conference on Applications of Physics in Financial Analysis. *Journal of Physics: Conference Series* 221, pp.01-13.
- Kaur, G., Dhar, J. and Guha, R.K. 2014. Stock market prediction from sectoral indices using an Adaptive Network Based Fuzzy Inference System. *International Journal of Management and Computing Sciences (IJMCS)*, 4(2):74-81.
- Khan, Z.H., Alin, T.S. and Hussain, M.K. 2011. Price prediction of share market using Artificial Neural Network (ANN). *International Journal of Computer Applications*, 22(2):42-47.
- Kim, K-j. and Han, I. 2000. Genetic algorithms approach to feature discretization in artificial neural networks for the prediction of stock price index. *Expert Systems with Applications*, 19:125–132.
- Mohapatra, P., Das, S., Patra, T.K. and Anirudh, M. 2013. Stock volatility forecasting using Swarm optimized Hybrid Network. *International Journal of Emerging Trends and Technology in Computer Science (IJETTCS)*, 2(3):78-85.
- Sheta, A. Faris, H. and Alkasassbeh, M. 2013. A Genetic Programming Model for S&P 500 Stock Market Prediction. *International Journal of Control and Automation*, 6(5):303-314.

Design and Development of a Four-Point Probe Technique to Detect Nondestructively the Internal Cracks in the Elements of Agricultural Machinery

Md Rostom Ali and Md Abdul Momin

Location: Department of Farm Power and Machinery, Bangladesh Agricultural University, Mymensing

Duration: Two years (2014-2016)

Expenditure of the project: Tk. 900000.00

Introduction

Farm machineries are major elements of farm mechanization in Bangladesh. Timeliness in farm operations is a crucial factor for successful agricultural operations. The failure of important parts like PTO/propeller shaft, spline shaft, tine/blade etc. of farm machineries especially tractor and power tiller during the peak season causes various large losses of revenue and inefficient utilization of labor. Therefore, it is necessary to routine check-up, inspection and diagnose of important parts of agricultural machinery for getting proper performance and timeliness operation. It is possible to adjust, repair and/or replace the defective component/parts according to diagnose results. Non-destructive testing is a key inspection criterion - across many fields of engineering. In agricultural machinery sector, reliable and accurate nondestructive testing of important parts of agricultural machineries and integrity assessment are both essential in order to maximize efficiency and minimize downtime and improve productivity. There is no available technique/method to nondestructively evaluate/diagnose the important parts of agricultural machinery in Bangladesh. The four-point probe voltage drop technique can be considered one of the best candidates for the purpose. This technique is simple in construction and easy to handle for conducting experiment (Dover *et al.*, 1991). The Voltage Drop technique is popular for material evaluation because of its advantages over other techniques, including sensitivity, flexibility, and simplicity of application. The main purpose of this study was to design and development of a laboratory based nondestructive testing set up for evaluating internal defects/cracks in the important parts of agricultural machinery using DC four-point probe measuring system.

Objectives

The specific objectives of the project were-

- to design and development of DC four-point probe measuring system
- to develop a method for identifying type & homogeneity of materials of the machine parts
- to detect internal cracks and defects of machine parts nondestructively.

Methodology

1) Site Selection

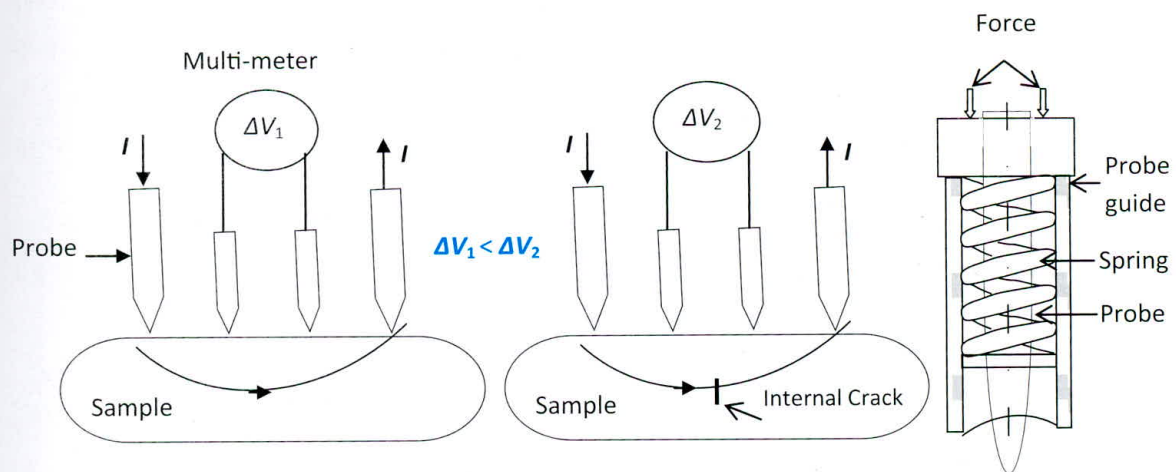
An experimental facility was developed in the workshop under the Department of Farm Power and Machinery, Bangladesh Agricultural University, Mymensingh. Different spare parts of agricultural machinery were collected from four regions like Bogra, Dhaka, and Jessore, Mymensingh and tested nondestructively as well as checked the homogeneity of final product.

2) Materials for experimental set up

For installing the experimental set up, many equipments and accessories were collected from local market. Here some major equipment and accessories are listed as follows: i. Digital multi-meter, ii. Switching system, iii. AC to DC converter, iv. High quality probes, v. Spring and vii. High tension cables, etc.

3) Experimental procedure

In this research, simple spring-loaded contact probes were used for both the purposes of current supply and measurement of voltage drop (VD). The contact of every probe with the test sample was considered as constant pressure by identical compression springs attached with probes. The current was injected into the material/test sample via the outer probes and inside two probes were used for measurement of VDs which were connected with a digital multi-meter as shown in Fig.1. The solid stainless steel probes were used for both the current supply and measurement of VD. A constant DC supply source was used to inject the required current to the test sample through the current input and output probes.



a) Test sample without defect/crack b) Test sample with defect/crack c) Probe contact with sample through compression spring.

Fig.1: Simplified arrangement of four probes on test sample.

4) Material sorting and detecting the internal crack

The following mathematical equation was used to determine the electrical resistivity of testing materials (Ali *et. al*, 2010). From the result, it is possible to mention the type material by

comparing with standard electrical resistivity of different materials.
$$\Delta\Phi = \frac{2\rho I}{\pi} \times \frac{S_2}{S_1^2 - S_2^2},$$

where, $\Delta\Phi$ is voltage drop, I is direct current, ρ is electrical resistivity, S_1 and S_2 are probe spacing. According to Fig.1, the VD will be larger for the test sample having an internal crack. On the other hand VD will be smaller for the crack free sample. From the experimental results, it is possible to identify the defective elements of agricultural machinery.

Results

1) Design of Probe

A stainless steel rod having 3.175 mm diameter is used as a probe. A spring was set between collar and tail (made of plastic fiber) to maintain rigidly contact and avoid sparking at the contact point between probe and sample. Direct current was used in the experiment. A 12V Battery was used as power source. The construction, design of probes and arrangement are shown in Fig 2.

2) Voltage Drop on Mild Steel Flat Bar

Comparison of voltage drop on different conditions of MS flat bar shown in Fig.3. Figure 3 indicates the voltage drop increases with increasing of corresponding current. These also show that the voltage drop of crackfree material is less than both of cracked and twisted samples. Crack makes resistance on the flow of current in material. So, both the resistance and voltage drop become higher in cracked specimen than crackfree. On the other hand, twisted material makes more resistance through the material due to several hair like crack generated in the twisted part, therefore it is higher than cracked one. The value of VD for the probe spacing 152.50 and 25 mm is less than that of 125.00 and 25mm. Similarly, VD for 177.50 and 50 mm spacing is less than that of 152.50 and 50 mm spacing.

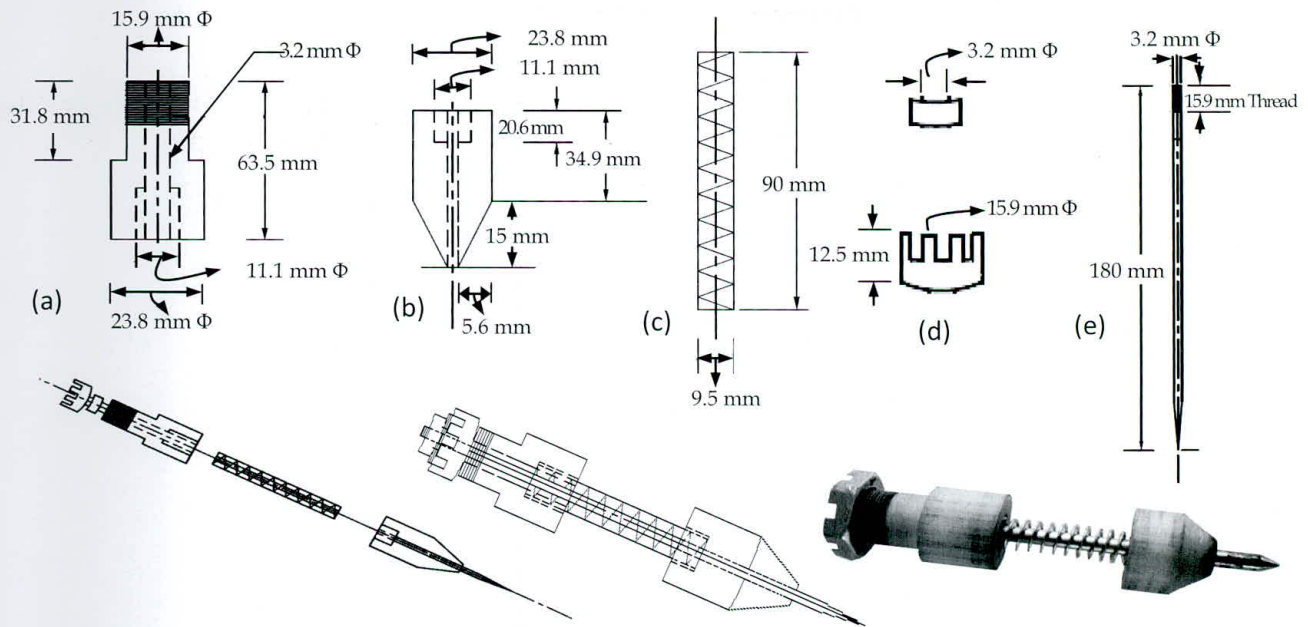


Fig. 2: (a) Collar (b) Tail (c) Spring (d) Nuts (e) Probe (f) Assembly Drawing (g) 2D View (h) Pictorial View of Probe

3) Voltage Drop on Stainless Steel Flat Bar

Comparison of voltage drop for different conditions of SS flat bar are also shown in Fig. 4. It shows 3 mm crack gives larger voltage drop than that of 1 mm and 2 mm cracks as it creates more resistance toward the current flow path. From the experimental results, it is possible to identify the defective elements of agricultural machinery.

4) Outcomes

Specifically, the following outcomes have been obtained at the end of the project:

- Suitable testing set up has been developed and it can be used to test internal crack and check the homogeneity of the materials of the spare parts of the agricultural machinery
- Disseminated technical information to graduate students
- One student got MS in Farm Power Machinery degree
- Produced report, thesis (by students) and published articles in the Journal of AMA-Agricultural Mechanization in Asia Africa and Latin America.

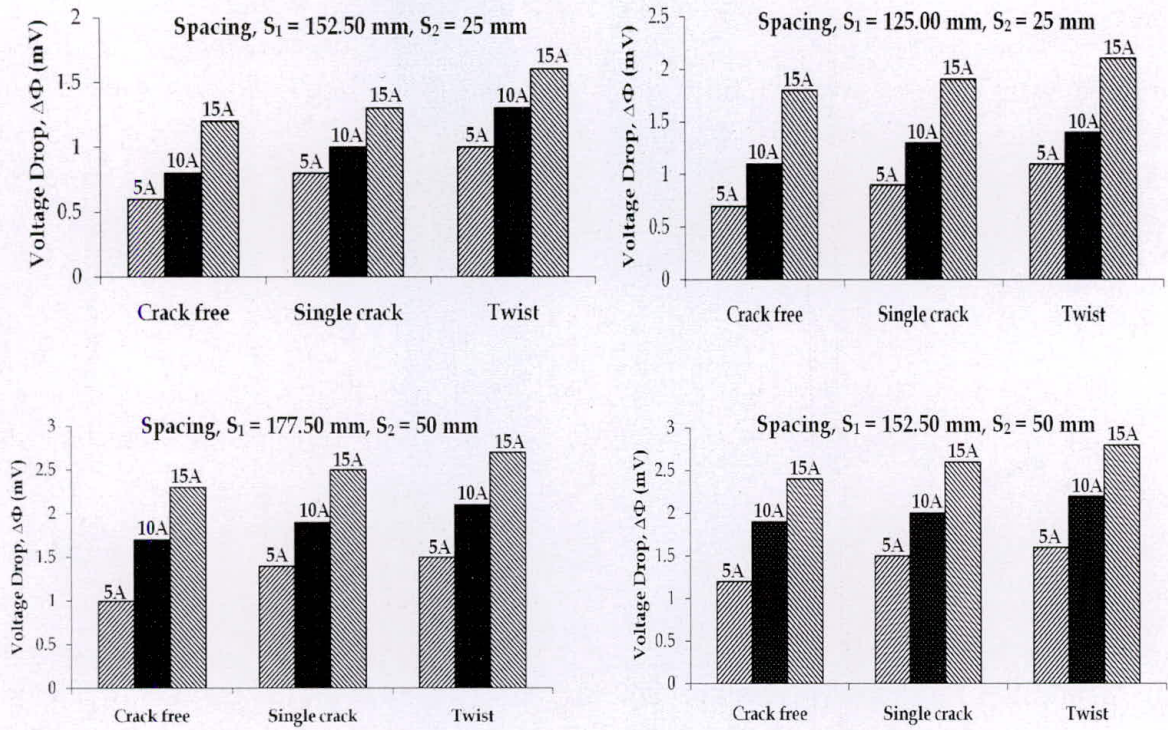


Fig. 3: Comparison of Voltage Drop on different conditions of MS Flat Bar

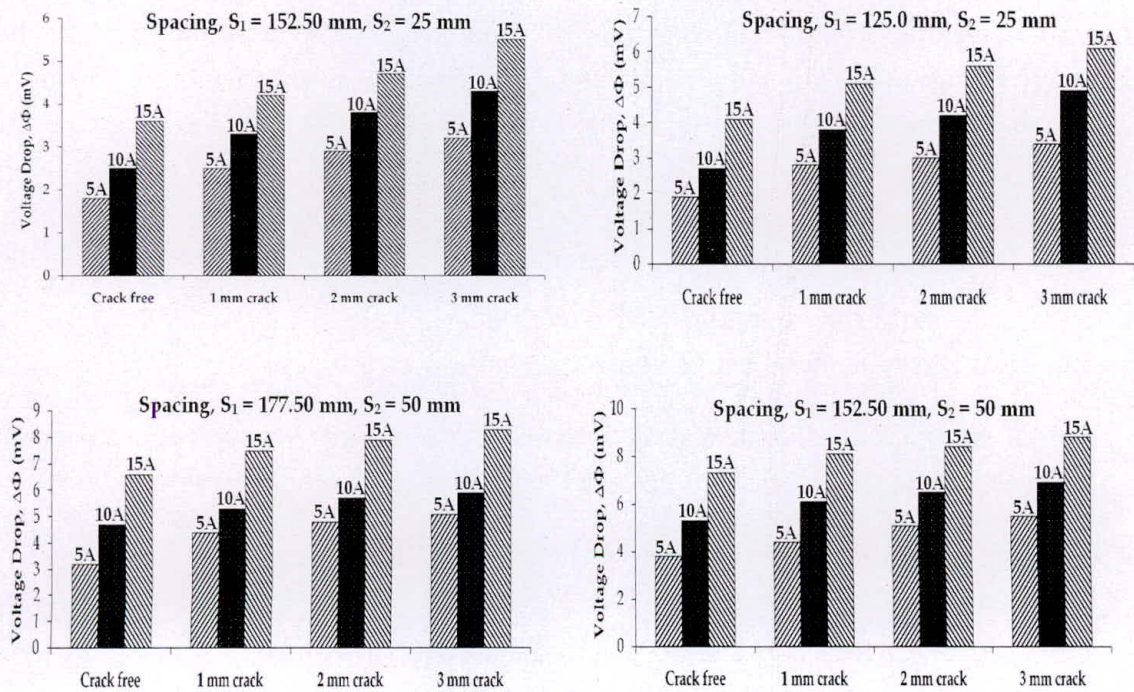


Fig. 4: Comparison of Voltage Drop on Different Conditions of SS Flat Bar

Conclusions

A low-current experimental setup for checking internal defects of metallic materials was developed using four-point probe technique. Variation of voltage drop on the same sample before and after cracking indicates the presence of defects of the material. The developed technique is capable to detect in homogeneity (defects) in the spare parts of the agricultural machinery.

Publication from this Research

M. Nur-A-Alam, Md. Rostom Ali and Murshed Alam. 2015. Nondestructive Approach to Evaluate Defects in Elements of Agricultural Machinery. AMA-Agricultural Mechanization in Asia Africa and Latin America. 46 (3), pp. 43-52, Japan.

References

- Ali, M. R., Saka, M. and Tohmyoh, H. 2010. Checking surface contamination and determination of electrical resistivity of oxide scale deposited on low carbon steel by DC potential drop method. *Materials Transactions*, 51(8): 1414-1419.
- Dover, W. D., Collins, R. and Michael, D. H. 1991. Review of developments in ACPD and ACFM. *British Journal of NDT*, 33(3):121-127.

An assessment of the Impacts of Hardrock Mining on Environments with Their Management Around the Maddhyapara Mining Area, Dinajpur, Bangladesh

M Farhad Howladar and Mohammed Omar Faruque

Location: Department of Petroleum and Mining Engineering, SUST, Sylhet.

Duration: Two years (2014-2016)

Expenditure of the project: Tk. 1000000.00

Introduction

The Maddhapara Hardrock Mine (MHM) is the first experience of hardrock mine and the second major mining project in the country, which is situated in Parbatipur Upazilla of Dinajpur district in the northern part of Bangladesh (Quamruzzaman *et. al.* 2012). As we know that underground mining operations and rock crushing plant bringing about changes to surface landforms, soil fertilities, Surface-underground water bodies, social environment and so on (Park 1987; Steve *et. al.* 2002). Although the same impacts due to man-made surface structures and other features are relatively well known and studied in Bangladesh, of environmental impacts related to underground hard-rock mining operations and rock crushing plant are not well known and have not yet been extensively performed in our country. Thus this present research work is one of the pioneer initiative to examine the occurrence of various impacts on surface landforms, soil fertilities, air qualities, surface-underground water bodies, social environment and so on through direct field investigations, laboratory analysis, community consultation, computer aided statistical analysis and Leopold matrix (1971) for impact assessment and so on in the area. At the end, the existing laws and recommendations have been examined and proposed the required recommendations and management plan to minimize the environmental dilemma and solve the recent mining operation related obligations and increase the mining operation for extracting natural resources

Objectives

The objectives of the research are:

- Interpreting land morphology before and after underground mining operation in the area;
- Clarifying the current water environment by analyzing the physical and chemical properties of surface and subsurface water samples;
- Assessing the soil standards in the area for viewing environmental quality;
- Investigating the noise level and recognize the present noise pollution around the area;
- Understanding the state of land, water level, availability of water, quality of water, mine water drainage system, water, sound level, and condition and also the socio-environmental development or degradation before and after the mine operations by extensive community consultation survey in the area.

Finally combining and comparing all of these results, the impacts of hardrock mining and rock crushing plant on environments and their management plan have been presented with necessary recommendation for safe and sound mining operations with avoiding the environmental and ecological damage around the area.

Methodology

The study employed two principal methods such as firstly the direct field investigation and secondly laboratory analysis and also other methods and techniques as specific purpose and circumstance necessitated to accomplish the objectives of the research. The summary of such methods are shown in Table-1.

Table 1. The summaries of different methods and their probable outputs of the research

Impact s on env ironment have been analyzed by the following methods	
Methods	Probable out put
Desk study and reviewing the previous reports	Understanding all about the research area, previous research and others
Direct field study	Understanding the field status around the research area, collecting various (field photographs, soil, water, sound and and research relevant samples and data
Laboratory analysis	Understanding the physical and chemical properties of soil, water and rock dust.
Questionnaires survey/community consultation	Understanding the state of land, water level, availability of water, quality of water, mine water drainage system, water condition and also the socio -environmental development or degradation before and after the mine operations by Questionnaires survey in the area.
Computer aided statistical analysis	Compare and correlating the lab oratory test results with their justification
Application of Leopold matrix (1971)	Assess the mining industries impacts on environment

Results

Physical and chemical impacts of this mine operation typically include those on geology, soils, topography, landforms, meteorology, climate, air and water quality, and noise. These impacts for this particular case are estimated as follows:

- Hard rock mining and its impact on topography and landforms: Still there is no detectable impact of mine operations on local topography and landform observed around the mining area. Based on the present mine design and state of extraction there will have very minor possibility to deform the landforms for future.
- Hard rock mining and its impact on soils: The results of all soil analysis show that all the major chemical parameters such as Na⁺, Mg₂⁺, Zn₂⁺, Ca₂⁺, K⁺ are slightly varies from sample to sample but there is very small deficiency in nitrogen, phosphorus and sulfur. The concentration of As, K, Organic matter, Fe, P, Cu and Ni are in good condition. But, pH and S of soil is little higher than the recommended value of FAO in few areas near the mine which might be the indicator of soil pollution for the days coming days.
- Hard rock mining and its impact on water: The overall chemical characteristics of water bodies are not so much deviated consequently not harmful for agricultural utilization. But for drinking, it's pretty harmful and a little bit harmful for aquatic creatures. The pH in the study area varied from 5.3 to 9.02. Amongst all categories of data analyzed 9.68% of the samples have exceeded the maximum permissible limit. So, precaution should be taken immediately. Water should be treated properly before exposed to the surface otherwise it will become a great hazard for the human being, aquatic creatures and also for the agricultural activities around the mining area.
- Hard rock mining and its impact on Noise: In the field, the noise impacts occurred as a result of operation of heavy equipment, pile drivers, and onsite power generators. Estimated noise level ranges from 56.8-86.6 db around this mine. However the comparison of the maximum tolerable limit of noise for human being it is clear that sound level of the surrounding mine area is under tolerable condition and do not exceed the tolerable limit proposed by national and World Bank standard.
- Hard rock mining and its impact on air quality: There is no mentionable surface activities such as burning of vegetation, blasting and/or other refuse except crushing of rocks were observed around this mine. Therefore, the impacts on air quality problems will not be high to short lived
- Hard rock mining and its impact on ecosystems: In fact there is no significant changes of land pattern, water and soil environment hence the declination of vegetation coverage, the change of biological species evolution, biomass and biological diversity, the risk of human health and the overall ecosystems around the mining area are considered to be in very minor scale.

- Hard rock mining and its impact on climate and meteorology: It is difficult to mention anything regarding the impacts of hard rock mining on climatic condition and Meteorology for local and short term case. Besides from the field visit and the previous operation records of the mine, it can be concluded that there is no noticeable activities to changes in surface environment and no aerodynamic disturbances except slight rock dusting. Thus the negative impacts on the climate and meteorology of the area might be negligible and not harmful to the surrounding environment.
- Hard rock mining and its impact on socio-economic environment: Scio-economic condition of the inhabitant of the study area had been determined by visiting the people around the study area and asking questions including their economic condition, physical condition, Income source, their view about the Granite mine, socio-economic development around the mine area etc. The results of this analysis replicates the overall socio-economic conditions are satisfactory according to their opinion.
- Overall environment impact assessment by Leopold matrix: The Leopold matrix analysis implied the impacting scores are +125 which is favorable for environmental safety as a whole. Moreover considering the overall situation of environment around the mine, this research recommends a possible management plan (Fig. 1) for the sound mine operation with safe and green environment.

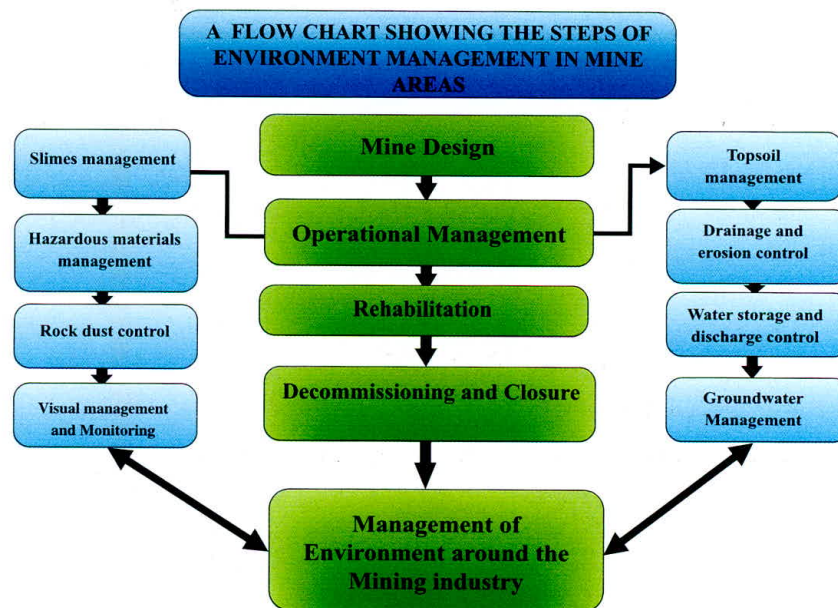


Fig. 1: A flow chart showing Sustainable environment management plan.

Conclusions

The research work has been conducted for understanding current state of environment around the hard rock mining area. To carry out the research, a number of methods have been applied and the results implied that the overall environmental state is good.

In addition, the Leopold matrix analysis reflected the positive impacting +125 which is significantly favorable for environmental development and safety as a whole. But mine has several minor impacts on the surrounding environment so some precautionary steps e.g. treatment of mine water is necessary and a proper infrastructure for rock stocking is also required. If proper steps are not taken in near future condition may get worse. The study area is fully agriculture dependent so the necessary steps should be taken quickly.

Publication from this Research

- Howladar, M. F., Rahman, M. M. 2016. Characterization of underground tunnel water hydrochemical system and uses through multivariate statistical methods: a case study from Maddhapara Granite Mine, Dinajpur, Bangladesh, *Environ Earth Sci.*, 75:1501, DOI 10.1007/s12665-016-6309-7 (Springer, IF: 1.57).
- Howladar, M. F., Numanbakth, M. A. A., and Faruque, M. O. 2017. An application of Water Quality Index (WQI) and Multivariate Statistics to evaluate Water Quality around Maddhapara Granite Mining Industrial Area, Dinajpur, Bangladesh, *Environ Syst Res.*, 6:13, DOI 10.1186/s40068-017-0090-9 (Springer).
- Numanbakth, M. A. A., Howladar, M. F., and Faruque, M. O. 2016. Water Quality Evaluation in Terms of Water Quality Index and Statistical Approach around the Maddhapara Granite Mine Industrial Area, Dinajpur, Bangladesh, International Conference on Botanical pesticides and environmental sustainability, Rajshahi University, 24-25 Sep, 2016, pp 43.
- Rahman, M. M., Howladar, M. F., and Faruque, M. O. 2016. Soil Quality Assessment for Agricultural Purposes Around the Barapukuria Coal Mine Industrial Area, Bangladesh: Insights from Chemical and Multivariate Statistical Analysis, International Conference on Botanical pesticides and environmental sustainability, Rajshahi University, 24-25 Sep, 2016, pp. 62.

References

- Park D.W., 1987. Effect of mine subsidence on ground water hydrology, 08. Society of Mining Engineers. AIME, pp 87-98.
- Quamruzzaman C., Kabir A.K.M.F., Malek M.A., Hossain B.M.R, and Woobaidullah A.S.M., 2012. Ventilation shaft construction by conventional freezing method in Maddhapara Granite Mine, Bangladesh, *Journal of Mechanical and Civil Engineering*, 2 (4), pp. 07-13.
- Steve Blodgett M.S., James R, Kuipers PE 2002. Technical report on underground hard-rock mining: subsidence and hydrologic environmental impacts, vol 50. Center for Science in Public Participation, Bozeman, pp 01-72.

Grants for Advanced Research in Education