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Preface

The Ministry of Science and Technology has been funding small projects for Research & Development (R&D) since the 70s. Experience has shown that there is a wide gap between the allocated research grants and the demand for the scientific community. The budget head for R&D was separated in 1993, but a large gap still remained between demand and allocation. Attempts have been taken to minimize this gap and special support to scientific and technological research has been emphasized over the years. The necessity of promoting scientific research for the development of Science and Technology was recognized and the Honorable Prime Minister Sheikh Hasina is increasing special research grants every year to encourage researchers and also handed over the cheque to the researchers formally with her kind presence. For this remarkable task, we are offering humble tribute to Honorable Prime Minister on behalf of the Ministry. We have also experienced a proactive outlook of the Ministry of Finance towards financing for R&D and the last year allocated was Tk. 15 Core. We express our gratitude and profound thanks to them.

Sustainable Development Goals targets 17.6 and 17.8 aim to enhance North-South, South-South and triangular regional and international cooperation on and access to science, technology and innovation. Present Bangladesh Government has given more emphasis on Science and Technology considering their direct association with development.

In this new millennium, the world has been experiencing a rapid transformation of society that is becoming knowledge based. In this context, scientific and technological knowledge, experiences and expertises have become the crucial elements in production system. Research has a significant role to improve the existing scientific and technological knowledge and enhance human's inexhaustible creativity.

The unique arrangement of providing fund for conducting scientific and technological research has created new opportunities and enthusiasm for research in Bangladesh. Ministry Science and Technology provided fund for research in Biological, Medical, Environmental, Engineering, Physical science and other Inter-disciplinary group to develop qualified manpower in crucial areas of science and technology.

The special allocation for science and technology research has generated a high level of enthusiasm among the scientific community in last financial year (2016-2017). Eleven crore twenty seven lacs (11,27,00,000) BDT are financed among 383 research projects. Among these research projects, 23 research findings are selected for the first time to publish as a Journal. The selection procedures are done by a learned editorial board. This Journal will add new information to research domain of the world and help scientists, academicians and other researchers in their future research

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SL	TABLE OF CONTENTS	PAGE NO

Ministry of Science and Technology

Govt. of The People's Republic of Bangladesh
Bangladesh Secretariat, Dhaka. www.most.gov.bd

Ministry of Science and Technology
Govt. of The People's Republic of Bangladesh
Bangladesh Secretariat, Dhaka. www.most.gov.bd

Bioremediation of Heavy Metals Using Air-Liquid Biofilms Produced by *Enterobacter asburiae, Vitreoscilla* sp., *Acinetobacter lwoffii and Klebsiella pneumoniae*

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Abstract

Bacterial biofilm plays a pivotal role in remediation of toxic heavy metals from wastewater. In this study, we quantified the effect of different concentrations (0, 500, 750, 1000, 1250, 1500, 1750 and 2000 mg/L) of Cu, Zn, Pb, Ni and Cr on biofilm formation by *Enterobacter asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *Acinetobacte*r *lwoffii* ENSG302 and *Klebsiella pneomoniae* ENSG303. It was observed that several concentrations of Cu, Zn, Pb, Ni and Cr significantly induced the biofilms at the *a*ir-*l*iquid (AL) or *s*olid-*a*ir-*l*iquid (SAL) interface in these bacteria. Minimum biofilm-inhibitory heavy metal concentration was also determined. When these biofilms were applied to remove these heavy metals, a significant amount of Cu (93.4 to 94.7%), Zn (84 to 91 %), Pb (83.1 to 94.2%), Ni (85.8 to 88.1%) and Cr (71 to 78.6%) were removed from the contaminated media. Thus, biofilm produced by *E. asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneomoniae* ENSG303 might be applied to remove the heavy metals from the wastewater.

Introduction

Indiscriminate discharge of heavy metals into soil and water bodies, pose threat to human health and overall biodiversity. Numerous physico-chemical methods are applied to remove the heavy metals from soil and wastewater worldwide. However, most of these techniques are very expensive and ineffective when the concentration of heavy metals is very low i.e., less than 100 mg/L (Dixit *et al.,* 2015). Besides, bioremediation (use of biocatalysts, i.e., enzymes or whole living microbial cells) is an innovative, effective, minimally hazardous, economical, versatile and eco-friendly promising technology. Whole cells as biocatalysts is often preferred over enzymes but it has also some drawbacks (Buchholz *et al.,* 2012). Biofilms are structured, surface-associated, multicellular bacterial communities, surrounded by the self-produced *e*xtracellular *p*olymeric *s*ubstances (EPS). EPS comprises of exopolysaccharides (mainly cellulose), proteins, and extracellular DNA (Flemming and Wingender, 2010; Haque *et al.,* 2009; 2017). Among them, the polysaccharide specifically binds to heavy metals (Li and Yu, 2014). Despite these advantages, bacterial biofilms have been appreciated and applied to remove the heavy metals (Singh *et al.,* 2006; Edwards and Kjellerup, 2013). However, only a few bacterial biofilms, including *Acinetobacter calcoaceticus*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli*, *Pseudomonas putida*, *P. aeruginosa*, and *Rhodococcus* sp. have been found to remove the heavy metals (Pal and Paul, 2008).

Bacterial biofilms in resistance to toxic heavy metals is well documented (Teitzel and Parsek, 2003). However, information regarding the role of different concentrations of toxic heavy metals on biofilm formation is only poorly understood (Koechler *et al.,* 2015). In this study, we quantified the effect of different concentrations (0, 500, 750, 1000, 1250, 1500, 1750 and 2000 mg/L) of Cu, Zn, Pb, Ni and Cr on biofilm formation by *Enterobacter asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *Acinetobacter lwoffii* ENSG302 and *Klebsiella pneomoniae* ENSG303. Minimum biofilm-inhibitory heavy metal concentration was also determined. We also applied these biofilms to remove these heavy metals from the contaminated media.

Materials and methods

Heavy metals on biofilm formation

Four AL biofilm producing bacteria viz., *E. asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneumoniae* ENSG303 were used in this study. In order to quantify the effect of different concentrations (0, 500, 750, 1000, 1250, 1500, 1750, 2000 mg/L) of Cu (copper sulphate), Zn (zinc sulphate), Pb (lead nitrate), Ni (nickel chloride) and Cr (potassium dichromate) on biofilm formation initially, a single colony of each bacterium was grown in *y*east extract *p*eptone (YP) broth overnight in shaking condition at 28ºC. Then 50 µL cultures of each bacterium were inoculated in the glass test tubes containing 5 mL of *s*alt *o*ptimized *b*roth [SOB (without magnesium)] plus *g*lycerol (SOBG) with varying concentrations of heavy metal salts as mentioned above. The test tubes were incubated at 28°C in static condition and the photographs were taken after 72 h incubation,. Biomass of the AL- and SAL biofilms was quantified as described in Haque *et al*. (2012; 2015).

Determination of minimum biofilm-inhibitory heavy metal concentration

*M*inimum *b*iofilm-*i*nhibitory *h*eavy *m*etal *c*oncentration (MBIHMC) is defined as the lowest concentration of a heavy metal that will inhibit the visible biofilm growth either AL biofilm or SAL biofilm of a bacterium. All these bacteria were inoculated in magnesium-deprived SOBG broth with different concentrations (0 to 2500 mg/L) of the heavy metal salts as mentioned above. MBIHMC of Cu, Zn, Pb, Ni and Cr was determined after 72 h incubation at 28°C in static condition.

Removal of Cu, Zn, Pb, Ni and Cr from contaminated media

A single colony of each bacterium was grown in YP broth overnight at 28°C in shaking condition. Afterword, 50 µL cultures (OD_{660} at 1.0) were inoculated in the glass test tubes containing 5 mL of SOBG (without magnesium) along with 500 mg/L of heavy metal salts as mentioned above. The test tubes were incubated at 28°C in static condition. After 72 h incubation, 1 mL suspension was collected from each treatment and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was collected and analyzed for Cu, Zn, Pb, Ni and Cr using atomic absorption spectrophotometer (VARIAN model AA2407). Each sample was analyzed three times to obtain representative results.

Statistical analysis

All the experiments were laid out in a complete randomized design with three replications and repeated at least three times unless otherwise stated. Analysis of variance, standard deviations and comparison of means were calculated with the statistical package "agricolae" of R software version 3.3.3. The means were compared by using Fisher's *l*east *s*ignificance *d*ifference (LSD) test (P value < 0.001).

Results and discussion

Several concentrations of Cu stimulates biofilm formation

E. asburiae ENSD102 produced the dense, robust and smooth AL biofilms in response to 500, 750 and 1250 mg/L of Cu, while they developed the skinny and delicate AL biofilms responding to 1500 and 1750 mg/L of Cu (Fig. 1A). This bacterium created a faint AL biofilm exposed to 2000 mg/L of Cu, (Fig. 1A). When quantified, compared to the absence of Cu, *E. asburiae* ENSD102 produced 3.39-, 4.61-, 5.4-, 7.53-, 3.38-, 3.37- and 2.07-fold more biomass biofilms responding to 500, 750, 1000, 1250, 1500, 1750 and 2000 mg/L of Cu, respectively (Fig. 1E). *Vitreoscilla* sp. ENSG301 constructed a tinny and rough surface AL biofilms responding to 750, 1000 and 1250 mg/L of Cu (Fig. 1B), whilst *Vitreoscilla* sp., generated the weak SAL biofilms in response to 1500 and 1750 mg/L of Cu (Fig. 1B). *Vitreoscilla* sp. ENSG301 developed 3.46-, 3.07-, 3.06-, 2.95-, 1.78-, and 1.57-fold higher biomass biofilms responding to 500, 750, 1000, 1250, 1500 and 1750 mg/L of Cu, respectively as compared to the absence of Cu (Fig. 1E). A thick, stout and smooth AL biofilm was also created by *A. lwoffii* ENSG302 increasing the Cu concentration from 0 to 500 mg/L (Fig. 1C), while they developed a thin and fragile AL biofilm in response to 750 mg/L Cu (Fig. 1C).

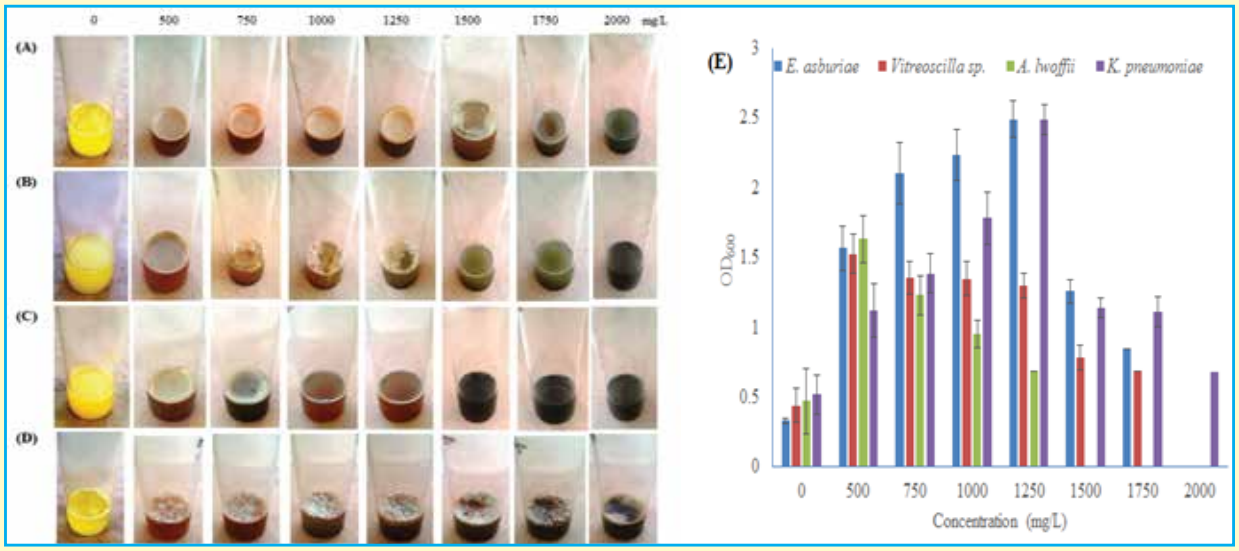

Fig. 1. Certain concentrations of Copper sulphate (mg/L) stimulates biofilm formation in *E. asburiae* ENSD102 (A), *Vitreoscilla* sp. ENSG301 (B), *A. lwoffii* ENSG302 (C) and *K. pneumoniae* ENSG303 (D). Amount of biomass biofilms produced by different bacteria (E). The photographs were taken after 72 h incubation at 28°C in static condition.

Conversely, 1000 and 1250 mg/L of Cu triggered the SAL biofilm formation (Fig. 1C) and 1500 mg/L of Cu prevented the biofilm formation in *A. lwoffii* ENSG302. *A. lwoffii* ENSG302 generated 3.47-, 2.62-, 2.03- and 1.46-fold increase biomass biofilms in response to 500, 750, 1000 and 1250 mg/L of Cu, respectively (Fig. 1E). Like *E. asburiae* ENSD102, increasing the Cu concentration from 500 to 1500 mg/L stimulated the rough surface AL biofilm formation in *K. pneumoniae* ENSG304 (Fig. 1D).

Nevertheless, the minimal biofilm inhibitory concentration (mg/L) of Cu for *E. asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneumonae* ENSG304 was 2100, 2000, 1500 and

2500, respectively (Table 1). Increasing the Cu^{2+} concentration from 50 to 100 µM increased the biofilm formation in *Xylella fastidiosa* strain Temecula, while higher concentrations (>200 µM) prevented the biofilm formation (Cobine *et al.,* 2013).

Numerous concentrations of Zn enhanced biofilm formation

E. asburiae ENSD102 produced the profuse and rough AL biofilms exposure to 500, 750, 1000, 1250 and 1500 mg/L of Zn (Fig. 2A), while *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneumoniae* ENSG304 developed the prolific and uneven AL biofilms responding to 500, 750, 1000 and 1250 mg/L of Zn (Fig. 2B-D). However, biofilm formation of *E. asburiae* ENSD102, *A. lwoffii* ENSG302 and *K. pneumoniae* ENSG304 was prevented by 2000 mg/L of Zn (Fig. 2A, C and D), while 1750 mg/L of Zn inhibited the biofilm formation in *Vitreoscilla* sp. ENSG301 (Fig. 2B). Thus, minimal biofilm inhibitory concentration (mg/L) of Zn for *E. asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneumoniae* ENSG304 was detected to 2000, 2000, 2000 and 1750, respectively. When quantified, biomass biofilm was also found to be significantly differed in these bacteria (Fig. 2E). *X. fastidiosa* also increased the biofilm formation when PD2 amended with 400 µM $ZnSO_4$ under flow conditions and with constant bacterial feeding (Navarrete and Fuente, 2014).

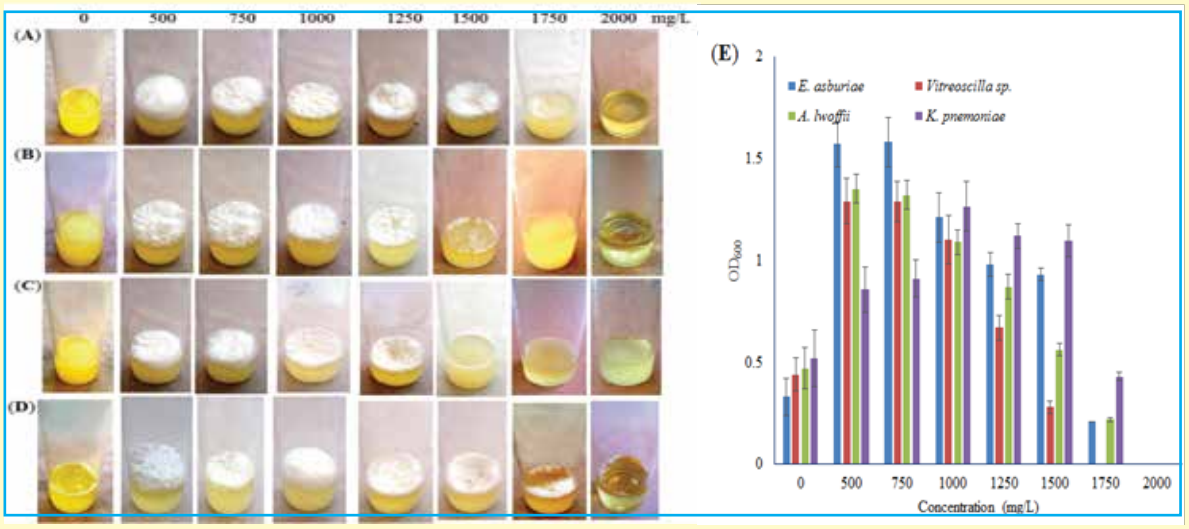

Fig. 2. Certain concentrations of zinc sulphate (mg/L) stimulates biofilm formation in *E. asburiae* ENSD102 (A), *Vitreoscilla* sp. ENSG301 (B), *A. lwoffii* ENSG302 (C) and *K. pneumoniae* ENSG303 (D). Biomass biofilms (E). The photographs were taken after 72 h incubation at 28°C in static condition.

Certain concentrations of Pb, Cr and Ni increased biofilm formation

None of the bacteria produced the AL biofilm in response to different concentrations of Pb, while certain concentrations of Pb triggered the SAL biofilm formation in these bacteria (Fig. 3). *E. asburiae* ENSD102 and *A. lwoffii* ENSG302 created the AL biofilms increasing the Ni concentrations up to 750 mg/L (Fig. 4A and E), while *Vitroscilla* sp. ENSG301 generated the AL biofilm in response to 500 mg/L of Ni (Fig. 4B). Conversely, *K. pneumoniae* ENSG394 formed the SAL biofilms up to 1750 mg/L of Ni (Fig. 4D). Certain concentrations of Cr also triggered the AL and/or SAL biofilm formation (data not shown). Thus, the effect of metal on biofilm formation might be depended on particular metal, concentration and bacterial strain.

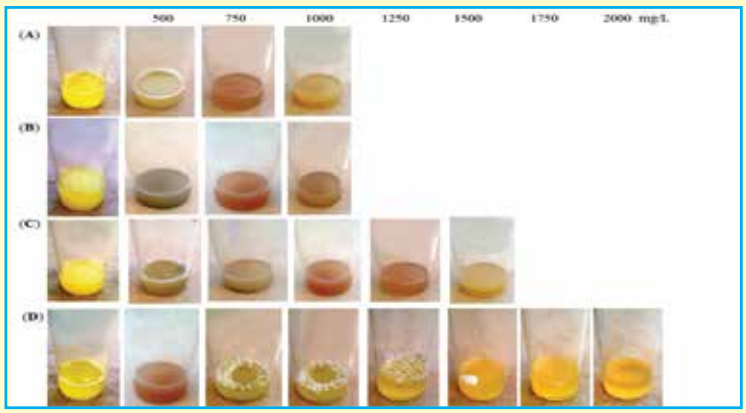

Fig. 3. Effect of different concentrations of Pb (mg/L) on biofilm formation in *E. asburiae* ENSD102 (A), *Vitreoscilla* sp. ENSG301 (B), *A. lwoffii* ENSG302 (C) and *K. pneumoniae* ENSG303 (D). Photographs were taken after 72 h incubation at 28°C in static condition.

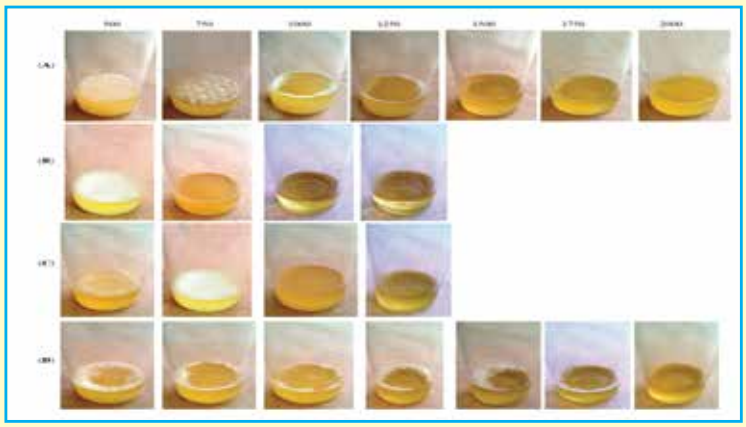

Fig. 4. Effect of different concentrations of Ni (mg/L) on biofilm formation in *E. asburiae* ENSD102 (A), *Vitreoscilla* sp. ENSG301 (B), *A. lwoffii* ENSG302 (C) and *K. pneumoniae* ENSG303 (D). Photographs were taken after 72 h incubation at 28°C in static condition.

Table I. Minimum biofilm-inhibitory heavy metal concentration (MBIHMC)

Bacterial strains	MBIHMC (mg/L)				
	Cu	Zn	Pb	Ni	Cr
E.asburiae	2000	2000	1000	1750	1250
Vitreoscilla sp.	2000	1750	1000	1000	1000
A. lwoffii	1500	1750	1500	1000	1000
K. pneumoniae	2500	2000	1750	2000	1750

Table II. Removal of heavy metals by different bacterial biofilms

Bacteria	% removal				
	Cu	Zn	Pb	Ni	Cr
E.asburiae	93.8	91.0	84.48	85.76	75.2
Vitreoscilla sp.	94.0	84.4	94.2	87.48	73.2
A. lwoffii	93.6	84.8	83.16	88.16	78.6
K. pneumoniae	93.4	84.4	84.32	87.32	71.0

Removal of Cu, Zn, Pb, Ni and Cr from contaminated media

A significant amount of Cu (93.4 to 94.7%), Zn (84 to 91 %), Pb (83.1 to 94.2%), Ni (85.8 to 88.1%) and Cr (71 to 78.6%) were removed by these bacteria (Table 2). *E. asburiae* ENSD102 removed 93.8, 91.0, 88.48, 85.76 and 75.2% of Cu, Zn, Pb, Cr and Ni, respectively. Per cent removal of Cu, Zn, Pb, Ni and Cr by *Vitroscilla* sp. ENSG301 was 94, 84.4, 94.2, 87.48 and 73.2, respectively. *A. lwoffii* ENSH201 removed 93.8, 84.4, 83.16, 88.16 and 78.6% of Cu, Zn, Pb, Ni and Cr, respectively. *K. pneumonae* ENSG304 also removed 93.4, 84.4, 84.32, 87.32 and 71% of Cu, Zn, Pb, Cr and Ni, respectively. Thus, these bacterial biofilms might be used in remediation of heavy metals from contaminated sites.

Conclusions

Several concentrations of Cu, Zn, Pb, Ni and Cr stimulated the biofilm formation in *E. asburiae* ENSD102, *Vitreoscilla* sp. ENSG301, *A. lwoffii* ENSG302 and *K. pneomoniae* ENSG303. These bacterial biofilms also remove a significant amount of Cu (93.4 to 94.7%), Zn (84 to 91 %), Pb (83.1 to 94.2%), Ni (85.8 to 88.1%) and Cr (71 to 78.6%) from the contaminated media. Thus, all these biofilms might be applied to remove the heavy metals from the wastewater.

Acknowledgement

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Synthesis of Chitin-based Derivatives for Pharmaceutical Uses

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Abstract

Carboxymethyl chitin (CMCT) and carboxymethyl chitosan (CMCS) were synthesized and characterized by FTIR, XRD and the surface morphology were studied by SEM. Antibacterial activity of chitosan (CS), CMCT and CMCS were tested against both gram negative (*S. flexneri*, *E.faecalis*, *P. aeruginosa*,*K. pneumoniae*, *V.paraheamolyticus*)and gram positive (*S. aureus*, *B. subtilis*, *B. cereus*) bacterial strains. It has been observed that CS showsantimicrobial activity against all tested pathogenic bacteria, whereas CMCS shows antimicrobial activity against *S. flexneri,V.paraheamolyticus*and *B.cereus*. CMCT showsantimicrobial activity against *B.cereus* and *B. subtilis.*

Introduction

Chitin (CT) 1, is ß-(1-4)-2-acetamido-2-deoxy-D-glucose, is a common constituent of insect exoskeletons, shells of crustaceans and fungal cell walls (Wang *et al.,* 2006). CS2 is a polymer obtained by deacetylation of CT, is a cationic polysaccharide with linear chain consisting of β-(1,4)-linked 2-acetamino-2-deoxy-β-Dglucopyranose and 2-amino-2- deoxy-β-D-glucopyranose (Mathur *et al.,* 1990). CT and CS are promising natural biopolymers as it is characterized by biocompatibility, biodegradability, and nontoxicity and they have many biomedical applications (Pillai *et al.,* 2012). The insolubility of CT and CS, in water or in common organic solvents, limit their utilization for a specific application in industry and pharmaceutical uses. Chemical modification of CT and CS affords new derivatives having promising biological activities and physiochemical properties and these chemical modifications may enhance solubility in slightly acid, neutral and alkaline media (Inmaculada *et al.,* 2010). Many researchers reported the chemical modification of CT1 and CS2 such as N,N,N-trimethylchitosan (Muzzarelli *et al.,* 1988), N,O-acetylchitosan (Sashiwa *et al.,* 2002), N-acetylatedchitosan (Lu *et al.,* 2004) and N-carbxymethylchitosan (Kumirska *et al.,* 2011) weresynthesized to yield soluble CS derivatives. The aim of this study is to synthesis of chitin derivatives and their biological study. In our laboratory, we are now focusing on valuable derivatives of CT and CS, their biological properties, CS-composite for water purification and others application possibility (Islam *et al.,* 2011; Islam *et al.,* 2011; Islam *et al.,* 2011; Islam *et al.,* 2011; Siraj *et al.,* 2012 and Kabiraz *et al.,* 2016).

Materials and methods

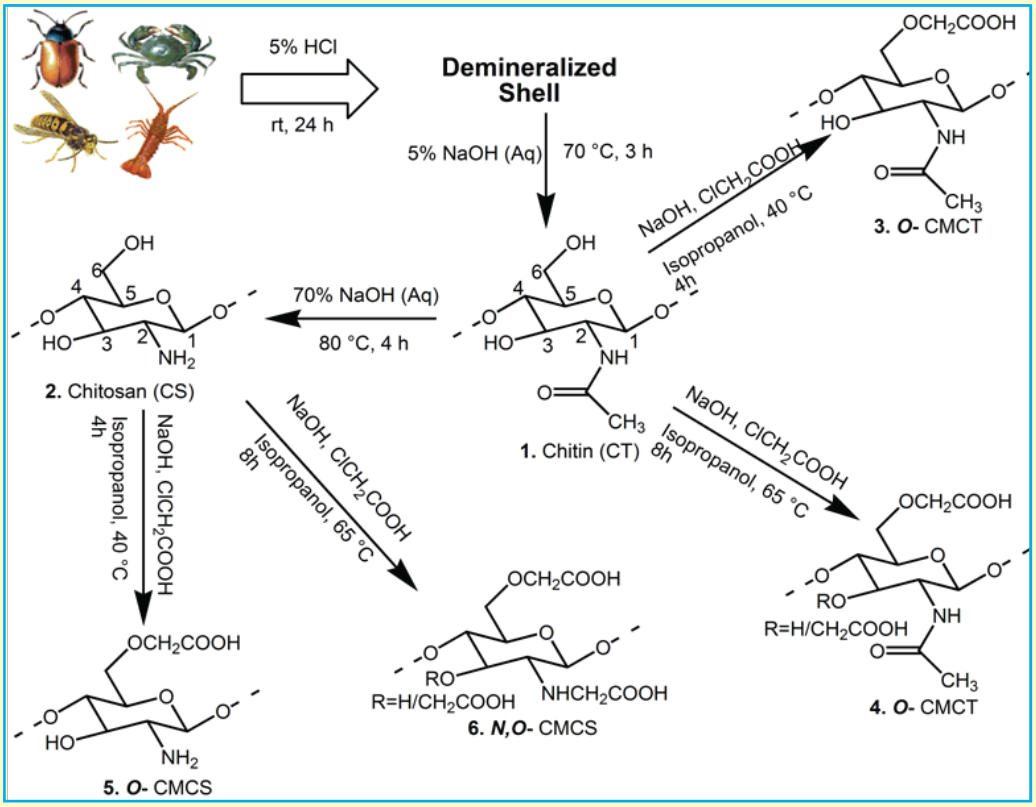
General

Fourier transform infrared spectroscopy (FTIR) was performed by using a Perkin Elmer Universal ATR spectrophotometer (UATR-FTIR, USA) equipped with a ZnSe crystal for the FTIR spectroscopy. X-ray diffraction (XRD) was collected on EMMA GBC, Australia at room temperature from 10º to 60º (2θ) in

0.02º step with a counting time of 4° per minute. Thermal properties were studied using different heating rate thermo-gravimeter (TG/DTA, 6300 Japan) and under heating rates of 5 (with data collecting rate of 40 points/K) and 10°C min^{-1} (collecting rate data of 60 points/K). SEM of all samples were taken by field emission scanning electron microscope (JSM-7610F, JAPAN); at voltage 10 kV.

Materials

Prawn (in Bengali Bagda) head peels were collected from Satkhira, Bangladesh. Head peels were scraped free of loose tissue, washed with cold water and dried in sun for 2 days. All chemicals were reagent grade and used without further purification.



Scheme 1: Preparation of CT **1**, CS **2** and their carboxymethyl derivatives **3**–**6**

Preparation of *O*-carboxymethyl chitin 3 (*O*-CMCT)

5 g CT was mixed thoroughly with 40 mL 40% (w/w) NaOH and kept overnight at -20ºC. 200 mL isopropyl alcohol (IPA)was added into the thawed chitin slurry. 28.8 g sodium monochloroacetate was added under stirring and the reaction system was stirred at 40ºC for 6 h and neutralized by HCl. The mixture was filtered, and the solid was collected, washed with 80% (v/v) alcohol/water and dried in oven.The dried product is *O*-CMCT **3**.

Preparation of *O*–carboxymethyl chitin 4 (*O*-CMCT)

2 g of CT was added to 50 mLIPAand stirred using magnetic stirrer at room temperature for 2 h in 500 mL round bottom flask. Then 80 mL of aqueousNaOH solution (60%) was added and refluxed at 85 ºC for 4

h.Then, 100 mLof aqueous sodium monochloroacetate (60% w/v) was added in five equal parts over a period of 10 min. The mixture was heated with stirring, at 65 ºC for further 8 h. The reaction mixture was then neutralized using HCl solution (4 M). After removalof the residue by filtration, the resulting carboxymethylchitin**4**was precipitated by adding MeOH. Theproduct was filtered, washed several times with a mixture ofCH_3OH/H_2O (1:1) and dried in oven.

Preparation of O-carboxymethyl chitosan 5 (O-CMCS)

5 g CS was mixed thoroughly with 40 mL 40% (w/w) NaOH and kept overnight at -20ºC. 200 mL IPA was added into the thawed CSslurry. 28.8 g sodium monochloroacetate was added under stirring and the reaction system was stirred at 40ºC for 6 h and neutralized by HCl. The mixture was filtered, and the solid wascollected, washed using 80% (v/v) alcohol/water and dried in oven. The product is *O*-CMCS **5**.

Preparation of N,O–carboxymethyl chitosan 6 (N,O-CMCS)

2 g of CS was added to 50 mLIPA and stirred using magnetic stirrer at room temperature for 2 h in 500 mL round bottom flask. Then 80 ml of aqueous NaOH solution (60%) was added and refluxed at 85 ºC for 4 h.Then, 100 ml of aqueous sodium monochloroacetate (60% w/v) was added in five equal parts over a period of 10 min. The mixture was heated with stirring, at 65 ºC for further 8 h. The reaction mixture was then neutralized using HCl solution (4 M). After removal of the undisclosed residue by filtration, the resulting carboxymethylchitosan was precipitated by adding MeOH. Theproduct was filtered, washed several times with a mixture ofCH_3OH/H_2O (1:1) and dried in oven.

Result and discussion

CT **1** and CS **2** were preparedfrom the indigenous shrimp processing waste as our previous reported procedure. Islam *et al.,* (2011) The typical production of CT **1** and CS **2** from crustacean shell generally consists of four basic steps: demineralization, deproteinization, discoloration and deacetylation. The C=O stretching region of the amide moiety, between 1700 and1500 cm^{-1}, yields different signatures for α- and β-chitin. For α-chitin, the amide I band is split into two components at 1660 and 1619 cm^{-1}due to the influence of hydrogen bonding or the presence ofan enol form of the amide moiety, Focher *et al.,* (1992) whereas for β-chitin it is at 1619 cm^{-1}. Theamide II band is observed in 1569 cm^{-1} for β-chitin. Infrared spectra of β-chitin reveal twoadditional bands for CHxdeformations at about 1416 and 1376 cm^{-1} and a greater number of narrowerbands in the C–O–C and C–O stretching vibration region (1150–950 cm^{-1}) observed in β-chitin. The efficiency of CS**2**production by the *N*-deacetylation of CT**1** was investigated by IR. During the *N*-deacetylation of CT, the band at 1619 cm^{-1} gradually decreased, while that at 1578 cm^{-1} increased, indicating the prevalence of NH_2 groups. The band at 1578 cm^{-1} displayed a greater intensity than the one at 1619 cm^{-1} and demonstrated the effective deacetylation of CT.The arising of a new band at 1578 cm^{-1} and the vanishing of the band at 1619 cm^{-1} are due to the NH_2 deformation, which predominates over the band at 1736 cm^{-1}. Thedegree of deacetylation (DA) of CS was calculated by using FTIR spectra from Eq. (1) Kaya *et al.,* 2015.

$$DA\% = 100 - [(A_{1655}/A_{3450}) * 100/1.33] \qquad (1)$$

where $A_{1655}$and A_{3450}were the absorbance at 1655 $cm^{-1}$of the amide I. The factor 1.33 denoted the value of the ratio of $A_{1655}/A_{3450}$for fully N-acetylated CS.

The average DA of CS samples were determined by using the following Eq. (2) Rahman *et al.,* 2015.

$$DA\ (\%) = C_{NaOH} \times (V_2 - V_1) \times 161/m \qquad (2)$$

Where C_{NaOH} was the concentration of sodium hydroxide; ($V_2 - V_1$) was the difference between the two inflection points; 161 was the molecular mass unit of CS; m was the mass of CS sample.The DA of CS**2** was calculated by using both IR spectrum (Equation 1) and titrimetric method (Equation 2) and the calculated DA is 80% and 78 % respectively. Average DA of CSassumed79%.FT-IR absorption bands (cm^{-1}) of product *O*-CMCT **3**were 3357 (O–Hstretching) and 3282 (N–H stretching), 2895 (C–H stretching), 1571 (C=O of –COOH antisymmetric stretching), 1416 (C=O of –COOH symmetric stretching), 1311 (C–N stretching), and 1055 (C–Ostretching). Compared with the CT spectrum, the new absorption bands of –COOH arestrong, and theO–HandN–Hbands become narrow and weak, both indicating a high carboxymethylation on –OH. Meanwhile, the bands at 1571cm^{-1} intensify significantly indicatingcarboxymethylation has occurred on the hydroxyl groups of CT.FT-IR absorption bands (cm^{-1}) of product *O*-CMCT **4**were 3257 (O–Hstretching) and 2865 (C–H stretching), 1579 (C=O of –COOH antisymmetric stretching), 1310 (C–N stretching), and 1056 (C–Ostretching). Compared with the CT spectrum, the new absorption bands of –COOH arestrong, and theO–HandN–Hbands become narrow and weak, both indicating a high carboxymethylation on –OH. This phenomena is similar to **3**.The broad peak of *O*–CMCT **4** at 3372 cm^{-1} is due to–OH stretching vibrations. The sharp absorption bands at 1372 cm^{-1} correspond to theCH_2bending vibration, respectively. The band 899 $cm^{-1}$is attributed to the C–C stretching vibration. The peak at 1724 cm^{-1} in the FTIR spectrum can be assigned to the C=O vibrational mode. Comparedwith the chitin spectrum, the new absorption bands of –COOHarestrong, and theO–HandN–Hbands become narrow and weak, both indicating a high carboxymethylation on –OH at 3 position.

The broad peak of *N,O*–CMCS**6** at 3410 cm^{-1} is due to–OH stretching vibrations. The sharp absorption bands at 1436 cm^{-1} correspond to theCH_2bending vibration, respectively. The peak observed at 1375 cm^{-1}isdue to the CH_2wagging vibration. The band 899 $cm^{-1}$is attributed to the C–C stretching vibration. The peak at 1737 cm^{-1} in the FTIR spectrum can be assigned to the C=O vibrational mode. The absorption band at 680 cm^{-1}isassigned to the out-of-plane bending vibration of carboxylate group.Comparedwiththe CS spectrum, the new absorption bands of –COOHarestrong, and theO–HandN–Hbands become narrow and weak, both indicating a high carboxymethylation on –OH or $–NH_2$. Meanwhile,the bands at 1737 $cm^{-1}$and 1216 cm^{-1} intensify significantly, thus indicating that carboxymethylation has occurred on both the aminoandthe hydroxyl groups of CS.[18] In addition, the bands corresponding to C=O of NH–C=O stretching and N–Hbending are overlapped with the much stronger C=O of –COOH antisymmetric stretching.

The degree of substitution (DS) was determined by using titrimetric method as reported procedure byusing the equation (3) for **3** and **4**; equation (4) for **5** and **6** (Ge and Luo 2005).

$$DS = 203A/(m - 58A) \quad (3); \qquad DS = 161A/(m - 58A) \quad (4)$$

where, $A = V_{NaOH} X C_{NaOH}$, V_{NaOH} is the volume difference of NaOH solution between the two inflection points and C_{NaOH} is the molarity of aqueous NaOH (0.05 mol/L) and m is the mass of **3**–**6** and 203, 161and 58 are the molecular weights of *N*-acetylglucosamine (CT skeleton unit), glucosamine (CS skeleton unit) and a carboxymethyl group, respectively.

The DS of **3** (0.66) is almost equal to that of **5** (0.68) indicating at same condition only C-6 hydroxyl hydrogen might be substituted by carboxymethyl group. In case of **4** (0.75) and **6** (0.89), at higher temperature (65 °C), for **4** both C-3, C-6 hydroxyl hydrogen have chance to substitute by carboxymethyl group. For **6** both C-2 (NH_2), C-3 and C-6 hydroxyl hydrogen have coincidental to substitute by carboxymethyl group and give higher value of DS (0.89).

XRD analysis was applied to detect the crystallinity of the isolated CT **1**and CS**2**. CT**1**show strong reflections at 2θ around 9–10º and2θ of 19–20º. CS**2**show reflections at 2θ around 10–11º and 2θ of 20–21º. The XRD pattern of both CT and CS are in good accordance with the literature data fromdifferent sources of CT **1**and CS **2**. Rahman *et al.,* 2015, **3** shows peaks on angle 2θ=26.8º,33.5º, 29.3º, 42.2º, 54.3º, 58.1º. **5** shows peaks on angle 2θ=32.2º, 45.8º, 56.2º,75.6º.The X-ray patterns of carboxymethyl chitin **4** shows peaks on angle 2θ=31.9º, 45.8º, 57.1º,77.9º. **6** did not show any crystalline peakand it is clear that, the carboxymethylation of CT and CS forced important changes in the arrayof the polymer chains in the solid state. In fact, the spectra of the **3**, **4**, **5** and **6** is exhibit poorly defined and less intense peaks as compared to the parent CT and CS. This is may be due to the presence of the carboxymethyl moieties which substitute the hydrogen atoms of the hydroxyl and amino groups of CS. Thus, as the carboxymethyl groups are much largerthan the hydrogen atoms, an important excluded volume effect occurs and a polyelectrolyte effect must also be considered due to the presence of charged groups in the chains of CMCS. The decrease in crystallinity of derivatives was considered tobe due to the deformation of the strong hydrogenbond in the CT and CS backbone. This result means thatcarboxymethyl of CT and CS derivatives were more amorphous than CT and CS. Kim *et al,* observed similar phenomena in case of water-soluble chitin derivatives and reported triethylaminoethyl–chitin (TEAE–chitin) didn't show any crystalline peak Kim *et al.,* 1997.

SEM was used to analyze the structure and morphology of the CT, CS and their derivatives **3**–**6**. CT**1** shows a more compact, denser structure, with layers of crumbling flake without porosity. CS**2** shows non homogenous and non-smooth surface with straps and shrinkage. **3** exhibits irregular, rough and wrinkle surface without smear layer and ice melting type.**4** shows a prominent arranged microfibrillar crystalline structure with porous surface. The surface morphology of the **5**hasnonsmooth porous surface. The SEM micrographs of the **6** showed an irregular non smooth surface.

TG and DTG curves of CT, CS and their derivatives **3**–**6** show the initial weight loss (below 120°C), observed in all compounds, can be attributed to the loss of moisturebecausepolysaccharides usually have a strong affinity for water and therefore may be easily hydrated. The second(main) step includes both decomposition and oxidation reactions of the prepared compounds. In the last stage, there is almost a complete degradation of intermediates generated earlier at lower temperatures. In case of **6**, we observed exceptional second stage from 120 °C. It is may be due to the change of thestructure of the material and the change of the mechanismof its thermal degradation process.

The antibacterial activity of the CS **2** and the prepared derivatives **3**–**6** were tested against eight bacterial strains as reported procedure. Islam *et al.,* 2011; Islam *et al.,* 2011. The five gram negative bacteria were *Shigellaflexneri* ATCC 12022, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853,*Klebsiella pneumoniae* ATCC 13883, *Vibrio paraheamolyticus* ATCC 17802, and gram positive three were *Staphylococcus aureus* ATCC 9144, *Bacillus subtilis* ATCC 11774 , *Bacillus cereus* ATCC 10876in Muller–Hinton (M–H) broth. CS **2** exhibited activity against all eight pathogens and the highest zone of inhibition was observed against*S. aureus*(35 mm). The *O*-CMCT**3** only shows antimicrobial activity against*B. subtilis* (13 mm), whereas *O*-CMCT **4** shows antimicrobial activity against three pathogens, *S. flexneri* (11 mm), *V. paraheamolyticus*(14 mm) and *B. cereus*(12 mm).*O*-CMCS **5** shows antimicrobial activity against*B. subtilis (12 mm)* and*B. cereus* (12 mm) and *N,O*-CMCS **6**shows antimicrobial activity against three pathogens such as *P. aeruginosa*(14 mm), *K. pneumoniae* (12 mm)and*S. aureus* (12 mm).M. Kaya *et al. also* observed similar phenomena and reported decrease of antibacterial activity of CMCS than CS Kaya *et al.,* 2215.

Conclusion

Synthesis of carboxymethyl, phthalic anhydride, trimellitic anhydride, succinic anhydride derivatives of CT and CS from indigenous shrimp processing waste are in progress in our laboratory to improve its solubility in different solvents, biological properties and would be published in elsewhere.

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Environmental Challenges and Solution of Increasing Solar Home System (SHS) in Bangladesh

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Abstract

The solar home systems (SHS) in Bangladesh have received applaud for its use of renewable energy. But the proponents of SHS largely ignored the problems of the recycling of used lead-acid battery (ULAB) that is employed in this system. Improper recycling of ULABs can result in environmental disaster and human health hazards. In this study, the current practices of the recycling of ULABs from SHS are examined. This study is explorative in nature and both qualitative and quantitative research methodology were applied to complete this. A semi-structured interview of 25 stakeholders of ULAB recycling was conducted and analyzed in Nvivo to find out the current practices in recycling of ULABs used in SHS followed by an excel modeling tool to find data on the generation of waste by the solar home systems (SHS). The study found that the ULAB recycling sector is largely dominated by informal recyclers. As the Informal recyclers don't comply with any environmental safety and regulations, a large amount of lead and electrolyte from each recycled ULAB is going to the earth every year. According to our calculation, up to 22,119 metric ton wastage will be dumped by ULABs of SHS by 2020. Besides, Informal recyclers use coal inefficiently to extract lead from the lead paste, which harms the surrounding environment. The study also found that there is reluctance by the battery producers in collecting ULABs and in their absence, there has been a prevalence of brokers in the collection of ULABs used in SHS. Some policy level decisions are needed to be taken to mitigate these risky and unsystematic practices.

Introduction

Throughout the world, the concept of generating electric power through solar energy has gained traction in the recent times (Modi, Bühler, Andreasen, & Haglind, 2017). Keeping up with the world trend, Bangladesh is also observing the rise in the use of solar energy to produce electricity. The use of solar home system (SHS) has received attention because its Photovoltaic (PV) based generation of energy using renewable energy source like sunlight (Taheruzzaman & Janik, 2016). The SHS program of Bangladesh is being led by Infrastructure Development Company Limited (IDCOL) which is a government subscribed non-bank financial institution (NBFI) (IDCOL, 2017, 2017). Currently, the total number of solar home systems (SHS) in Bangladesh is close to 4.5 million and the total power generated by these is amounted to 179 MW (SREDA, 2017). The number of total SHS is expected to rise to 6 million by 2020 (Pavel, 2016) and it will contribute to 10% of the total electricity production of Bangladesh by that year (SREDA, 2016).

Solar Home Systems (SHS) installed in Bangladesh are basically standalone solar power systems. The main objective of SHS is to provide off-grid electric energy to the households in rural areas. The essential components of an SHS consists of a solar PV panel, a 12 V battery and a charge controller (Chowdhury & Mourshed, 2016, p. 586; IDCOL, 2017d). The proliferation in the use of solar powered energy has received some scrutinization in the literature, especially regarding the improper recycling of ULABs in the SHS(Gottesfeld & Cherry, 2011). The improper recycling can eventually result in the cognitive deficiencies among the adults and children. In particular, the children living close to the area of ULAB recycling is vulnerable (Albalak, Rebecca, Noonan, & Buchanan, 2003; De freitas *et al*., 2007).It has been observed that people living around battery repair workshop, especially children are significantly prone to high blood lead levels (T. Ahmad, Mumtaz, Ahmad, & Rashid, 2017). Also, high blood lead level and illnesses attributable to lead toxicity were observed among the workers of the lead acid battery manufacturing industries which included "frequent headache, numbness of the limbs, colic pain, nausea, tremor, and lead line on the gum" (S. A. Ahmad *et al*., 2014, p. 1)manufacture of LAB is increasing. Most of the lead used by these industries comes from recycling of LAB. Workers in LAB industry are at risk of exposure lead and thus development of lead toxicity. OBJECTIVE The objective of this study was to measure the blood lead concentration and to assess the magnitude of health problems attributable to lead toxicity among the LAB manufacturing workers. METHODS A cross-sectional study was conducted among the workers of LAB manufacturing industries located in Dhaka city. RESULT Mean blood lead level (BLL. Lead sulfate contamination in lands and water bodies were identified by IDCOL of Bangladesh as a direct result of improper disposal and recycling of lead-acid batteries. According to a report by Green Earth, used lead-acid battery (ULAB) recycling industry is the world's top polluting industry in 2016. Lead smelting industry was also ranked in the top three most pollution causing industries (Pure Earth & Green Cross Switzerland, 2016).This is to be kept in mind that Lead sulfate has the ability to contaminate groundwater. In a densely populated country like Bangladesh, lead contamination through improper recycling has the potential to become an emergency as a similar case was observed in the heavily populated Uruguayan capital where lead contamination became "an environmental, sanitary, and social emergency" (Mañay, Cousillas, Alvarez, & Heller, 2008, p. 95). Except for few reports, this concern has been mostly ignored by the researchers in the context of Bangladesh (GDI, 2015; Waste Concern, 2005).

This research has tried to shed light on this long-ignored topic of major concern. Considering the significance of proper recycling process of ULAB, this study aims to find out the current practices of recycling batteries used in the solar home systems in Bangladesh, forecast the amount of wastage by the year 2020 and some policy level suggestions has been provided to mitigate the risks of current situations.

Materials and methods

Due to the lack of previous studies on this topic in Bangladesh, an exploratory descriptive qualitative research was deemed necessary. Three stakeholders were selected from the literature(Clean Energy Solutions Centre, 2016). They were customers, Distributors/retailers, and recyclers. Only Informal recyclers were chosen because they are known for not complying with the environmental standards. And also because there are few studies on their recycling practices in contrast to formal recyclers. Another major type of stakeholders were identified during the study referred to as "brokers". Brokers were also interviewed in this research.

A semi-structured interview was conducted using a purposive sampling procedure. The interview was administered to 25 stakeholders. Among them there were 6 Customers, 11 distributors/retailers, 3 brokers and 5 informal recyclers. Although this research is constrained by small sample size of the respondents, data was collected from all the major parties involved in the recycling process of used lead acid battery. The interview was conducted in three Upazilas of Bhola – Bhola Sadar, Char Fasson and Lalmohan. The district of Bhola was selected because there is an abundance of SHS in this area (IDCOL, 2017). For the interview of the informal recycler of battery, the industrial hub of southeast Bangladesh, Khulna was selected. Khulna was selected because the brokers told the researchers that they carry the used lead-acid battery to Khulna for the purpose of recycling. Thematic data analysis technique was employed to find out the emerging themes from the data. A Computer-assisted qualitative data analysis software (CAQDAS) named NVivo was used to identify the themes. The effectiveness of using the software in the data analysis process has been recognized in the literature(Richards & Richards, 1991).As for the quantitative analysis of data, a simple excel worksheet was used as a modeling tool. Data on the generation of waste by the solar home systems (SHS) was considered only from Bangladesh. Various government reports and articles published in international journals served as the primary data source for the quantitative analysis.

Results and discussion

Apart from four major stakeholders identified in previous research (Clean Energy Solutions Centre, 2016), our study identified another major kind of stakeholder in the ULABs recycling process. We refer to them as “Brokers”. Their main tasks involve collecting ULABs from distributors/retailers and getting them to recyclers, both formal and informal. The major findings of the study is discussed below.

Producers don’t collect their used lead-acid batteries

It has been observed from the field survey that producers don't collect their ULABs from the distributors/retailers. As one distributor put it, “Big battery companies just sell their batteries and they couldn’t care less about the old batteries”.

Collection of used lead-acid solar batteries are dominated by brokers

A prevalence of brokers was found in the collection of ULABs. Customers are interested in selling their old batteries from SHS and there is an abundance of brokers who will pay money to buy those old batteries or ULABs.

Electrolyte from used lead-acid battery is dumped on open space

Our study revealed that electrolyte from ULABs used in SHS was being dumped in open spaces such as open roads, drains and water bodies. This practice commenced when brokers started to throw away the electrolyte before weighing and buying ULABs from distributors/retailers.

Used lead-acid battery recycling activities are highly centralized

Although IDCOL’s SHS program has been established in all the districts of Bangladesh (IDCOL, 2017), majority of the battery recycling activities are taking place in the capital Dhaka and in the major industrial hubof Khulna in southeastern Bangladesh.

Many users are switching from SHS to only battery based system

An alarming trend for SHS is that many users are switching from SHS to only battery. Three general reasons for this have been identified from the study. Firstly, ULABs sellers are more interested in selling batteries to users than brokers because they can get good price that way. Secondly, users think it is costly to set up a solar home system. Thirdly, users think that using only battery is more convenient than using a SHS.

The life cycle of the lead acid battery is unpredictable

An important cause for consumer dissatisfaction as identified in our study is the unreliable nature of the lifetime of solar batteries. According to the consumers, distributors/retailers and brokers, It can range from 1-2 years to longer than 5 years.

Distributors/retailers wants a new battery recycling system in place

Distributors/retailers want a new system in place and also a better pay scheme for selling ULABs used in SHS. This demand indicates the growing dissatisfaction of distributors/retailers. As one of them put it, "Not every battery should sell for the same price, there is a question of quality. They (the brokers) come and buy everything on the basis of weight."

A large amount of ULABs are finding their way to the informal sector

Distributors/retailers are saying that almost half of the ULABs find their way to their original selling point. And the whereabouts of other half aren't known to them. Also, Brokers are selling ULABs to informal recyclers. No brokers told the researchers otherwise.

Informal recyclers are harming the environment with inefficient recycling technologies and disposal system and these practices are indirectly incentivized by the producers.

The Informal recyclers don't follow any environmental safety standards

The informal recyclers break the battery in their shop's backyard. Every part of the battery such as plastic case, separator, lead alloy and lead plates are reprocessed there for selling.

The informal recyclers use inefficient recycling technologies

The real problems the informal recyclers face is the extraction of lead from lead paste. It resembles ash so an earlier study referred it as lead ash (Waste Concern, 2005). The process of lead ash extraction can get messy so the recyclers do it at night in the open field of Gollamari, a significantly distant place from the city. "We do it at the dead of the night, mostly on weekends so that no one can see what we are doing." Coal is used in this process inefficiently to generate heat.

The disposing of waste by the informal recyclers isn't safe

Among the 450 kg solid waste collected from the Khulna city each day, 50-200 kg are from retail and sale market which includes Ismail Metal market and Sheikhpara market (Ahsan, Islam, & Shahriar, 2009). These markets are hub for informal recyclers. The informal recyclers said that for every 40 kg ash collected from the ULABs, 5kg ash is thrown directly to the dustbin. In that ash, at least 60% or 3 kg is lead.

Major buyers of recycled parts of ULAB are Producers, thus incentivizing the informal recyclers

The recovered lead is sold to some battery producers in Khulna; mainly Hamko battery and Lucas Battery. Sometimes, informal recyclers just sell out the lead ash instead of recycling. It depends on the quality. By buying the recycled parts of ULABs from the informal recyclers, the producers are indirectly incentivizing the environmentally unsafe practices of the informal recyclers.

In our quantitative research we estimated that approximately 22,119 metric tons of ULAB waste from SHS will go to earth by 2020. In the waste,approximately12, 403 metric ton is electrolyte, 840 metric ton is lead & lead alloy grid, and 8876metric tonis Lead Paste which will pollute the environment of Bangladesh.

Conclusion

Although there are many problems regarding the collection and proper recycling of used lead-acid battery, it can very well be solvable. The solutions to this problem can be the use of lithium ion batteries and solar mini-grid. An economic analysis of PV stand-alone system showed that lead-acid battery is cheaper than that of lithium-ion battery. But in terms of energy density, maintenance, environment friendliness and lifecycle, lithium-ion battery is more suitable (Anuphappharadorn, Sukchai, Sirisamphanwong and Ketjoy, 2014). It has been calculated that Li-ion battery, especially Li Fe SO4, can be a better replacement of conventional lead acid battery in the context of SHS(Podder & Khan, 2016). It has been observed from a study that solar PV minigrid is a viable technical option for rural electrification of rural markets (Khan *et al.,* 2016). Not only is that, grid-connected SHS is also a good options which reduces the need for a lead-acid battery and thus the reduction of complication associated with the recycling of those solar batteries (Mirhassani, Ong, Chong and Leong, 2015; Mohammed, Mohammed and Ibrahim, 2017). For the remote areas of Bangladesh, solar PV hybrid-grid has already been suggested back in 2009 (Bala & Siddique, 2009). A non-government utility company named PGEL has already implemented this technology to the remote Sandwip Island since 2010. However, we have to carefully observe the pros and cons of each of the approach before deciding on one or combining several solutions. We have observed several laws/guidelines and incentives related to the proper controlling of the recycling process of used lead acid battery (IDCOL, 2014). But the abundance of a large number of informal battery recyclers otherwise points to the ineffectiveness of those preventive measures. What we need right now is the proper placement and strict monitoring of the acts/laws already established. Above all, formalized method of the collection of Lead acid battery for the recyclers is needed to be ensured by the law.

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Carbon Sequestration Potentiality and It's Economic Analysis of Different Land Use Systems in the Northern Part of Bangladesh

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Abstract

This study was conducted to evaluate the carbon sequestration potentiality in different land use systems in the Northern part of Bangladesh. Common land use system (Cropland, roadside, homestead and orchard) were used. Data were recorded from tree growth parameters (height and diameter at breast height) and under storied vegetation (herbs, shrubs and crops) in order to estimate the total land use biomass accumulation. The results showed that there was significant difference of carbon sequestration potentiality of different land use systems. The highest total land use carbon sequestration (325.33 t/ha) was recorded from double roadside and the lowest (36.51 t/ha) was obtained from cropland. In case of the agro-ecological zones (AEZs), the highest carbon sequestration (155.23 t/ha) was obtained from AEZ- 25 and the lowest (109.28 t/ha) was recorded from AEZ-3. However, in terms of economic of carbon sequestration, double roadside gave maximum (4879.95$ t/ha) monitory returned. So, double roadside tree plantation is a better land use option for reducing atmospheric carbon. Therefore, more emphasis should be given in roadside plantation for mitigating the green house effects.

Introduction

The problem of global climate change is one of the most important to the environment. Again the elevated CO_2 of atmosphere is the main culprit for global climate change or Global warming. On the other hand, increasing demand for food, fodder, fuel and timber is increasing the pressure on land-use systems. Now sustainable development of land-use systems are critical for meeting those demands sustainably and stabilizing CO_2 concentration in the atmosphere to mitigate global climate change (Ravindranath and Madelene, 2008). The carbon storage capacity in different land use systems varies across species and geography (Newaj and Dhyani, 2008). Trees and shrubs in land use systems act as carbon sinks. Moreover, the amount of carbon in any land use system depends on the structure and function of different components within the systems put into practice (Schroeder, 1993; Albrecht and Kandji 2003). It is, therefore, important to take serious steps which contribute in fighting climate change through the role of land use practices to mitigation the climate change. However, in Bangladesh, the amounts of carbon sequestration by different land use system are unknown. The study therefore seeks to assess the

potentiality of different land use systems for carbon sequestration as a way of mitigating climate change through reduction of carbon dioxide in the atmosphere.

Materials and methods

The study was conducted in the northern part of Bangladesh located in the districts of Dinajpur. A stratified random sampling method was used in a randomized complete block design (RCBD) with three (3) replications as representative areas where different agroforestry land use systems like homestead agroforestry, road side agroforestry, cropland agroforestry and orchard based agroforestry are practiced. Indeed, Dinajpur district includes three Agro-ecological Regions: Old Himalyan Piedmont Plain (AEZ-1), Tista Meander Flood Plain (AEZ-3) and Level Barind Tract (AEZ-25) and land use practices vary among the AEZs. Therefore, one site from each of the AEZs was selected. The simple random sampling design was used to collect data from each sampling units. The biomass of tree is the sum of aboveground and belowground biomass content. The aboveground biomass (AGB) has been calculated by multiplying volume of biomass and wood density the volume were calculated based on diameter and height (Pandya *et al*., 2013). Belowground Tree biomass (BGB) was calculated by multiplying the above ground biomass (AGB) with a default value of 0.26, provided by Hangarge *et al*., 2012. Generally, for any plant species 50% of its biomass is considered as carbon storage (Pearson *et al.,* 2005). So, estimation of carbon stock in trees (tC/ha), this idea was used. Finally, to estimate the carbon sequestration in tree, the carbon biomass weight was multiplied with a factor of 3.67 as per by Rajput (2010).

Results and discussion

The study found that, the total land use system on carbon sequestrations per hectares (TLUCseq) was highly influenced by the effects of different land use systems (Table 1). The highest TLUCseq (325.33t/ha) was recorded from Double Roadside (T_3) which was followed by Single Roadside (T_2) and Homestead (T_4). However, the lowest TLUCseq (36.51t/ha) was recorded from Cropland (T_1) which was followed by Litchi orchard (T_5) and Mango orchard (T_6).

Table I. Effect of different land use system on Carbon sequestrations

Land Use System	NT/ha	TTCS (tC/ha)	LHG/RCS (tC/ha)	TTCseq (t/ha)	LGCseq (t/ha)	TLUCseq (t/ha)
Boundary Cropland (T_1)	158.9e	6.39d	3.56d	23.47d	13.05d	36.51c
Single Roadside (T_2)	420.0c	30.68b	11.52b	112.58b	42.29b	154.87b
	1166.7a	75.43a	13.22a	276.83a	48.51a	325.33a
Double Roadside (T_3)	988.9b	33.51b	7.69c	122.99b	28.22c	151.27b
Homestead Plant. (T_4)	216.6d	10.74cd	2.19e	39.62cd	8.03e	47.65c
	220.0d	14.64c	2.61e	53.72c	9.58e	63.30c
Litchi Orchard (T_5)						
Mango Orchard (T_6)						
Level of Sig.	***	***	***	***	***	***
CV%	21.7	19.9	4.5	19.9	4.5	16.1

In a column, figures having similar letter(s) do not differ significantly where as figure's bearing different letter(s) differ significantly (as per DMRT).
*** = significant at 0.1% level of probability.

The economic value of carbon sequestration provides market for GHG reduction in monetary value (Fig 1). According to Vivian (2010) 1 ton of carbon was sold at US$ 15. So, the highest carbon price (4879.95 $ t/ha) was recorded from Double roadside (T_3) which was followed by single roadside (T_2) and Homestead (T_4). On the other hand, the lowest carbon price (547.65 $ t/ha) was obtained from Cropland (T_1) which was followed by Litchi orchard (T_5) and Mango orchard (T_6). Vivian (2010) estimated the economic value of carbon trading for Kakamega forest and its environs. He observed that the carbon sequestration potential for Kakamega forest was 334Mg C/ha, then the economic value of carbon trading was US$ 5010 per hectare. On comparison to that of the farms which was US$ 3045 per hectare, it implies that the forest has a higher capacity to generate revenue to the country if it participated in carbon trading.

Conclusions

From the finding of this study it may be concluded that since forest plantations cannot be extended to many large areas of Bangladesh due to high population pressure and demand of agricultural land, roadside agroforestry land use system will be a better option for larger tree plantation coverage and reduction in GHGs effects.

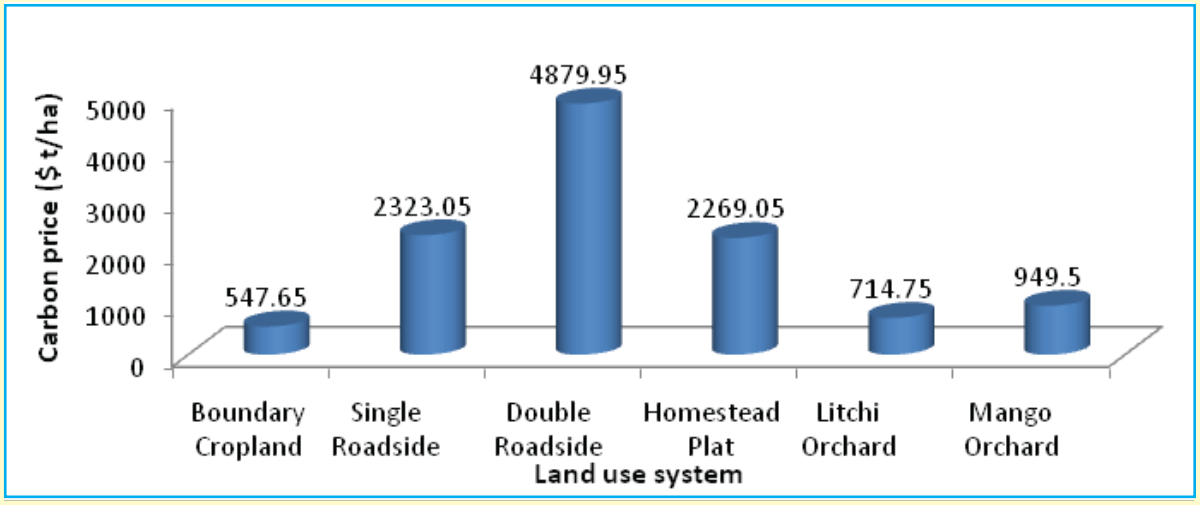

Fig. 1. Economic value of carbon sequestration (US$ t/ha)

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Integrated Rice Duck Farming to Ensure Food Security and Improving Flood Water Paddy Ecosystem through Reducing Methane Emission

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Abstract

Two field experiments were conducted (Boro season, 2016-17), at Showair and Tetulia Unions of Mohanganj Upazila of Netrokona district, nearby the Dingaputa haor, with a view to ensure local food security through increasing the overall productivity of rice duck farming and reducing CH_4 emissions from low lying wetland paddy conditions. The selected soil amendments bioslurry, oystershell, vermicompost and Azolla compost were applied in field plots one week prior to rice transplanting and Cyanobacterial mixture was inoculated in field plots after one week of rice transplanting. Fourteen days after rice transplanting in the field, ducklings (20-day-old) were released in the plots at the rate of 5 birds/ plot. Ducklings were kept in the plots for 2–4 hours a day (1st week), then allowed to remain in the plots from morning to evening and removed from the rice fields when the plants reached at flowering stage. At milking stage of rice growth, (April, 2017) the flash flood from the upper stream, probably Indian Meghalaya state and unnatural excessive rainfall severely damaged rice crop and grain yield. The organic C, T-N, Av.P, Av.S, exchangeable K, exchangeable Ca, TDS, dissolved iron, NH_4^+-N, and NO^-_3 –N concentrations in the flood water samples at milking stage of rice growth were significantly higher in all treatments compared to the permissible limits, whereas DO levels drastically dropped at1.5 ppm and the redox potential (Eh value) towards -250 mV to 300 mV, which caused significantly higher **CH_4** emissions from rice rhizosphere to the atmosphere. It is assumed that the fermentative bacteria consumed oxygen for the decomposition of organic materials in flood water paddy ecosystem, thereby caused intensive reductive conditions and released CO_2, CH_4, and H_2S gases which badly affected the aquatic fish population and ducklings' mortality rate (10-15%). Therefore, the integrated rice duck farming through combined application of vermicompost, azolla-cyanobacterial mixture and NPKS fertilizers (50% of the recommended doze) could be a feasible technique for food production as well as higher net returns compared to conventional sole rice cropping in the low lying haor areas of Bangladesh.

Keywords: Rice duck farming, **CH_4** emissions, food security Dingaputa haor

Introduction

Bangladesh is a rice-based country, where 80 % rice is grown under irrigated and rain fed conditions. However, methane (CH_4) emission is a major environmental problem for the low land haor paddy ecosystem. Bangladesh not yet developed any feasible bio-environmental friendly technology to mitigate methane emissions from wetland paddy ecosystem. The overall goal of the study is to minimize the methane emission from rice duck farming and sustaining agricultural productivity which will ultimately accelerate the socio-economic development and livelihood of the rice growers.

Materials and methods

Two field experiments were conducted at Showair and Tetulia Unions of Mohanganj Upazila of Netrokona district, nearby the Dingaputa haor. The experiment was designed with Randomized Complete Block Design (RCBD), having five (05) treatments, each replicated 3 times. There were fifteen (15) plots, each unit area 48 m^2. The rice cultivar used BRRI Dhan-28. The selected soil amendments such as bioslurry, Oystershell, Vermicompost and Azolla compost were applied in the selected rice field one week before rice transplanting (Ali *et al,* 2009). Standard basal fertilizer applied: N: P_2O_5: K_2O = 90: 45: 60 kg ha^{-1}

Experimental treatments:

T_1 : Control (Only basal doze of N,P, K,S applied)

T_2 : NPKS (100%) + Ducklings

T_3 : NPKS (50%) + Bioslurry with Oyster shell+ Ducklings

T_4 : NPKS (50%) + Vermicompost + Ducklings

T_5 : NPKS (50%) + Azolla compost with cyanobacteria + Duckling

Rice duck farming system

28 days old rice seedlings of cultivar BRRI Dhan-28were transplanted in the field at 25 cm x 25 cm spacing with two seedlings $hill^{-1}$. Bioslurry, Azolla compost and Vermicompost were applied in field plots one week prior to rice transplanting and Cyano-bacterial mixture was inoculated in field plots after one week of rice transplanting. Ten days after transplanting, 20-day-old ducklings were released in the plots at the rate of 400 birds/ ha. For the first three to five days, ducklings were kept in the plots for 2–4 hours a day. Later they were allowed to remain in the plots from morning to evening.

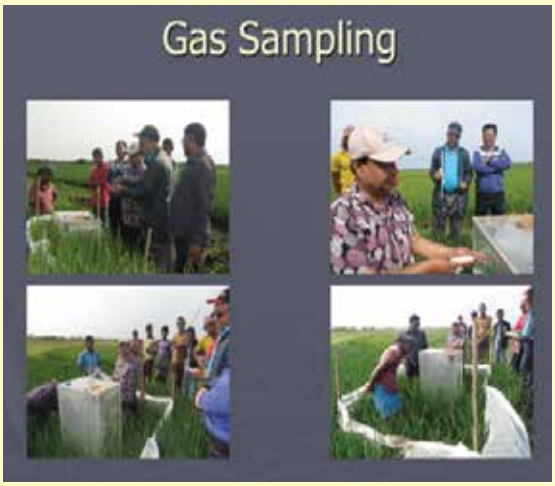
Methane Gas sampling and analysis

Gas samplings were conducted through closed-chamber method (Ali *et al.,* 2009) during rice cultivation.

Investigation of soil and flooded water properties

Soil redox potential (Eh), flood water pH, EC, TDS, iron conc. and DO conc. were measured at every week interval during rice cultivation. After rice harvesting, soil organic carbon (Walkley and Black method; Allison 1965), total-**N %** (Micro–Kjeldahl method, Keeney and Nelson, 1982), available P (Colorimetric method, Watanabe and Olsen, 1985) and available S (by the calcium chloride (0.15%) extraction method, (Williams and Steinbergs, 1959) were determined following standard methods. Ammonium (NH^+_4) concentration in water samples were determined by Indophenol blue method . NO^-_3 concentration in water samples were determined at 410 nm using a UV spectrophotometer (Brucine-sulfanilic acid method, Jenkins and Medsken, 1964).

Experimental Field Activities performed



Results and discussion

CH_4 flux measured at 21 days after rice transplanting was low, which increased significantly with plant growth and the development of soil reductive condition in both locations of Showair and Tetulia rice fields, respectively (Figure 1 a and 1b).The highest CH_4 peak was observed at flowering to milking/booting stage (77-91 days after rice transplanting) of rice plant in both locations. This was most probably due to the development of intense reduced conditions, e.g., Eh -200 mV to -230 mV (Figure 1a and 1b) in the rice rhizosphere. At ripening stage, methane emission rate decreased sharply in both locations, even though soil redox value was low enough to produce CH_4. This decline in CH_4 emission could be related to plant aging. Application of soil amendments such as bioslurry, vermicompost and Azolla compost released large amount of water soluble iron oxide, nitrate and sulfate which acted as oxidizing agent and electron acceptor, eventually reduced CH_4 emissions in amended plots compared to the control plots during the rice cultivation.

Table I. Rice yield, total seasonal CH_4 Flux and GHGI

Treatments	Flood water paddy field (Showair Union)				Flood water paddy field (Tetulia Union)			
	Grain yield (kg/ha)	Yield Index	Total CH4 flux (Kg CH4 /ha/season)	GHGI CH4 evolved (Kg CH4/Kg grain yield)	Grain yield (kg/ha)	Yield Index	Total CH4 flux (Kg CH4 /ha/season)	GHGI CH4 evolved (Kg CH4/Kg grain yield)
T1	2210	100	239	0.108	2190	100	253	0.116
T2	2570	116	195	0.076	2450	112	208	0.085
T3	2430	110	247	0.101	2660	121	263	0.098
T4	2750	124	176	0.064	2830	129	185	0.065
T5	2620	119	192	0.0732	2780	127	182	0.065

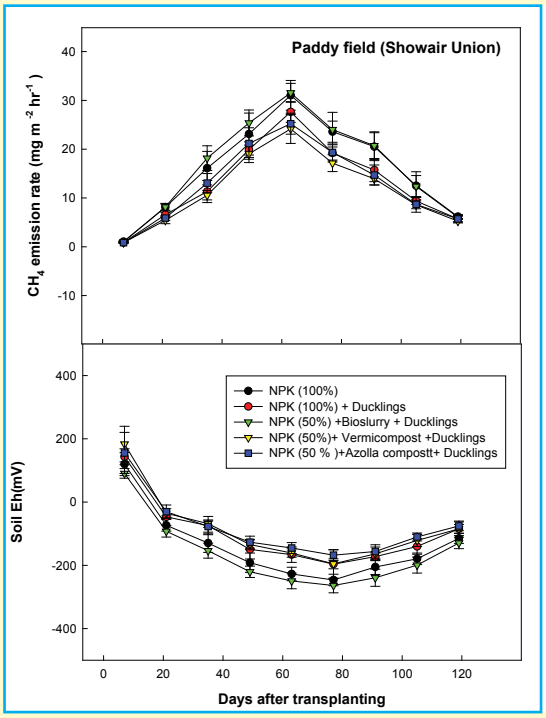


Fig.1(a). Trends of CH4 emission rate and soil redox potentials (Eh) during rice growing season (Showair Union)

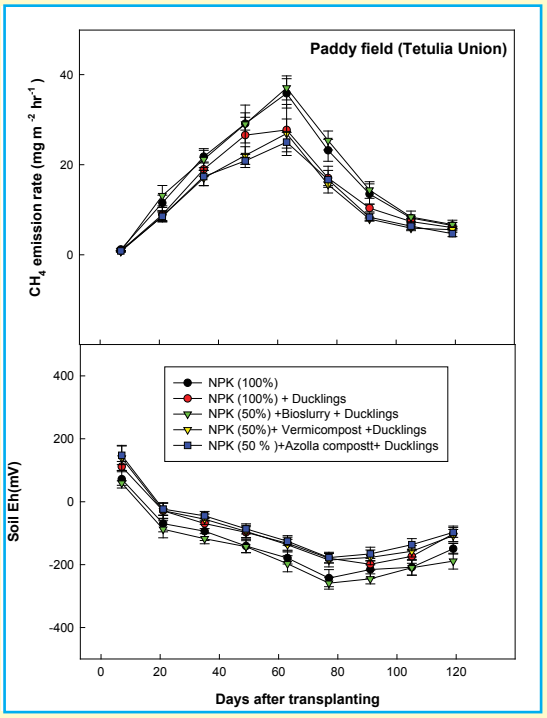


Fig.1(b). Trends of CH4 emission rate and soil redox potentials (Eh) during rice growing season (Tetulia Union)

Table II. Rice duck farming productivity, net profit and benefit cost ratio

	Flood water paddy field (Showair Union)						Flood water paddy field (Tetulia Union)					
Treatments	Grain yield (kg/ha)	Straw yield kg/ha	Gross return (Tk./ha)	Total variable cost (Tk./ha)	Net return (Tk./ha)	BCR	Grain yield (kg/ha)	Straw yield (kg/ha)	Gross return (Tk./ ha)	Total variable cost (Tk./ha)	Net return (Tk./ha)	BCR
T1	2143	4146	44940	30333	14606	1.48	2106	4126	44196	30266	13930	1.46
T2	2576	5106	104086	49450	54636	2.12	2496	4840	100353	49450	50903	2.04
T3	2446	4850	101358	49750	51608	2.04	2433	4883	9910	49750	49558	2.0
T4	2776	5516	108291	507333	57558	2.14	2693	5370	104515	47400	57151	2.20
T5	2650	5283	105641	48333	57308	2.20	2603	5183	102658	48334	54325	2.14

The aquatic living organisms such as fish population might have badly affected under the critical O_2 levels (DO value 1.5 ppm, below the critical value 4.0 ppm). In this study large amount of organic matter formed from the rotten green biomass of rice plant under flooded and submerged conditions. The decomposition

of this organic materials and the dead organisms at the bottom of haor basin produced ammonia, which diffuses from the sediment into the water column. To take in fresh oxygen in their blood stream fish must discharge CO_2, which might slow down or badly affected under higher CO_2concentration in anaerobic flood water paddy ecosystem. Significant amount of NH_4^+-N formed under the intensive reductive conditions of flood water paddy ecosystem, which might have blocked oxygen transfer in the gills of fish population, thereby damaged gills of fish and caused mortality of fish population. In this April of 2017 the Haor people of Bangladesh lost their only food security crop-the Boro rice (winter rice) due to flash flood caused by water of Indian Meghalaya state and unnatural excessive rainfall.

Conclusion

It was found that the rice-duck system is superior to the traditional system of rice production (sole rice or rice only) in terms of rice productivity and overall economic benefits, as well as better environmental services. Combined application of organic fertilizers (bioslurry, vermicompost and azolla compost) with the 50% of the recommended chemical fertilizers (NPKS) in rice-duck system is a feasible strategy to sustain overall productivity and enhancing economic gain, while minimizing methane emissions from wetland paddy ecosystem.

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Development of Production Package and Adaptation of Bt. Brinjal in Different Agro-Ecological Zone as Changing Climate of Bangladesh

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Abstract

Four experiments were executed during 2016-2017 to fulfill the objectives of the project. Earlier planting (November to January) was found better for proper growth and phenological development producing higher fruit yield of Bt. Brinjal (24.93-34.22 t/ha in BARI Bt. Begun-1 at Rangpur and 48.84-53.69 t/ha in BARI Bt. Begun-2 at Gazipur). Fruit yield showed strong and negative correlation ($r=-0.96$ at $p<0.01$ in Rangpur and $r=-0.98$ at $p<0.01$ in Gazipur) with total rainfall during growing season. Effect of growing degree days (GDD) for flowering on the fruit yield of Bt. Brinjal at Rangpur can be explained 86% by the functional model of $Y = 1847.6 - 2.115X + 0.0006X^2$ ($R^2 = 0.86$). Similarly effect of growing degree days (GDD) for flowering on the fruit yield of Bt. Brinjal at Gazipur can be explained 89% by the functional model of $Y = 2271.1 - 2.4908X + 0.0007X^2$ ($R^2 = 0.89$). Nutrients dose, 180-54-135-22-3-1.50 kg/ha of N-P-K-S-Zn-B +3 t/ha poultry manure was required for higher yield of bt. Brinjal (63.23 t/ha in BARI Bt. Begun-2 at Gazipur and 35.89 t/ha in BARI Bt. Begun-1 at Dinajpur) representing higher gross return (Tk. 632300/ha in BARI Bt. Begun-2 at Gazipur and Tk. 287120 /ha in BARI Bt. Begun-1 at Dinajpur) and BCR (3.71 in BARI Bt. Begun-2 at Gazipur and 3.10 in BARI Bt. Begun-1 at Dinajpur). Fruit yield was significantly correlated with applied nutrients ($r=0.97$ at $p<0.01$). Functional relationship between applied nutrient and fruit yield of Bt. Brinjal indicated that effect of nutrient on the fruit yield of Bt. was 92% ($Y=0.31x-56.37$, $R^2 = 0.92$). Higher fruit yield was found in two hand weeding (42.89 t/ha) at 25 DAP and 50 DAP and weeding by soil mulching (46.87 t/ha) during fertilizer side dressing at 20 DAP, 40 DAP, 60 DAP and 80 DAP representing better economic returns (Gross return of Tk. 428900-468700/ha and BCR of 2.51-2.80) where weed growth was minimum. In adaptation study the results revealed that fruit yield was recorded higher in BARI Bt. Begun-2 (38.71-42.66 t/ha) and BARI Bt Begun-1(33.25-35.98 t/ha). Economic returns were computed higher in BARI Bt Begun-1and BARI Bt Begun-2 irrespective of Agro-ecological zone. Farmers are in interested to cultivate Bt. brinjal except Jessore region. Rainfall in later planting caused damage of plant in the field of Pabna, Comilla and Gazipur. Infestation was zero in shoot but negligible (0.00-0.05%) in fruit of Bt. Brinjal by brinjal shoot and fruit borer (BSFB) insect. BARI Bt. Begun-1 for northern parts (Dinajpur, Rangpur), BARI Bt. Begun-2 for Gaibbandha, Bogra & Gazipur regions and BARI Bt. Begun-4 for Pabna and Comilla were found more suitable in respect of agro-climatic adaptation, farmers' interest and market demand. Farmers' of Jessore are not interested to cultivate Bt. Brinjal because of no demand in the market. Local popular varieties (like Chaga) of Jessore are more demanding.

Introduction

Brinjal is a nutritious and tasteful vegetable. It is popularly cultivated everywhere in Bangladesh (BARI, 2014a). Crop productivity of brinjal can be increased in many ways (Islam *et al.,* 2016). But brinjal shoot and fruit borer (BSFB) is a devastating pest of brinjal (BARI, 2014b). Farmers spayed poisonous insecticide about 160-180 times in a brinjal field (BARI, 2014b). This pollutes environments and causes human health hazard. Sometimes, non targeted and beneficial insects, and pollinators are killed. Moreover, non judicious and frequent spay increase cost of brinjal cultivation (Islam *et al.,* 2016). The vegetable is delicious and it has high demand in the market (Islam *et al.,* 2016). BARI and Colonel Universality, USA, jointly developed four Bt. brinjal varieties (BARI, 2014b). The name of Bt. Brinjal varieties are BARI Bt. Begun 1, BARI Bt. Begun 2, BARI Bt. Begun 3 and BARI Bt. Begun 4. Scientists have transferred Cry 1 Ac gene from a bacterium named *Bacillus thuringiensis* to four local varieties of brinjal (Anonymous, 2010). Thus these four Bt. Brinjal varieties have been developed. Bt. Brinjal is hygienic and not poisonous for human health (BARI, 2014c). As GMO crop the nature of crop growth, fruit bearing, nutrients requirement, growing time, crop management practices may vary from non GMO brinjal (Mian, 2016). Variation of climatic factors at different agro ecological zone also may affect the growth and yield of Bt. Brinjal (Mian *et al.,* 2013). Therefore, production package with location specific variety of Bt. Brinjal is needed to be developed. Hence, the project was undertaken.

Materials and methods

Total four experiments were conducted to fulfill the objectives of the project during 2016-2017. The experiment on "Phenology and performance of Bt. Brinjal at different agro-ecological zones as influenced by date of planting" was conducted at Bangladesh Agricultural Research Institute (BARI), Gazipur and Regional Agricultural Research Station(RARS), Ranpur. Plating of 34 days old seedling was started from 1 November 2016 and continued to 15 June 2017 as one month interval using treatments (treatment no. 8). BARI Bt. Begun-2 was used in Gazipur and BARI Bt. Begun-1 was used in Rangpur as per demand in local market. The treatments were T_1= STB Recommended dose (120-36-90-15-2-1 kg/ha of N-P-K-S-Zn-B+ 3 t/ha poultry manure) (FRG' 2012), $T_2 = T_1$ + 25% of NPK (150-45-112-18-2.5-1.25 kg/ha of N-P-K-S-Zn-B +3 t/ha poultry manure), $T_3 = T_1$ + 50% of NPK (180-54-135-22-3-1.50 kg/ha of N-P-K-S-Zn-B +3 t/ha poultry manure), $T_4 = T_1$ + 25% of NPK + 3 t ha poultry manure (150-45-112-18-2.5-1.25 kg/ha of N-P-K-S-Zn-B+6 t/ha poultry manure), $T_5 = T_{1+}3$ t/ha poultry manure (120-36-90-15-2-1 kg/ha of N-P-K-S-Zn-B + 6 t/ha poultry manure) in the experiment "Effect of fertilizer management on fruit yield of bt. Brinjal at Gazipur and Dinajpur". The experiment on "Development of weed control method for Bt. Brinjal" was conducted at BARI, Gazipur. The treatments were T_1= Paraxon as pre planting (7 days before planting), T_2=Paraxon as post planting (25 days after planting=DAP), T_3=Paraxon as post planting (25 DAP)+ one hand weeding (HW) at 50 DAP, T_4=Two HW at 25 DAP and 50 DAP, T_5= Weeding by soil mulching during fertilizer side dressing (at 20 DAP, 40 DAP, 60 DAP and 80 DAP) and T_6=Control. Crop management was done as per requirement. Adaptation status of Bt. Brinjal was studied in different agro-ecological zone (AEZ) of Bangladesh through focal group discussion and field monitoring of Bt. Brinjal. Selected AEZ were AEZ-3 (Dinajpur), AEZ-2 (Rangpur and Gaibandha), AEZ-4 (Bogra), AEZ-9 (Gazipur), AEZ -11(Jessore), AEZ-12 (Pabna) and AEZ-16 (Comilla). Relevant crop data and weather data in different AEZ was recorded. Farmers' opinion was evaluated. Collected data was analyzed and reported.

Results and discussion

Growing degree days for flowering (GDD) and phenological duration of Bt. Brinjal showed variation in different dates (Table 1). Fruit yield of Bt. Brinjal produced higher (24.93-34.22 t/ha in BARI Bt. Begun-1 and 48.84-53.69 t/ha in BARI Bt. Begun-2) in earlier planting (November to January). Similar results have been described by Matiur et al. (2011). Fruit yield variation was occurred in different planting dates due to variation of prevailing weather condition (data not shown) in two locations. Fruit yield showed strong and negative correlation ($r=-0.96$ at $p<0.01$ in Rangpur and $r=-0.98$ at $p<0.01$ in Gazipur) with total rainfall (data not shown) during growing season. Effect of growing degree days (GDD) for flowering on the fruit yield of Bt. Brinjal at Rangpur can be explained 86% by the functional model of $Y = 1847.6 - 2.115X + 0.0006X^2$ ($R^2 = 0.86$). Similarly effect of growing degree days (GDD) for flowering on the fruit yield of Bt. Brinjal at Gazipur can be explained 89% by the functional model of $Y = 2271.1 - 2.4908X + 0.0007X^2$ ($R^2 = 0.89$). Nutrients dose like 180-54-135-22-3-1.50 kg/ha of N-P-K-S-Zn-B +3 t/ha poultry manure produced higher fruit yield of BARI Bt. Begun-2 (63.23 t/ha) with higher economic return (Gross return of Tk. 632300/ha and BCR of 3.71) at Joydebpur and higher fruit yield of BARI Bt. Begun-1 (34.95-35.89 t/ha) with higher economic return (Gross return of Tk. 287120/ha and BCR of 3.10) at Dinajpur (Table 2). Functional relationship between applied nutrient and fruit yield of Bt. Brinjal indicated that effect of nutrient on the fruit yield of Bt. was 92% ($Y=0.31x-56.37$, $R^2 = 0.92$). The results are in agreement with findings of Mian et al.(2017). Higher fruit yield was found in T_4 (42.89 t/ha) and T_5 (46.87 t/ha) representing better economic returns (Gross return of Tk. 428900-468700/ha and BCR of 2.51-2.80) where weed growth was minimum (Table 3). Fruit yield was recorded higher in BARI Bt Begun-2 (38.71-42.66 t/ha) and BARI Bt Begun-1(33.25-35.98 t/ha) (Table 4). The results have been supported by the report of BARI (2017). Economic returns were computed higher in BARI Bt Begun-1and BARI Bt Begun-2 irrespective of AEZ (Table 4). Farmers are in interested to cultivate Bt. brinjal except Jessore region. Rainfall (data not shown) in later planting caused damage of plants in the field of Pabna, Comilla and Gazipur. Infestation was zero in shoot but negligible (0.00-0.05%) in fruit of Bt. Brinjal by brinjal shoot and fruit borer (BSFB) insect.

Table I. Growing degree days (GDD) and fruit yield of Bt. Brinjal at different locations as influenced by sowing date

Sowing date		Growing degree days (GDD)		Fruit yield (t/ha)	
Rangpur	Gazipur	Rangpur	Gazipur	Rangpur	Rangpur
1November	15 November	1497(82)	1513(89)	34.22(48)	53.69(21)
1 December	15 December	1547(91)	1601(97)	33.86(40)	49.41(20)
1 January	15 January	1576(97)	1725(103)	24.93(38)	48.84(19)
1 February	15 February	1584(96)	1512(84)	12.11(23)	15.39(16)
1 March	15March	1598(90)	1600(79)	9.17(17)	10.55(13)
1 April	15 April	1600(81)	1617(77)	8.92(11)	9.95(9)
1 May	15 May	1660(80)	1471(68)	8.55(11)	8.89(7)
1 June	15 June	1615(76)	1452(67)	8.31(10)	8.84(5)
$\bar{x}$	-	1585(87)	1561(83)	34.22(25)	53.69(6)

Flowering duration (days) with GDD and number of fruit with fruit yield are in parenthesis

Table II. Fruit yield and gross return of Bt. Brinjal at different locations as influenced by fertilizer level

Treatment	Yield (t/ha)		Gross return (Tk.)	
	Gazipur	Dinajpur	Gazipur	Dinajpur
T_1	17.45	32.05	1745001(1.44)	256400(2.89)
T_2	46.77	32.26	467700(3.21)	258080(2.84)
T_3	63.23	35.89	632300(3.71)	287120(3.10)
T_4	48.28	34.95	482800(3.18)	279600(2.98)
T_5	34.35	31.11	343500(2.69)	248880(2.71)
$LSD_{(0.05)}$	4.01	4.08	-	-
CV(%)	5.07	6.57	-	-

Details of treatments are given in materials and method. Market price of Brinjal: Tk. 10/ kg

Table III. Weed parameters and fruit yield of Bt. brinjal as influenced by weed control method

Treatments	Weed (no.)	Weed dry matter (g/m^2)	Weed control Efficiency (%)	Fruit yield (t/ha)
T_1= Paraxon as pre planting (7 days before planting)	268	346	13	4.68
T_2=Paraxon as post planting (25 days after planting=DAP)	247	321	20	5.26
T_3=Paraxon as post planting (25 DAP) + one hand weeding (HW) at 50 DAP	68	79	77	29.95
T_4=Two HW at 25 DAP and 50 DAP	52	61	84	42.89
T_5= Weeding by soil mulching during fertilizer side dressing	19	22	93	46.87
T_6=Control	307	356	-	3.15
$LSD_{(0.05)}$	51	43	-	4.11
CV (%)	9.28	5.93	-	7.63

Table IV. Fruit yield and economic returns of Bt. Brinjal at different Agro-ecological zone in Bangladesh

Location	AEZ number	Variety	Fruit yield (t/ha)	Gross return (Tk.)	BCR
Dinajpur	3	BARI Bt Begun-1	35.98	359800	2.39
Rangpur	2	BARI Bt Begun-1	33.25	332500	2.21
Bogra	4	BARI Bt Begun-2	39.65	396500	2.47
Gaibandha	2	BARI Bt Begun-2	38.71	387100	2.42
Gazipur	9	BARI Bt Begun-2	42.66	426600	2.66
Jessore	11	BARI Bt Begun-4	27.62	276200	1.84
Pabna	12	BARI Bt Begun-4	28.09	280900	1.87
Comilla	16	BARI Bt Begun-4	26.44	264400	1.76
$\bar{x}$			34.05	-	-
Sd			6.17	-	-

Conclusion

Bt. brinjal is eco-friendly technology. BARI Bt. Begun-1 for northern parts (Dinajpur, Rangpur), BARI Bt. Begun-2 for Gaibbandha, Bogra & Gazipur regions and BARI Bt. Begun-4 for Pabna and Comilla were found more suitable in respect of agro-climatic adaptation, farmers' interest and market demand. Production technology should disseminate in suitable areas of Bangladesh.

Plate: BARI Bt. Begun-1

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Selection Of Tolerant Vegetable Varieties For Selected Drought Prone Char Lands Of Mymensingh District

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Abstract

Twenty field trials were set up in the farmer's field of Charnoupara village of Ishwarganj upazila under Mymensingh district in 2016-17. The varieties that were incorporated in the trials included ash gourd: BARI chalkumra-1 and Durbar; ridge gourd: BARI jhinga-1; snake gourd: BARI chichinga-1 and Dhaka green; bottle gourd: BARI lau-4; cucumber: Green king; yard long bean: BARI borboti-1 and Toki and brinjal: BARI begun-8. Results revealed that the chalkumra variety Durbar yielded the highest (34.3 t/ha) followed by BARI chalkumra-1 (32.8 t/ha). BARI jhinga-1 yielded the highest (24.8 t/ha) compared to the local variety (19.7 t/ha). Among the chichinga varieties, BARI chichinga yielded the highest (32.6t/ha) followed by Dhaka green (29.1t/ha) and local (24.1 t/ha). The other vegetable varieties that yielded the highest included BARI lau-4 (55.9 t/ha), Green king cucumber (35.8 t/ha), BARI borboti- 1 (19.3 t/ha) and BARI begun-8 (38.5 t/ha). Considering yield and other parameters, BARI jhinga-1, BARI chichinga-1, BARI lau-1 and Toki may be selected for large-scale cultivation in drought prone charlands of Mymensingh district.

Introduction

Bangladesh is one of the most climate vulnerable countries in the world and will become even more so as a result of climate change. Floods, tropical cyclones, storm surges and droughts are likely to become more frequent and severe in the coming years. Droughts in Bangladesh are seasonal and can devastate crops, causing hardship to poor agricultural labourers and others who cannot find work. If the crop totally fails because of drought, the situation for poor people can become critical. Droughts most commonly affect the northern region followed by northeastern region, which generally has lower rainfall than the rest of the country (MoEF, 2009).

Total vegetable production in Bangladesh is 3357 thousand MT (BBS, 2016); whereas, in Mymensingh it is 283 thousand MT in 2013-14. In last two years, total production of vegetable in Mymensingh has been increasing by an average 9.05% per annum. Close proximity with Dhaka is one of the major comparative advantages of Mymensingh which also played significant role with the growth process. Moreover, increased demand from Dhaka and establishment of large industries in Gazipur-Mymensingh Highway also created a large customer base for vegetables (Anon., 2007).

In the Mymensingh division, there are some drought prone areas that are mainly spread to the eastern side of Brahmmaputra River. These are called *Charlands* and are characterized by prevailing high temperature with sandy loam soil. These drought prone areas are located in several upazilas of Jamalpur district and Sadar, Ishwarganj, Nandail and Gaffargaon upazila of Mymensingh district (Anon., 2007). Almost all kinds of vegetables are cultivated particularly bitter gourd (karala), cucumber, bottle gourd, bean, ribbed gourd, snake gourd, lady's finger, stem amaranth, potato and brinjals are abundantly grown (Hossain, 2016).According the Department of Agricultural Extension, 5.54% (18605 hectare) of the cultivated land in Mymensingh district is under vegetable cultivation excluding the *Char* area. Consultation with the experts acknowledged that if *Char* vegetable area would included, the total vegetable area would increase by around 5500 hectare more. Recently, vegetables are cultivating in some areas of Ishwarganj, Nandail and Gaffargaon upazila which were previously under rice cultivation or fallow. But yield are very low compared to other vegetable growing areas. Among the possible reasons of low yield, use of local varieties, lack of awareness and unavailability of quality seeds of drought tolerant HYV's and lack of technical knowledge on improved cultivation practices of vegetable are noticeable. Different research organization, agricultural universities and some other seed company has been developed a lots of high yielding varieties of different vegetables. So, it is very much essential to validate the available high yielding vegetable varieties under farmers' condition with a view to select drought tolerant varieties for those areas to boost up the production. Therefore, the research work has been initiated to validate and select high yielding vegetable varieties under farmers' field condition of *Charlands* of Ishwarganj upazila of Mymensingh district.

Materials and methods

Twenty field trials were set up in the farmer's field of Charnoupara village of Ishwarganj upazila under Mymensingh district. Each farmer cultivated in 5 decimals of land. Seeds of high yielding varieties of seven different vegetables were given to each farmer. The varieties that were incorporated in the trials included ash gourd: BARI chalkumra-1 and Durbar; ridge gourd: BARI jhinga-1; snake gourd: BARI chichinga-1 and Dhaka green; bottle gourd: BARI lau-4; cucumber: Green king; yard long bean: BARI borboti-1 and Toki and brinjal: BARI begun-8. Recommended doses of fertilizers alongwith recommended practices were employed in all trials (Anon., 2005). Local varieties of each crop were used as check. Randomized Complete Block Design was followed in dispersed location; where, each farmer was considered as one replication. Data on different characters were recorded and analyzed as per necessity.

Results and discussion

Results regarding performance of different vegetables varieties are presented in Table I-VII. While studying the performance of different ash gourd varieties under farmer's field condition, it is revealed that the variety Durbar yielded the highest (34.3t/ha) followed by BARI chalkumra-1 (32.8t/ha) and local (28.3t/ha); although, the number of fruits per plant was the lowest (6.2) in Durbar. Range of individual weight of fruit was the highest (1.07-1.39) in Durbar, which might be the cause of highest yield of this variety. The local variety performed the lowest in case of individual fruit weight, number of fruits/plant and also in average yield (Table I). The farmer liked the local variety as the size was smaller compared to other two varieties. Therefore, they expect to have variety that is high yielding but fruit size is smaller.

Table I. Performance of different ash gourd varieties under farmer's field condition

Variety	Individual fruit weight (Kg)	No. of fruits/ plant	Yield (t/ha)
BARI chalkumra-1	1.04- 1.31	7.1	32.8[a]
Durbar	1.07- 1.39	6.2	34.3[a]
Local	0.87- 1.10	6.4	28.3[b]

During studying the performance of different ridge gourd varieties under farmer's field condition, it is revealed from the Table 2 that the variety BARI Jhinga-1 yielded the highest (24.8 t/ha) and local variety the lowest (19.7 t/ha). Number of fruits per plant (53.2) and range of individual fruit weight (110-132 g) was also the highest in BARI Jhinga-1, which might be the cause of highest yield of this variety. The local variety performed the lowest regarding individual fruit weight and number of fruits/plant (Table II).

Table II. Performance of different ridge gourd varieties under farmer's field condition

Variety	Individual fruit weight (g)	No. of fruits/ plant	Yield (t/ha)
BARI jhinga-1	110-132	53.2	24.8
Local	96-117	46.5	19.7

In case of performance of different varieties of snake gourd (Table III), it is revealed that BARI Chichinga-1 produced the highest yield (32.6 t/ha) under farmer's field condition and this was statistically similar with the yield of other high yielding variety Dhaka green (29.1 t/ha). The local variety produced the lowest yield (24.1 t/ha). Although, BARI chichinga-1 produced the lowest range (145-151g) of individual fruit weight but it produced the highest number of fruits per plant (57.5). This might be the reason behind the highest yield of the variety. Similar results regarding the highest yield of BARI chichinga-1 have also been reported (Anon., 2015). Minimum number of fruits per plant (41.2) was recorded in the local variety, which was very close to the Dhaka green (43.2).

Table III. Performance of different snake gourd varieties under farmer's field condition

Variety	Individual fruit weight (g)	No. of fruits/ plant	Yield (t/ha)
BARI chichinga-1	145-151	57.5	32.6[a]
Dhaka green	178-189	43.2	29.1[a]
Local	165-178	41.2	24.1[b]

Performance of different bottle gourd varieties under farmer's field condition are presented in Table IV. The variety BARI lau-4 produced the lowest range (2.14-2.53 kg) of individual fruit weight compared to the local variety (2.31-2.76 kg) but BARI lau-4 produced the highest number of fruits per plant (15) in comparison to the local (10). The highest number of fruits per plant yielded the highest in BARI lau-4 (55.9 t/ha) than the local (45.9 t/ha). Similar results regarding the highest yield of BARI lau-4 have also been reported (Anon., 2012).

Like other varieties, different cucumber varieties performed differentially under farmer's field condition (Table V). The hybrid variety Green king performed better in all aspects. In Green king, the individual fruit weight was ranged from 1.38-1.45 kg and it was much higher than the local (0.18-0.35 kg). More

Table IV. Performance of different bottle gourd varieties under farmer's field condition

Variety	Individual fruit weight (Kg)	No. of fruits/ plant	Yield (t/ha)
BARI lau-4	2.14-2.53	15	55.9
Local	2.31-2.76	10	45.8

fruits per plant was found in the local variety (29) compared to the Green king (18). More numbers of fruit per plant in the local variety failed to produce higher yield as because the individual fruit weight was lower. The yield of Green king (35.8 t/ha) was much higher than the local variety (25.9 t/ha). The farmer opined that large fruit had low demand in the market. The consumers want to have smaller fruits.

Table V. Performance of different cucumber varieties under farmer's field condition

Variety	Individual fruit weight (Kg)	No. of fruits/ plant	Yield (t/ha)
Green king	1.38-1.45	18	35.8
Local	0.18-0.35	29	25.9

In case of yard long bean, it was found that BARI borboti-1 performed better than other two varieties in respect of average yield. A range of 42-49 cm long fruits were produced by BARI borboti-1, which was shorter than the variety Toki (51-55 cm) and longer than the local variety (23-34 cm). Number of pods per plant was recorded the highest in the variety BARI borboti-1 (63.6) followed by Toki (57.8) and the local (53.9). Yield range was the highest in BARI borboti-1 (15-22 t/ha) followed by Toki (17-21 t/ha) and local (13-15 t/ha). Although, average yield was the highest in BARI borboti-1 (19.3 t/ha) but it was statistically similar with the yield of Toki (18.5 t/ha) but significantly differed with the local (13.4 t/ha) variety (Table VI). The farmers preferred the deep green colour of Toki than BARI borboti-1, which was light green in colour.

Table VI. Performance of yard long bean varieties under farmer's field condition

Variety	Individual fruit length (cm)	No. of pods/ plant	Yield (t/ha)	Average yield (t/ha)
BARI borboti-1	42-49	63.6	15-22	19.3[a]
Toki	51-55	57.8	17-21	18.5[a]
Local	23-34	53.9	13-15	13.4[b]

BARI begun-8 was put to farmers' field trial and performed better than the local regarding yield and other characters. Range of individual fruit weight, number of fruit per plant and yield was higher in BARI begun-8 (75-82 g, 23 and 38.5 t/ha, respectively) compared to local (66-79 g, 16 and 29.9 t/ha) (Table VII). Several farmers did not like a long fruited variety rather they prefer to have a variety that produces round or oval shaped fruit.

Table VII. Performance of different brinjal varieties under farmer's field condition

Variety	Individual fruit weight (g)	No. of fruits/ plant	Yield (t/ha)
BARI begun-8	75-82	23	38.5
Local	66-79	16	29.9

Conclusion

From the above results and discussion, it can be concluded that BARI jhinga-1, BARI chichinga-1, BARI lau-1 and Toki may be selected for large-scale cultivation in drought prone charlands of Mymensingh district. However, the trial may be repeated including these varieties along with other high yielding varieties of different vegetables. Small sized high yielding ash gourd variety may be developed as the farmers expected. Besides, small sized varieties of cucumber and round or oval fruited brinjal varieties may be put to trial for observing farmers acceptance of that locality.

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Studies on the Potential of Environmental Bacteria as a Reservoir of Antibiotic Resistance Genes, Screening of Possible Antibiotic Efflux Mechanism, and the Effect of Efflux Pump Inhibitors on Enhancing Therapeutic Efficiency of Antibiotics

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Abstract

Antibiotic resistance is an increasing global problem resulting from selective pressure , inappropriate usage, greater mobility of the population, and industrialization. *Escherichia coli* is a widespread bacterium encompassing a variety of strains, ranging from highly pathogenic strains, causing worldwide outbreaks of severe diseases to a virulent, well characterized safe laboratory strains. From March 2015 to June 2017, a total 310 surface water samples were collected from 20 sampling sites. and cultured onto MacConkey agar plates for selective isolation of Gram negative bacteria. Of 197 representative isolates, 110 were confirmed as *E. coli* and rest 87 were non-*E.coli* belonged to fifteen different species under eight genera . All these isolates were subjected to antibiotic susceptibility against antibiotics belonging to aminoglycoside, cephalosporin, fluoroquinolone, penicillin, sulfonamide and tetracycline group. Majority of the isolates showed resistance to one or more antibiotic in disc diffusion and MIC assay. A notable number of isolates showed multi-drug resistance (resistance to three or more antibiotic) phenotype. Antibiotic susceptibility test revealed that resistance to amoxicillin was most prevalent (65%) followed by cefixime, sulfamethoxazole-trimethoprim ciprofloxacin , tetracycline and gentamicin . Plasmid extraction of all Gram negative isolates revealed that majority of them carried plasmids with diverse profiles. Multidrug resistant 58 bacteria were subjected to ethidium bromide efflux assay for presence of efflux pump. About 45% of them showed omeprazole induced intense fluorescence, indicating the presence of efflux mechanism. MIC assay was done against all resistant bacteria in presence and absence of omeprazole. Omeprazole induced MIC depression by 2 to 10 fold further indicate the presence of efflux mechanism. This study shows that environmental *E. coli* may functions as a reservoir of antibiotic resistance against therapeutically important antibiotics

Introduction

Studies on the prevalence of antibiotic resistance phenomenon in environmental bacteria is scanty to understand antibiotic resistance gene pool in Bangladesh. Resistance scenario against therapeutically important antibiotics is required to formulate antibiotic policy for proper and effective use of antibiotics. This study may help in future efficacy testing of these agents in humans, particularly omeprazole as this is extensively used in human as a proton pump inhibitor.

We have been studying the resistance pattern of intestinal *E. coli* isolated from healthy human subjects, their plasmid profile for possible association in antibiotic resistance phenomenon. In our previous studies, about 50 *E. coli* isolates was tested for their susceptibility to therapeutically important antibiotics .The plasmid profile analysis, MIC determination and preliminary studies on the effect of proton pump inhibitor on efflux of antibiotics such as ampicillin, ciprofloxacin, chloramphenicol and tetracycline have been carried out. MIC depression by proton pump inhibitor varied from 5 to 100-fold in case of ampicillin, chloramphenicol, azithromycin and tetracycline in some of the *E. coli* isolates. Our preliminary studies carried out at Jahangirnagar University indicate that normal intestinal flora accumulated antibiotic resistance gene against clinically important antibiotics and may serve as the reservoir of antibiotic resistance gene[1,2]. Many strains of *E. coli* isolated from stool samples of healthy human subjects possibly possess significant drug efflux capability that can expel common clinically important antibiotics[3]. As surface water play vital role in the dissemination of diverse enteric infections, further study in this field is being planned with the environmental bacteria which largely constitutes the subject of the present project proposal.

Methodology

Multidrug resistant *E.coli* with apparent presence of efflux pump are available as our laboratory collection additional control strains were collected as a gift from domestic biomedical research institution. For selective isolationof *E. coli and* other gram-negative bacteria,each of the samples were inoculated onto MacConkey agar/ Eosin methylene blue agar plates and were identified by conventional biochemical tests according to Bergey's Manual of Systematic Bacteriology[4]. Antibacterial succeptibility was carried out by the disc diffusion assay[5]followed by MICs assay using agar dilution method.Plasmid profiling of the resistant bacteria was carried out by alkaline lysis method of Birnboim and Doly[6] followed by agarose gel electrophoresis.Efflux potential of the test strains were determined by Ethidiumbromide (EtBr) effluxassaymethod[7]. Efflux potential was also determined byinhibitor induced MIC depression.

Result and discussion

A total of 310 surface water samples from 50 different ponds at 25 sites were cultured onto McConkey plates for selective isolation of Gram–negative environmental. Of 197 isolates 110 were identifiedas *E. coli* while other 87 were non-*E.coli* belonged to fifteen different species under eight genera (Data not shown). All 197 isolates were tested for sensitivity against commonly used antibiotics. Majority of the Gram-negative isolates showed resistance to one or more antibiotic in antimicrobial susceptibility test. In general, highest incidence of resistance phenomenon in diverse community of Gram-negative bacteria was observed against amoxicillin (66.5%), followed by cefixime,sulfamethoxazole-trimethoprim , ciprofloxacin, tetracycline and gentamicin.*E. coli* isolates showed higher percentage of resistance against clinically important under investigation than non-*E. coli* population. Multi-antibiotic resistance phenomenon was also more frequent in *E. coli* than that of *non-E.coli*isolates of environmental bacteria (data not shown). Plasmid extraction of 197gram-negative isolates revealed that majority were found to contain plasmids with heterogeneous origin.

Multidrug resistant 58 isolates were tested for their efflux potential by ethidium bromide efflux assay. Of 58 *multi-drug resistant bacteria*, 26 were found to show enhanced fluroscence in presence of omeprazole.We examined environmental 197 gram-negative isolates for possible efflux potential against amoxicillin, ciprofloxacin, gentamicin and tetracycline by omeprazole induced MIC depression. Results

of this assay indicate that omeprazole caused significant reduction in MIC of different antibiotics in many of these isolates. Reduction of MIC by the presence of omeprazole indicates that in the absence of omeprazole, efflux mechanism is operational. However, in case of ciprofloxacin and tetracycline only 2 fold decreasein MIC value was found. With the antibiotic gentamicin 2-10 fold reduction in MIC value was found

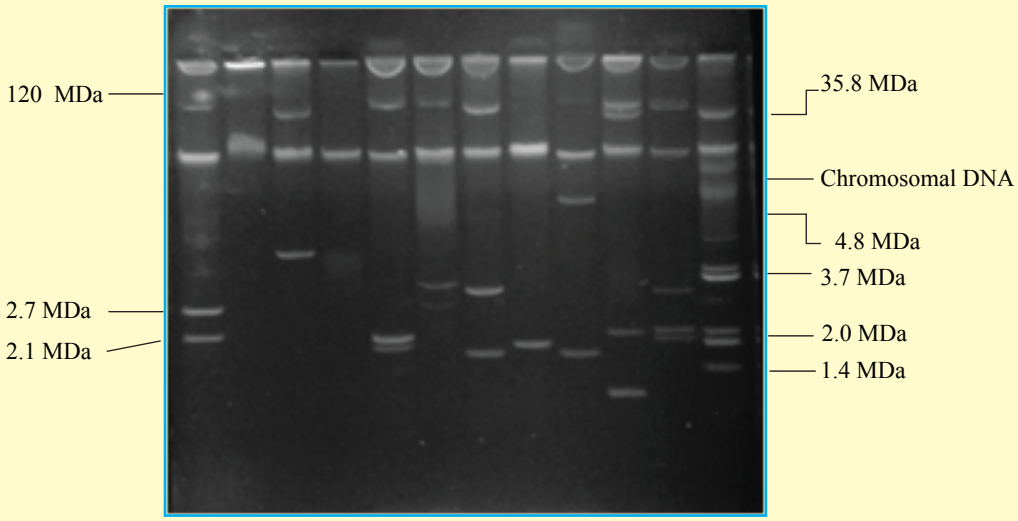

Fig. Plasmid profiles of environmental isolates of *E. coli* obtained from surface water. Lane 1 and 12 represent plasmid DNA markers obtained from *E. coli* K-12 strains PDK-9 and V-517, respectively

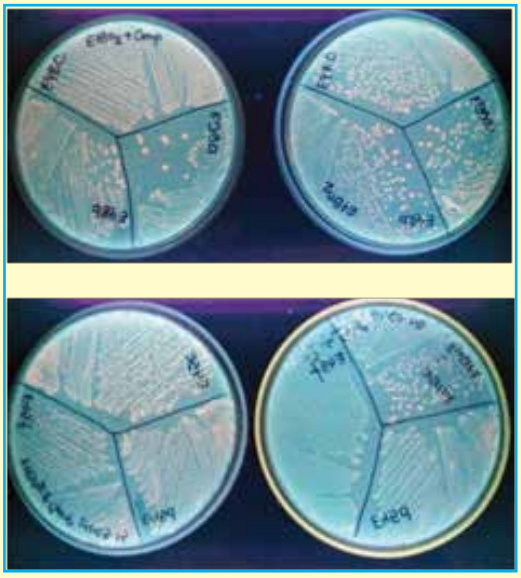

Fig. Fluoremetric determination of EtBr-agar efflux assay of multidrug resistant E39a, E48b, E48c isolates. Here, much less fluorescence is exhibited by E39a, E48b, E48c isolates shows in right side plate in absence of omeprazole compared to much intense fluorescence in presence of omeprazole by same set of plate at the left side.

Conclusion

In the first phase (1st year) of our present study was to describe the species diversity of environmentalgram- negative bacteria and their potential as reservoir of antibiotic resistance gene.This time we report plasmid diversity and possible presence of efflux phenomenon through ethidium bromide efflux assay and omeprazole induced MIC depression assay. Results of this study suggest that environmental*Gram-negative*bacteria also carry efflux phenomenon against clinically relevant antibiotics.

Acknowledgement

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Development Of A Multi-purpose Hybrid & Portable Surveillance Drone For Security and Disaster Management

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Abstract

We demonstrate the design and development of a low-cost multi-purpose autonomous hybrid & portable autonomous dronesuitable for surveillance and disaster management. The vertical landing capabledrone flies at a maximum speed of 75 km/hour, where the maximum altitude is ~ 200 meter. The portable drone comprises five distinct parts those are easily installable within ten minutes and can be fit in a small portable kit.The integrated high definition camera sends real-time video stream of desired area to the ground control station using video transmitter. In addition, the drone is capable of carrying ~ 2 kg of payload along with it.We believe that, the hybrid (quad-plane) drone will be valuable in wide range of applications areas.

Introduction

From technological point of view, drones are considered as unmanned aircraft. Beyond military applications, drones have been deployed in dynamic civil and commercial applications: crowd monitoring, surveillance, aerial surveying, delivery of products, spraying insecticides, sowing seeds, acrobatic aerial footage in filmmaking, search & rescue operations, inspection of power lines & pipelines, counting wildlife, detection of forest fire & illegal hunting [1–8]. Typically, drones are classified as non-autonomous and autonomous depending on the controlling system. Non-autonomous drones are controlled manually by means of a remote controller. In contrast, flight of the autonomous drones is precisely controlled from the remotely spaced programmable computers. On the contrary, from structural point of view, the drones are classified as aircraft type and copter type. Aircraft type drones are popular for their high speed flying ability. On the contrary, the copter type drones are required for precise flying and vertical landing. An aircraft type drone cannottakeover or land in a small area. As a result, a hybrid type drone, having the advantages of both the aircraft and copter type drones, is required for effective application in surveillance and disaster management. Because of the size of the conventional surveillance drones, it is difficult to carry them without carrying the attention of the general people. Portable drone can be considered as the solution of the problem.

The prime objective of our research is to develop a low-cost hybrid (quad-plane) autonomous portable drone targeting surveillance and disaster management. The drone is not only portable but also capable of landing vertically without any requirement of runway. Most importantly, parts of the drones are fit in a small portable kit. The quad-plane is capable of flying at a high speed under different altitude, where the

range is several kilometres from the ground control station. The built-in high-definition (HD) camera and video transmitter are responsible for real-time video streaming of the desired area to the ground control station for surveillance.Furthermore, the drone is capable of carrying 2 kg of payload, suitable for disaster management.

Materials and methods

The first step of the research is to survey the current drone technologies and available devices required to integrate with the autonomous hybrid & portable drone. Based on the surveyed information, we designed the system architecture of all the modules of the hybrid &portable drone including ground control system, communication system, power unit, autopilot & GPS unit, and camera & video transmission unit. We also designed the structure of the drone, circuit diagrams for electrical & electronic modules, and their interfacing.Before finalization of the design and development, the electrical and electronics modules were designed and simulated using PSpice and PROTEUS simulators. The structural model of the portable drone was designed using SOLID WORKS simulator.After convincing simulation results, we developed various modules of the hybrid &portable drone. After the performance of all the modules passed the desired value, all modules were integrated to develop the complete drone and the corresponding ground control station. After satisfactory results in the laboratory, we went for real life performance analysis. The system architecture of the drone is illustrated in Figure 1.

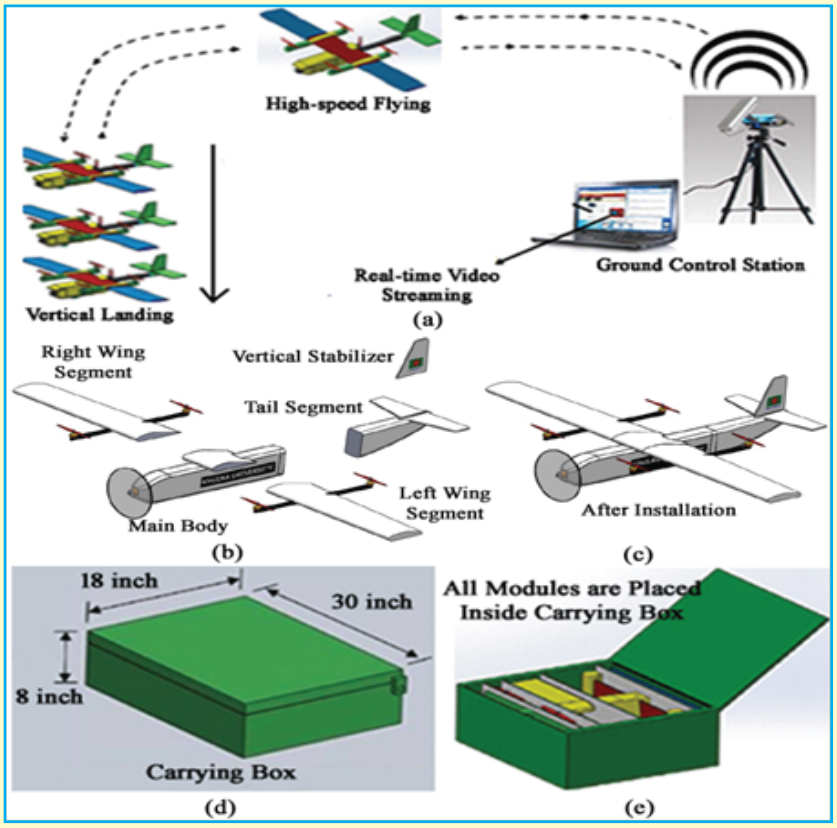

Fig. 1(a). System architecture of the multi-purpose autonomous hybrid & portable drone; (b) various parts of the drone; (b) appearance of the drone after the parts are assembled; (c) dimension of the carrying box; (d) placement of various modules inside the carrying box.

Result and discussion

The drone was autonomously controlled from the ground control station, as shown in Figure 1(a). A high power long range transmitter antenna (T_x) of the ground control station communicates with the integrated high-speed receiver (R_x) of the drone. Figure 2 shows the image of the developed hybrid & portable drone.As mentioned before, the hybrid drone has the characteristics of both aircraft type and copter type drones. The front propeller of the drone is responsible for high speed flying of the drone, which is the normal operational mode of the drone. The additional four propellers, representing the copter-type section,are responsible for vertical landing in a small area, which is helpful in hilly/forest regions and during disaster management.The architecture of the drone is divided into fundamental three sections: mechanical section, electrical & electronic section, and communication section.

Fig. 2. Left and right view of the autonomous hybrid & portable drone.

Mechanical section

The airframe of the drone was constructed using glass fiber on top of light weight plywood and foam so that it can carry the power unit, GPS & autopilot unit, telemetry, camera & video transmission unit, and receiver unit. As mentioned before, the whole body of the drone was divided into five (05) parts those are installable within ten minutes. The empty weight and the payload capacity of the drone were ~ 2.6 kg and 2 kg.

Electrical and electronic section

We used five highly efficient brushless motors along with propellers to drive the portable drone. For proper coordination of the motors and controlling the motor speed, we connected highly efficient electronic speed controllers with the motors. Two high efficiency lithium polymer batteries having high current rating was considered as power unit of our drone. We considered a laptop as our ground control station. An HD camera and 3-axis gimbal was installed with the drone.

Communication section

Primarily, the drone is guided autonomously from the ground control station. In parallel with autonomous flight, the drone can be controlled by long range remote controllers. For autonomous flight, the flying path

was transmitted from the ground control station using the Mission Planner software, as illustrated in Figure3(a). The operating frequency of the drone for sending the control command is 2.4 GHz. The real-time video wastransmitted by the video transmitter using 5.2 GHz. A high precision GPS unit allows us to direct

Fig. 3. (a) Flying path programmed from the ground control station for autonomous flight; (b) Top view of Khulna University pond, captured using the HD camera of the drone at an altitude of 100 meter and 100 meter from the ground control station.

the drone to the appropriate location.The autopilot unit accepts the flying path from the ground control station and location information from the GPS unit, and converts the incoming signals to appropriate commands for autonomous flight of the drone.We captured real-time video of Khulna University campus using the integrated HD camera of the drone. Top view of Khulna University pond, captured from our drone, is illustrated in Figure 3(b). The HD quality video confirms the applicability of our drone for surveillance. The drone is hand launched, the specifications of which are summarized in Table I.

Table I. Summary of various parameters of the Autonomous Hybrid &Portable Drone

Parameter	Type/Value	Parameter	Type/Value
Type	Hybrid (Quad-plane)	Empty Weight	2.6 kg
Takeoff	Hand Launch	Payload	1.8 kg
Construction Material	Foam, ply wood, and glass fiber	HD Camera	1080 P; Viewing Angle: 170°
Airfoil	Clark Y	Propeller	5 pieces: 9×3.8 SF
Battery	2 pieces: Lithium Polymer;8000 mah	Camera Gimbal	3-axis; Rotation Angle: 180°
Wing	2 pieces: 1.8 meter	Cruise Speed	75 km/hour (maximum)
Motors	5 pieces: 790 kV brushless motor	Electronic Speed Controller (ESC)	5 pieces: 60 Ampere
Altitude	200 meter (maximum)	Flight Time	19 minutes (@60% throttle)

Conclusion

In summary, we developed an autonomous hybrid &portable drone for surveillance and disaster management. The high speed drone can land vertically in a small area, which fits in a small carrying box. The drone can fly at a maximum speed of 75 km/hour and altitude of 200 meter with a payload of ~ 1.8 kg for maximum 19 minutes. The drone can transmit real-time video from approximately 2 km.

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Microwave Assisted Synthesis and Biological Evaluation of Flavones

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Abstract

The microwave assisted synthesis of ten flavones (11-20) was carried out by irradiating the chalcones in the presence of DMSO and catalytic amount of I_2. The corresponding chalcones (1-10) were obtained by the Claisen-Schmidt condition under the microwave-assisted heating technique. The reaction is clean with shorter reaction time, mild reaction condition, eco-friendly and excellent yield. The antimicrobial activity, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these synthesized compounds were determined.

Introduction

Organic synthesis is one of the important branches of chemical synthesis, which is connected with the construction of organic compounds, possess high level of complexity. It has been playing major role to produce beneficial products, such as pharmaceuticals, agricultural pesticides and others. Besides, advanced technology in synthetic procedure promotes the organic synthesis and widen up the study of interest. Chalcones, one of the major class of natural products with widespread distribution in fruits, vegetables, spices, tea and soy-based foodstuff, was recently highlighted due to their interesting pharmacological activities (Carlo *et al.,* 1999). Flavones have antioxidant, anti-proliferative, anti-tumor, anti-microbial, estrogenic, acetyl cholinesterase, anti-inflammatory activities and are also used in cancer, cardiovascular disease, neurodegenerative disorders, etc. (Verma *et al.,* 2012). These compounds show varied biological activities including activity against HIV (Wang *et al.,* 2003), dengue (Kiat *et al.,* 2006), influenza virus (Nagai *et al.,* 1995) as well as antitumor (Hsu *et al.,* 2006) and antioxidant (Montana *et al.,* 2007) effects. Beneficial effects of flavonoids on human health have gained increasing interest among researchers over the last few years. Several strategies for the synthesis of flavones have been reported (Ghiya *et al.,* 1986; Patonay *et al.,* 1997; Lokhande *et al.,* 2005). However, many of these methods suffered from harsh reaction conditions, toxic reagents, strong acidic / basic conditions, prolonged reaction time, poor yield and low selectivity.

Recently microwave radiation has gained the attention of chemists due to its unique advantages, such as shorter reaction times, cleaner reaction products, higher yields and better selectivity (Hayes *et al.,* 2002; Bhuiyan *et al.,* 2011). Keeping in view of these findings, herein we describe a simple and convenient method for the synthesis of chalcones and corresponding flavones under microwave irradiation in solvent free environment, with improved yields and short reaction time.

Materials and methods

Representative procedure for the Synthesis of 2'-hydroxy-4-methylchalcone (1)

An equimolar mixture of 2-hydroxyacetophenone and 4-methylbenzaldehyde dissolved in minimum amount of rectified spirit and KOH (10%) was irradiated under 320 watt microwave irradiation for 100-140 sec. The progress of the reaction was monitored by TLC (*n*-hexane: ethyl acetate, 6:1) every after 30 sec. After the reaction mixture was diluted with water, acidified with dil. HCl and extracted with ether. The ether layer was washed with water and dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure. The obtained solid after evaporation was recrystallized from ethyl acetate and *n*-hexane solvent mixture. yellow crystals (96.72%), mp. 111-112 °C, IR (KBr) ν_{max} (cm^{-1}): 3433.29 (C-OH), 2916, 1635.64 (>C=O), 1592.34 (C=C), 1485.19 (C=C, Ph), 1199, 1026, 748.38; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H (ppm): 2.43 (s, 3H, C_4-CH_3), 6.96 (m, 1H, $C_{4'}$-H), 7.05 (d, 2H, *J*=8 Hz, C_3-H and C_5-H), 7.27 (d, 1H, *J*=8 Hz, $C_{3'}$-H), 7.50 (m, 1H, $C_{5'}$-H), 7.59 (d, 2H, *J*=8 Hz, C_2-H and C_6-H), 7.65 (d, 1H, *J*=16 Hz, C_α-H), 7.94 (d, 1H, *J*=16 Hz, C_β-H), 7.96 (d, 1H, *J*=8 Hz, $C_{6'}$-H), 12.88 (s, 1H, $C_{2'}$-OH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C (ppm): 21.9 (C_4-CH_3), 117.2 (C-3'), 119.6 (C-5'), 121.9 (C-1'), 124.2 (C-α), 126.8 (C-2 & C-6), 129.9 (C-3 & C-5), 130.1 (C-1), 132.1 (C-6'), 136.2 (C-4'), 137.3 (C-4), 143.9 (C-β), 158.7 (C-2'), 186.4 (>C=O).

Similarly 2'-hydroxy-4-methoxychalcone (2), 2'-hydroxy-4-ethoxychalcone (3), 2'-hydroxy-2-chlorochalcone (4), 2'-hydroxy-4-chlorochalcone (5), 2'-hydroxy-3,4-methylenedioxychalcone (6), 2'-hydroxy-2,4-dimethoxychalcone (7), 2'-hydroxy-2,4,5-trimethoxychalcone (8), 2'-hydroxy-4-nitrochalcone (9) and 2'-hydroxy-3-nitrochalcone (10) were prepared.

Representative procedure for the Synthesis of 4'-methylflavone (11)

A suspension of 2'-hydroxy-4-methyl chalcone (1 mmol) in (DMSO, 2 ml) and iodine (0.02 mmol) was irradiated under 320 watt microwave irradiation for 300-325 sec. After completion of reaction the mixture was diluted with water, extracted with diethyl ether, washed with aqueous 20% sodium thiosulphate, dried over anhydrous Na_2SO_4 and the solvent was evaporated. The obtained solid was recrystallized from ethyl alcohol to give flavones. Flavones gave blue fluorescence in UV light. Light brown needles, Yield: 99.04%, mp. 75-76°C; IR (KBr) ν_{max} (cm^{-1}): 3035.96, 2920, 1639.49 (>C=O), 1566.20 (C=C), 1465.90 (C=C, Ph), 1373.32, 1122.57 (C-O), 1199, 1026, 748.38; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H (ppm): 2.46 (s, 3H, $C_{4'}$-CH_3), 6.82 (s, 1H, C_3-H), 7.34 (d, 2H, *J*=8 Hz, $C_{3'}$-H and $C_{5'}$-H), 7.43 (t, 1H, *J*=8 Hz, C_6-H), 7.58 (d, 1H, *J*=8 Hz, C_8-H), 7.71 (m, 1H, C_7-H), 7.84 (d, 2H, *J*=8 Hz, $C_{2'}$-H and $C_{6'}$-H), 8.25 (d, 1H, *J*=8 Hz, C_5-H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C (ppm): 23.1(C-4' CH_3), 97.8 (C-3), 117.5 (C-8), 123.3 (C-6), 124.6 (C-4a), 127.8 (C-2' & C6'), 129.6 (C-5), 130.2 (C-3' & C-5'), 132.3 (C-1'), 135.9 (C-7), 137.8 (C-4'), 158.3 (C-8a), 168.9 (C-2), 178.3 (>C=O).

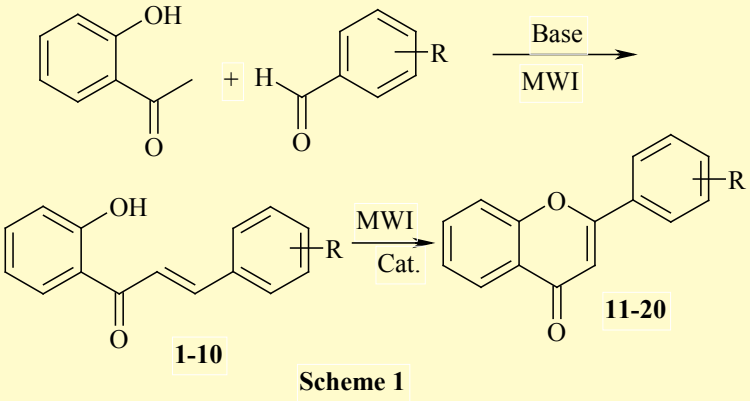
Similarly 4'-methoxyflavone (12), 4'-ethoxyflavone (13), 2'-chloroflavone (14), 4'-chloroflavone (15), 3',4'-methylenedioxyflavone (16), 2',4'-dimethoxyflavone (17), 2',4',5'-trimethoxyflavone (18), 4'-nitroflavone (19) and 3'-nitroflavone (20) were prepared from their corresponding chalcone.

Antimicrobial activities

The antibacterial activity of the synthesized compounds 1-20 were studied against six human pathogenic by filter paper disc diffusion method (Arima *et al*., 2002). The antifungal activity of compounds 1-20 were evaluated towards four plant pathogenic and mold fungi, by food poison technique (Bauer *et al.,* 1999). The MIC and MBC of the tested compounds in comparison to ampicillin were determined by broth micro-dilution method (Amsterdam *et al.,* 1996).

Results and discussion

Alkaline condensation of 2-hydroxyacetophenone with 4-methylbenzaldehyde gave the corresponding chalcone 1 (Scheme 1). The IR absorption band at 3433.29 cm^{-1} indicated the presence of hydroxyl group. A positive ferric chloride test also indicated that compound 1 has a free hydroxyl group and a band at 1635.64 cm^{-1} showed the presence of a conjugated carbonyl group (>C=O). The ^{1}H NMR spectrums of 1 explained the presence of a methyl proton of B-ring integrating for three protons from the presence of one singlet at δ 2.43 (C_4-CH_3). The olefinic protons of an α,β unsaturated ketone were clearly observed at δ 7.65 (*J*=16 Hz) and δ 7.94 (*J*=16 Hz) corresponding to C_α-H and C_β-H, respectively. The higher coupling value shows that the olefinic protons are in *trans* orientation. Compound 1 also showed typical two doublets integrating for two protons each respectively of B- ring at δ 7.05 (C_3-H and C_5-H; *J*= 8 Hz) and δ 7.59 (C_2- H and C_6-H; *J*= 8 Hz). One doublet resonated at δ 7.27 (*J*=8 Hz) corresponding to one aromatic proton, $C_{3'}$-H of A ring. This proton coupled with proton $C_{5'}$-H. A multiplet signal resonated at δ 6.96 corresponding to one aromatic proton, $C_{4'}$-H. Another doublet signal at a lower field, δ 7.96 (*J*=8 Hz) was attributed to one aromatic proton, $C_{6'}$-H. A characteristic singlet at δ 12.76 indicated the presence of a chelated phenolic proton at $C_{2'}$-OH integrating for one proton. The ^{13}C-NMR spectrum of compound 1 showed the presence of fourteen signals attributed to sixteen carbons corresponding molecular formula $C_{16}H_{14}O_2$. The existence of a carbonyl group (>C=O) was indicated at δ 186.4. At the same time the olefinic carbon of C_α and C_β resonated at δ 124.2 and δ 143.9, respectively. Remaining signals were assigned to the rest of the aromatic carbons in the molecule.



Scheme 1

Oxidative cyclization of chalcone 1 into the corresponding flavone 11 was carried out using DMSO/I2 reagent under microwave irradiation. The IR absorption at 1639.49 cm-1 showed the presence of a conjugated carbonyl group (>C=O) and the absence of a hydroxyl group band confirmed the oxidation of chalcone 1 into flavone 11. Signal of -OH was also not observed in 1H-NMR spectrum. The spectrum of 11 also displayed a singlet at δH 6.82 for one proton corresponding to C3-H and showed that the Cβ-H of the corresponding chalcone 1 involved in cyclization of chalcone to form corresponding flavone. The rest of the 1H- and entire 13C- NMR spectral data were in accordance with the structure of 4'-methylflavone 11 (see experimental section). Similarly, the structure of the compounds 2-9 and 12-20 have been elucidated by using IR, 1H and 13C NMR data.

Some of the synthesized compounds showed low antibacterial activities and some were unable to show inhibition. Amongst all the compounds tested some compounds showed lower MIC values against both

the gram- positive and gram-negative bacteria strains. The MBC of some compounds showed lower values and some compounds showed higher values for all bacteria strains. In the antifungal activity flavones are somewhat effective than their corresponding chalcones towards the selected organisms (fungi). From the result it can be concluded that the flavone ring system and presence of various groups are responsible for the antifungal effects.

Conclusion

In this work, we have demonstrated the synthesis of flavones using microwave irradiation. The advantages of this method are high yields, relatively short reaction times, low cost, simple experimental and as isolation procedures, and finally, it is in agreement with the green chemistry protocols. The activity data obtained during the study will be certainly useful to go for further research for drug designing and synthesizing new flavone derivatives.

Acknowledgment

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Regioselective Synthesis and Reactions Of Some New Uracil Riboside Derivatives for Potential Antimicrobial Agents

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Abstract

A novel series of uracil riboside i.e., uridine (1) derivatives were synthesized using the direct acylation method. Firstly, uridine was selectively converted to 5/-*O*-(2-bromobenzoyl) derivative by treatment with 2-bromobenzoyl chloride in dry pyridine with fair yields. Using a wide variety of acylating agents, a series of 2′,3′-di-*O*-acyluridine derivatives (scheme-1) of this product were also prepared in order to gather additional information for structure elucidation. Another new octanoylation series of uracil riboside (uridine) were also successfully carried out using the same acylation method and afforded the methyl 5/-*O*-acyl uridine in good yields. In order to obtain newer products, the 5/-*O*-acyl derivative was further transformed to a series of 2′,3′-di-*O*-acyluridine derivatives (scheme-2). The structure of the all acylation products were ascertained by analyzing their elemental data, IR, ^{1}H-NMR and Mass spectra. All synthesized products were screened for *in vitro* antibacterial and antifungal activities against a number of human pathogenic bacteria and plant pathogenic fungi. The study revealed that the tested acylated uracil ribose derivatives exhibited moderate to good antibacterial and antifungal activities. Encouragingly, a number of test compounds showed better antimicrobial activity compare with the standard antibiotics employed. For comparative studies, the antimicrobial activities of two standard antibiotics, Ampicillin and Nystatin, were also measured. Another noteworthy observation was that the uridine derivatives were found comparatively equally effective against both Gram positive microorganisms and Gram negative microorganisms. It also observed that the test compounds were more effective against fungal phytopathogens than those of the bacterial organisms.

Keywords: Uracil riboside, Acylation, Synthesis, Spectroscopy, Antibacterial, Antifungal

Introduction

Nucleosides are glycosylamines consisting of a nucleobase which β-glycosidic bonded to a sugar and the nitrogen-containing compound is either a pyrimidine base or a purine base. Nucleosides and nucleotide derivatives are necessary for life, as they are building blocks of nucleic acids and have thousands of other roles in cell metabolism and regulation. Nucleosides are required for DNA and RNA synthesis, and the nucleoside adenosine has a function in a variety of signaling processes (Wen and Xia 2012). In medicine several nucleoside analogues are used as antiviral or anticancer agents (Jordheim *et al.,* 2013). Infectious diseases worldwide have been known to be a cause of morbidity, disability and mortality. Approximately 15 million people die each year due to infectious diseases-nearly all live in developing countries (WHO, 2004).

Selective acylation is very important in the field of nucleoside chemistry because of its usefulness for the synthesis of biologically active products. During the last few years, many workers have investigated selective acylation and alkylation of hydroxyl groups of the carbohydrate moieties of nucleosides and nucleotides using various methods (Williams and Richardson 1967; Hiroyuki *et al.,* 1990; Willard *et al.,* 1964). Different methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully (Wagner *et al.,* (1974) ; Kim *et al.,* 1985). Of these, direct method has been found to be the most encouraging method for acylation of carbohydrates and nucleosides (Ichinari *et al.,* 1988). From literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nucleus and acyl substituents (Gawande and Shingare 1987; Gupta *et al.,* (1997). It is also known that, if an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity (Singh *et al.,* 1990). The benzene and substituted benzene nuclei play important role as common denominator of various biological activities (Kawsar *et al.,* 2013). Results of an ongoing research work on selective acylation of carbohydrates (Kawsar *et al.,* 2014a; Kabir *et al.,* 1998). and nucleosides (Kabir *et al.,* 2003; Kawsar *et al.,* 2014). and also evaluation of antimicrobial activities reveal that in many cases the combination of two or more aromatic or heteroaromatic nuclei [16]. It is also found that nitrogen, sulfur and halogen containing substitution products showed marked antimicrobial activities of the parent compound (Kawsar *et al.,* 2013; Kawsar *et al.,* 2012; Kabir *et al.,* 2009; Kabir *et al.,* 2008a; Kabir *et al.,* 2004; Bauer *et al.,* 1966). Encouraged by literature reports and previous project research findings, we synthesized a series of uracil riboside i.e., uridine (1) derivatives (Schemes 1 and 2). Antibacterial and antifungal screening of these compounds were carried out using a variety of bacterial and fungal strains against a number of human and plant pathogens and the results are reported here as first time.

Materials and methods

FTIR spectra were recorded by KBr disc at the Chemistry Department, University of Chittagong, Bangladesh, with an IR Affinity Fourier Transform Infrared Spectrophotometer (SHIMADZU). Mass spectra of the synthesized compounds were done by liquid chromatography electrospray ionization-tandem mass spectrometry in positive ionization mode (LC/ESI(+)-MS/MS) by using a system that consisted of a JASSO LC (JASCO, Tokyo, Japan) at the Yokohama City University, Yokohama, Japan. All reagents used were commercially available (Aldrich) and were used as received, unless otherwise specified. Melting points were determined on an electro-thermal melting point apparatus (England) and are uncorrected. Evaporations were carried out under reduced pressure using VV-1 type vacuum rotary evaporator (Germany) with a bath temperature below 40^{o}C. ^{1}H-NMR spectra (400 MHz) were recorded for solutions in deuteriochloroform ($CDCl_3$) with a Bruker DPX-40C spectrometer. Thin layer chromatography (t.l.c) was performed on Kieselgel GF_{254}. Column chromatography was performed with silica gel G60 (Silicycle, 60-200 μm, 60 Å).

Synthesis of uracil riboside i.e., uridine derivatives (Scheme-1 & Scheme-2)

Synthesis of 5′-O-(2-Bromobenzoyl) uridine (2)

A solution of uridine (**1**) (200 mg, 0.82 mmol) in dry pyridine (3 ml) was cooled to -5^{0}C whereupon 2-bromobenzoyl chloride (0.11 ml, 1.1 molar eq.) was added to it. The mixture was stirred at this temperature for 5 hours and then stirred overnight at room temperature. The progress of the reaction was monitored by T.L.C. (CH_3OH-$CHCl_3$, 1:5), which indicated full conversion of the starting material into a single product (R_f = 0.50).

A few pieces of ice was added to the flask and then extracted the product mixture with chloroform

(3×10=30 ml). The combined chloroform layer was washed successively with dilute hydrochloric acid (10%), saturated aqueous sodium hydrogen carbonate ($NaHCO_3$) solution and distilled water. The chloroform layer was dried with anhydrous magnesium sulphate ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to leave a syrup. The syrup was passed through a silica gel column chromatography and eluted with methanol-chloroform (1:5) provided the 2-bromobenzoyl chloride derivative (**2**) (138 mg, 69%) as crystalline solid. Recrystallization from chloroform-*n*-hexane gave the (**2**) as needless, m.p. (210-212^{0}C). The compound was sufficiently pure for use in the next stage without further purification and identification.

Anal Calcd. for $C_{16}H_{15}O_7N_2Br$ (427.23)

C, 44.94%, H, 3.51%; found: C, 44.98%, H, 3.55%. FTIR data: ν_{max} 1735 (-CO), 3505 (-OH) cm^{-1}. H -NMR (400 MHz, $CDCl_3$) data: δ_H 9.21 (1H, s, -NH), 7.88 (1H, d, J = 7.8 Hz, Ar-H), 7.76 (1H, d, J = 7.8 Hz, H-6), 7.67 (2H, m, Ar-H), 7.42 (1H, m, Ar-H), 6.26 (1H, d, J=5.8 Hz, H-1′), 6.20 (1H, s, 2′-OH), 5.96 (1H, dd, J = 2.2 and 12.2 Hz, H-5′a), 5.86 (1H, dd, J=2.3 and 12.4 Hz, H-5′b), 5.75 (1H, d, J = 8.2 Hz, H-5), 5.28 (1H, s, 3′-OH), 4.70 (1H, dd, J = 2.3 and 5.6 Hz, H-4′), 4.40 (1H, d, J = 5.8 Hz, H-2′), 4.03 (1H, dd, J = 7.6 and 5.6 Hz, H-3′).

*Synthesis of 5′-O-(2-Bromobenzoyl)-2′,3′-di-**O**-pentanoyluridine (3)*

To a cooled (0^0 C) and stirred solution of the 2-bromobenzoyl derivative (**2**) (64.3 mg, 0.16 mmol), in anhydrous pyridine (3 ml) was added pentanoyl chloride (0.066 ml, 5 molar eq.). The mixture was stirred at 0^{0}C for eight hours and then overnight at room temperature. T.L.C. examination (methanol-chloroform, 1:20) showed complete conversion of reactant into a single product (R_f = 0.52). A few pieces of ice were added to the reaction flask in order to destroy the excess reagent and the reaction mixture was processed as usual. Percolation of the resulting syrup by passage through a silica gel column with methanol-chloroform, (1:20), as eluant afforded the pentanoyl derivative (**3**) (58 mg, 90.20%) as a semi-solid mass which could not be crystallized.

Similar reaction and purification procedure was applied to prepare compound **5** - compound **25**, successfully.

Anal Calcd. for $C_{26}H_{31}O_9N_2Br$ (595.40)

C, 52.40%, H, 5.21%; found: C, 52.42%, H, 5.24%. FTIR data: ν_{max} 1758 (-CO) cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) data: δ_H 9.35 (1H, s, -NH), 7.90 (1H, d, J = 7.8 Hz, Ar-H), 7.85 (1H, d, J = 7.9 Hz, H-6), 7.75 (2H, m, Ar-H), 7.59 (1H, m, Ar-H), 6.22 (1H, d, J = 5.8 Hz, H-1′), 5.97 (1H, dd, J = 2.4 and 12.4 Hz, H-5′a), 5.88 (1H, dd, J = 2.2 and 12.4 Hz, H-5′b), 5.75 (1H, d, J = 7.9 Hz, H-5), 5.58 (1H, d, J = 5.4 Hz, H-2′), 4.69 (1H, dd, J = 7.8 and 5.8 Hz H-3′), 4.57 (1H, m, H-4′), 2.40 {4H, m, 2×$CH_3(CH_2)_2CH_2$CO-}, 1.62 (4H, m, 2×$CH_3CH_2CH_2CH_2$CO-), 1.24 {4H, m, 2×$CH_3CH_2(CH_2)_2$CO-}, 0.89 {6H, m, 2×$CH_3(CH_2)_3$CO-}. Mass spectra (MS) (positive ion LC-ESI): *m/z* $[M+H]^+$ 596.40 (29), 581.4 (26), 539.4 (19), 495.4 (7), 452.6 (14), 411.3 (10), 372.4 (22), 226.4 (11), 200.0 (8), 186.0 (19), 112.0 (14), 85.6 (100).

Antimicrobial activity

The synthesized test compounds (Scheme 1 and 2) were subjected to antibacterial screening against five bacterial strains viz., *Bacillus subtilis* BTCC 17, *Bacillus cereus* BTCC 19, *Escherichia coli* ATCC

25922, *Pseudomonas aeruginosa* CRL (ICCDR,B) and *Salmonella typhi* AE 14612. The name of two phytopathogenic fungi viz., *Aspergillus niger* ATCC 16404 and *Rhizopus nigricans* ATCC 6227b. In all cases, a 2% solution (in $CHCl_3$) of the chemicals was used. The *in vitro* antibacterial activities of the synthesized compounds were detected by disc diffusion method (Miah *et al.,* 1990; Grover and Moore 1962). The *in vitro* antifungal activities were done by Poisons Food technique with some modification.

Results and discussion

Characterization of uracil riboside i.e., uridine derivatives (Scheme-1)

In the scheme-1 of this research work presented in this project was to carry out regioselective 2-bromobenzoylation of uridine (1) with 2-bromobenzoyl chloride using the direct method. A series of derivatives of the resulting 2-bromobenzoylation product were prepared using non-traditional twelve acylating agents.

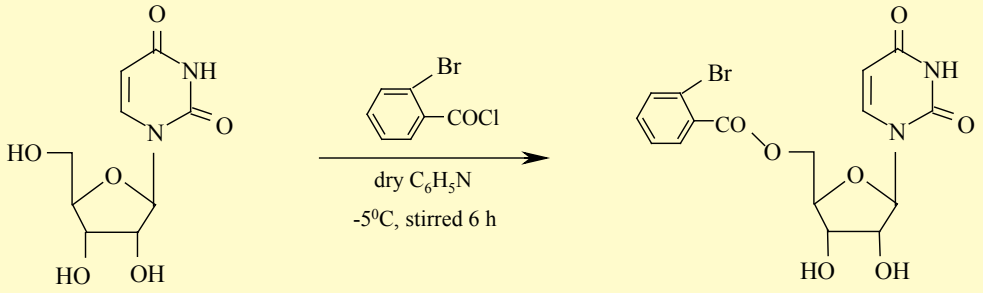
Our initial effort was to react uridine (**1**) with unimolecular amount of 2-bromobenzoyl chloride as acylating agent in dry pyridine at freezing temperature, followed by removal of solvent and silica gel column chromatographic purification, furnished the 2-bromobenzoyl derivative (**2**) in 69% yield as needless, m.p. 210-212^{0}C (chloroform-*n*-hexane). This compound was sufficiently pure for use in the next stages. However, an analytical sample was prepared by recrystallisation from chloroform-*n*-hexane. In its FTIR spectrum, the absorption bands at 1735 and 3505 cm^{-1} corresponded to carbonyl (C=O) and hydroxyl (-OH) stretching, respectively. The formation of a monosubstitution product was clearly revealed by its ^{1}H-NMR spectrum which showed one one-proton doublet at δ 7.88 (as d, J = 7.8 Hz), one two-proton muliplet at δ 7.67 (2H, m) and one one-proton muliplet at δ 7.42 (1H, m) corresponding to the aromatic ring protons of one 2-bromobenzoyl group in the molecule. Also, the C-5′ proton was deshielded considerably (i.e., downfield shift) to δ 5.96 (as dd, J = 2.2 and 12.2 Hz, 5′a) and 5.86 (as dd, J = 2.3 and 12.4 Hz, 5′b) from their usual value suggested the introduction of the 2-bromobenzoyl group at position 5′. The rest of the ^{1}H-NMR spectrum was in conformity with the structure accorded to it. The formation of compound **2** may be explained by assuming that 2-bromobenzoyl chloride attaches with the most reactive and less sterically hindered primary hydroxyl group of the ribose moiety at 5′ position, thereby forming the 5′-*O*-2-bromobenzoate (**2**) as the sole product. Complete analysis of the IR, ^{1}H-NMR of this compound was in agreement with the structure accorded as 5′-*O*-(2-bromobenzoyl)uridine (**2**).

Further support for the structure of the 5′-*O*-2-bromobenzoate (**2**) was achieved by its conversion to the dipentanoate **3**. By complete analysis of the FTIR, ^{1}H-NMR, mass spectra and elemental data (please see experimental section for details), the structure of this compound was assigned as 5′-*O*-(2-bromobenzoyl)-2′,3′-di-*O*-pentanoyluridine (**3**).

Hexanoylation of the 2-bromobenzoyl derivative (**2**) with hexanoyl chloride in dry C_5H_5N provided the dihexanoate (**4**) in 88.63% yield as liquid syrup. The rest of the FTIR, ^{1}H-NMR, mass spectra and elemental analysis (please see experimental section for details) enabled us to assign the structure of the hexanoyl derivative as 5′-*O*-(2-bromobenzoyl)-2′,3′-di-*O*-hexanoyluridine (**4**). The same 2-bromobenzoyl derivative **2** was then converted to the octanoyl derivative (**5**) in 94.93%. The structure of the octanoyl derivatives (**5**) was confidently established by completely analyzing their FTIR, ^{1}H-NMR, mass spectra and elemental data (please see experimental section for details) as 5′-*O*-(2-bromobenzoyl)-2′,3′-di-*O*-octanoyluridine (**5**).

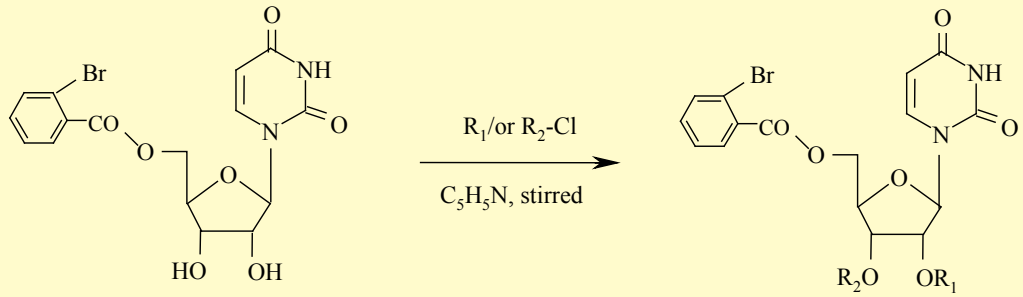
We then derivatized the 2-bromobenzoyl derivative 2 with a number of fatty acid chlorides, such as decanoyl chloride, lauroyl chloride, myristoyl chloride and palmitoyl chloride using similar reaction and work-up procedures. The corresponding fatty acid derivatives (**6, 7, 8** and **9**) were isolated in reasonable yields. The structures of these derivatives were established confidently by completely analyzing their ^{1}H, ^{13}C NMR and mass spectra.

Finally, confirmation of the structure of compound **2** was provided by preparation of its derivatives 10, 11, 12 and 2′,3′-di-*O*-4-chlorobenzoyl derivative (13). In its ^{1}H-NMR spectrum, the two four-aromatic proton multiplets at δ 8.02 (as m), δ 7.44 (as m) are characteristic of *p*-substituted two benzoyl groups. The resonance of other protons in their anticipated positions confirmed the structure of this compound as 5′-*O*-(2-bromobenzoyl)-2′,3′-di-*O*-(4-chlorobenzoyl)uridine (13).



Uridine (**1**)

5′-*O*-(2-Bromobenzoyl)uridine (**2**)



5′-*O*-(2-Bromobenzoyl)uridine (**2**)

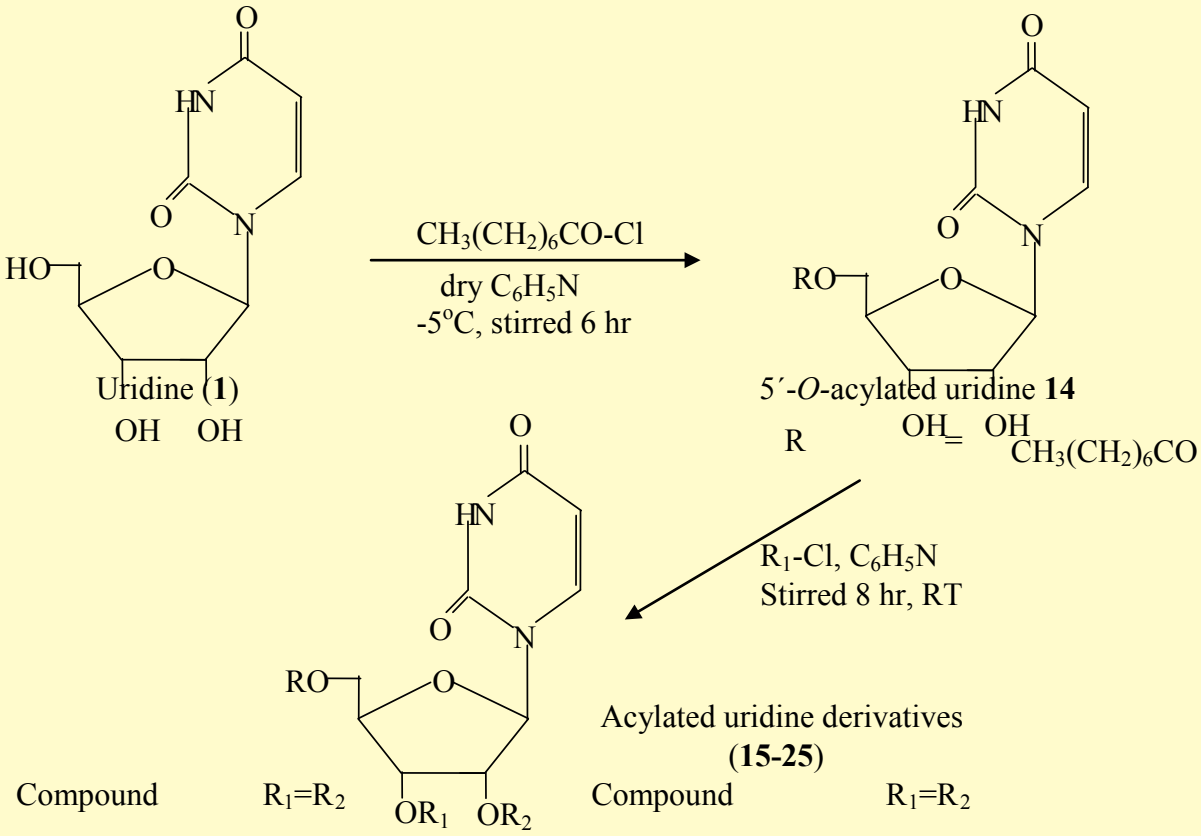
2′,3′-di-*O*-acyl uridine derivatives (**3-13**)

Compound no.	$R_1 = R_2$	Compound no.	$R_1 = R_2$
3.	$CH_3(CH_2)_3CO$-	**9.**	$CH_3(CH_2)_{14}CO$-
4.	$CH_3(CH_2)_4CO$-	**10.**	$(CH_3)_3CCO$-
5.	$CH_3(CH_2)_6CO$-	**11.**	CH_3SO_2-
6.	$CH_3(CH_2)_8CO$-	**12.**	$C_6H_5SO_2$-
7.	$CH_3(CH_2)_{10}CO$-	**13.**	4-Cl.C_6H_4CO-
8.	$CH_3(CH_2)_{12}CO$-		

Scheme-1: The structure of synthesized uracil riboside i.e., uridine derivatives (1-13).

Synthesis and characterization of uracil riboside derivatives (Scheme-2)

The main objective of the research work described in this project was to carry out regioselective acylation of uracil riboside i.e., uridine (**1**) with octanoyl chloride using the direct acylation method (Scheme-2).



Compound	$R_1=R_2$	Compound	$R_1=R_2$
15	$CH_3(CH_2)_3CO$-	21	$(CH_3)_3CCO$-
16	$CH_3(CH_2)_4CO$-	22	CH_3SO_2-
17	$CH_3(CH_2)_8CO$-	23	$C_6H_5SO_2$-
18	$CH_3(CH_2)_{10}CO$-	24	2-Br.C_6H_4CO-
19	$CH_3(CH_2)_{12}CO$-	25	4-Cl.C_6H_4CO-
20	$CH_3(CH_2)_{14}CO$-		

Scheme-2: The structure of synthesized uracil riboside i.e., uridine derivatives (14-25).

Antibacterial Activities of Scheme-1 and 2

In this project, our synthesized and reported chemicals have not been tested before against the selected bacterial and fungal pathogens. The results of the present investigation showed that some of the newly synthesized acylated derivatives of uracil riboside i.e., uridine (1) may be tested against a wide range of

phytopathogenic fungi and bacteria, before sending them to pesticide producing companies for further tests. So it is hoped that the acylated uridine derivatives (Scheme-1 and Scheme-2) might show potential anticancer, antiviral, antituberculatic and anti-inflammatory activities. It is also expected that this piece work employing uridine derivatives (2-25) as test chemicals will help further work to the development of pesticides and medicine for plant and human disease control and also hoped to find clinical and microbial possibilities for future studies as antimicrobial agents.

Conclusion

In current project research work, we successfully synthesized and characterized uracil riboside i.e., uridine (1) derivatives by the direct acylation method. This method demonstrates a very simple and efficient method for the synthesis with excellent yields and short reaction times. The piece of work is being reported for the first time. The compounds 5′-*O*-(2-bromobenzoyl)-2,′3′-di-*O*-octanoyluridine (94.93%), 5′-*O*-(2-bromobenzoyl)-2′, 3′-di-*O*- methanesulphonyluridine (93.24%), 5′-*O*-(2-bromobenzoyl)-2′, 3′-di-*O*- (4-chlorobenzoyl) uridine (95.01%) and 2′,3′-di-*O*-(2-bromobenzoyl)-5′-*O*- octanoyluridine (93%) were found to be encouraging in terms of high selectivity and excellent yields. All the compounds were subjected to biological screening studies. Antibacterial activities of the newly synthesized compounds bearing benzene and various acyl moieties including sulphur, halogen revealed that some tested compounds showed good to moderate activities against selected human and phytopathogenic strains. The tested compound 2′,3′-di-*O*-methanesulfonyl-5′-*O*-octanoyluridine (22) (17 mm) and 2′,3′-di-*O*- (4-chlorobenzoyl) -5′-*O*-octanoyluridine (25) (16 mm) exhibited highest inhibition activity against *Escherichia coli*. The results also showed some of the tested compounds to be the most active antibacterial agents on the test organisms. The antifungal activities of the tested compounds displayed the moderate to good activities. This proves the high therapeutic value of these compounds and encourages further study to explore their biological potential applications may have to clinical and microbial possibilities.

Acknowledgement

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Using the Green Nanotechnology for Synthesis of Titanium Di Oxide (TiO_2) Nanoparticle and Its Used for Fabrication of Dye Sensitized Solar Cells

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Abstract

In this project we developed very large scale (>10g) TiO_2 anatase nanoparticle using the green and clean environment nanotechnology. For the synthesis of highly crystalline nanoparticle we choose EISA (evaporation induced self- assembly) method. For crystallinity and purity measurement we used highly sophisticated PXRD, for surface area measurement using nitrogen adsorption-desorption isotherm, BET instruments and average pore size distribution we chose BJH technique. This would be a significant step in the context of catalyst development for dye - sensitized solar energy and environmental pollution controlled in Bangladesh.

Introduction

Bangladesh stands at the cross roads at the beginning of the 21st Century. It has huge potential, but it must overcome many challenges especially the challenges of global warming and energy crisis, to realize its full potential. Many of the rural people of the country are still not connected to the national grid. Our industries are failing to get the power, they need to function thus influencing in GDP.

Modern civilization relies heavily on electric energy. It is recognized that the pace of power sector development has to be accelerated in order to achieve overall economic development of the country. To upgrade the socio-economic condition and to lighten poverty, electricity sector has been prioritized by all the Governments. One of the main challenges Bangladesh currently facing is the shortage of electric power primarily in rural areas. Recently Bangladesh has given a significant thrust to tapping of solar energy and to install a set target 500MW solar energy in Bangladesh. A huge number of 'green jobs' are also being created with the growth of this newly developed sector. Currently, over 100,000 people work in the solar energy sector across Bangladesh. The country is moving ahead to lay out a mission, Bangladesh Solar Mission. The Government has set a target of making Bangladesh a middle- income country by 2021 by generating electricity from various sources. Renewable Energy is a key tool to make this target come to a reality. Above this background in mind choice of semiconducting material is very important Hossain *et al.,* 2015; Hossain *et al.,* 2015; Aziz ; 2016; Dylla *et al.,* 2013; Dylla 2012; Ren *et al.,* 2012; Brutti 2012; Armstrong *et al.,* 2005; Etacheri *et al.,* 2014; Etacheri *et al.,* 2013; Giannuzzi *et al.,* 2014; Zhang *et al.,* 2014). From the literature point of view TiO_2 is one of the smart candidates to

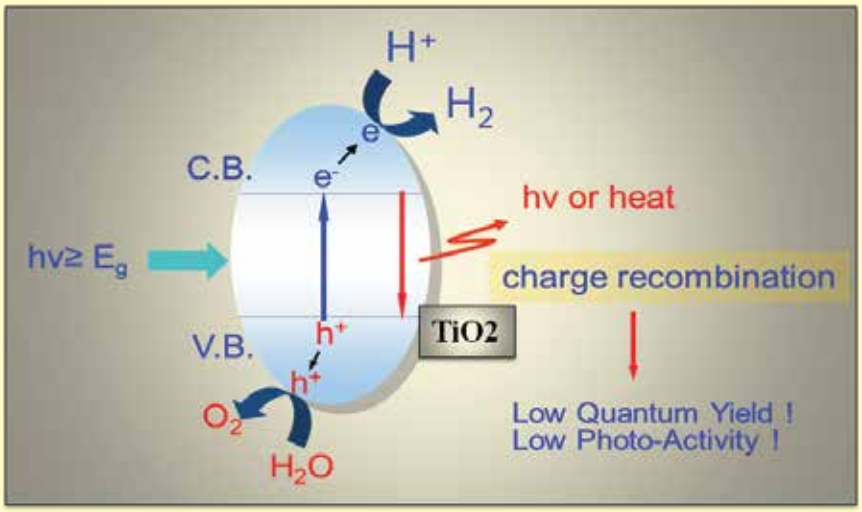

Fig. 1. Schematic illustration of semiconductor excitation leading to the creation of "electrons" in the conduction band and "holes" in the valance band.

meet the criteria. Because TiO_2 has been recognized as excellent materials because of their exceptional electronic and optical properties with high photosensitivity,no toxicity and suitable band gap [1-5]. From the figure 1 clearly illustration proposed mechanism how TiO_2 work as a catalyst to harvest the solar energy mainly production of H_2 from water.

Materials and methods

Titanium isopropoxide [>98%, $Ti(OCH(CH_3)_2)_4$, TIP] and commercial diblock copolymer Pluronic P123 were purchased from Sigma Aldrich (Germany). Absolute ethanol, hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) were purchased from Samchun (Korea).The reagents were used as received without further purification.

Wide angle X-ray (WAX) diffraction patterns of the samples were recorded on a Rigaku D/MAX-2500/pc diffractometer, using Cu-K radiation (200 kV, 40 mA for WAX). N2 adsorption-desorption isotherms were mea-sured at 77 K on a BELSORP-max. Prior to measurements, the samples were degassed under vacuum at 200°C for 6 h. The Brunauer–Emmett–Teller (BET) method was used to calculate the surface areas. The pore size distributions were obtained using the BJH method. Elemental analyses were performed on a Thermo Sci-entific Elemental Analyzer (Flash EA 1112 series).

Synthesis procedure

As-made titania nanoparticle samples were prepared via the ethanolic EISA method by using titanium isopropoxide (TIPO) as a precursor and commercial amphiphilic triblock copolymers (Pluronic P123,) as a surfactant template and a mixture of HCl and H_2SO_4 as the acidic catalyst[1-2]. For a typical synthesis, Pluronic P123 (1.0 g) was dissolved in ethanol (30 g) with vigorous stirring, concentrated HCl (1.4 g) and 98 wt% H_2SO_4 (0.46 g) were added to the solution. After being heated at 40 ^{0}C for 3 h, TIPO (3.0 g) was added with vigorous stirring for 20 h at 40 ^{0}C. The solution was poured into the dishes and evaporated at 40 ^{0}C in air under 50–60% relative humidity for about 2 d, and the resultant membranes were dried at 100 ^{0}C for 2 d. The surfactant sulfuric acid carbonization method was adopted to form crystalline anatase frameworks and then the templates were removed by burn out at 400 ^{0}C in air. The as-made products were

subsequently heated at 400 ^{0}C in air atmosphere for 6 h to burn out the carbon. Finally white powder samples were obtained. figure 2 shows the flow diagram of synthesis of TiO_2 nanoparticle.

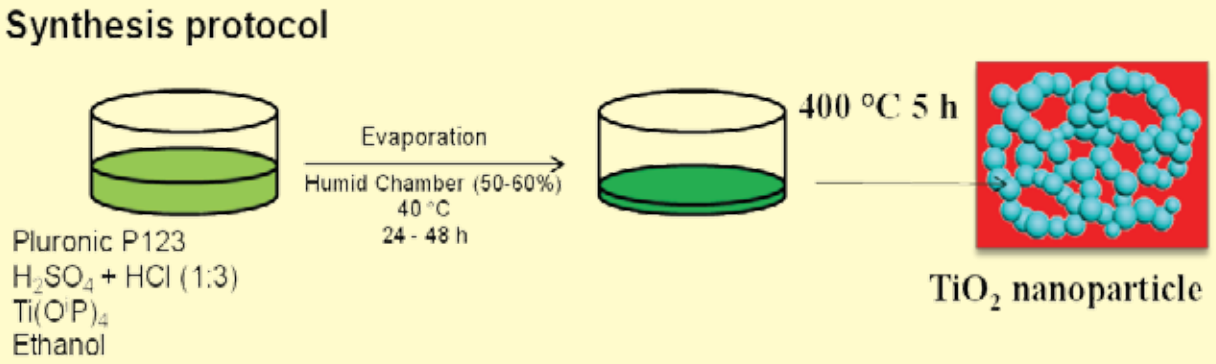

Fig. 2. Schematic illustration of synthesis protocol of TiO_2 nanoparticle

Results and discussion

The desired morphology of TiO_2 nanoparticle has ensured by SEM images of figure 3a and 3b. Insets of figure 3, photos of vivid white powder clearly demonstrated that as synthesis materials was highly pure which was good agreement of XRD pattern of our materials (figure 4a). Powder XRD pattern and nitrogen absorption-desorption isotherm and pore size distribution (insets) which are shown in figure 4 a and figure 4b

respectively. From the XRD pattern it has clearly demonstrated that as synthesize nanoparticle has highly crystalline and there is no extra peaks of XRD pattern means sample was highly pure. From the nitrogen absorption desorption isotherm (figure 4b) indicate that hysteresis loop was sharply increase means nanoparticle was highly uniform and average surface area calculate 198 m^2/g and figure 4 (insets) pore size distribution graph and surface morphology (SEM images, figure 3) clearly shows one an average particle diameter are below 10 nm in size which is highly potential for solar cells application.

Figure 3. Scanning Electron Microscopic(SEM) images (figure 3a and 3b) and insets white powder of as synthesized highly crystalline TiO2 nanoparticle

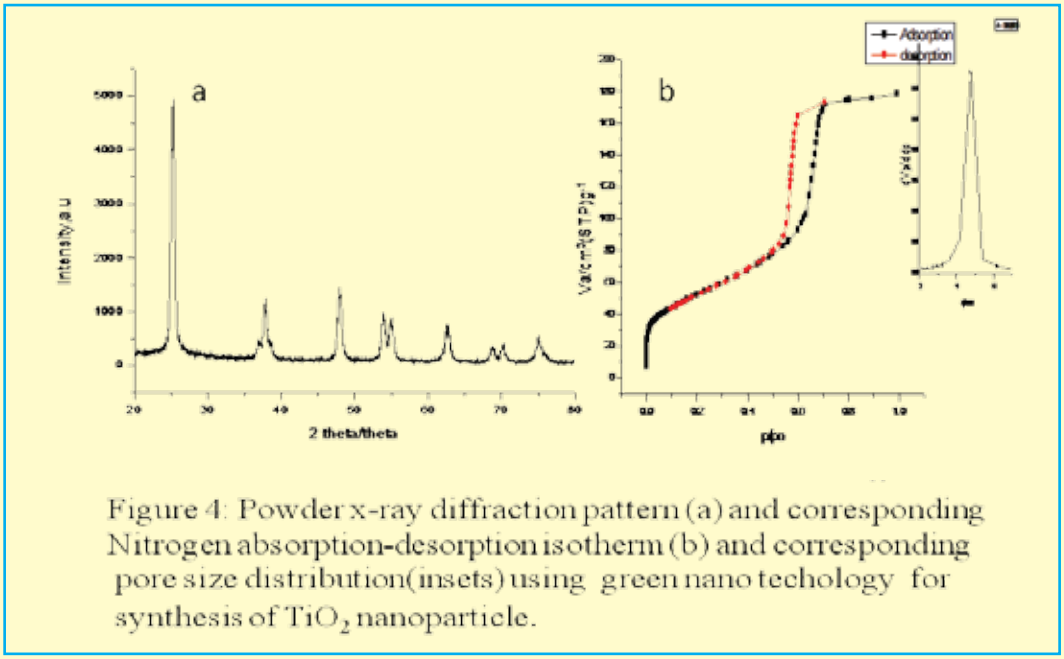

Figure 4: Powder x-ray diffraction pattern (a) and corresponding Nitrogen absorption-desorption isotherm (b) and corresponding pore size distribution(insets) using green nano techology for synthesis of TiO_2 nanoparticle.

Conclusion

In this project we had successfully synthesize large scale (>10g) TiO_2 anatase nanoparticle using the green and clean environment nanotechnology. This nanoparticle might be suitable for dye sensitized solar cells and other potential application for environmental pollutants like waste water degradation and contaminated soil remediation. This would be a significant step in the context of catalyst development for dye sensitized solar energy and environmental pollution controlled in Bangladesh.

Acknowledgment

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Antibody Titer against Hepatitis B Virus Surface Antigen (Hbsag) among the Children Having Hepatitis B Virus Vaccination through EPI Program in Some Areas of Brahmanbaria District of Bangladesh

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Abstract

This study evaluated anti-HBs levels and sero-protection rates and observed sero-prevalence of hepatitis B virus infection measured by anti-HBc among vaccinated children 5 to 12 years after completing their primary HBV vaccination schedule as part of the EPI in Brahmonbaria district. Five hundred (500) children who had received the hepatitis B vaccine as part of their primary infant immunization were followed up 5 to 12 years after their primary immunization schedule. Blood samples were taken and tested for anti-HBs, and anti-HBc Among the sero negative children 1 dose of HBV vaccine was given as a booster. Samples from booster vaccinee were taken one month later and tested for anti Hbs titer. Anti HBs titer was found below protective level in about 46% (230/500) participants. Sero-protection rate decreased up to 72.2% in 5 to 6 year's age group which than further decreased to 58.3% in 7 to 9 years age group and increased again to 69.5% in 10 to 12 years age group children. On the other hand, the mean anti Hbs titer was 97.72 IU/L initially and then increased with the increasing of age from 165.40 IU/L to 196.67 IU/L. Breakthrough infection of HBV was seen in 1.2% (6/500) participants measuring by anti HBc which indicated protective efficacy of HBV vaccine was about 98.8% (494/500). Among the sero negative participants who were given a booster dose, about 6.43% (9/140) were found non responsive to booster dose; 93.57% (131/140) participants showed boosting of mean anti HBs titer upto 804.92 IU/L which was below protective level (<10 IU/L) before booster dose. Anti-HBs titer goes below with the increase of age after completion of primary vaccination. Response to booster dose indicated that most of the participants had immunological memory which will boost antibody titer after any exposure, so routine booster dose is not needed at least 12 years after primary vaccination. But non-responder to vaccination should screen after primary vaccination because of chance of breakthrough infection and chronic carriage.

Keywords: Hepatitis B Virus, Immune response, Antibody titer

Introduction

Hepatitis B Virus infection causes more than 50% of all cases of hepatocellular carcinoma and approximately 620,000 deaths per annum (Goldstein *et al.,* 2005). One of the principal factors that influence the incidence and occurrence of the chronic carriage status is the age at which Hepatitis B Virus infection occurs. Approximately 90% of babies born to HBV surface antigen- (HBsAg) and hepatitis B e antigen (HBeAg)-positive mothers and almost 30% of children infected before 6 years of age turn out to be chronic carriers, matched with less than 10% of adults or grown-up children (Hyams *et al.,* 1995; Ip *et al.,* 1989), Children in early childhood can be horizontally infected or vertically infected from carrier mothers (perinatally). Three modes of Hepatitis B Virus transmission from HBsAg-positive mothers to babies have been proposed: (i) Trans placental intrauterine transmission; (ii) Contact with maternal infected fluids in the birth canal during delivery and (iii) Transmission through breast feeding and childcare during postnatal age Ghendon 1987; Shepard *et al.,* 2006). The only most effective way of controlling hepatitis B disease is by immunization. The development of anti-HBs level greater than or equal to 10 mIU/mL is considered as protective immunity and any level less than 10 mIU/mL as non-protective is directly associated with protective immunity of HBV following vaccination (Mahoney *et al.,* 1995). After a complete vaccination, most individuals develop antibody titers greater than 100 IU/L within 6-8 weeks. Some healthy individuals seemingly do not demonstrate anti-HBs response or respond poorly to the vaccine and are considered as non-responders or hypo responders with titer less than 10 IU/L and 10-100 IU/L respectively (Zuckerman 1996). In agreement with references made by the World Health Organization (WHO 2015) 110 countries have incorporated the Hepatitis B Virus vaccine into their Expanded Programme on Immunization (EPI) by April 2000. In Bangladesh the vaccination coverage rate by the age of 12 months nationally was 82.5 percent, and in Brahmanbaria district is 81.7 percent. Particularly for pentavelent vaccine coverage of 3rd dose completed was found 94.1, 94.0 and 93.8 percent respectively (Bangladesh EPI survey coverage 2015; Civil surgeon Brahmanbaria). There is no comprehensive and authoritative data regarding the prevalence of Hepatitis B infection and the sero-protective rate of the HBV vaccine 10 years after the integration of the HBV vaccine in to the EPI in Bangladesh and specifically, in Brahmonbaria district. The aim of this study is to detect the antibody titer against Hepatitis B virus after vaccination in EPI program, to evaluate the efficacy of the vaccine, the seroprotection rate and duration of immunity among recipients of the vaccine even after 5 to 12 years of vaccination to HBV.

Materials and methods

The study was a descriptive cross sectional study. Children of 5 -12 years who were vaccinated against HBV through EPI program of Bangladesh and have EPI vaccination card was considered for the study. Five hundred (500) samples were collected purposively from Sadar Hospital and other nongovernment hospital of Brahmonbaria district. All the laboratory works were done at Department of Virology, Bangabandu Sheikh Mujab Medical University, Dhaka. During data analysis the samples were divided into groups depending on the age, sex, vaccination history. After receiving verbal and written consent from guardian, 3 ml of blood sample was taken from anti-cubital vein with full aseptic precaution from each child. Serum was separated at collection place. Samples were stored at -20 ^{0}C temperature until transported to virology laboratory BSMMU.

Results and discussion

Table I showing antibody titer is significantly increased among male children then female. Seroconversion rate is about 99.8%, but about 45.8% children have got antibody titer below protective level i.e. 10 IU/ml. Antibody titer within different age group showed that titer increased as age increases.

Table I: showing antibody titer according to age category, sex and level of protection

Mean Antibody titer according to sex who are sero -positive (Anti HBs > 10 IU/ml)			
Sex	N	Mean Anti HBs titer (range)	P value
Female	106	106.9321 (SD±173.93681)	0.004
Male	164	190.0085 (SD±298.99036)	

Level of Protection		
Category	Number	%
No sero-conversion < 1IU/ml	1	0.2%
No protection <10 IU/ml	229	45.8%
Moderate protection 10 -100 IU/ml	182	36.4%
Strong protection >100 IU/ml	88	17.6%
Total	500	100%

Mean Antibody titer according to age category among seropositive children		
Age group	N	Mean Anti HBs titer
Group A (5 -6 years)	68	97.7250
Group B (7 -9 years)	124	165.4048
Group C (10 -12 years)	78	196.6756
Total	270	157.3933

Total 270 157.3933Among the 140 children vaccinated with booster dose who had anti HBs below protection level (< 10 IU/ml), 9 subjects did not show any response to booster dose. Among the rest of the 131children who have responded to booster dose antibody titer was seen after one month and mean anti-Hbs was found 804.92 IU/ml which ranged from minimum 11.90 IU/ml to maximum > 1000 IU/ml (Table: II). This difference was statistically significant.

Table II. Antibody titer among participants before and after giving booster dose (n=131)

	N	Minimum	Maximum	Mean	P value
Anti-HBs titer before booster dose	131	5	9.9	5.89	0.000
Anti-HBs titer after booster dose	131	11.90	1000.00	804.9275	

The immunization with HB vaccine has been in practice irregularly in our country before introduction in the EPI schedule since 2004 to cover all children. Now 3 doses of recombinant HB vaccine are given to every child along with DPT in the EPI program. The present study was designed to evaluated seroconversion and seroprotection status by measuring the anti-HBs titer among the individuals of different age and sex, vaccinated in EPI schedule 5 to 12 years after vaccination. Result of the study indicated that one but all 500 children had seroconversion (i.e. had anti-HBs >1 IU/L) after three doses of hepatitis B vaccination, so the seroconversion rate was 99.8% (Table-1). The rate of sero-conversion was

found to be 92.20% in a study conducted in Bangladesh among EPI-vaccinated children (Khan 2006). The results of the present study are consistent with a longitudinal study by Freitas da Motta *et al.* in Brazil (Freitas *et al.,* 2002), who reported, 98% of individuals seroconverted. Moreover, Chakraborty et al. reported 100% seroconversion in a cross-sectional study in Bangladesh (Chakraborty *et al.,* 2001).

Some of the antibody levels however, did not reach a concentration that confers protection (< 10IU/L). Out of 500 vaccinated persons, who received 3 doses of Hepatitis B vaccine, 270 developed seroconversion with protective level of immunity in the current study. The overall sero-protectivity rate (anti-HBs titer >10 IU/L) among the vaccinated individuals was found to be 54%.

In the present study prevalence of HBV infection was confirmed by detecting anti Hbc among vaccinated children that should not be present if protection is adequate by vaccination. The prevalence of HBV infection among general population found in different previous studies in Bangladesh was about 5.5%, 5.39%, 4.4%, 3% and 2.35% (Mahtab *et al.,* 2008; Shil *et al.,* 2015; Rukunuzzaman *et al.,* 2015; Munshi *et al.,* 2008). In this study, among the vaccinated 500 children only 6 children were found to have anti Hbc in serum indicating breakthrough infection which was about only in 1.2% (6/500). This significant decrease in HBV infection among the vaccinated children might be as a result of the effectiveness of the HBV infant immunization in preventing the horizontal transmission of the virus to the vaccinated children. In one case, who was positive for anti Hbc, was positive for HBsAg also. Mother of that child was tested and found positive for HBsAg which indicated vertical transmission of HBV to child. In this study, it was evident that across age groups seroprotection rate was found 51.51% at 5 to 6 years age group after which it increased up to 56.36% with increasing age among 7 to 9 years age group and then declining again with increasing age through 10 to 12 years age group up to 52.70% among the vaccinated children; but this findings was not supported by the mean anti-HBs titer (GMT) levels which was found gradually increasing with age including 97.72 IU/L 5 to 6 years age group then increased up to 165.40 IU/L and 196.67 IU/L among 7 to 9 years and 10 to 12 years age group respectively (Table-I). This decline in seroprotection rates and increase in anti HBs levels in this study with increasing age from 5 to 6 years, 7 to 9 years and 10 to 12 years age group is in line with findings by (Issaka Koray 2016; Nuhaila 2016). In contrast to the overall generally low anti HBs levels among the vaccinated group can be attributed to waning immunity with increasing age, (declining anti-HBs titre values with age). The findings in this study and the findings by (Lu, CY *et al,* 2008; Lee *et al.,* 1995; Lu *et al.,* 2004; Lee *et al.,* 1995; Lin *et al,* 2003; Lin *et al.,* 2003). showed that although the vaccination program has been very successful in reducing carriage rate of HBV in the world, a gradual yearly decline in antibody titers against the HBsAg among vaccinees has been noted in several follow-up studies. In this study it was found that males have higher antibody titer than females among seropositive subjects (Table-I) which is similar to a study done by Behairy *et al.,* who reported that males retain higher anti-HBsAb titres values than females (Mazahi *et al.,* 2014). In our study, response to booster dose among children who were sero negative (n=140) seen to responsive in 131(93.57%) cases and 6.43% cases were non responsive. In contrast to our study, Lu *et al.,* reported 29.2% non-responsiveness to a booster dose by vaccinees that initially sero-converted with non-protective levels (Lu *et al.,* 1979). The need for booster dose for long term protection into adolescence and adulthood need to be well investigated and a consensus reached on them large pool of non-responders being reported across the globe.

Conclusion

It can be concluded that HBV vaccine given at 6, 10 and 14 weeks as part of routine immunization for HBV infection, is highly effective within the framework of the EPI in Brahmonbaria district since it have

successfully reduced the prevalence of HBV infection to 1.2% among vaccinated children from 3-6.25% among the entire study population. The results clearly indicate that universal childhood HBV vaccination can drastically reduce the rate of new infections, and thereby reduce the burden of HBV infection and HBV related liver disease, especially cirrhosis and hepatocellular carcinoma in Bangladeshi children.

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Magnesium Sulphate Versus Sildenafil in the Treatment of Persistent Pulmonary Hypertension of Newborn: A Randomized Clinical Trial

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Abstract

One of the common causes of respiratory distress in neonate is persistent pulmonary hypertension of Newborn (PPHN) and has been estimated to occur in 2 per 1000 of live born term infants. To evaluate the effect of injectable Magnesium Sulphate and oral Sildenafil in the Treatment of Persistent Pulmonary Hypertension of the Newborn. It was a randomized controlled clinical trial, conducted from August 2015 to July 2017 among 50 neonates having moderate to severe PPHN in the Pediatric Cardiac Intensive Care Unit (CICU) of Dhaka Shishu (Children) Hospital. Neonates were randomized into Magnesium Sulphate (Group-A) and Sildenafil group (Group-B). Side effects were observed and outcome was recorded. Data were analyzed by using SPSS version 17. Mean age in hour in Group A was 29.12±14.26 hours and in Group B was 34.36±10.38 hours. There was significant improvement of oxygenation and decrease in pulmonary vascular resistance at 72 hours after treatment in both study groups ($p<0.05$). Significant improvement of oxygenation was found in Sildenafil study group than Magnesium Sulphate group after 72th hour of treatment (91.95±7.96 vs 96.39±1.58%, $p=0.01$). Limited complications and comparable survival rates were observed in both groups. There was no significant difference in time taken to improve and hospital stay between two groups ($p>0.05$). Magnesium Sulphate and Sildenafil both are effective in improvement of oxygenation and reduction of pulmonary vascular resistance. Sildenafil was more effective than Magnesium Sulphate in the treatment of PPHN with regard to improvement of oxygenation.

Keywards: Magnesium Sulphate, Sildenafil, PPHN, Neonate

Introduction

Respiratory distress is one of the most common presenting problems of newborn.[1] Newborns with respiratory distress must be evaluated promptly and accurately. As occasionally, neonatal respiratory distress is life-threatening and requires immediate intervention. It is a common emergency responsible for 30-40% of admissions in the neonatal period (Edwards *et al.,* 2013; NNF Recommended Nomenclature 1998). Fifteen percent of term infants and 29% of late preterm infants admitted to the neonatal intensive care unit develop significant respiratory morbidity (Hibbard *et al.,* 2010). One of the common cause of respiratory distress in neonate is persistent pulmonary hypertension of Newborn (PPHN) and has been estimated to occur in 2 per 1000 of live born term infants, and some degree of pulmonary hypertension complicates the course of more than 10% of all neonates with respiratory failure (Walsh *et al.,* 2000).

PPHN may result when pulmonary vascular resistance does not fall after birth, hypoxemic respiratory failure. It is characterized by increased pulmonary vascular resistance and right-to-left shunting through the foramen ovale, with or without a patent ductus arteriosus, causing arterial hypoxia even with 100% FiO2 (Askin 2009). Primary treatment of the neonate with PPHN depends on the underlying disorder. A variety of treatment options includes surfactant, sedation, alkalinization, vasodilatation e.g (tolazoline, inhaled nitric oxide, magnesium sulfate, adenosine, bosentan, sildenafil), high frequency jet-ventilation (HFJV) and extracorporeal membrane oxygenation (ECMO) (Konduri *et al.,* 2009). The aim of treatment is to lower pulmonary vascular resistance, maintain systemic blood pressure, reverse right to left shunt, and improve arterial oxygen saturation (Kinsella *et al.,* 2005), There is strong evidence for the use of inhaled nitric oxide (NO) and ECMO in the treatment of PPHN. However, many developing countries and resource limited centers do not have the funds or the technical expertise required for these expensive therapies (Chambers *et al.,* 2006). Sildenafil is a potent and selective inhibitor of cGMP-specific phosphodiesterase 5 (PDE5). This isoenzyme metabolizes cGMP which is the second- messenger of NO and a principle mediator of smooth muscle relaxation and vasodilatation. By inhibiting the hydrolytic breakdown of cGMP, sildenafil prolongs the action of cGMP. This results in augmented smooth muscle relaxation and cause pulmonary vasodilatation (Reffelmann *et al.,* 2003). Sildenafil decreases pulmonary vascular resistance in pulmonary hypertensive neonate (Humbert *et al.,* 2004) Magnesium sulphate is a natural Ca channel blocker that antagonizes Ca ion entry into smooth muscle cell thus promoting vasodilatation. It is safe and cheaper alternative for first line treatment in moderate PPHN (Shaltout *et al.,* 2012). A recent study concluded that where nitric oxide facilities are not available, magnesium sulphate is a cheap alternative for first line treatment of moderate PPHN. However, pre-term neonates are at high risk for respiratory depression due to magnesium sulphate. Oral sildenafil use may therefore be preferable in pre-term neonates (Raimondi *et al.,* 2008). Magnesium sulphate and sildenafil have been studied independently in the treatment of PPHN but randomized controlled trials comparing both are limited. Very few data are available in our country regarding outcome and treatment of PPHN in Bangladesh. Current evidence indicates that different approaches such as with sildenafil and magnesium sulphate may improve oxygenation in PPHN. Early awareness of predisposing conditions and proper identification along with alternative and less expensive treatment leads to better outcome in PPHN in countries with limited resources.

Materials and methods

It was a randomized controlled trial, conducted from August 2015 to July 2017 in the Cardiac Intensive Care Unit (CICU) of Dhaka Shishu (Children) Hospital. During this period 461 neonates having respiratory distress and/or cyanosis were screened and 137 with high index of suspicion with pre and post ductal O_2 saturation difference >10% were undergone echocardiography. Moderate to severe PPHN were identified among 50 neonates and were randomized into Magnesium Sulphate treatment group (Group-A) and Sildenafil treatment group (Group-B). Informed written consent was taken from parents. In Magnesium Sulphate group Magnesium Sulphate was started with a loading dose of 100mg/kg over 30 min followed by 20-50 mg/kg/h for 5 hours for 3 days. Sildenafil treatment group was treated with oral Sildenafil in a dose of 2 mg/kg/day in three divided doses by nasogastric tube for 3 days. Pre-ductal and post ductal SpO_2 was monitored. Outcome measures include drop of pulmonary vascular resistance measured by right ventricular systolic pressure (RVSP <25 mmHg), increase PaO_2 >30 mmHg and time interval to improve O_2, ABG, decrease ventilator duration etc. Side effect was observed in the patient and outcome was recorded. Data were analyzed by using SPSS version 17.

Result and discussion

Mean age in hour in Group A was 29.12±14.26 hours and in Group B was 34.36±10.38 hours and there was no statistically significant difference between mean age in two groups ($p>0.05$). There was significant improvement of oxygenation in each study group from diagnosis to 72 hour after treatment ($p<0.05$) [Table I].

Table I. Assessment of improvement of oxygenation in each study group from diagnosis to 72th hour after treatment

Group A (n=25)			Group B (n=25)		
SpO 2 (mean ±SD)		P value	SpO 2 (mean ±SD)		P value
At diagnosis	64.56±7.04	0.08	At diagnosis	66.08±7.34	0.04
At 6th hour	70.72±8.49		At 6th hour	75.44±11.43	
At diagnosis	64.56±7.04	0.000	At diagnosis	66.08±7.34	0.000
At 12th hour	72.84±6.37		At 12th hour	79.08±9.88	
At diagnosis	64.56±7.04	0.000	At diagnosis	66.08±7.34	0.000
At 24th hour	79.12±6.53		At 24th hour	84.32±8.59	
At diagnosis	64.56±7.04	0.000	At diagnosis	66.08±7.34	0.000
At 36th hour	82.64±5.83		At 36th hour	89.36±7.82	
At diagnosis	64.56±7.04	0.000	At diagnosis	66.08±7.34	0.000
At 48th hour	87.80±7.91		At 48th hour	93.65±3.32	
At diagnosis	64.56±7.04	0.000	At diagnosis	66.08±7.34	0.000
At 72th hour	91.95±7.96		At 72th hour	96.39±1.58	

Group - A: Magnessium sulphate, Group - B: Sildenafil treatment group, Paired samples t test

There was statistically significant improvement of oxygenation in Sildenafil study group from 12th hour to 72th hour of treatment ($p<0.05$) [Table II].

Table II. Assessment of improvement in both study groups from 6 hours to 72 hours hour after treatment

Assessment		Grou p A (n=25)	Group B (n=25)	P value
SpO_2(mean± SD)%	at 6 hours	70.72±8.49	75.44±11.43	0.10
	at 12 hours	72.84±6.37	79.08±9.88	0.01
	at 24 hours	79.12±6.53	84.32±8.59	0.02
	at 36 hours	82.64±5.83	89.36±7.82	0.001
	at 48 hours	87.80±7.91	93.65±3.32	0.002
	at 72 hours	91.95±7.96	96.39±1.58	0.01

Statistically significant improvement was found at 72th hour regarding drop of right ventricular systolic pressure (RVSP) in both treatment groups [Table III].

Table III. Assessment of comparison in improvement regarding RVSP after treatment in both groups at 72th hour

Drug	RVSP (mean ± SD)	P value
Magnessium sulphate (n=23)	At diagnosis = 53.03±9.20	0.000
	At 72th hour = 34.69±9.88	
Sildenafil (n=21)	At diagnosis = 53.75±7.90	0.000
	At 72th hour = 34.51±7.60	

RVSP - Right ventricular systolic pressure, Paired Sample Test; Group - A: Magnesium sulphate Group - B: Sildenafil treatment group

No significant statistical difference in comparison of complications, time taken to improve, hospital stay and outcome between two study groups (p>0.05) [Table IV].

Table IV. Comparison of time taken to improve and hospital stay between two study groups

		Group A (n=13)	Group B (n=13)	p value
Complications	Present	7	6	0.44
	Absent	18	19	
Time taken to improve (hours) [mean ± SD]		56.57±12.66	47.56±18.38	0.06
Hospital Stay (days) [mean ± SD]		7.32±2.54	6.68±1.81	0.31
Outcome	Improved	21	23	0.33
	Died	4	2	

Group - A: Magnessium sulphate, Group - B: Sildenafil treatment group, chi-square test

Our primary outcome measure was drop in right ventricular systolic pressure (RVSP) by echocardiographic evaluation which drops remarkably in both $MgSO_4$ and Sildenafil group. Shaltouta *et al*., also found similar result. Blood pressure was monitored and hypotension was observed in two of our patients (13.3%) in $MgSO_4$ group. In response to hypotension MgSO4 infusion was temporarily discontinued and saline infusion was given. (Shaltouta *et al.,* 2012), reported 20% hypotension in their study in case of $MgSO_4$. However, other side effects of $MgSO_4$ (flaccidity, hypocalcaemia and GIT disturbance) were not found in the present study. Secondary outcome measure was improvement of oxygenation measured by changes in partial pressure of oxygenation and ventilatory requirements where a significant improvement of patients' oxygenation parameters was noted at 72 hours which also shown by previous studies (Chandran *er al.,* 2004). One Cochrane collaboration, published in 2011 showed improvement in oxygenation.

Khorana *et al.,* 2011), in 2011also reported improvement of oxygenation in full term babies with the use of sildenafil. However, hypotension was a concern in their study and it was not found on sildenafil in the present work. In a recent randomized clinical trial sildenafil was found more effective in the treatment of PPHN then $MGSO_4$ (Uslu *et al.,* 2011). This study also found that oxygenation was more improved in Sildenafil group. No significant statistical difference in comparison of time taken to improve, hospital stay and outcome between two study groups were found in present study.

Conclusions

Magnesium Sulphate and Sildenafil both are safe and effective in improvement of oxygenation and reduction of pulmonary vascular resistance. Sildenafil was more effective than Magnesium Sulphate in the treatment of PPHN with regard to improvement of oxygenation.

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Synthesis of Antimicrobial Drug like Fused Thiazole-Chalcone Schiff Base Derivatives

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Abstract

In our study Nine thiazole 2-(5-acetyl-4-methyl-2-thiazolyl) hydrazonebenzaldehyde Schiff base derivatives were synthesized via (a) condensation of benzaldehyde and thiosemicarbazones followed by (b) cyclization with 3-chloroacetylacetone and characterized by various spectroscopic methods (IR, UV, ^{1}H NMR, ^{13}CNMR & HRMS**)**. Antimicrobial activities of these compounds were evaluated against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* gram positive bacteria and *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* gram negative bacteria and *Aspergillus niger*, *Tricodarma harzianum* fungal strains. All the synthesized compounds showed good antifungal activity test against *Aspergillus niger.*

Introduction

A thiazole heterocyclic ring system originates naturally in the crucial water soluble Vitamin B_2 which supports the discharge of energy from carbohydrates through the course of metabolism. On the other hand, Schiff bases are important class of compounds in medicinal and pharmaceutical field. They show Biological activities such as antibacterial, antifungal, antitumor activities (Azza *et al.,* 2006; Pandeya *et al.,* 1999). Thiazoles contain one sulfur atom which play vital role in drugs because sulfur atom containing compounds are universal and crucial in living organisms (Fontecave *et al;* 2003). They show wide varieties of biological applications including the treatment of allergies, hypertension, inflammation, microbial infections, HIV infections and cancer treatment where a thiazole containing commercial drug dasatinib is used for the treatment of leukemia (Omar *et al.,* 2010; Bell *et al.,* 1995). They have also been used as fibrinogen receptor antagonists with antithrombotic activity and as new inhibitors of bacterial DNA gyrase B (Rudolph *et al.,* 2001). There is an urgent need to discover new drugs with novel mechanisms of action, higher activity and improved selectivity to address the severe challenge of multidrug resistance in treating bacterial infections and cancer. Biochemical reactions can easily metabolize thiazoles which are non-carcinogenic in nature. All the available penicillins contain thiazole nucleus which have revolutionized the therapy of bacterial diseases[8]. In order to bestow different electronic environment to the molecules, the substitution pattern of the thiazole was carefully selected. Aldehydes or Ketones form Schiff base product which is a reversible reaction and generally takes place under acid or base catalysis, or upon heating (Cozzi *et al.,* 2004). Nine new

thiazole Schiff base derivatives were synthesized, characterized by various spectroscopic methods (IR, UV, ^{1}H NMR, ^{13}C NMR & HRMS**)** and tested for their antimicrobial activities against gram positive and gram negative bacteria and also against two fungi.

Materials and methods

All chemicals used are of analytical grade purchased from Sigma Aldrich and TCI chemical co. All the solvents were used after distillation. TLC was run on the silica coated aluminium sheets and visualized in UV light.IR spectra were recorded on the FT-IR Perkin Elmer spectrum BX spectrophotometer.NMR spectra were obtained by using Brucker NMR instrument 400 MHz.

Synthesis of Benzaldehydethiosemicarbazone (2)[10]

A mixture of Thiosemicarbazide (i) (0.005 mole) & benzaldehyde (**1**) (0.005 mole) in ethanol was heated under reflux with stirring at 80 ^{0}C temperature until the completion of the reaction. After cooling, the reaction mixture was filtered and recrystallized with methanol to produce Benzaldehydethiosemicarbazone.

Synthesis of 2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde (3)

Benzaldehydethiosemicarbazone (2) (0.002mole) was dissolved in 10 mL of acetone with 3-chloroacetylacetone (ii) (0.002 mole). The mixture was refluxed with stirring at 60 ^{0}C temperature for 2-20 hours. Then the precipitate was filtered and recrystallized from ethanol.

Synthesis of Chalcone

2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde (3) (0.001 mole) was dissolved in 6.0 mL of methanol and 10% of NaOH with Benzalldehyde (1) (0.001 mole). The mixture was stirred at 25^{0}C temperature for 12-72 hours. But the reaction did not show prominent progress. The synthesis of chalcone is under progress.

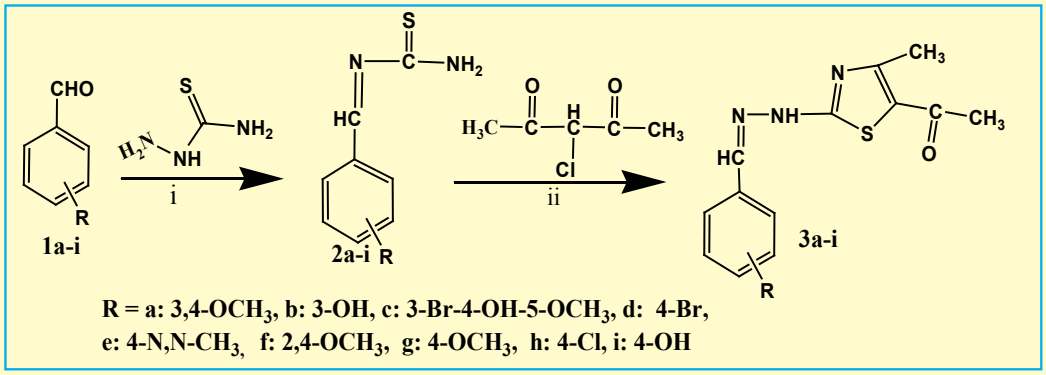
Antimicrobial evaluation

A previously described filter paper disc diffusion method[11] against eight strains was used to determine the *in vitro* antibacterial effects of all compounds and two strains for *in vitro* antifungal effects. Briefly, nutrient agar (NA) media (Difco Laboratories, Lawrence, KS, U.S.A.) was used as a basal medium for test. These agar media were inoculated with 0.2 mL of 24-hr liquid cultures containing the microorganisms. The sample discs were placed gently on pre-inoculated agar plates and then incubated aerobically at 37°C for 24 h. Discs with only dimethyl sulfoxide (DMSO) were used as a control, and nalidixic acid was used as a positive control. Inhibitory activity was assessed (in mm) by measuring the diameters of observed inhibition zones. These evaluations were performed in triplicate for each compound at a concentration of 300 µg $disc^{-1}$.

Results and discussion

All of the compounds (2) were previously synthesized[10]. We have synthesized nine new compounds (3) with 71-98% yield. Among all the compounds 3c was yield with highest 98% and 3b with lowest 71%.

Reaction Scheme:



R = a: 3,4-OCH_3, b: 3-OH, c: 3-Br-4-OH-5-OCH_3, d: 4-Br, e: 4-N,N-CH_3, f: 2,4-OCH_3, g: 4-OCH_3, h: 4-Cl, i: 4-OH

Fig. Synthesis of thiazole Schiff base derivatives

Table I. Reaction of Chloroacetylacetone (ii) & Benzaldehydethiosemicarbazones (2) to synthesis 2-(5-acetyl-4-methyl-2-thiazolyl) hydrazonebenzaldehydes (3)[a].

Thiosemicarbazone(2)	Time(h)	Thiazole (%)[b]
2a	6	3a (81%)
2b	8	3b (71%)
2c	8.5	3c (98%)
2d	20	3d (81%)
2e	7.5	3e (93%)
2f	4	3f (91%)
2g	3	3g (95%)
2h	2	3h (95%)
2i	9.5	3i (95%)

*[a]Refluxed at room temperature with 1:1 ratio. Acetone (10 mL) was used as solvent. [b]The yield based on (2) used.

Antimicrobial activity study:

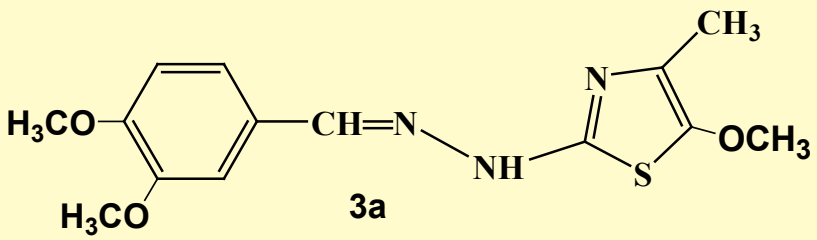
Table 2: *In Vitro* Bactericidal Profiles of 3a–i in Terms of Zone of Inhibition

	Microbial species									
	Bacteria								Fungi	
Comp	*B. ccereus*	*L. monocytogenes*	*S. aureus*	*B. subtilis*	*S. typhimurium*	C. freundii	*E. coli*	*P. aeruginosa*	*T. harzianum*	*A. niger*
3a	-	-	-	-	-	-	-	-	10	-
3b	-	-	-	-	-	-	6	-	11	-
3c	6	-	-	-	8	-	6	10	11	-
3d	-	-	-	-	-	-	-	-	6	10
3e	-	-	-	-	-	-	-	-	16	8
3f	8	-	6	-	-	-	-	6	15	10
3g	-	-	-	-	-	-	-	-	13	-
3h	-	-	-	-	-	10	-	-	12	-
3i	14	13	-	11	10	-	-	12	10	-

* 25 µL dose used & concentration was 300 µg/ml. *Michonazole* used as Standard antifungal agent which showed 25 mm zone of inhibition for *Aspergillus niger* and 28 mm against *Tricodarma harzianum*. *Ciprofloxacin* used as standard antibacterial agent.

Characterization of Compounds

3a: 3,4-Dimethoxy-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde



3a

Amorphous solid,Yield: 80.97%,mp: 248 ^{0}C. R_f value: 0.24 (EtOAc : Hexane = 4:6), IR (KBr) ν max/cm^{-1}:1650, 1423, 1278, 1212, 1163, 1125, 1035; 836 ^{1}H NMR (δ ppm, DMSO-d6, 400 MHz):8.35 (s, 1H, CH-N), 7.72 (s,1H benz), 6.62 (s, 2H benz), 3.84(s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 2.50 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3).FAB HRMS (acetone/NBA) m/z: Calcd for $C_{15}H_{18}N_3O_3S$: 320.0991 (M + H); Found : 320.1072.

3b: 3-Hydroxy-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde

White amorphous,Yield: 70.77%, mp: 240^{0}C. R_f value: 0.21 (EtOAc:Hexane 4:6), IR (KBr)ν max/cm^{-1}:3300, 1664, 1593, 1448, 1360, 1287, 793, 691.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):8.03 (s, 1H, CH-N),7.22,7.14,7.06,6.81 (s 4H benz), 2.40 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3). FAB HRMS (acetone/NBA) m/z: calcd for $C_{13}H_{14}N_3O_2S$276.0728 (M + H); Found:276.0809.

3c: 3-Bromo-4-hydroxy-5-methoxy-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde

White amorphous, Yield:97.93%, mp: 239^{0}C, R_f value: 0.40 (EtOAc:Hexane=4:6),), IR (KBr)ν max/cm^{-1}: 3350, 1652, 1597, 1505, 1365, 1296, 1044. ^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):10.00(s 1H OH), 8.00 (s, 1H, CH-N), 7.41, 7.27 (s, 2H benz), 3.88 (s, 3H, OCH_3), 2.50 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3).FAB HRMS (acetone/NBA) m/z: calcd for $C_{14}H_{16}BrN_3O_3S$ 383.9939 (M + H); found: 384.002.

3d: 4-Bromo-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde

White amorphous, Yield: 84.26%, mp: 260^{0}C. $R_f$value: 0.50 (EtOAc:Hexane= 4:6),), IR (KBr)ν max/cm^{-1}:1652, 1287, 1068, 821.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):8.11 (s, 1H, CH-N), 7.63 (s, 4H benz), 3.80 (s, 6H, OCH_3), 2.49 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3).FAB HRMS (acetone/NBA) m/z: calcd for $C_{13}H_{13}BrN_3OS$ 337.9884 (M + H); found 337.9951.

3e: 4-(N, N-Dimethyl-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde

Orange amorphous, Yield: 92.92%, mp: 254^{0}C. R_f value: 0.56 (EtOAc:Hexane= 4:6),), IR (KBr)ν max/cm^{-1}:1652, 1623, 1530, 1296, 1186.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):7.99 (s, 1H, CH-N), 7.50 (d, 2H benz,*J*=6Hz), 6.78 (d, 2H benz,*J*=6Hz), 2.98 (s, 6H, OCH_3), 2.50 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3). FAB HRMS (acetone/NBA) m/z: calcd for $C_{15}H_{19}N_4OS$303.1201 (M + H); found 303.1308.

3f: 2, 4-Dimethoxy-2-(5-acetyl-4-methyl-2-thiazolyl)hydrazonebenzaldehyde

White amorphous, Yield: 91%, mp: 261^{0}C. R_f value: 0.39 (EtOAc:Hexane=4:6),), IR (KBr)ν max/cm^{-1}:2649, 1645, 1622, 1519, 1335, 1270, 1140, 1021.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):8.07 (s, 1H, CH-N), 7.28 ,7.20,7.02 (s, 3H benz), 3.80 (s, 6H, OCH_3), 2.49 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3).FAB HRMS (acetone/NBA) m/z: calcd for $C_{15}H_{18}N_3O_3S$320.0991 (M + H); found 320.1065.

3g: 4-methoxy-2-(5-acetyl-4-methyl-2-thiazolyl) hydrazonebenzaldehyde

Cream amorphous, Yield: 95%, mp: 255^{0}C. R_f value: 0.51 (EtOAc:Hexane=4:6),), IR (KBr)ν max/cm^{-1}:2597, 1623, 1509, 1318, 1261, 1172, 668.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):8.12 (s, 1H, CH-N),7.63 (s, 2H,benz), 6.99(s,2H,benz), 3.79 (s, 3H, OCH_3), 2.49 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3). FAB HRMS (acetone/NBA) m/z: calcd for $C_{14}H_{16}N_3O_2S$290.0885 (M + H); found:290.0965.

3h: 4-Chloro-2-(5-acetyl-4-methyl-2-thiazolyl) hydrazonebenzaldehyde

Cream amorphous, Yield: 95%,mp: 256^{0}C. R_f value: 0.45 (EtOAc : Hexane= 4:6), IR (KBr)ν max/cm^{-1}:1652,1499,1288, 1258, 1069, 1010, 928, 841.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):9.89 (s, 1H, CH-N), 7.97 (d, 1H), 7.49 (d, 1H, J=7.6 Hz), 7.79(d, 1H, *J* = 7.6 Hz), 2.46 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3).FAB HRMS (acetone/NBA) m/z: calcd for $C_{13}H_{13}ClN_3OS$294.0390 (M + H); found:294.0466.

3i: 4-Hydrodxy-2-(5-acetyl-4-methyl-2-thiazolyl) hydrazonebenzaldehyde

Cream amorphous, Yield: 95%, mp: 288 ^{0}C. R_f value: 0.24 (EtOAc:Hexane=4:6), IR (KBr)ν max/cm^{-1}:1579, 1428, 1247, 1237, 1041, 946.^{1}H NMR(δ ppm, DMSO-d6, 400 MHz):8.01 (s, 1H, CH-N), 7.51 (d, 1H, *J*=7.6 Hz), 6.82 (d, 1H, *J* = 7.6 Hz), 2.50 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3).

Conclusion

We have synthesized nine new Schiff base thiazole derivatives with high yield. Amongst them 3a-3i showed very good antifungul activity against *Tricodarma harzianum* and 3d-3f showed moderate activity against *Aspergillus niger*. 3i showed good antibacterial activity against almost all eight bacteria.

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The Changes in Chemical Properties of Flora at Increasing Salinity in the Sundarbans Mangrove Forest

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Abstract

A study was conducted in the Department of Agricultural Chemistry, Patuakhali Science and Technology University to assess the changes in chemical properties of flora in the Sundarbans Mangrove Forest of Bangladesh. The plant samples were analyzed for P, K, S, Ca, Mg, Cu, Mn, Zn, Fe and B. The pH and EC values of soils were also analyzed. Almost all the soils at Chandpai range were found slightly alkaline (pH 7.5-7.9) and moderate to strongly saline (EC 2.46-8.54). The uptake of Mg and Mn were found higher by the plants and the accumulation of P, K, Ca, S, Cu, Zn, Fe and B were detected lower with lower soil EC levels. The accumulations of some micro elements in plants were found very poor and this might be the cause of plant top-dyeing in the Sundarbans. The chemical properties, mainly the ionic uptake of plants were decreasing with increasing salinity.

Introduction

The Sundarbans is the largest contiguous mangrove forest in the world. Along the mouth of the Bay of Bengal, it extends over 10,000 square kilometres in Bangladesh and India. Some 60 percent of the forest lies in Bangladesh and the rest in the Indian state of West Bengal. The Sundarbans is a globally significant ecosystem rich in bio-diversity providing habitat for around 334 plant and 453 animal species, including the world famous Royal Bengal Tiger. Several endangered species refuge in this forest containing Sundari, Gewa, Goran, Keora, Passur, Amur and many other trees and plants. The Sundarbans (Bengali: Shundorbôn) is a natural region in the Bengal region comprising Eastern India and Bangladesh. The name may have been derived from the Sundari trees (the mangrove species *Heritiera fomes*) that are found in Sundarbans in large numbers.

The mangrove ecosystem provides living support to nearly 3,00,000 coastal people through fishing, collecting honey, wax and timber, hunting and so on. It has also a buffer function, protecting the densely settled agricultural areas at the north from the full force of cyclonic storms and tidal waves. Every year tidal surges hit Bangladesh's south and southwestern coastline and the Sundarbans is acting as a vital barrier against all such natural calamities to protect the country's southwestern coastlines including the regional towns and cities like Mongla and Khulna. Sundari, the most dominating trees of the Sundarbans, is thought to suffer from salinity-induced top-dying disease (Hasan *et al*., 2013). Over the past 50 years, approximately one-third of the world's mangrove forests have been lost. Nutrient enrichment is one of the most serious threats to near shore coastal ecosystems. The mangrove forest is richer in floristic

composition than any other mangrove forest in the world. But extensive uses of forest resources, indiscriminate felling, contamination of forest water and soil affected the floristic composition (Maniruzzaman and Zaman, 2009). Increase in salinity and sea level, and outbreak of diseases like top dying of trees occur in the recent time that pose serious threats to the rich biodiversity of Sundarbans.

Very little or no study was undertaken in Bangladesh on chemical properties of plants at increasing salinity in the Sundarbans mangrove forest. So it is essential to identify the chemical properties of plants. Therefore, research work was conducted on the Sundarbans to acquire knowledge on this vital information to be needed for framing guidelines for proper management of mangrove trees and as well as biodiversity conservation.

Materials and methods

Site selection

The soil and plant samples were collected from Joymoni, Karamjol, Boiddomari, Dashervani, Kolomteji, Vola camp and Nandabala at Chandpai station area of the Sundarbans in Bagerhat district to achieve the target of getting representative samples.

Analytical methods for plant and soil samples

The ionic constituents of plant samples were measured by the following methods:

Elements	Methods of Analyses
Total N	Micro-Kjeldahl method. (Page *et al.* 1982).
P	Colorimetric method (Tandon, 1995).
K and Na	Flame photometric method (Golterman, 1971 and Ghosh *et al.,* 1983).
S	Turbidimetric method (Tandon, 1995).
Ca, Mg, Zn, Cu, Mn, Fe and B	Atomic absorption spectrometric method (APHA, 2005).

Results and discussion

Ionic constituents of different plant leaves at Bagerhat area of the Sundarbans mangrove forest

Potassium and Ca contents in plant leaves: The concentrations of K and Ca varied from 323708.0 to 92940.9 and 12754.6 to 2828.2 $mgKg^{-1}$, respectively. The highest amount of K and Ca was accumulated by sundari tree in Jaymoni at 4.10 EC level and kalomteji at soil EC 4.43 level and the lowest amount of K was uptake by Karamja tree in Karamjal at 4.1 EC level and the lowest Ca was found in Tiger fern plant in Boiddamari at 8.54 EC level. The plants uptake higher amounts of K and Ca at higher EC levels.

Phoshorus and S contents in plant leaves: The concentration of P and S varied from 13.61 to 2.64 and to 19.16 to 7.44 mgKg-1, respectively. The highest P and S Concentrations of were found in Dhonchey in Kalamteji at soil EC 4.43 and Karamja tree in Karamjal at soil EC 5.73. The lowest amount of P was uptake by Hetal plant in Karamjal and the lowest amount of S was found in Geora plant at Dasher Varani. The concentrations of P and S found higher at higher EC level.

Table I. Ionic constituents of different plant leaves at different locations of Bagerhat area of the Sundarbans mangrove forest

Sl. No	Location	Tree	Soil pH	Soil EC	P	K	Ca	Mg	S	Cu	Mn	Zn	Fe	B
					mgKg^{-1}									
1	Joymoni	Sundari	7.9	4.01	4.6	192859.0	12755.0	37.44	11.26	0.058	0.2	0.15	0.66	0.5
2	Karamjol	Karomja	7.6	4.93	4.1	92941.0	9038.4	397.8	19.16	0.032	0.1	0.11	0.80	1.0
3	Boiddamari	Tiger Fern	7.5	8.54	11.0	167080.0	2828.2	63.6	18.77	0.042	0.1	0.16	0.88	0.3
4	Karamjal	Hetal	7.6	4.93	2.6	112382.0	3282.6	160.3	14.42	0.051	0.2	0.03	0.67	0.8
5	Karamjol	Singra	7.6	4.93	4.2	146519.0	7400.2	456.8	7.65	0.052	0.2	0.06	0.60	0.5
6	Dasher Varani	Geoa	7.9	3.97	4.7	138220.0	10844.0	200.2	7.44	0.049	0.2	0.03	0.30	0.2
7	Kalomteji	Dhonchey	7.8	4.43	14.0	323708.0	12507.0	423.4	13.77	0.054	2.2	0.15	1.04	0.5
8	Vola Camp	Choila	7.9	2.46	7.9	195452.0	7707.8	52.56	13.72	0.034	6.4	0.07	0.74	0.3
9	Nandabala	Amur	7.7	5.73	4.3	169272.0	11703.0	760.7	8.59	0.042	0.1	0.02	0.36	0.4
Range			7.5-7.9	2.46-8.54	2.6-14	92941.0-323708.0	2828.2-12755.0	37.44-760.7	7.44-19.16	0.032-0.058	0.1-6.4	0.02-0.16	0.3-1.04	0.2-1.00
Mean					6.4	170937.0	8674.0	283.6	12.75	0.046	1.1	0.09	0.67	0.5
SD					3.8	66724.0	3727.6	243.6	4.413	0.009	2.1	0.06	0.23	0.3
CV%					59	39.034.0	42.974	85.88	34.61	19.6	195	64.4	34.7	53

Copper, Mn and Zn contents in plant leaves

The accumulation of Cu, Mn and Zn was varied from 0.058 to 0.032, 0.406 to 0.062 and 0.16 to 0.023 mgKg^{-1}, respectively. The highest concentration of Cu was found in sundari tree at Joymoni, Mn was in Choila at Vola camp and Zn in Tiger fern at Boiddamari at soil EC levels 4.1, 2.46 and 8.54, respectively. The lowest amount of both Cu and Mn was ccumulated by Karamja tree in Karamjal locaation, and Zn was in Amur plant in Nandabala at soil EC 5.73 level. The concentrations of Cu, Mn and Zn were found in higher EC level.

Iron and B contents in plant leaves

The concentration of Fe and B varied from 1.042 to 1.048 and 0.304 to 0.228 mgKg^{-1}. The highest concentration of Fe was found in Dhonchey plant at Kalomteji and B was found in karamja plant at Karamjal location at soil EC levels 4.43 and 5.73, respectively. The lowest concentration of both Fe and B was found in Geoa plant at Dashervarani at soil EC 3.97.

Conclusion

Phosphorus, K, Ca, S, Cu, Zn, Fe and B accumulation in mangrove plants were higher at high salinity prone areas of the forest whereas uptake of Mg and Mn was found lower at this area. Therefore salinity has little impacts on the uptake of most of the essential plant nutrients by mangrove plants. Lower uptake of Mg and Mn due to increasing salinity level indicates some top dying symptoms in mangrove plants. This might be due to the insufficient chlorophyll formation as Mg is one of the basic elements for chlorophyll in plant leaf.

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Design and Characterization of Vanadium and Zinc Complexes With Flavones, Isoflavones, Chalcones and Other Biogenic Chelators As New Classes of Therapeutic Insulin-mimetic Agents and Their *Invivo* Evaluation In Stz-induced Rats

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Abstract

The novel oxovanadium(IV) complexes, $[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ and $[V^{IV}O$ (5-hydroxyflavone$)_2]$ have been synthesized and their structure were determined by FT-IR, UV-Vis and ^1H NMR spectroscopic measurements. The bioactivity of the above mentioned complexes and their corresponding ligands were also determined. The complexes adopt a square pyramidal structure, in which the two L-Asn, L-Leu, L-Met and 5-hydroxyflavone ligands coordinate to vanadium (IV) center in bidentate fashions as homoleptic compounds. The amino nitrogen and a carboxylato oxygen atom coordinates the vanadium (IV) center from both sides making the seven (with L-Asn ligand), five (with L-Leu ligand) and five (with L-Met ligand) members chelates by one side. The complexes are stable in amorphous state and in aerobic solution. To the best of our knowledge, the homoleptic L-asparaginato, L-leucinato, L-methionato and 5-hydroxyflavonato oxovanadium (IV) complexes are the first examples of corresponding L-Asn, L-Leu, L-Met and 5-hydroxyflavone respectively with oxovanadium (IV) complexes matched by various counter anions. Interestingly, the complex, $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ exhibits antibacterial activity against *Shigella dysenteriae* but ineffective against *Salmonella typhe, Shigella boydii* and *Escherichia coli.* The complex $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ and $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ exhibit antifungal activity against *Aspergillus niger* and *Penicillium notatum,* but ineffective against Candida tropicalis. Unfortunately, the above mention complexes are cytotoxic.

Introduction

Vanadium chemistry with multidentate ligands is based on the remarkable biological and pharmacological properties (Rehder 2012; Vilter 1995 and Butler 1998) of vanadium. Vanadium, an essential biometal, is involved in various catalytic and inhibitory processes (Narla *et al.,* 2000). It is present in many abiotic as well as biotic systems. It also plays several roles such as cofactors in Metalloenzymes (Fern *et al.,* 2009; Rehder *et al.,* 2003; Robson *et al.,* 1986) and metalloproteins (Ueki *et al.,* 2003). Many oxovanadium complexes are known to possess potent insulin-mimetic effects (Shechter and Karlish 2001) and anticancer activity (John *et al.,* 2011), which merits the increasing application of vanadium complexes to biomedical sciences (Saha and Mukherjea 2015), Although there is extremely small requirement of vana-

dium uptake as a nutrient on organisms and higher animals it plays a major role as metalloenzyme and metallo protein like vanadium nitrogenase (V–Nase) and haloperoxidase (VHPO).[8] Oxovanadium(IV) (VO^{2+}) ions, also known as Vanadyl ions and its complexes dominate the chemistry of V^{IV}. A wide range of complexes have been reported containing this oxo group with five coordinated (tetragonal pyramidal, square pyramidal, trigonal bipyramidal) and six coordinated (octahedral) geometry.[8] Asparagine is an α-amino acid that is used in the biosynthesis of proteins.Asparagine is required for development and function of the brain (Ruzzo 2013). Asparagine is required by the nervous system to maintain equilibrium and is also required for amino acid transformation from one form to the other which is achieved in the liver (Berg *et al.,* 2012; Berg *et al.,* 2002; Hames *et al.,* 2005; Wise *et al.,* 2010). Among the essential amino acids, leucine is essential in promoting growth in infant and regulating nitrogen concentration in adults. It is generally used as a flavor enhancer. It facilitates skin healing and bone healing by modulating the release of natural pain-reducers, Enkephalins. It is also a precursor of cholesterol and increases the synthesis of muscle tissues by slowing down their degradation process (Rosenthal *et al.,* 2008 and Combaret *et al.,* 2008). Leucine potently activates the mammalian target of rapamycin kinase that regulates cell growth (Cota *et al.,* 2006).

Methionine helps the breakdown of fat and reduces blood cholesterol levels. It is an antioxidant that neutralizes free radicals and removes waste in the liver (Metayer *et al., 2008; Deth et al., 2008 and* Mato *et al., 2008).* Synthesis of DNA and RNA requires the presence of Methionine. It is also a precursor of several critical amino acids, hormones, and neurotransmitters in human body. Its AUG codon also serves as a "start" signal for ribosomal translation of messenger RNA; this means that every peptide chain began with a methionine residual at its N-terminal. It may however be removed later on by cleavage.[10-13, 19] Deficiency and Excess Methionine deficiency can be seen in chemical exposure and vegetarians. Severe liver disease can also result from having excessive methionine.[10-13] Flavonoids have a basic structure of 2-phenyl-benzo-γ- pyrones, mostly polyphenolic in nature (Cornard *et al.,* 2006). Among the flavonoids, flavonol is known to chelate metal ions with great affinity owing to the presence of an α-hydroxy-carbonyl group (Prasath *et al.,* 2001). It was observed that some flavonoids possess antidiabetic properties (Venkatesan *et al.,* 2012 and Cohen *et al.,* 1995). Vanadyl complexes have been extensively studied as the orally effective insulin mimetic compound in treatment of diabetes mellitus,[24] and the oxovanadium(IV) complex with a histidine derivative, [VO(pmH)(ClO_4)] (pmH = N-2-pyridylmethyl-(S)-histidine), has been found to have insulin-mimetic activity (Kawabe *et al.,* 1998). The first homoleptic oxovanadium(IV)-histidine complex $[V^{IV}O(L\text{-his})(L\text{-Hhis})]^{+}ClO_4^{-}.H_2O$ has been synthesized and structurally characterized by X-ray crystallography (Islam *et al.,* 2001). The behavior of asparagine as ligand towards Cd(II), Pb(II), Zn(II), has been studied in the constant ionic medium (Bottari and Festa 1996). Fe(III) and Cu(II) complexes of leucine were synthesised and characterized (Asemave *et al., 2015).* Cu(II) complex formation of L-Leucine leads to favor stable trans conformation rather than unstable hydrated cis conformation which is confirmed by XRD (Yokota *et al.,* 2016). Ternary copper (II) complexes [Cu(L-met)B(-Solv)](ClO_4) (1–4), where B is a N,N-donor heterocyclic base like 2,2′ bipyridine (bpy, 1), 1,10-phenanthroline (phen, 2), dipyrido [3,2-d:2′,3′f]quinoxaline (dpq, 3) and dipyrido[3,2-a:2′,3′-c] phenazene (dppz, 4), are prepared and Complex 2, structurally characterized by X-ray crystallography (Patra *et al., 2005).* Recently, it has been found that only vanadium with 3-hydroxyflavone complex has been synthesized, characterized and it's toxic as well as insulin mimetic potential has evaluated in STZ-induced experimental diabetes in rats. The results indicate that the complex is non-toxic and possess anti-diabetic activity (Pillai *et al., 2013).*

However, a very few work have been conducted in this issue, we have successfully synthesized and characterized three homoleptic bis amino acid and flavone oxovanadium(IV) complexes as biogenic chelators in this report.

Materials and methods

Vanadium oxosulfate ($VOSO_4$),L-Leucine, L-Methionine, 5-Hydroxyflavove, barium perchlorate and cellite-64834 cellite®R566 were purchased from Sigma-Aldrich, Munich, Germany. Asparagine was purchased from Merck Germany. All chemicals were used without further purification.

Measurements

FT-IR spectra were taken as KBr discs in the range 4000-400 cm^{-1} on a Perkin-Elmer infrared spectrophotometer, Department of Chemistry, Mawlana Bhashani Science and Technology University, Bangladesh. Electronic absorption spectra were recorded on a Perkin Elmer Lambda-25 spectrophotometer under anaerobic and aerobic condition, Department of Chemistry, Mawlana Bhashani Science and Technology University, Bangladesh. ^{1}H-NMR spectra were measured in DMSO solution on a Bruker AVANCE III 400 MHz NMR instrument at Wazad Miah Science Research Center, Jahangirnagar University, Bangladesh. Chemical shifts are given in ppm relative to tetramethylsilane as an internal reference. Melting points of the complexes were measured on a Stuart SMP10 melting point (range: upto 300°C) apparatus.

Synthesis

Synthesis of $[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$ (**1**)

Vanadyl sulfate (0.4890 g, 3.0 mmol) was dissolved in 12 cm^3 of deaerated water. To this solution $Ba(ClO_4)_2$ (1.0090 g, 3.0 mmol) was added and the mixture was stirred at ambient temperature for 2 h. Barium sulfate precipitated was filtered off using cellite-64834 cellite®R566. The blue filtrate was deaerated and then saturated with argon. Aqueous solution (12 cm^3) of L-Asparagine (0.9008 g, 6.0 mmol)was added to the filtrate in an argon atmosphere. The pH of the mixture was 3.35. The deep blue solution obtained was evaporated to some extent and allowed to stand at ambient temperature. After four days, the deep blue color solution is changed to green color. The green product deposited was filtered off and argon-dried after stored in desiccator.

Synthesis of $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ (**2**)

Vanadyl sulfate (0.4890 g, 3.0 mmol) was dissolved in 12 cm^3 of deaerated water. To this solution Ba(ClO4)2 (1.0090 g, 3.0 mmol) was added and the mixture was stirred at ambient temperature for 2 h. Barium sulfate precipitated was filtered off using cellite-64834 cellite®R566. The blue filtrate was deaerated and then saturated with argon. Aqueous solution (12 cm^3) of L-Leucine (0.7870 g, 6.0 mmol) was added to the filtrate in an argon atmosphere. The pH of the mixture was 4.25. The deep blue solution obtained was evaporated to some extent and allowed to stand at ambient temperature. After four days, the deep blue color solution is changed to green color. The green product deposited were filtered off and argon-dried after stored in desiccator.

Synthesis of $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ (**3**)

Vanadyl sulfate (0.4890 g, 3.0 mmol) was dissolved in 12 cm^3 of deaerated water. To this solution $Ba(ClO_4)_2$ (1.0090 g, 3.0 mmol) was added and the mixture was stirred at ambient temperature for 2 h. Barium sulfate precipitated was filtered off using cellite-64834 cellite®R566. The blue filtrate was deaerated and then saturated with argon. Aqueous solution (12 cm^3) of L-Methionine (0.8953 g, 6.0 mmol) was added to the filtrate in an argon atmosphere. The pH of the mixture was 4.13.

The deep blue solution obtained was evaporated to some extent and allowed to stand at ambient temperature. After four days, the deep blue color solution is changed to green color. The green product deposited were filtered off and argon-dried after stored in desiccator.

Synthesis of [$V^{IV}O$(5-hydroxyflavone)$_2$] (**4**)

To an anaerobic warm methanolic solution (10 ml) of 5-hydroxyflavone (0.050 gm, 0.210 mmol), 5 mL KOH (0.0118 g, 0.210 mmol) solution in methanol was added under argon atmosphere to deprotonate the 5-hydroxyflavone. The solution was stirred for at least 3 hours. Vanadium sulfate (0.017 g, 0.105 mmol) dissolved in warm methanol (10mL) was added drop wise to the above solution under constant stirring. The resulting solution was refluxed for 4 hour at 75 ^{o}C. At this time, the green colored amorphous product was formed. This was filtered off and washed with methanol and argon dried after stored in desiccator. Product is soluble in chloroform, melting point 233 ^{o}C.

Results and discussion

All the oxovanadium(IV) compounds were green in color as expected. The infrared spectra of these compounds exhibitνC-Hbands at around 2960-2963 cm^{-1}, νC-N bands at around 1180-1263 cm^{-1}, νC-O bands at around 1328-1344 cm^{-1} and other characteristic νN-H, νCOO and νV=O bands in the expected regions (table 1). All three complexes show νOH stretching bands at around 3424-3432 cm^{-1} and νH-OH bending bands at around 1624-1632 cm^{-1} which confirm the presence of lattice water molecule. The stretching bands at around 690-692 cm^{-1} for νV-O and 552-553 cm^{-1} for νV-N indicate the coordination mode of oxygen (O) & nitrogen (N) atoms of asparagine, leucine and methionine molecule with vanadium (IV) metal respectively. The perchlorate group is ionic in the complex, since there is no splitting of the perchlorate band around 1100 cm^{-1}.

Table I. IR spectral data* (cm-1) for complexes.

Complex No.	νC-H	νC-N	νC-O	νN-H	νCOO	νV=O	νOH	νH-OH	νV-O	νV-N	Other bands
1	2957 s	1260 m	1376 w	3178 vs	1622 m, 1462 m	1018 m	3432 s	1638 s	804 W	544 vw	1096 s, νClO_4
2	2917 s	1263 m	-	3259 s	1635 m, 1401 m	991 s	3424 s	1624 s	691 W	553 vw	1121 s, νClO_4
3	2960 s	1121 m	1340 w	3170 s	1625 m, 1406 m	984 s	3431 s	1632 s	691 w	538 vw	1121 s, νClO_4
4	2929 s	-	1121 vs	-	-	979 s	-	-	618	-	1578& 1561s νC=C 3424 s νSO_4

*vs, very strong; s, strong; m, medium; w, weak; vw, very weak.

UV Visible spectra of all the complexes were observed in DMSO solution under aerobic and anaerobic condition at pH 4.84. Two absorption bands were detected for all the complexes as shown in Fig. 1. One band is at around 780-800 nm and another is about 595-609 nm (Fig. 1.) which are characteristic of oxovanadium(IV) species.

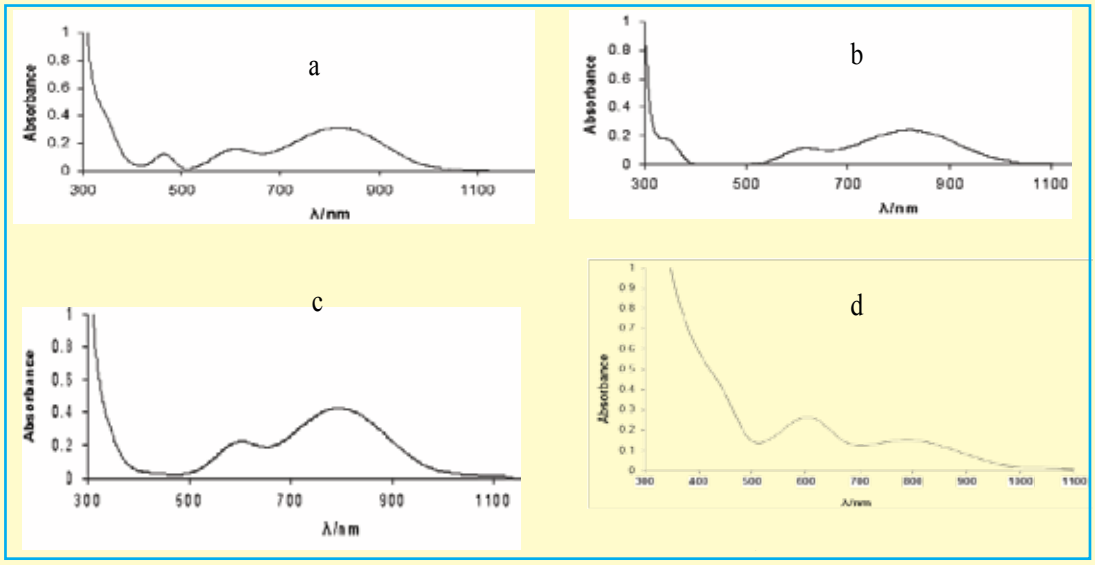


Fig. 1.: UV Visible absorption spectra of (**a**) $[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$, (**b**) $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ (**c**) $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ and (**d**) $[V^{IV}O(5\text{-}Hydroxyflavone)_2]$

$[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$ (1)

^{1}H-NMR (400 MHz, DMSO, δ, ppm): 4.92 & 7.216 (d, 4H, C3′-H, ^2J= 16 Hz, due to anomeric effect), 5.755 (s, 4H, NH_2).

$[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ (2)

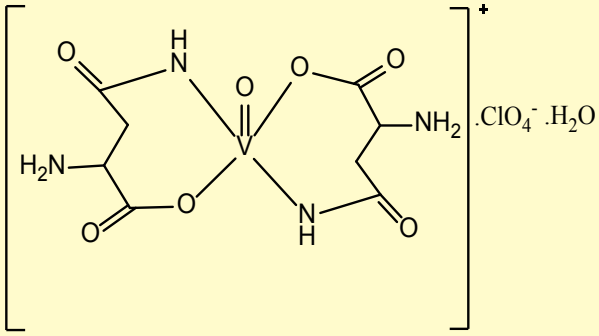
^{1}H-NMR (400 MHz, DMSO, δ, ppm): 0.92 (d, 12H, CH_3, J= 16 Hz), 1.23 – 1.75 (m, 2H, $CH(CH_3)_2$), 5.74 (s, 2H, ring CH), 2.5 - 2.6 (m, 4H, CH_2), 7.879 (s, 2H, NH).

$[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ (3)

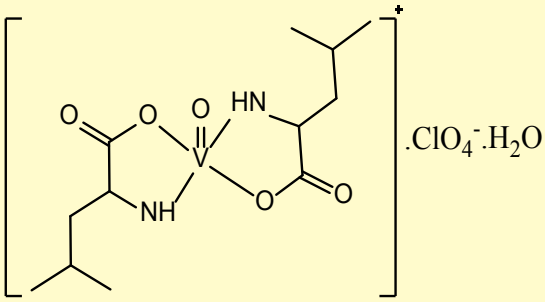
^{1}H-NMR (400 MHz, DMSO, δ, ppm): 2.055 (s, 6H, SCH_3), 2.503 – 2.511 (m, 10H, CH_2, CH), 5.748 (s, 2H, ring CH), 8.138 (s, 2H, NH).

$[V^{IV}O(5\text{-}hydroxyflavone)_2]$ (4)

^{1}H-NMR (400 MHz, DMSO, δ, ppm): 6.52 (dd, 2H,J = 7.5 Hz and 1.5 Hz, C6′-H), 6.1 (s, 2H, C3′-H), 7.11 (dd, 2H,J = 7.5 Hz and 1.5 Hz, C8′-H), 7.33 (dd, 2H,J = 7.5 Hz and 1.5 Hz, C7′-H), 6.52 (dd, 2H,J = 7.5 Hz and 1.5 Hz, C6′-H), 6.52 (dd, 2H,J = 7.5 Hz and 1.5 Hz, C6′-H),7.46 (t, 2H,J = 7.5 Hz, 1.5 Hz and 1.5 Hz, C14′-H), 7.48 (t, 4H,J = 7.5 Hz, 1.5 Hz and 1.5 Hz, C13′-H, C15′-H), 7.77 (t, 4H,J = 7.5 Hz, 1.5 Hz and 1.5 Hz, C12′-H, C16′-H). From the above discussion the following structures as Fig. 2. can be assigned for $[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ and $[V^{IV}O(5\text{-}hydroxyflavone)_2]$ respectively.



$[V^{IV}O(^{L}\text{-Asn})_2]^{+}.ClO_4^{-}.H_2O$



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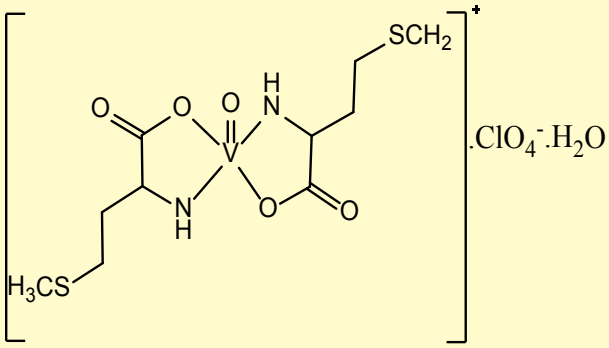
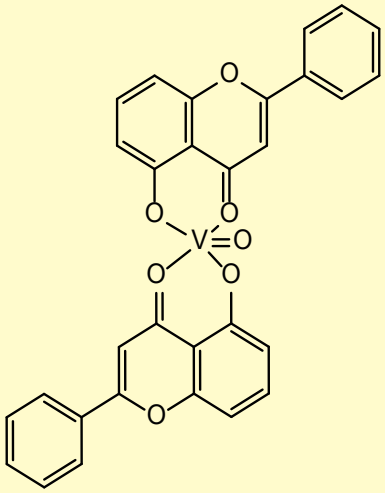


$[V^{IV}O(^{L}\text{-Met})_2]^{+}.ClO_4^{-}.H_2O$



$[V^{IV}O(5\text{-Hydroxyflavone})_2]$

Fig. 2. Proposed structure of the complexes

Antibacterial activity

Assessments of antibacterial activities of biogenic chelators, leucine, methionine and their corresponding oxovanadium(IV) complexes were carried out against some pathogenic bacteria by disc diffusion method and compared with the standard Ciprofloxacin (10 µg/disc) antibiotic disc.

Antibacterial effects of the salt, ligand and complexes are summarized in Table II.

The results showed that the, the solvent, DMSO; salt, $VOSO_4$; ligand, leucine as well as the complexes, $[V^{IV}O(L\text{-}Leu)_2]^{+}.ClO_4^{-}.H_2O$, have no activity against the gram-negative bacteria*Salmonella typhe*, *Shigella dysenteriae*, *Shigellaboydii* and *Escherichia coli*at the said concentrations. The complexes, $[V^{IV}O(L\text{-}Met)_2]^{+}.ClO_4^{-}.H_2O$, have no activity against the gram-negative bacteria*Salmonella typhe*, *Shigellaboydii* and *Escherichia coli*but have activity against *Shigella dysenteriae*, at the said concentrations. The results have been compared with commercially important bactericidalCipofloxacin. The inhibition by different complexes is also shown by Fig. 3.

Table II. Diameter (mm) of zone of inhibition by solvent, salt, ligand, complexes and standard against some gram-negative bacteria

Complexes and other components	Zone of inhibition (mm)			
	Shigella boydii (K-10244)	*Shigella dysenteriae (K-12992)*	*Salmonella typhi (K-323130)*	*E. coli (K-3203)*
DMSO	-	-	-	-
$VOSO_4$	-	-	-	-
Leucine	-	-	-	-
Methionine	-	-	-	-
4a	-	-	-	-
4b	-	-	-	-
4c	-	-	-	-
5a	-	7	-	-
5b	-	12	-	-
5c	-	14	-	-
Ciprofloxacin (10 µg/disc)	32	30	28	32

"-": no inhibition; a: 2 mg/mL; b: 1 mg/mL; c: 0.5 mg/mL; 4:$[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$and 5:$[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$. *E coli* ATCC 25922 was used as control strain during characterization of the mentioned strain.

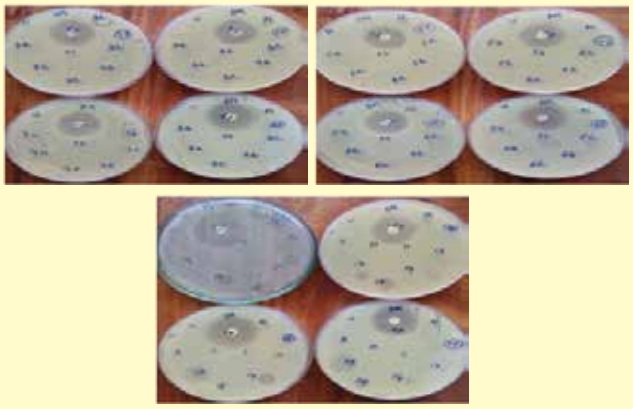


Fig. 3. Antibacterial activity of complexes against Salmonella typhe, Shigella dysenteriae,Shigella boydii andEscherichia coli.

Antifungal activity

The antifungal activities of recent biogenic chelator, leucine, methionine and their oxovanadium(IV) complexes were studied against the selective fungi, *Aspergillus niger*, *Penicillium notatum* and *Candida tropicalis* by disc diffusion method in potato dextrose agar (PDA) media and compared with the standard antifungal drug griseofulvin (5 µg/disc).

The table 3 depicts the antifungal activities of the compounds. It is clear that all the complexes were inactive against *Candida tropicalis* but show good activity against fungi *Aspergillus niger* and *Penicillium notatum*. From the data we can explicitly say that inhibition of mycelial growth decreases with decline of the concentration of the compounds as expected. In case of *Aspergillus niger*, the complex $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ show highest activity (12 mm) compared to the complexes $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ (10 mm). In case of *Penicillium notatum*, the complex $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ show highest activity (14 mm) compared to the complexes $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ (12 mm).

Table III. *in-vitro* antifungal activity of salt, ligand and their complexes.

Complexes and other components	Zone of inhibition (mm)		
	Aspergillus niger	*Penicillium notatum*	*Candida tropicalis*
DMSO	-	-	-
$VOSO_4$	-	-	-
Leucine	-	-	-
Methionine	-	-	-
4a	10	14	-
4b	10	11	-
4c	9	11	-
5a	12	12	-
5b	10	12	-
5c	10	10	-
Griseofulvin (5 µg/disc)	16	15	16

"-": no inhibition; a: 2 mg/mL; b: 1 mg/mL; c: 0.5 mg/mL; **4**: $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$; and **5**: $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$.

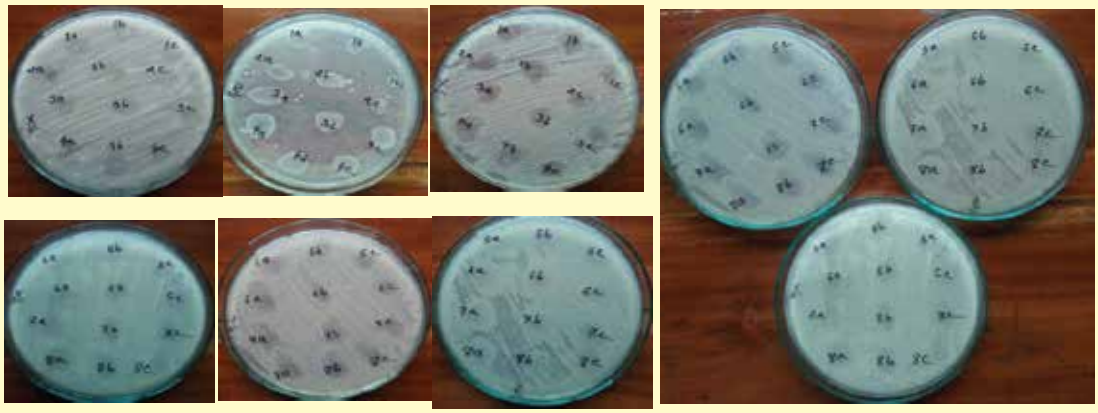


Fig. 4. Antifungal activity of complexes against X= *Aspergillus niger,* Y = *Penicillium notatum*, Z= *Candida tropicalis)*

Cytotoxic bioassay (*in vitro*)

The vanadyl complexes were screened for their cytotoxic (brine shrimp bioassay) activity using (Meyer *et. al.,* 1982; Finney, 1971) protocol. It is shown from the data recorded in table 4 that all the complexes possess potent cytotoxicity.

Table IV. *in*-vitro cytotoxicity of the oxovanadium(IV) complexes

Compound s ID	After 4 hours		After 8 hours		Remarks
	Dead	Live	Dead	Live	
H_2O with 3.8% NaCl	0	10	0	10	Not cytotoxic
DMSO	0	10	0	10	Not cytotoxic
4a	10	0	NA	NA	Highly cytotoxic
4b	10	0	NA	NA	Highly cytotoxic
4c	10	0	NA	NA	Highly cytotoxic
5a	10	0	NA	NA	Highly cytotoxic
5b	10	0	NA	NA	Highly cytotoxic
5c	10	0	NA	NA	Highly cytotoxic

N.B. a: 2 mg/mL; b: 1 mg/mL; c: 0.5 mg/mL; 4: $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$and 4: $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$.

Conclusion

The novel oxovanadium (IV) complexes, $[V^{IV}O(L\text{-}Asn)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$, $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ and $[V^{IV}O(5\text{-hydroxyflavone})_2]$ have synthesized and their structure were determined by FT-IR, UV-Vis and ^1H NMR spectroscopic measurements. The complexes adopt a square

pyramidal structure, in which the two L-Asn, L-Leu, L-Met and 5-hydroxyflavone ligands coordinate to vanadium(IV) center in bidentate fashions as homoleptic compounds. The amino nitrogen and a carboxylato oxygen atom coordinates the vanadium(IV) centerfrom both sides making the seven (with L-Asn ligand), five (with L-Leu ligand), five (with L-Met ligand) and five (5-hydroxyflavone ligand) members chelates by one side. The complexes are stable in amorphous state and in aerobic solution.To the best of our knowledge, the homoleptic L-Asparaginato, L-Leucinato, L-Methionato and 5-hydroxyflavone oxovanadium(IV) complexes are the first examples of corresponding L-Gly, L-Asn, L-Leu and L-Met respectively with oxovanadium(IV) complexes matched by various counter anions.The $[V^{IV}O(L\text{-}Leu)_2]^+.ClO_4^-.H_2O$ and $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ have antifungal activity against Aspergillus niger and Penicillium notatum but ineffective Candida tropicalis. The $[V^{IV}O(L\text{-}Met)_2]^+.ClO_4^-.H_2O$ complex is found to be antibacterial activity against Shigella dysenteriae but it is ineffective against Salmonella typhe, Shigella boydii and Escherichia coli. The rest of the above mention compounds showed no antibacterial activity against all organisms. Unfortunately, the above mention complexes are cytotoxic.

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Short Communication

Evaluation of Different Graded Brahman Calves in Local Environment of Bangladesh

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Executive summary

The weight at different stages of growth of 25% Brahman cross calves at Bhabakhali were higher than that of other two areas, which indicating that farmers of Bhabakhali may provide better feeding and management to their calves. Strong phenotypic correlations were found between birth weight and weight at three-month (0.65), between weights at six- and nine-month (0.65), which indicate that selection for body weight at one stage of growth will also help to improve the body weight at other stages. The birth weight, twelve-month weight and average daily gain of 50% Brahman crossed calves were higher than that of 25% Brahman cross calves in Bangladesh as well as major part of the world. With some exception, higher the birth weight eventually greater in average daily gain and twelve-month weight, although management system of calves might have affected on these traits. As the Brahman crossed cattle are a new introduction to Bangladesh. Considering the overall performance of Brahman crossbred calves, it may be concluded that the studied factors should be considered in making decisions for implementing breeding program.

Distinct output of the research

The present study was conducted using growth performance data on 624 Brahman cross (25%) calves collected under three villages adjacent to Bangladesh Agricultural University, Mymensingh, Bangladesh. Growth performance data of 289 Brahman cross (50%) calves were also collected from the record sheet maintained at the 12 Upazila Livestock Offices under the "Beef breed development project" of Department of Livestock Services to compare the performance between 25% and 50% Brahman cross calves to local environment of the country. Growth performance and adaptability traits considered were birth weight, weight at three-, six-, nine-, twelve-month of age, average daily gain from birth to twelve-month of age, calving difficulties (dystocia) and calf mortality under the study area. The birth weight, weight at three-, six-, nine- and twelve-month average daily gain for

25% Brahman cross calves were 19.79±0.20, 52.72±1.31, 86.61±2.02, 129.90±3.08, 172.60±3.48 kg and 426.00±4.99g, respectively. The average birth weight (21.40±0.24 kg) and year weight (229.62±2.08 kg) of 50% Brahman cross calves were significantly higher ($p<0.05$) than those of 25% Brahman cross calves. Average daily gain was significantly higher (570.52±5.19g) in 50% Brahman cross and the lower (529.98±4.54g) was in 25% Brahman cross calves. Birth weight, three-, six-, nine-, twelve-month weight and average daily gain all are positively correlated to each other. Strong correlations were found between birth weight and weight at three-month (0.65), between weights at six- and nine-month (0.65). There was no report of calving difficulties or abnormal calf birth in the study areas. It indicates that Brahman cross calves are well adapted to this climatic condition. However, further study with larger sample sizes covering more different management systems would be required to draw a better conclusion in this regard.

Small holder farmers and fattening farmers are benefitted to rear their cattle for beef purpose, which will help to fulfill the gap between the supply and demand of animal protein in Bangladesh. The outcome of this project will also help the breeder to take selection decision on how much blood will be more suitable for breeding purpose.

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Short Communication

Development of *In Vitro* Grown Buffalo Oocytes

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Executive summary

The major problem of in vitro embryo production (IVEP) technology in buffalo lies in the low number of ovulatory follicles comparing cattle. To overcome the shortage of large follicles, attempt should be paid to grow up small oocytes in vitro. This study was aimed to know the effect of Stem Cell Factor (SCF), a growth factor that regulates the folliculogenesis in mammalian ovaries, on growth of buffalo oocytes in early antral follicles in vitro. Cumulus oocyte complexes were dissected from early antral follicles (1 mm) of slaughtered buffaloes and cultured in Dulbecco's Minimum Essential Medium (DMEM) supplemented with fetal bovine serum (FBS), sodium pyruvate, gentamycin, hypoxanthine, dexamethasone, cysteine, polyvinylpyrolidione, L-ascorbic acid, estradiol-17β and androstenedione in 96-well culture plate at 38.5 ºC under an atmosphere of 5% CO2 in air for 6 days. The culture media were supplemented with 0, 50 and 100 ng/ml SCF.

Distinct output of the research

Animal protein from milk and meat are essential for human nutrition where the daily requirement of milk and meat for an adult person is 250 ml/h/d and 120 g/h/d but the availability in Bangladesh is only 55 ml/h/d and 25 g/h/d, respectively with more than 80% huge deficit (DLS, 2013). In Bangladesh, buffaloes are producing 3500 and 22400 ton meat and milk, respectively per year (DLS 2010). There are about 188 million buffaloes in the world and around 96.4 percent of them are found in the Asian region (FAO, 2010). Buffalo population in Bangladesh is 1.62 million (DLS, 2013). They are supplying power in the form of ploughing and traction, organic manures, industrial raw materials in the form of hide, skin, bone and hooves. In Bangladesh, the role of buffaloes was not emphasized and the species did not receive the attention of the policy makers and the researchers in accordance with its merits. They can utilize low quality roughage to produce more protein and to gain more body weight. They are known to have high disease resistant power compared with cattle. Buffalos are reported to have low reproductive performance with inherent reproductive problems of silent estrus, seasonal anestrous, delayed puberty, and delayed first calving, late post-partum conception, and a long calving interval. Due to the low and inconsistent response to multiple ovulation and embryo transfer (MOET) treatments, there has been an increasing interest in the in vitro embryo production (IVEP)

technology in buffaloes. The major limitation of IVEP technology in buffalo lies in the low number of immature oocytes that can be recovered per donor. This limitation is arisen from physiological peculiarities of the species, such as the low number of primordial and antral follicles present on the buffalo ovary, as well as the high incidence of follicular atresia. To overcome this problem, in vitro growth of oocytes is essential to support the embryo transfer technology in buffaloes. This study will involve in the further development of buffalo oocytes for embryo production. This study will help reproductive biotechnology specially embryo transfer technique in buffaloes. The proposed study will be useful to accelerate buffalo production. Ultimately, the new technology developed by this project will increase livestock production and eliminate poverty. The results will provide valuable information for assisted reproductive biotechnology for buffaloes. The results will be helpful to increase the buffalo production from which poor farmers will be benefitted ensuring food and nutrition security.

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Short Communication

Conservation of Red Jungle Fowl as a Potential Genetic Resources in Bangladesh

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Executive summary

To meet up the objectives a special type of poultry shed has constructed to build up a flock with this birds and permission has taken to the ministry of Forest and environment to collect this wild bird. This birds are found at Moulovibazar, Habigang Sylhet sader and other forest areas of Bangladesh. In this concerned some birds has been collected (Figure.1,2,3,4,5) and their mophometric, productive and reproductive characteristics (Table. 1,2,3,4) were studied and to meet up the other objectives activities is going on.

Objectives

i. To identify signatures of natural and sexual selection existing in rural indigenous domesticated and wild chicken (Red Jungle Fowl) populations in Bangladesh

ii. Determine genetic diversity in the RJF in Bangladesh.

iii. To build up cryopreservation of germ plasm (sperm and gonad tissue) for gene banking purposes and the maintenance the variation for both RJF and indigenous chicken for research and breeding.

Distinct output of the research

Taking up RJF as a research matter really seems to be important, because, as far as we concerned, the population of RJF are decreasing in a dramatic manner in recent years. As we all know that existence of every species in this world is crucial for the ecosystem, for the existence of human race. We all are humans and we all have been destroying the Mother Nature from the very beginning of the human kind. We are cutting down our trees leaving a scar on the lungs of the world every time for expanding our habitats, for augmenting the speedometer of our economy and at the end we are doing it for nothing actually. This type's of research works are really concerned about the environment and trying to relocate the nature where it belongs to. Thanks to the MOST for accepting on a matter that really needs to bring under light. Always we are creating our new homes, real estates and industries more and more, annihilating the natural home of many species. RJF is one of them. On the top that, for achieving a lucrative profit margin, the people of our country are becoming curios to bring up animals and birds in an industrial scale. It has numerous privileges though, the farmers are losing their interests on indigenous scavenging and free ranging chicken. RJF is the forefather of modern day chicken. Though it has been

domesticated, it's still now found wild in many forests of different countries. Every successful research may become a blessing for the world and the investigation this tends to complete is not devoid of them.

I hope and believe, it will be a perfectly choreographed research resulting in a marvelous outcome. The exploration of the genome of RJF and comparing it to the indigenous chicken may unveil the cause of the existence of RJF in wild from the ancient time fighting with adverse climatic conditions. Moreover, I am optimistic about the fact that it will be able to identify the root cause of their immunity and imply the possible solutions to different physical problems.

Study the Present Status of Wastewater Use and Its Effect on Crop

Fig. 1. Red Jungle Fowl

Fig. 2. Red Jungle Fowl

Fig. 3. Female Red Jungle Fowl

Fig. 4. A pair of Matured Red Jungle Fowl

Table I. Qualitative Traits in Red Jungle Fowl

Traits	Sex	n	Characteristic Features
Shank color	M/F	15	M: Blackish; F: Whitish
Shank feathering	M/F	15	Absent
Spur	M	7	Developed (M; 2 inch)
No. of toes	M/F	4	3 Toes
Wattles	M	7	Medium
	F	8	Rudimentary
Beak color	M/F	15	M: Upper portion black; F:Smaller and whitish border
Ear lobe shape	M	7	Small
	F	8	Medium
Ear lobe color	M/F	15	M:Reddish; F:Whitish
Comb type	M/F	15	M: Single; F: Absent
Skin color	M/F	15	Whitish
Eye color	M/F	15	M: yellowish and black in centre; F: deep yellow and black in centre
Egg color	F	8	Light brown

n= No. of Observation; M= Male; F= Female

Table II. Distribution and frequency of feather in RJF

Name of Feather	Feather color frequency										
	Male					Female					
	Black	White	Red	Black & white	Black & red	Black	White	Red	Pale brown	Black & white	Black & red
Neck/hackles feather color	4.00	1.00	74.14	10.23	11.10	17.11	5.00	6.20	55.01	3.20	22.00
Sickle feather color	95.12	2.02	1.52	1.13	1.20	6.03	3.25	3.22	80.50	5.00	3.10
Saddle feather color	17.40	10.20	50.60	12.40	9.62	14.10	3.00	-	70.20	10.10	3.50
Breast feather color	80.30	2.01	3.50	5.00	10.10	25.25	6.40	2.00	54.04	2.30	10.10
Wing bow feather color	3.50	12.10	18.30	2.04	64.05	14.34	20.30	2.12	83.63	-	-
Wing bar feather color	5.41	5.06	30.41	-	64.52	7.20	10.45	10.22	60.10	3.00	10.02
Primary feather color	65.10	10.30	10.50	5.04	10.05	3.10	3.34	10.20	79.10	2.04	3.31

Table III. Growth Performance of RJF

Trait	Body weight(g)							
	Male				Female			
	n	Min	Max	Mean±S.E	n	Min	Max	Mean±S.E
Egg weight					8			28.35± 0.01
Day old chick wt (gm) as straight run	7			22.25± 0.01	8			21.10± 0.01
4 weeks	2			45.00± 0.01	2			43.00± 0.02
8weeks	2			95.00± 0.01	2			84.00± 0.02

N= No. of chicks

Table IV. Reproductive performance of RJF

Trait	n	Minimum	Maximum	Mean± S.E
Age at 1st lay (weeks)	8	29	31	30.00±1.50
Number of clutch per year	8	0	2	-
Number of eggs per clutch	8	4	7	5.30± 0.20
Total number of eggs per year	5	6	20	8.00± 2.50
Egg weight (g)	5	27	35	31.50± 1.10
Hatchability of eggs (%)	6	0	85	60.00± 2.00

n= No. of fowl

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Short Communication

Study the Present Status of Wastewater Use and Its Effect on Crop Production in Surrounding Areas of Mymensingh Municipality

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Executive summary

This study was carried out during the period from January to June 2017 to investigate the wastewater parameters at field level and utilization for irrigation and its effects on crop production. Mymensingh Periurban area was selected for this study as because the whole wastewater of Mymensingh Municipal area passed through this Periurban area. The study area consists of four places, namely: Maskanda area, Cantonment area, Vatera area and Torar Beel area. After Vatera, wastewater flows to Chorar Beel. From this point wastewater discharged into the Brahmaputra river. In order to achieve the objectives, an intensive field survey was conducted in the study area. In this regard, one hundred respondents of the study area were interviewed based on the pre-tested interview schedule. The yield data also collected from the farmers of the study area during the survey. The wastewater parameters such as pH, EC, TDS, NH_4, PO_4 were measure in the field during the study.

Objectives

i. Investigate the wastewater parameters at field level in the study area.

ii. Study the wastewater utilization for irrigation and its effects on crop production.

Distinct output of the research

i) The wastewater discharge into the different exit locations of Mymensingh municipality is suitable for irrigation purpose.

ii) Farmer is using wastewater successfully in their fields to grow crops. But the yield is low in comparison to other fields where wastewater is not used.

iii) The growth of paddy is very high under wastewater cultivation method in comparison to normal water use. This luxuriant growth of stems and leaves hampers the overall yield.

iv) Because of lack of knowledge of farmers, they sometimes use urea in their fields with the hope of getting higher yields. But the result is negative.

v) Natural purification of wastewater is being done by water hyacinth.

vi) Wastewater after biological treatment can be used as irrigation in crop field and also in non-fruit fast growing plants.

vii) Wastewater should be released in open places like rivers, canals or ponds after necessary treatments.

Social impact of research findings

The main objective of this study was to identify the status of wastewater utilization in Mymensingh Periurban areas. From the study it was observed that, farmer's first impression when goes towards the fertilizer cost savings but many of them have no idea about the toxicity of wastewater irrigation and how it can bring about their economic losses. The wastewater of the area increased pesticide cost and potential impact of weed problem. It increased the growth of plant but reduced the grain yields and delayed its maturities. Wastewater also increased crop diseases and soil pollution. However, the wastewater reuse does not bring green environment and well socio-economic condition and so we should have to focus on a sustainable wastewater management procedure.

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Short Communication

Ensure Food Security of Bangladesh: Analysis of Post-Harvest Losses of Maize and Its Pest Management in Stored Conditions

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Executive summary

Most of the farmers identified pests to be the main problem at the time of cultivation, whereas storage problem being the main in case of post-harvest productions. About 33% maize was found infested from the seed collected at farm level after 6 months of storage. Among the pests infested, maize weevil was found to be the most abundant in the stored grains. On a separate experiment, different botanicals, bio-rational insecticides, and types of containers were evaluated in order to observe the post-harvest infestation of pests in stored conditions. Among botanicals and insecticides, 1% Neem-leaf powder and 0.01% Spinosad was found to be the best for controlling insect infestation. Performance of different types of containers were also evaluated, and it was found that the earthen pots provided with the best protection, storing maize seeds up to 6 months.

Objectives

i. To identify post-harvest problems through survey of the farmers of the research area.
ii. To find out present on farm post-harvest losses of maize in Bangladesh.
iii. To conduct an IPM practices for reducing post-harvest pests of stored maize.

Distinct output of the research

1) Problems of maize cultivation were identified through questioner from three selected research areas (such as Rangpur, Bogra and Kustia) of Bangladesh and found that insect pest is the important problem of maize cultivation, whereas storage problem being the main in case of post-harvest productions.
2) About 33% maize were found infested from the seed collected at farm level after 6 months of storage, Among the pests infested, maize weevil was found to be the most abundant in the stored grains.
3) Finally an IPM was developed for protecting of maize in storage conditions.

Social impact of research findings

Farmer will benefit directly from the research because they get message or method, how can preserve maize seed in stored conditionat farm level. The farmers also get a suitable bio-rational IPM package for controlling maize pests in storage. This technology will be transferred and sustained in rural areas for improving maize production.

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Short Communication

Weed Suppressing Ability of Buckwheat And Marshpepper Debris and Their Subsequent Effect on Yield Performance of Rice

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Executive summary

Weed management by mechanical method is very expensive and is almost out of reach to the resource poor farmers. Chemical control, on the other hand, exhibits residual effects of herbicides in crop produces and leads to environmental pollution. Current agricultural practices involved in weed management rely strongly on the use of synthetic herbicides. Weed control with herbicides, although effective, can be costly and is increasingly problematic due to public concerns about health and environmental issues. Modern agriculture is now challenged to reduce environmental damage and health hazards from chemical inputs and yet maintain a high level of production. The potential for undesirable environmental contamination from herbicides is relatively high, and there is a need for environmentally safe herbicides that are equally or more effective and selective than currently available synthetic herbicides. Hence, an alternative way is needed to control weeds in crop fields which have no adverse effect on the environment. So the effort was to develop an alternative weed control strategy allelopathic activity of crop residues to common weeds and selectivity of crops were studied in upland farming. All crop residues applied in the experiments suppressed weed growth and inhibition at satisfactory level. Three experiments were conducted to evaluate varieties and various crop residues on weed suppression and crop performance. Among the crop residues used in this study, buckwheat was the most effective followed by marshpepper. It was noticed that broadleaf weed species were more susceptible to rotation crop residues than grass weed species. In this respect, highest growth inhibition (78.81%) was observed in controlling Chesra (*Scirpus juncoides*). In respect of yield and yield contributing characters, cultivar and crop residues respond significantly. The results indicated that highest grain and straw yield (4.21 t/ha and 7.09 t/ha) produced by BR11. Highest grain and straw yield was observed in crop residues application @ 2 t/ha buckwheat crop residues along with variety BR11. Variety and rate of marshpepper residues application significantly influenced weed growth and inhibition. Marshpepper residues application @ 2.0 t ha^{-1} and BRRI dhan33 showed superior performance and inhibition was maximum for all weed species.

In respect of yield and yield contributing characters, BR 11 performed best and highest grain and straw yield along with 0.5 t/ha buckwheat + 1.0 t/ha marshpepper residues.

Objectives

i) to explore the possibility of utilizing allelopathic properties of crop residues for weed management
ii) to determine the suppressing ability of weeds through buckwheat and marshpepper debris
iii) to find out the combined effect of buckwheat and marshpepper debris to control weeds in rice
iv) to enrich the soil organic matter through the cheapest source of crop residues and
v) to develop a sustainable and cost-effective weed control method using buckwheat and marshpepper debris for rice production.

Distinct output of the research

Strong allelopathic abilities crop species especially buckwheat and marshpepper debris was used which helped to offer interesting possibilities for effective and an ecologically, economically and environmentally sustainable approach to controlling weeds.

Social impact of research findings

Sustainable crop production is the main theme in agricultural production system all over the world. To make sustainable crop production it is important to increase the yield with the technology which are acceptable by the society and are environmentally friendly. By using phytotoxic crop residues like buckwheat and marshpepper debris, our resource-poor farmers will be benefited through reduction of weed control cost as well as maintains the good soil condition. It will be acceptable by the society since the technology will help to maintain the healthy environment. No technical knowledge is needed to adopt this technique.