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Preface

The special allocation for science and technology (S&T) research has generated a high level of enthusiasm among the scientific community. Last year (2017-2018), 475 Prejects were financed from the Ministry to universities out of a large number of research programmes. It is therefore, important to select the best evaluated programmes in the priority areas. Successful implementation of the programme will accelerate the momentum in Science and Technology research. The ongoing processes of human resource development in critical areas of S&T including spin off benefits like academic advancement and promotion of our industrial base. The programme, therefore needs to special attention and concerted efforts by all concerned for strengthening the fabric of our S&T research through creativity and imagination. Creativity and imagination are deeply associated with the edifice of scientific research any where in the world. It cannot be any different for us.

In the past, resources from the Government and International Agencies have been spent for scientific research. But the impact is yet to be felt except perhaps in agriculture and some other specific areas Resources have been spent to continue the existing R&D system and to maintain the infrastructure. Additional resources are, therefore needed for a meaningful and specific result oriented thrust. In allocating resources, we should ensure that the best and the brightest among the most useful to the nation gets due support.

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 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 of Science and Technology provided fund for research in Biological, Medical, Enveronmental, Engineering, Physical science and other Inter disciplinary group to develop qualified manpower in crucial areas of science and technology.

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Characterization of fungal stress-responsive miRNAs against *Macrophomina* infection in jute (*Corchorus* sp.)

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Abstract

Jute is predominantly a cash crop yielding bast fiber and is well regarded for its long tensile strength. The cultivation of the farmer popular jute species namely *C. olitorious* and *C. capsularis* is rigorously threatened by infection of *Macophomina phaseolina*, a devastating fungal pathogen which causes stem-rot disease of jute and thus diminishes the fiber yield. However, a wild and poorly characterized jute species, *C. trilocularis*, is found to exhibit resistance against this fungal attack. The genetic basis of susceptibility and resistance in response to infestation by this fungus has remained poorly elucidated. One reason of this resistance may be a group of small RNAs called micro RNAs (miRNA), the non-coding small RNAs. These miRNAs have been reported to be instrumental in the modulation of defense gene expression in plants in response to fungal attack. In this context, this study aimed at unveiling the effect of differential expression of stress responsive miRNAs and their targets in plant immunity conducted by systemic acquired resistance (SAR) pathway in *M. phaseolina* sensitive *C. olitorious* in comparison to that of *C. trilocularis*. Three canonical (miR 167, miR 319 and miR 408) and a non-canonical (miR 6300) miRNA reported to be responsive to fungal stress in other plant species, were evaluated in both and *C. trilocularis* upon *M. phaseolina* infection. Targets of thee miRNAs-ARF4 for miR 167; TCP4 and TCP 9 for miR 319; laccase and plantacynin for miR 408 and serine/threonine kinase and AVR for miR 6300 have been analysed for their expression profile. In this study, these stress responsive miRNAs involved in disease progression were found to be highly expressed with concomitant down regulation of their targets in the sensitive species *C. olitorius*. However their expressions were found to be irregular in *C. trilocularis*. This study therefore signifies a major perspective of plant defence mechanism which may help in improving the farmer popular plant variety and allow for the development of a bio-control system against this devastating fungus in jute as well as other plant species.

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Introduction

In natural environments, plants are under continuous biotic stress caused by different assailants (e.g., bacteria, fungi, viruses, oomycetes, and insects) that compromise plant survival. If the stress is severe enough, it may prevents flowering, seed formation, and induces senescence that leads to plant death. One such factor is fungal stress imparted by microorganisms from diverse groups. *Macrophomina phaseolina* is one of the fungal pathogens that infects more than 500 species including jute to many vegetable crops. It is a common causative agent for diseases like: seedling blight, charcoal rot, stem rot, and root rot etc (Islam et al., 2012)

However, a wild type jute species, *C. trilocularis*, having a short bushy appearance is known to exhibit resistance against this pathogenic fungus, compared to the farmer popular species (*C. olitorius* and *C. capsularis*) (de los Santos-Villalobos et al., 2012). However, the reasons for this resistance at the molecular level are yet to be elucidated.

Micro RNAs, a major class of short non-coding RNAs are known to guide gene silencing in most eukaryotes by triggering sequence-specific cleavage or translation repression of the target transcripts. In jute, a recent study has revealed the existence of a total of 227 known microRNAs candidates in jute, of which 164 belong to 23 conserved families and the remaining 63 belong to 58 non-conserved families (Islam et al., 2015). In the recent years, data generated by high throughput sequencing platforms have elucidated the distinct role of miRNAs in plants during fungal invasion and miRNAs have emerged as a potential means to study the complex regulatory networks operated during plant-fungus interaction. It has been observed that host miRNAs along with their targets participate in disease modulation in plants upon fungal infection by either up or down-regulation (Gupta et al., 2014).

This study therefore aimed to objectify deeper insights into the effect of differential expression of the previously reported fungal stress responsive miRNAs, namely: miR 167, miR 319, miR 408 and miR 6300 (Jin and Wu, 2015) and their corresponding targets in plant immunity. The change in expression profile of these four miRNAs have been analyzed upon *M. phaseolina* infection in both *C. olitorius* and *C. trilocularis*.

Among these four miRNAs, miR 319 is a highly conserved miRNA that plays important roles in plant growth, morphogenesis, and reproduction and regulates transcription factors of the TCP family. The balance between miR319 and its targets controls leaf morphogenesis and several other plant developmental processes (Schommer et al., 2012). miR 408 is reported to be a negative regulator of plantacyanins and laccase (Abdel-Ghany and Pilon, 2008). Although, the precise function of plantacyanins in plants is unknown; however, they have been proposed to function in cell-to-cell signalling, lignin formation, stress responses and its downregulation in *C. olitorius* is thus predicted to contribute to its susceptibility to the fungal stress (Romo et al., 2001). Consequently, laccases are involved in regulating diverse functions in plants such as lignin synthesis, wound healing, iron acquisition, response to stress and maintenance of cell wall structure and integrity (Hoopes and Dean, 2004). miRNA 6300 a non-canonical miRNA is reported to undergo upregulation in response to fungal stress, although its target is not documented due to the non-canonical nature of its biogenesis (Hoopes and Dean, 2004).

So in order to comprehend the underlying mechanism of resistance of a wild *Corchorus* species- *C. trilocularis* against the necrotrophic fungus *Macrophomina*, it was necessary to understand the roles of the specific miRNAs mentioned above. For this purpose, fungal responsive miRNAs and their corresponding targets were evaluated in both the resistant and sensitive jute species.

Methods and materials

Plant material and fungal stress

Two jute species namely *Corchorus olitorius* variety O4 and *Corchorus trilocularis* were used in this study. Seeds were obtained from the collection of Molecular Biology Lab at Department of Biochemistry and Molecular Biology, University of Dhaka and were surface sterilized with 70% ethanol, subsequently washed in distilled water, and allowed to germinate on sterile petri dishes containing 3mm moist filter paper (Whatman) at 30 ± 1°C and simply in presence of water (for maintaining 65% relative humidity). Seeds were allowed to grow for different time intervals under the specified conditions. *M. phaseolina* was cultured in PDA medium as described previously (Aboshosha et al., 2007). 1% solution of *Macrophomina phaseolina* was sprayed on the seven day old seedlings, subsequently samples were collected at different time intervals starting from 0 days to 7 days (which includes 0, 3, 5 and 7days) for expression analysis (Sharmin et al., 2012). Two independent biological replicates were collected for each of the samples.

RNA isolation and cDNA construction

Total RNA was isolated from collected seedlings at different time intervals after *Macrophomina* infestation using TRIzol reagent (Invitrogen, USA) by following the manufacturer's instructions (Hummon et al., 2007). First strand cDNA synthesis was made to amplify desired genes for quantitative PCR data using oligodT primer mix (includes: 12T, C12T, G12T, A12T) to reverse transcribe all messenger RNAs (mRNAs). Two thousand nanograms of total RNA was used to perform RT reactions with Thermo Scientific™ RevertAid™ RT Kit - Thermo Fisher Scientific according to manufacturer's instructions, which were further modified for jute for a reaction volume of 10 µL which was carried out in a Mastercycler (Eppendorf, Germany). For the miRNA's expression analysis Superscript III First-Strand Synthesis System (Invitrogen, USA) was used to perform the stem-loop RT PCR according to the manufacturers' protocol using the miRNA specific primers for jute (Varkonyi-Gasic et al., 2007).

Real-time expression analysis

To validate the differential expression of desired genes and fungal stress responsive micro RNAs at different time intervals after *M. phaseolina* infection in *C. olitorius* and *C. trilo*cularis, real-time PCR was performed in a 32-well plate Roche LightCycler Nano System with Roche SYBR Green Master I (Roche Diagnostics, Germany) and Applied Biosystem 7500 Fast version 2.3. The real-time data analysis was completed by the log $2^{-\Delta Cq}$ method (Schmittgen and Livak, 2008a) for the desired genes using gene-specific primers. The primer sets amplified 100-200 bp within the region of interest for each gene. Tubulin was used as a reference sample for evaluating the differential expression of targeted genes in different time intervals after *Macrophomina* infection (Ferdous et al., 2015) setting the untreated sample of 0 day with respect to the fungal stress as endogenous control whereas U6 sno RNA was used as a reference for differential miRNA expression analysis (Kou et al., 2012). Thermo cycling conditions were different for miRNAs and the desired genes in the two different systems of real time conditions. All samples were amplified in technical triplicates and the mean of Cq (quantification cycle) value of each sample was used day-wise for qRT-PCR data analysis using excel data sheets (Schmittgen and Livak, 2008b).

In silico method of target identification

In this study, computational tools had been used to predict possible targets of miR 6300 in jute. The first attempt by *in silico* identification of the potential targets of miR 6300 was conducted by using the web-based tool psRNA Target (Dai and Zhao, 2011). The tool was used with default cut off values. Serine/threonine-protein kinase-like protein CCR4, mRNA of *Vitis vinifera* was returned as a possible target for miR 6300. The complete coding sequence of serine/threonine-protein kinase-like protein CCR4, mRNA of *Vitis vinifera* was subject to local BLAST against the jute genome database (Islam et al., 2017b) using LINUX 16.1 system. Highest identity of the query sequence was observed with contig18717 (AWUE01018684.1) of *Corchorus olitorius* cultivar O-4. Subsequent sequence searches in the NCBI database revealed seven different protein coding sequences which were later retrieved and aligned with the sequence of miR 6300 using the NCBI Nucleotide BLAST server setting BLOSUM 62 matrix as default parameters for this analysis.

Data analysis

For expression profiling randomly selected seedlings were collected from each treated and untreated samples after *M. phaseolina* stress given to each of the two species, *C. olitorius* and *C. trilocularis* at different time intervals. Two randomly selected biological replicates and three technical triplicates for each of the samples were taken into account in expression profiling and morphological study. Standard errors were calculated by square root of standard deviation using Microsoft excel tool pack 2013. Physiological conditions regarding plants' height and diameter were measured. For each parameter, the means, and one way ANOVA tests were performed using MS excel.

Results and discussion

To elucidate a possible role of miRNAs in defence, previously reported miRNAs (miR 167, miR 319, miR 408 and miR 6300) were evaluated with an assessment of their targets.

miRNA 167 and its target ARF 6: In the sensitive species *C. olitorius* expression of miR 167 was found to gradually increase (Figure 1 (a)) with a concomitant downregulation of its target, the auxin responsive factor (ARF 6) (Figure 1 (a)). Overall downregulation of miR 167 and a coherent high expression of its target ARF 6 were observed for *C. trilocularis* (Figure 1 (a)).

miRNA 319 and its target, a family of transcription factors, TCPs: Expression of miR 319 was seen to decrease gradually over the 7 days of fungal stress in *C. olitorius,* whereas its expression in *C. trilocularis* appeared to be markedly elevated, particularly on days 3 and 5. miR 319 expression was found to drop moderately on day 7 in *C. trilocularis* (Figure 1(b)). Under *M. phaseolina* stress, differential expression of TCP 4 was observed to follow the expected inverse expression of miRNA 319 in both the species (Figure 3b). In *C. olitorius* expression of another target, TCP 9 was found to increase with a progressive downregulation of miR 319 expression, during the interval between day 3 to day 7 of stress but in *C. trilocularis* expression of TCP 9 appeared to increase significantly from its initial state of downregulation (Figure 1 (b)).

miRNA 408 and its targets: From days 0 to 7, expression of miR 408 was found to be upregulated in *C. olitorius* and downregulated in *C. trilocularis* (Figure 1 (c)). Two targets of miR 408, plantacynin and laccase were upregulated in *C. trilocularis* with a peak in expression on day 7. This is significant regarding the down expression of miRNA 408 (Figure 1 (c)). In *C. olitorius*, there was a drastic reduction in the expression of laccase on day 3 after infection followed by a gradual

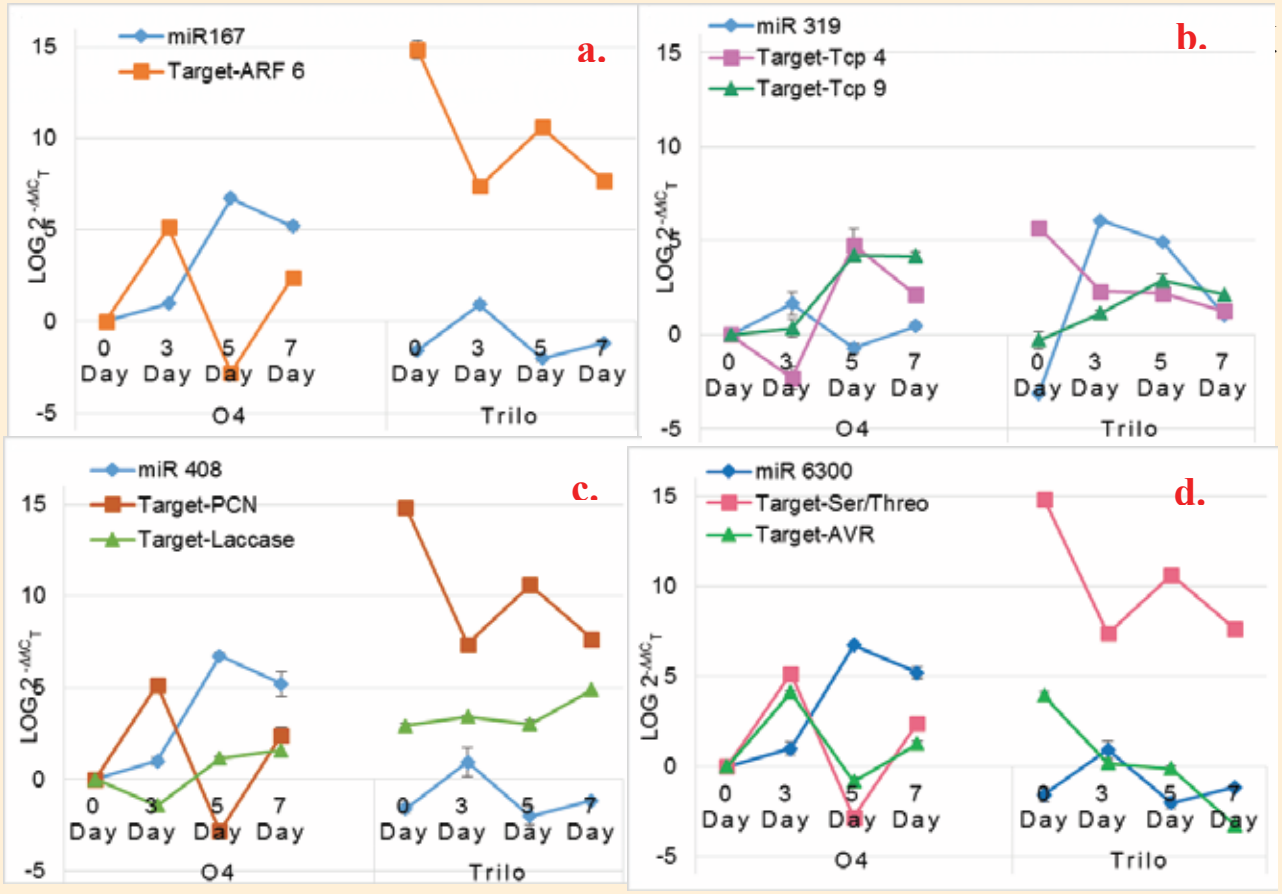

Fig. 1. Expression of miRNA and their targets in the fungal resistant and sensitive species of jute species at different time intervals after *Macrophomina* infection. (a) miRNA 167 and its target ARF 6; (b) miRNA 319 and its targets, a family of transcription factors, TCP 4 & 9; (c) miRNA 408 and its targets, plantacynin and laccase; (d) miRNA 6300 and its targets serine/threonine kinase and AVR.

miRNA 6300 and its targets: miR 6300 in *C. olitorius* showed high levels of expression throughout the 7 day interval with a peak on day 5, following *M. phaseolina* stress. On the other hand, expression of miR 6300 in *C. trilocularis* peaked on day 3 and reduced gradually on days 5 and 7 (Figure 1 (d)). miR 6300, a microRNA considered non-canonical and whose target is yet to be defined was studied in an attempt to connect the same to jute response and fungal attack. In this study, computational tools were used to predict two potential targets of miR 6300 in jute, namely: serine/theorinine protein kinase like CCR4 and avirulence induced family protein. Their expression profiles at different time intervals after fungal stress were validated through real time PCR. In case of serine/threonine kinase it was found to be upregulated with an increase in the length of fungal infection in *C. trilocularis*. This is coherent with the expression of miR 6300 and the opposite was true for the sensitive species (Figure 1 (d)). The level of basal expression of avirulence induced protein like gene was found to decrease upon fungal infection in *C. trilocularis*. In *C. olitorius* the expression increased on day 3 and downregulated upon further infection. Concomitantly an increased expression of miR 6300 was observed for *C. olitorius* (Figure 1 (d)).

Among other signaling mechanisms, host endogenous miRNAs are crucial in rearranging gene expression as a response to stress in plants (Mendgen and Hahn, 2002). In this study, four such miRNAs (miR167, miR 319, miR 408 and miR 6300) were evaluated together with an assessment of their targets. In *C. trilocularis*, miR319 expression was overall up-regulated with a reduction in expression at day 7 after application of fungal stress. This is similar to what was

found in tomato (Jin and Wu, 2015), poplar (Zhao *et al*., 2012), galled loblolly pine stem (Myburg et al., 2006) where a concomitant down-regulation of its targets, transcription factors, TCP 4 and TCP 9 were observed. On the other hand, expression of miR 319 was drastically down-regulated in *C. olitorius* with an upsurge of its targets, TCP 4 and 9 (He and Hannon, 2004). TCP 4 acts on lepoxinase 2 (LOX 2) a gene involved in jasmonic acid biosynthesis and functions in wound healing (Schommer *et al.,* 2008). Over-expression of TCP 4 causes a loss of LOX 2 function which results in impairment of wound healing and subsequent disease progression in *C. olitorius*. This loss of LOX 2 function may be surpassed by high miR 319 expression in *C. trilocularis*. Expression of another target-TCP 9, a key transcription factor regulates both the jasmonic and salicylic acid pathway (both involved in plant defense) (Danisman, 2016) with antagonistic effects on each other (Martínez-Medina *et al.,* 2017). However, the pattern did not show classical reversal of expression in *C. trilocularis*. This discrepancy in expression between the miRNA and its target can be explained by a report which states that miRNA concentration needs to increase to its threshold level for an effective downregulation of the target mRNA (Mukherji *et al*., 2011). In the light of this study, it may also be mentioned that miR319 confers drought tolerance in plants (Liu *et al.,* 2008) and *M. phaseolina* is active under drought conditions. So an up-regulation of miR319 in *C. trilocularis*, in addition to its effect on pathogen resistance related pathways, can provide an environment of drought tolerance thereby, protecting the plant from fungal damages.

miR167 which targets the auxin-responsive factors (e.g. ARF 6) was found to be upregulated in *C. olitorius* with a respective downregulation of its target ARF 6 gene. ARF 6 triggers defense signaling through WRKY transcription factors and interacts with the NBS-LRR domain. The latter leads to a hypersensitive response triggered by pathogen-associated molecular pattern (PAMPs) (Navarro *et al.,* 2006). Down regulation of ARF 6 may result in poor signalling through WRKY. However, increased expression of ARF 6 which is highly relevant to decreased expression of miR167 satisfies the signaling triggered by auxin-responsive genes in *C. trilocularis*.

miR 408 was found to be up-regulated in *C. olitorius* with an adjacent down regulation of corresponding targets: plantacyanin and laccase and the reverse was observed for *C. trilocularis*. Expression of miR408 is reported to be responsive to fungal stress and upregulated in wheat (Inal et al., 2014), poplar (Zhao *et al.*, 2012) and Arabidopsis (Sunkar and Zhu, 2004). Involvement of plant laccases in lignification, catalyzing the final step of the process together with its role in plant defense and wound healing has been reported (Ranocha *et al.,* 1999). When compared across the two jute species, the level of up-regulation of laccase was found to be higher in *C. trilocularis*. In order to corroborate this, expression of three lignin biosynthetic genes, namely: cinnamic 4 hydroxylases (C4H), ferrulate 5 hydroxylase (F5H) and catecholamine O methyltransferase (COMT) were previously studied. They were seen to be gradually down-regulated in *C. olitorius* but up-regulated in *C. trilocularis*. It appears that increased lignin biosynthesis in *C. trilocularis* allows for cell wall fortification, a mechanism not seen in *C. olitorius*. Decreased miR408 which leads to an up-regulation of its target, plantacyanin (Maunoury and Vaucheret, 2011) may play an important role in *C. trilocularis* in order to confer resistance by modulating the activities of oxidoreductase in the electron transport chain (Chen *et al*., 2017). On the other hand, high miR 408 expression indicates stressed conditions in *C. olitorius*. However, the expression of plantacyanin was also found to increase in *C. olitorius* (Fig. 1(c)). This discrepancy is possibly due to its involvement in other regulatory networks (De Paepe *et al*., 2004) functional in active cells but unheeded when a whole plant (seedling in our case) is assayed.

Because of its non-canonical nature of biogenesis, target(s) of miR 6300 has not been well reported in plant species till date. In this study, we used computational tools to predict two potential targets of miR 6300 in jute, namely: serine/threonine protein kinase-like CCR4 and avirulence induced family protein. We also analyzed their expression at different intervals after fungal stress. Expression of miR 6300 was found to be higher in the control samples of *C. trilocularis* and down-regulation was observed from day 5 onwards. But up-regulation of miR 6300 was observed in *C. olitorius* and the same has previously been observed in tomato upon similar necrotrophic fungal infection (Jin and Wu, 2015). This apparent down-regulation of miR6300 upon fungal stress in *C. trilocularis* may lead to a subsequent up-regulation of its predicted target: serine/threonine kinase. Serine/threonine kinase protein kinase-like CCR4 is an evolutionarily conserved protein complex which in conjugation with CCR4 associated factor 1 protein (CAF1) has been reported to play distinct roles in mediating defense responses in plants (Liu *et al.*, 2001). In CAF 1 transgenic plants, overexpression of these conserved proteins was found to correspond to the up-regulation of downstream genes activated by salicylic acid (Sarowar *et al.*, 2007) and cinnamic acid 4-hydroxylase, which is involved in a crucial step of lignin biosynthesis leading to subsequent plant cell fortification (Sarowar *et al*., 2007). Again the expression of the predicted targets: serine/threonine kinase and avirulence inducing family protein were found to be antagonistic. This concomitance with the expression of miRNA 6300, may be implicated in terms of host-pathogen interaction. The result of an interaction between a plant and a pathogen is often determined by the presence of resistance genes in the plant host that correspond to pathogen avirulence (AVR) genes in a gene-for-gene relationship (Flor, 1971, Reuber and Ausubel, 1996) . In *C. olitorius*, however, the expression of miR 6300 found at an up-regulated level with a coherent downregulation of its targets attests to its sensitivity towards *M. phaseolina* infection.

Due to worldwide climatic variations and environmental stressors potential plant diseases are predicted to become more severe and widespread in the future. A better understanding of stress resistance mechanisms should shed light on management strategies for crop diseases. In this study, genes involved in the systemic acquired resistant pathway as well as cell wall fortification were found to be highly expressed in *C. trilocularis*, a jute species resistant to *Macrophomina*. On the other hand, the sensitive species *C. olitorius* showed aberrant expression of these genes. Moreover, miRNA mediated defence response also significantly ascertained the overexpression of defence genes in the resistant species and the pattern was found to be reverse for *C. olitorius*. Again, lignification, a key defence adaptation was observed upon *M. phaseolina* infection in the resistant species. However, additional investigation is necessary for a thorough understanding of the interplay between regulatory sequences and their network that allow a privileged expression of defence genes in *C. trilocularis*.

Conclusion

Stress responsive miRNAs involved in disease progression in plants were found to be highly expressed with concomitant down regulation of their targets in fungal sensitive species *C. olitorius.* However their expressions were found to be irregular in *C. trilocularis*, a jute species which is resistant to *Macrophomina phaseloina*.

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Influence of planting dates on grafted tomato production during late summer

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Abstract

To evaluate the influence of planting dates on grafted tomato production during late summer, a field experiment was carried out at the experimental field of Sylhet Agricultural University, Sylhet, Bangladesh during August 2017 to February 2018. To improve the availability of tomato during late summer, two types of grafted tomato seedling of BARI hybrid tomato-4 viz., tomato grafted with the help of jute fiber and grafted with the help of plastic tube were used as planting material and evaluated at three different planting dates viz., 20 August, 20 September and 20 October, 2017. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The number of fruit per plant (38.83) and individual fruit weight (43.0 g) was higher on 20 October planting compared to other planting dates. The highest yield was obtained from 20 October (56.21 t/ha) planting followed by September (45 .56 t/ha) and August (39.16 t/ha) planting. Bacterial wilt incidence was the lowest on 20 October (2.08 %) planting. The two types of grafted seedlings didn't show any significant variation in any of the parameters though seedlings grafted with the help of jute fiber performed well then rope-grafted seedlings. Tomato harvested from plants grown from 20 August planting had higher market price (90 Taka/kg). The highest BCR was also calculated from the crops grown from 20 October planting (3.18). Therefore, BARI hybrid tomato-4 can be recommended for late summer cultivation in Sylhet region.

Keywords: Tomato, Bacterial wilt, Summer, Planting time and Sylhet region.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables grown in Bangladesh. It is an essential component of human diet for the supply of vitamins, minerals and certain hormone precursors in addition to protein and energy (Boamah *et al*., 2010; Kallo, 1993). It is largely grown during winter season in all parts of Bangladesh (Rashid, 1999). Therefore, there is a glut of ripe tomato in the market during winter season and thus sometime price of tomato got down to a

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minimum level. Almost all people like this vegetable crop and its demand remain equally high around the year. Due to adverse climatic condition during summer or late summer season tomato production is very difficult in Bangladesh and as well in Sylhet region. Scientists have already developed some heat tolerant tomato varieties and production technologies which are very popular in the country. In Sylhet region high rainfall, longer duration rain fall, high humidity, soil acidity, absentee farmer etc are prime causes for lower dissemination of summer tomato production. Production of tomato in summer or late summer in Sylhet region is very difficult due to infection of bacterial wilt disease which causes severe damage of the crop. High humidity, high rainfall etc. are most suitable condition for bacterial wilt hence in Sylhet region is considered as very vulnerable for this disease for tomato production. To improve tomato production during summer or late summer use of grafted seedling is the most alternative to protect the plants from bacterial wilt disease. Wild brinjal is resistant to bacterial wilt disease and thus tomato seedlings can be grafted onto wild brinjal to produce a grafted tomato seedling. This seedling can survive against bacterial wilt as was reported by several researchers (Rashid *et al.,* 2007, Hosain, 2016). To improve availability of tomato during off-season especially late summer planting might be an excellent option. In this regard necessary information is not available. Therefore, the present study was undertaken to evaluate grafted tomato seedling at different planting dates.

Materials and methods

The experiment was carried out at the experimental field of Horticulture Department, Sylhet Agricultural University, Sylhet, Bangladesh during August 2017 to February 2018. The climate of the experimental area is characterized by high temperature, heavy rainfall and high humidity. It belongs to the "Khadimnagar" soil series of Eastern Surma-Kushiara Floodplain under the Agroecological Zones-20 (FAO, 1988). The pH of the soil is around 4.98, soil organic matter 1.79% and Soil EC is 0.47 ds/m. Two different type of seedling BARI hybrid tomato-4 viz., tomato grafted with the help of jute fiber and grafted with the help of plastic tube were used as planting material. Pictorial view of two types of grafted tomato seedlings were showed in plate 1. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The grafted seedlings were transplanted in the main field on 20 August, 20 September and 20 October 2017. The unit plot size was 2.3 m × 2.4 m having four rows per bed and 6 plants per row and 24 plants per plot. Plants were transplanted with spacing at 60 cm × 40 cm. The unit plots and blocks were separated by 50 cm and 75 cm, respectively. The land was acidic in nature hence, lime (Dolomite) was applied in the field @ 4 kg decimal^{-1}. Each plot was fertilized with well decomposed cow dung 15ton, urea 300kg TSP200 kg, MOP 150 kg per hectare, respectively (Ahmad *et al*., 2008). Half

Plate 1. Two types of grafted tomato seedling.

of the quantity of cow dung and the entire amount of TSP were applied during final land preparation. The remaining cow dung and half of MOP were applied 5 days before of planting. The whole of urea and remained half of MOP were applied in 3 equal splits as top dressing at 15, 30 and 50 days after transplanting. The crop was protected from rain providing polythene tunnel. Irrigation, pruning, mulching, weeding and other intercultural operations were done as and when necessary. Data were recorded and mean yield and yield attributing characters were measured for interpretation of the result.

Results and discussion

Effect of planting dates

Most of the parameters in relation to yield and yield attributes of tomato grown during late summer in Sylet were found significant except days to flower and days to harvest (Table 1). Days to first flower was ranged from 49.0 to 50.17 days. Hossain et al. (2014) reported that tomato seedlings take 52.40 days to 71.73 days for days to 50% flowering while working with 3 different sowing dates of October. Around 85 days was required for first harvest of ripe tomato from all sowing dates. Yesmin (2011) observed that 82 to 88 days were required to maturity or first harvest after sowing of seed.

Number of fruits per plant was significantly different among the three sowing dates (Table 1). The highest number of fruits per plant was recorded from the plants grown from 20 October planting (38.83) followed by 20 September planting (32.83) while it was the lowest for 20 August planting (31.0). Abdul and Harris (1978) reported that low temperature reduced the level of different gibberellins in young leaves and this causes an increase in the number of flowers. The plants grown from later planting experienced cooler and congenial atmospheric condition which might caused higher number of flower as well as fruit production per plant. Significant variation in number of fruit per plant was also reported by Hossain *et al.* 2014 and Ahammad *et al.* 2009 while working with different sowing dates and planting dates, respectively. The highest (43.0 g) individual fruit weight was recorded from the plants grown from 20 October planting followed by 20 September planting (41.0 g).

Significant variation in fruit yield per plant and as well per hectare was observed among the planting dates (Table 1). The highest fruit yield per plant was measured from 20 October planting (1.65 kg) which was gradually decreased in earlier planting. The higher yield in 20 October planting was owing to higher number of fruit per plant and individual fruit weight. This also might be due to the plants grown from 20 October planting experienced more congenial environment then the previous planting dates. Yield of tomato at 20 August and 20 September (1.15 kg and 1.34 kg per plant, respectively) were also appreciable since the price of tomato remains high in the market at early winter. Ahammad *et al.* (2009) also reported yield variation while working with five planting dated from December to February. The corresponding fruit yield (t/ha) the highest fruit yield was recorded from 20 October planting (56.21 t/ha) followed by 20 September planting (45.56 ton). It was the lowest for 20 August planting (39.16 ton). High rainfall and high humidity caused during the August month caused bacterial wilt disease and lower plant growth and development might be the reasons for lower yield at 20 August planting. Singh and Tripanthy (1995) showed variation in yield of tomato when sown in different dates from June to August at Orissa of India. Several researcher of Bangladesh also reported variation in fruit yield per hectare while working with different sowing dates (Hossain *et al.* 2014, Ahammad *et al.* 2009).

Significant variation was noticed in bacterial wilt incidence among the planting dates although grafted seedlings were used in this study (Table 1). Around 5% plants were infected with bacterial wilt disease at 20 August planting which was decreased in 3.67% and 2.08% at 20 September and 20 October planting. Boro *et al.* (1996) mentioned that bacterial wilt incidence was significantly correlated with soil temperature, air temperature and total rainfall but no influence of relative humidity on bacterial wilt incidence. In August, presence of high rainfall in which rain splash might caused contamination of bacterial inoculums just above grafted union of the seedling and affected the seedling with the disease.

There was significant variation in term of total soluble solids due to different planting dates (Table 1). The variation of TSS (%) among the planting dates ranged from 4.53% to 4.93%. the highest (4.93%) TSS was recorded from 20 October planting and the lowest (4.53%) was from 20 August planting.

Table I. Influence of planting dates on tomato production during late summer

Planting date	Days to flower	Days to harvest	No. of fruit/plant	Individual fruit weight (g)	Fruit yield/plant (kg)	Fruit yield (t/ha)	Bacterial Wilt (%)	TSS (%)
20 Aug	49.0	85.17	31.0b	38.0b	1.15b	39.16b	5.12a	4.53b
20 Sep	49.17	86.67	32.83ab	41.0ab	1.34b	45.56b	3.67ab	4.78ab
20 Oct	50.17	85.83	38.83a	43.0a	1.65a	56.21a	2.08b	4.93a
F-test	NS	NS	**	*	**	**	*	**
CV%	3.67	1.94	10.10	5.89	8.71	8.71	42.45	2.0

NS indicates non-significant, * indicates significant at 5% level of probability and ** indicates significant at 1% level of probability. Values of a column having same letter did not differ significantly.

Effect of seedling types

None of the parameters was significantly affected due to seedling types (Table 2). Seedling produced using rope or tube equally performed for growth and yield of tomato during late summer. Ahmad *et al.,* (2011) reported 25.5 to 48.5 t/ha yield variation while working with 12 tomato hybrids under Gazipur condition. Use of grafted seedlings of same tomato genotype might be the reason for non-significant variation in this study. Number of fruits per plant ranged from 33.22 to 35.22. Hosain (2016) reported significant difference in number of fruits per plant while working with three types of tomato seedling viz., polybag seedling, normal and grafted seedling. Fruit yield per plant and fruit yield per hectare were significantly at par for both types of grafted seedlings. However, fruit yield per plant (1.41kg) was little higher in plants grown from grafted seedling produced with the help of rope than that of plastic tube made grafted seedling (1.35 kg). Bacterial wilt incidence was ranged from 3.50% to 3.74%. Hosain (2016) reported that grafted seedlings showed 3.5% bacterial wilt incidence whereas non grafted seedlings showed 20% incidence of bacterial wilt. Hossain et al. (1999) reported that 3.33 to 36.76% incidence of bacterial wilt can be occurred in tomato in non-grafted condition. Mina (2006) reported no incidence of bacterial wilt in grafted condition while working with BARI Tomato-3, BARI Tomato-4 and BARI Tomato-6.

Table II. Influence of grafting type on tomato production during late summer

Type of grafting	Days to flower	Days to harvest	No. of fruits/plant	Individual fruit weight (g)	Fruit yield/plant (kg)	Yield (t/ha)	Bacte rial Wilt (%)	TSS (%)
Rope*	50.33	86.0	35.22	40.33	1.41	47.94	3.74	4.70
Tube	48.56	85.78	33.22	41.0	1.35	46.01	3.50	4.80
F-test	NS	NS	NS	NS	NS	NS	NS	NS
CV	3.67	1.94	10.10	5.89	8.71	8.71	42.45	2.0

NS indicates non-significant.

*Rope: grafting united with the help of jute thread, Tube: grafting united with the help of plastic tube.

Interaction effect between planting dates and seedling types

There were no significant interactions found between planting dates and seedling types for different characters studied (Table 3). However, days to first flower was around 50 days for all treatment combinations. First harvesting of ripe fruits was made at around 86 days after sowing for all treatment combinations. Fruit yield was ranged from 37.74 to 57.91 t/ha of which the highest was recorded from T3G2 (20 October planting with tube made grafted seedling) followed by T3G1 (20 October planting with rope made grafted seedling) (54.51 t/ha).

Table III. Interaction effect on growth and yield of tomato during late summer

Type of grafting	Days to flower	Days to harvest	No. of fruits/plant	Individual fruit weight (g)	Fruit yield/plant (kg)	Yield (t/ha)	Bacte rial Wilt (%)	TSS (%)
T1G1	49.67	85.0	31.33	38.33	1.19	40.57	5.90	4.40
T1G2	48.33	85.33	30.67	37.67	1.11	37.74	4.33	4.67
T2G1	50.33	87.0	34.67	41.67	1.43	48.73	3.17	4.80
T2G2	48.0	86.33	31.0	40.33	1.25	42.39	4.17	4.77
T3G1	51.0	86.0	39.67	41.0	1.60	54.51	2.17	4.90
T3G2	49.33	85.67	38.0	45.0	1.70	57.91	2.0	4.97
F-test	NS	NS	NS	NS	NS	NS	NS	NS
CV	3.67	1.94	10.10	5.89	8.71	8.71	42.45	2.0

NS indicates non-significant.

G1: Rope G2: Tube, T1: 20 August, T2= 20 September, T3= 20 October

Economic analysis

Mean comparison for economic profitability among three different planting dates for summer tomato production is presented in Table 4. Yield of tomato at 20 October planting was higher (227.5 kg/decimal) than that of 20 September planting (184.5 kg/decimal) and 20 August

planting (158.5 kg/ decimal). Market price of tomato was also found variable at different months of the year. Price of tomato during summer was much higher than that of winter. In this study, tomato harvested from plants grown from 20 august planting had higher market price (90 Taka/kg) while it was 80 Taka and 70 Taka for September and October plantings, respectively. The highest BCR was calculated from the crops grown from 20 October sowing (3.18) followed by 20 September (2.95) and 20 August planting crops (2.85).

Table IV. Economic analysis of tomato production at varied planting dates

Planting dates	Yield (kg/ decimal)	Price of tomato	Gross income	Cost (Tk/decimal)	Gross margin	BCR
20 August	158.5	90	14265	5000	9265	2.85
20 September	184.5	80	14760	5000	9760	2.95
20 October	227.5	70	15925	5000	10925	3.18
Average	190.1667	80	14983.33	5000	9983.33	2.99

Conclusion

Among the discussion, it may be concluded that tomato production during late summer using BARI hybrid tomato-4 might be more useful for nutritional aspects and economic profitability. Because there is acute shortage of tomato and other vegetables supply during late summer in Bangladesh. Calculated BCR for all planting dates were also around 3.00. One can earn around 8000-9000 taka per decimal of land through growing heat tolerant tomato hybrids during late summer in Sylhet.

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Contamination of heavy metals in water, sediments and fish is a consequence of paddy cultivation: focusing river pollution in Bangladesh

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Abstract

Brahmaputra is a seasonal river around the Jamalpur, Sherpur and Mymensingh districts. The main livelihood of the people living in the immediate vicinity of the river is paddy cultivation. Farmers utilize different types of agricultural chemicals in their field expecting bumper harvest. Several agricultural chemicals have been reported to contain toxic trace elements in Bangladesh. Therefore, arsenic and cadmium might end up in the river water. The presence of these trace elements in the river water and sediments can result in their bioaccumulation in fish tissues. The main purpose of the study was to investigate the present of trace elements in water and sediments, as well as in fish tissues. Water and sediment samples were collected from selected 3 places (total 9 water samples and 9 sediment samples with 3 replications). In this study three fish species (Mastacembelus armatus, Channa punctatus and Mystus vittatus) were collected from the Brahmaputra River. The heavy metals (Pb, Cr, Cd, Cu, Ni and Zn) were analyzed with the help of Atomic Absorption Spectrophotometer (AAS). ni erew retaw revir eht ni dnuof latem yvaeh fo noitartnecnoc naem ehT 300.0) dC < (L/gm 8800.0) nM <(L/gm 6430.0) rC <(L/gm 4630.0) nZ<(L/gm 4220.1 eF :fo redro 120.97) nZ < (L/gm 695.244) nM < (L/gm 614.1821 eF :fo redro eht ni stnemides eht ni dna (L/gm iN dna bP .(L/gm 0320.0) dC < (L/gm 2074.9) bP < (L/gm 937.13) rC < (L/gm 611.67) iN < (L/gm revir artupamharB eht ,sgnidnfi eht no desaB .retaw revir eht ni timil noitceted woleb dnuof erew fo noitartnecnoc saerehw ,nZ dna nM ,rC ,dC ot tcepser htiw detullopnu sa deredisnoc eb nac retaw stnemideS .senilediug dradnats dednemmocer eht ot gnidrocca eulav dradnats eht evoba saw eF rof detullop ylivaeh dna nM dna rC rof detullop yletaredom ; nZ dna bP ,dC rof detullop ton erew .iNFurther, the level of bioaccumulation of the trace elements in fish tissues in relation to the contamination level of water and sediments was assessed. Arsenic and cadmium concentration in water showed a significant seasonal variation. Generally, the two trace elements in the river water were highest during the cultivating season than in other seasons. In all fish species, two trace elements in the gills highly depended on the concentration in the water. In all species, two trace

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elements in water and sediment did not significantly affect the levels in muscle tissue. Therefore, the trace element levels in the edible parts of the three fish species were well below the maximum permissible levels of the international institutions.

Keywords: Heavy metal; River water; River sediment; Paddy cultivation

Introduction

River sediments are a major carrier of heavy metals in the aquatic environment. Sediments are mixture of several components of mineral species as well as organic debris, represent as ultimate sink for heavy metals discharged into environment (Abbas *et al*., 2009; Bettinentti *et al*., 2003). Chemical leaching of bedrocks, water drainage basins and runoff from banks are the primary sources of heavy metals (Raju *et al*., 2012). Heavy metals are serious pollutants because of their toxicity, persistence and nondegradability in the environment (Brunner *et al*., 2008; Idris *et al*., 2007).

Heavy metals are widely found in the environment (Patrick, 2003) both from natural occurrence and due to anthropogenic activity (EFSA, 2009). Use of agricultural chemicals has been indicated as the main anthropogenic source of metal pollution in aquatic environments of Bangladesh. The most of the trace elements are potentially toxic at some bioavailability (Luoma and Rainbow, 2008), their presence in aquatic environments can result in deleterious effects on aquatic organisms (Mason *et al*., 2000). The toxic elements can exist in both abiotic (water and sediments) and biotic (organisms) components of aquatic environments at different concentrations. However, toxicity occurs in aquatic animals when the rate of uptake of a trace element exceeds the combined rates of efflux and detoxification of metal into metabolically inert forms (Luoma and Rainbow, 2008). In fish, metal uptake differs fundamentally from that of terrestrial animals as they are constantly submerged in the solution of metal ions (Perera *et al*., 2015). As a result, metal distribution in fish is determined mainly by its content in water and food (Farkas *et al*., 2000; Mohamed, 2008). Further, fish have a tendency to accumulate heavy metals depending on their position in the food chain and their feeding habits. Therefore, the concentration of heavy metals in water and bottom sediment, as well as the trophic position of fish can have significant effects on bioaccumulation of these elements in fish.

Brahmaputra River run across distinct land use types, paddy lands, people living in the upper area of the bank mainly depend upon paddy cultivation as their livelihood. All paddy farmers in the area utilize different types of pesticides, fungicides, inorganic fertilizers expecting high yield. The water utilized in their paddy lands diverts directly or indirectly to the Brahmaputra river. Several studies reported the prevalence of overusing agricultural chemicals by farmers in many parts of Bangladesh (Islam *et al*., 2015; Rahman *et al*., 2016; Uddin *et al*., 2016). Many studies have been carried out to investigate the disease among farmers due to impacts of inorganic fertilizers and other chemicals use in crop field that can contribute heavy metals into aquatic environment (Illeperuma, 2000; Jauasumana *et al*., 2011; Kumar and Singh, 2010). As a result, metals can appear in water, sediment and fish tissues. However, accumulation of metals in fish tissues can result in deleterious effects on fish. As freshwater fish is one of the sources of animal protein for human being, the elevated levels of heavy metals in edible fish tissues can cause health risks (EFSA, 2009) as well.

Rapid urbanization and industrial development during last decade have provoked some serious concerns in environment. Heavy metal contamination in river is one of the major quality issues in developing countries (Silambarasan *et al*., 2012). Rivers are a dominant pathway for metals transport (Mohiuddin *et al*., 2010) and metals enter these aquatic systems manly through natural

inputs such as weathering and erosion of rocks and anthropogenic sources including urban, industrial and agricultural activities, terrestrial runoff and sewage disposal (Barakat *et al*., 2012). As industrial activities, domestic wastes, urbanization and land development all contribute to the heavy metal pollution of rivers. The identification and uantification of the heavy metal in water and sediments are important environmental issues (Manoj *et al.,* 2012). Contamination of aquatic ecosystems with heavy metals has received much attention due to their toxicity, abundance and persistence in the environment and subsequent accumulation in aquatic habitats (Arnason and Fletcher, 2003). Heavy metals entering natural water become part of the water sediment system and their distribution processes are controlled by a dynamic set of physical and chemical equilibria.

The contamination of surface water by heavy metals is a serious ecological problem as some of them are toxic even at low concentrations, are non-degradable and can bio-accumulate through food chain (Abullah, 2013). Heavy metals undergo a global ecological cycle in which natural water are the main pathways (Saha and Hossain, 2011). Sediments act as sink of heavy metals can become immediate source of metal pollution of the water bodies (Manoj *et al*., 2012). Data from sediments can provide information on the impact of distant human activity on the wider ecosystem. Exposure to heavy metals has linked to several human diseases such as development retardation or malformation, kidney damage, cancer, abortion, effect on intelligence and behavior and even death in some cases of exposure to very high concentrations.

Bangladesh is constituted by a large delta at the confluence of three major rivers of the World, the anges, the Brahmaputra and the Meghna (Alam, 2003). The Brahmaputra river is pollution at different sites from agricultural and industrial activities which situated on the banks of the river or very close to the river system. The river receives municipal wastes directly in the water body, few industrial wastes water directly from industries and also domestic and agro-chemical wastes sediment. But no significant studies have been undertaken to investigate the pollution of the Brahmaputra. So it is necessary to assess the state of the water and sediment quality of the river.

To understand the effects, the current study mainly focuses on heavy metals in water and sediments, and common fish species available the study sites. Further, this study assessed the level of accumulation of trace elements in fish tissues in relation to the contamination level of water and sediments and tried to predict the suitability of these fish species for use as bioindicators.

Materials and methods

Site selection: The study was conducted at Brahmaputra river of Mymensingh district where three sampling sites were selected based on higher intensity of paddy cultivation.

Water sampling & preparation: Water samples were collected from selected 3 places (total 9 water samples with 3 replications). Water samples were collected in 250 ml plastic bottles, prior to sample collection all bottles were cleaned with dilute acid followed by distilled water. Before sampling, the bottles were rinsed again three times with the water to be sampled. About 500 ml of water sample from each sampling point was done. Samples contained 5ml 1M Nitric acid solution for the analysis of heavy metals. Nitric acid solution was used to protect water samples from any fungal and other pathogenic attack. After collection the samples were sealed immediately to avoid exposure to air. To provide necessary information for each sample such as date of collection, location, time etc. in a bottle was labeled separately with a unique identification number and then samples were placed in ice box. The water samples were then carefully carried to the laboratory of environmental science, BAU, Mymensingh. In the laboratory the water samples will be filtered using fine filter paper. The

bottles were kept in a clean, cool and dry place. The samples for the analysis of heavy metals were carefully transported to the Laboratory of Soil Science Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh.

Fish sampling & Preparation: In this study three fish species (*Mastacembelus armatus*, *Channa punctatus* and *Mystus vittatus*) were collected from the Brahmaputra River. The fish species were collected directly from fisherman during fishing. The samples were brought to the laboratory with ice box. Fish samples were kept in freeze for preservation until further analysis.

Analysis of heavy metals in water sample: At first 100ml water sample was taken in a beaker and then 4 ml HNO_3 was added. After mixing, the solution was kept into hot plate for evaporation until the volume become 50ml. Then the 50 ml volume sample was kept in 100ml volumetric flask and it was made 100ml volume by adding distilled water. Then this concentrated sample was filtered and standard solution was made for analyzing heavy metals (Pb, Cr, Cd, Cu, Ni and Zn) with the help of Atomic Absorption Spectrophotometer (AAS).

Heavy metal analysis in fish sample: Before analysis fish samples were cleaned. Then small portions of fish muscle were collected and weighted and dried. In drying procedure portion of muscle was kept in 100^0C oven overnight or in microwave oven. When dry, heat on hot plate until smoking ceases, and then placed dish in 525^0C furnace for minimum time necessary to obtain ash that was white and free from Carbon, normally 3-5h, but ≤8h. Remove dish from furnace and let cool. Ash should be white and free Carbon. But ash may be contained with Carbon particles. To remove Carbon particles ash from sample was wetted with water and added with 0.5-3 ml HNO_3. Then dried on hot plate and returned dish to 525^0C furnace 1-2h (Sultana *et al.,* 2014). The sample was digested in open beakers on a hot plate. 10g of weighted sample was kept on in an open beaker and 10ml of freshly prepared nitric acid was added the beaker was covered with a watch glass till initial reaction subsided in about 1 hour. The beaker was placed on a hot plate and temperature gradually allowed to rise 160^0C and the content boiled gently for about 2 hours to reduce the volume to between 2-5ml. The digest was allowed to cool and transferred to 50 ml volumetric flasks and made up to mark with water. The digested fish sample was kept in plastic bottles and preserved for laboratory analysis. Digested samples were analyzed by atomic absorption spectrophotometer (Model- PG-990, Made in England) method described by Association of Official Analytical Chemist (AOAC), 18^{th} edition. Atomic Absorption Spectroscopy was used for determination of heavy metals. The standard solutions of elements were prepared before every analysis. Samples were diluted in three different concentrations to obtain calibration curve for quantitative analysis.

Heavy metal analysis in sediment: Three sampling sites were chosen for collection of sediments along the Brahmaputra river. The auger tube was used for sediment sampling. The sediments samples were collected in winter and spring season. The samples were placed in polyethylene bags and transported to the laboratory under frozen condition. The samples were dried in the laboratory at 104^0C for forty eight hours, ground to a fine powder and sieved through 106 µm stainless still mesh wire. The samples were then stored in a polyethylene container ready for digestion and analysis. About 0.5 gram of sediment sample was put into the reference vessel. Then 25 ml of mixture ($HCL:H_2SO_4:HNO_3$,= 3:2:2) were added to reaction vessel which will be inserted into the microwave unit. The digested solution was cooled and filtered. The filtered sample was then made up to 50 ml with distilled water and stored in special containers. AAS (Atomic absorption Spectrometry) instrument was used to detect and measure heavy metal content in the sediment samples.

Statistical analysis: The collected data were compiled and tabulated in proper form and will subjected to statistical analysis. The Microsoft Office Excel software was used to present and interpret the collected data. The results of the study were presented in charts and tabular forms.

Results and discussion

Physical-chemical properties of river water and sediment

The values of physic-chemical parameters measured in the Brahmaputra river water at selected 3 sites are given in Table 1. The mean values temperature, pH, TDS, EC, turbidity, DO, BOD and COD were 25.45^{0}C, 6.85, 43.65 mg/L, 87.23 µS/cm, 14.97 FTU, 6.97, 5.3 and 57.31 mg/L on the right bank, respectively and 25.15^{0}C, 7.48, 43.69 mg/L, 87.34 S/cm, 11.61 FTU, 7.64, 4.34 and 66.68 mg/L on the Left bank, respectively. The result showed no significant differences in physic-chemical parameters values between both banks. Physic-chemical parameters play an important role into system restoration maintenance and self-regulation of water quality (Bharti and Singh, 2013). The measured pH values of the Brahmaputra river water were between slightly acidic to moderately alkaline during the study period. The pH has relationship with the solubility and accumulation of heavy metal in river water as well as sediments according to Singh et al. (2012). Temperature, pH, DO, TDS and EC of the river water were within the acceptable limit during the study period but turbidity, BOD and COD values were higher than the acceptable limit of DoE (1997) standard of Bangladesh for drinking water.

Table I. Values of physicochemical parameters of Brahmaputra river water.

Sampling site	Temp (^{0}C)	pH	TDS	EC	Turbidity	DO	BOD	COD
R-1	25.01	7.03	40.70	61.30	12.27	6.24	5.74	46.98
R-2	27.30	6.07	91.10	132.80	24.10	6.44	5.94	93.87
R-3	25.40	7.03	46.00	81.90	18.98	5.04	4.06	60.20
R-4	25.10	6.90	34.30	88.60	14.57	7.09	6.08	66.22
R-5	25.00	6.92	45.80	71.50	9.69	8.54	5.06	42.07
R-6	24.80	7.10	44.00	77.90	9.93	7.04	4.40	20.48
L-1	25.00	7.28	34.40	68.80	15.72	7.08	3.96	66.95
L-2	25.00	7.92	34.10	78.20	10.46	6.43	2.76	71.88
L-3	25.80	8.01	70.60	161.20	9.76	8.71	3.60	113.97
L-4	25.10	7.35	35.30	70.50	11.02	9.40	4.28	62,32
L-5	24.90	6.75	44.00	68.00	16.56	5.69	3.54	33.14
Mean	25.30	7.23	43.66	87.28	13.44	7.28	4.86	61.57
DoE (1997) Standard	20-30	6.5-8.5	1000.00	350.00	10.00	6.00	0.20	4.00

The physic-chemical parameters values of the present study were compared with other rivers of Bangladesh (Table 1). The measured mean values of pH, DO, TDS, EC and turbidity were lower but BOD and COD were higher than the other rivers recommended by Tareq *et al.* (2013). According to Rahman and Huda (2012), the mean values of temperature, pH, TDS, EC, turbidity, DO were higher but BOD were lower in the Padma river than the present study. The mean values of temperature, pH, TDS, EC were higher but DO, BOD were lower of the present study than Buriganga river (Hasan *et al*., 2009). Islam *et al*. (2012) investigated that temperature, TDS, EC were higher but pH, DO were lower in the Surma river than the present study.

The range of pH and Organic Matter (OM) in the Brahmaputra river sediment were 6.95-7.56 and 0.2121-5.9189% with mean values of 7.2382 and 1.7216% respectively (Table 2). The mean values of pH and OM were found 7.17 and 2.2631%, respectively on the right bank sediment and 7.32 and 1.0719%, respectively on the left bank. The result showed that pH was nearly same on the both bank but OM was higher alkaline pH, probably related to carbonate nature of the sediment (Barakat *et al*., 2012) and the presence of organic matter can influence the accumulation of heavy metals in the sediments (Suthar *et al*., 2009; Mohiuddin *et al.,* 2010; Manoj *et al*., 2012). pH mean value in the Buriganga river was lower but in the Shitalakhya river was nearly same and organic matter in the both rivers were lower as reported by Islam *et al.* (2014) than the present study.

Table II. Value of pH and OM in the Brahmaputra river sediment.

Sampling Reference	pH	OM (%)
R-1	6.80	1.58
R-2	6.85	5.81
R-3	7.15	3.04
R-4	7.05	3.11
R-5	7.18	0.42
R-6	7.22	0.74
L-1	7.32	1.94
L-2	7.45	0.76
L-3	7.50	0.82
L-4	7.40	0.73
L-5	7.00	1.29
Mean	7.24	1.47

Heavy metals in river water

The heavy metal concentrations for each sampling site found in water in this study and different standard and guidelines are shown in Table 3. The mean heavy metal concentrations were observed in water in decreasing order of Fe>Zn>Cr>Mn>Cd but Pb and Ni were found below detection limit. The mean concentrations of Cd, Cr, Fe, Mn and Zn were 0.0027, 0.0202, 1.0848, 0.0124 and 0.0357

mg/L, respectively on the right bank and 0.0033, 0.0520, 0.9475, 0.0045 and 0.0374 mg/L, respectively on the left bank. The results showed that Fe and Mn were found higher but Cd, Cr and Zn were lower on the right bank water than the left bank. The variation of concentration of heavy metals from location to locations may be correlated with the flow of the rivers and location of industries and their waste disposal system (Alam, 2003). The average concentration of Cd, Cr, Mn and Zn were found lower but Fe was higher in Brahmaputra river than DoE (1997) standard, WHO (1993) and USEPA (2008) guidelines. In the Buriganga river, the mean concentrations of Cd, Cr and Zn were investigated higher but Fe was lower than the present study according to Bhuiyan *et al*. (2015). The mean concentrations of Cd and Mn were found higher but Zn was lower as reported by Mokaddes *et al*. (2013) in the Shitalakhya, Turag and Balu river than the present investigation. According to Ahmed et al. (2012), the mean concentration of Cd was lower but Cr was higher in the Dhaleshwari river than present investigation.

Table III. Concentration of heavy metals in the Brahmaputra river water

Sampling sites	Fe	Mn	Zn	Pb	Cd	Ni	Cr
R-1	1.54	0.020	0.038	BDL	0.003	BDL	0.023
R-2	0.76	0.017	0.036	BDL	0.003	BDL	0.019
R-3	1.47	0.016	0.027	BDL	0.001	BDL	0.009
R-4	1.36	0.025	0.086	BDL	0.005	BDL	0.006
R-5	1.08	0.005	0.032	BDL	0.003	BDL	0.015
R-6	0.81	0.003	0.042	BDL	0.002	BDL	0.049
L-1	0.90	0.009	0.014	BDL	0.002	BDL	0.044
L-2	0.51	0.008	0.025	BDL	0.003	BDL	0.025
L-3	1.06	0.001	0.035	BDL	0.002	BDL	0.069
L-4	1.79	0.002	0.120	BDL	0.007	BDL	0.073
L-5	1.06	0.002	0.015	BDL	0.003	BDL	0.047
Mean	1.242	0.098	0.034	BDL	0.004	BDL	0.035

Pearson's correlation coefficients of heavy metals studied using statistical software SPSS (version 22.0) in the Brahmaputra river water have been summarized in the Table 4. The relationship between the heavy metals may offer remarkable information on the sources and pathway of heavy metals. Correlation analysis shows significant positive correlation between Zn-Cd ($r=0.864$) at $p<0.01$ level where as Mn is significantly but inversely correlated with Cr ($r=-0.715$) at $p<0.05$ level. The high significant correlations between heavy metals may reflect the fact that these heavy metals had similar pollution levels and similar pollution sources (Armah *et al*., 2010). On the other hand the rest elemental pairs show no significant correlation with each other, suggesting that these metals are not associated with each other and lack of their identical behavior transport in aquatic environment.

Table IV. Correlation coefficient of water samples of Brahmaputra river.

Sample: Water	Cr	Fe	Cd	Zn	Mn
Cr	1.00				
Fe	0.016	1.00			
Cd	0.240	0.467	1.00		
Zn	0.260	0.577	0.846**	1.00	
Mn	-0.605*	0.228	0.021	0.053	1.00

** Correlation is significant at the 0.01 level (2-tailed);
* Correlation is significant at the 0.05 level (2-tailed).

Heavy metals in Sediments

The heavy metal concentrations in the river sediments at all sampling sites and comparison with different sediment quality guidelines are given in Table 5. The mean concentrations of analyzed heavy metal were observed in sediment in decreasing order of Fe>Mn>Zn>Ni>Cr>Pb>Cd. Ni was found in all sediment samples but was not found in water samples because Ni is mainly transported in the form of a precipitated coating on particles and in association with organic matter. Ni may also be absorbed on to clay particles and via uptake by biota. Absorption process may be reversed leading to release of Ni from the sediments (Ahmad *et al*., 2010). The mean concentrations of Cd, Cr, Fe, Mn, Pb, Ni and Zn in sediments were 0..2808, 35.7464, 1293.85, 411.7323, 12.6384, 74.4498 and 96.6593 mg/Kg, respectively on the right bank of the river and 0.1698, 26.9322, 1266.496, 479.6334, 78.1162 and 57.8557 mg/Kg, respectively on the left bank. The results showed that Cd, Cr, Fe, Pb and Zn concentrations were higher but Mn and Ni were lower on the right bank than left bank. Among all sampling sites most all measured heavy metals release from base material (e.g., steel, stainless steel, galvanized steel, aluminum, copper-nickel and other copper alloys), abrasive blasting materials (e.g., coal slag, copper slag, nickel slag, glass, steel grit, garnet, silica sand), surface coatings (e.g., pre-construction primers, anticorrosive and antifouling paints) and welding materials in shipyard (Kura *et al*., 2006; OSHA, 2006) and deposited in river sediment. The mean concentrations of Cd, Pb and Zn in the Brahmaputra river sediments were found lower but Ni was found higher than WHO (2004), USEPA (1999) and CCME (1999) Sediment Quality Guidelines (SQG) (Table 7).

Pearson's correlation analysis shows significant correlation between Zn-Cd (r=0.894), Zn-Fe (r = 0.736), Ni-Pb (r = 0.930), Pb-Cd (r =0.977) and Fe-Cr (r = 0.0736) at $p<0.01$ level whereas significant correlation between Zn-Ni (r = 0.674) and Ni-Cd (r = 0.679) at $p<0.05$ level (Table 6 and Table 7). These highly significant positive correlations between heavy metals suggest the possibility of common sources of origins which may be anthropogenic Armah *et al.,* 2010). On the other hand the rest of elemental pairs show no significant correlations with each other in sediments that could be indication of separate source input or sources of pollution.

Table V. Concentration of heavy metals in sediments of Brahmaputra river.

Sampling sites	Fe	Mn	Zn	Pb	Cd	Ni	Cr
R-1	1274.40	616.40	202.46	26.47	0.202	70.53	42.70
R-2	1324.42	249.04	70.56	14.78	0.330	46.85	57.00
R-3	1298.04	361.08	88.92	7.54	0.210	75.16	33.86
R-4	1288.46	385.80	72.57	48.55	0.710	218.05	53.06
R-5	1310.22	406.80	119.75	5.94	0.182	82.54	21.10
R-6	1262.80	480.42	34.88	2.72	0.106	48.25	25.65
L-1	1324.66	702.04	35.06	8.44	0.174	186.00	18.24
L-2	1272.66	240.86	47.54	6.95	0.172	45.21	34.98
L-3	1198.80	790.02	86.22	4.04	0.204	67.44	38.06
L-4	1286.42	169.84	76.04	6.68	0.197	66.70	41.63
L-5	1302.48	496.44	64.80	4.20	0.113	26.08	12.50
Mean	1265.20	440.64	82.04	12.08	0.34	77.62	37.30

Table VI. Correlation coefficent of heavy metals content in sediment samples of Brahmaputra river.

Sediment	**Cd**	**Cr**	**Fe**	**Mn**	**Pb**	**Ni**	**Zn**
Cd	1.00						
Cr	0.208	1.00					
Fe	0.420	0.637**	1.00				
Mn	-0.163	0.544	0.667	1.00			
Pb	0.967**	0.245	0.425	-0.054	1.00		
Ni	0.675*	0.116	0.452	-0.07	0.657**	1.00	
Zn	0.893**	0.532	0.7360**	0.219	0.860**	0.764*	1.00

Table VII Acceptable limits of heavy metals in sediments and water samples based on reference standards.

Reference standard		Cd	Cr	Fe	Mn	Ni	Zn	Pb
WHO (2012)	River water	0.0050	0.0500	0.3 - 1.0	0.1000	0.10	5.00	0.05
	Sediment	6.000	25.000	NA	NA	20.0	123.0	NA
USEPA (2008)	River water	0.0300	0.0500	NA	0.5000	0.02	3.00	0.01
	Sediment	0.6000	25.000	30.000	30.000	16.0	110.0	40.0
CCME (2009)	River water	0.0500	0.1000	0.3000	0.0500	NA	5.00	NA
	Sediment	0.60	37.3	NA	NA	NA	123.0	35.0

Conclusion

This research reveals that the measured concentrations of Cd, Cr, Mn and Zn in the Brahmaputra river water are lower but Fe is higher than standard guidelines. Pb and Ni are found below detection limit in all sampling sites. Based on sediment quality guidelines, sediments are heavily polluted for Ni. According to contamination factor, all the sampling sites are low polluted for all studied heavy metals except Ni which is moderately polluted. These results clearly indicate the quality of the Brahmaputra river water and sediments to be unpolluted to low polluted. Continuous monitoring and assessment will keep checking the pollution status of the river water and sediments.

Paddy cultivation has been identified as the main anthropogenic source that affects river water quality. Arsenic and cadmium concentration in water showed a bimodal pattern which coincided with the bimodal rainfall of the dry zone and the paddy cultivation pattern in the study area. The presence of arsenic and cadmium in sediments and water affect bioaccumulation in fish. However, the trace elements levels in fish varied as *Mastacembelus armatus* > *Channa punctatus* > *Mystus vittatus*. Accumulation of As does not change according to the feeding habits of fish. Nevertheless, the feeding habits and habitat preferences of fish affect the accumulation tendency of Cd. Arsenic accumulation pattern in different organs (i.e., kidney > liver > gill > muscle) stress that the waterborne pathway is more important and Cd accumulation pattern (i.e. liver >kidney>gill>muscle) reflects the importance of dietary pathways. Further comprehensive assessment on the gills of *Mastacembelus armatus*, *Channa punctatus* and *Mystus vittatus* is necessary to understand the suitability of the species for biomonitoring studies. Water and sediment trace element levels do not considerably affect the muscle tissue. Therefore, fish consumption is not affected by paddy cultivation.

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Role of GSTT1, GSTM1 gene polymorphism in the development of pre-eclampsia in Bangladeshi women

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Abstract

Worldwide, pre-eclampsia (PE) ranks as a major obstetric problem leading to substantial maternal and perinatal morbidity and mortality, especially in developing countries (Aris et al; 2009). Clinically it appears as a maternal syndrome, including hypertension and proteinuria after second half of pregnancy (Zahra et al; 2011). Ten to twenty percent of the cases of preeclampsia leads to maternal death (Who et al; 2007). In developing countries, the prevalence rate of preeclampsia ranges from 1.8% to 16.7% (Osungbade ko et al; 2011). Half a million women dies of pregnancy related complications over the world each year and 99% of the cases occur in developing countries . Around the world, approximate rate of preeclampsia ranges from 2% to 10% (Osungbade ko et al; 2011). The number of baby dies of preeclampsia over the world is 500,000 per annum (Khklina E V et al; 2009). Study conducted by khan et al. (2013) reported that about 3.4% of the pregnant women was suffering from preeclampsia in Bangladesh.PE is a complex disorder affected by genetic trait (Mostafa et al; 2012). It affects only pregnant women and is diagnosed when hypertension and proteinuria occur after 20 weeks' gestation (NIH, 2004) and itis associated with oxidative stress due to imbalance between the production of reactive oxygen species and the ability of antioxidant process. The glutathione S-transferases (GST) are a group of enzymes which are involved in protecting cells from oxidative stress.

Objectives: Preeclampsia is widespread disease impact mostly on maternal death and child health retardation In our study, we evaluated the risk of developing preeclampsia with respect to GSTT1 and GSTM1 genotypes.

Study design: Total 100 patients having preeclampsia and 100 healthy controls having no history of preeclampsia was recruited for study. DNA was extracted from the blood. Polymerase Chain Reaction (PCR) method was used to identify genotypes of GSTT1 and GSTM1 in study subjects.

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Results: Genotype frequency of GSTM1 nul genotype showed significant difference between case and control group ($p<0.001$). Also combined null genotype for GSTT1 and GSTM1 showed significant difference between two groups when compared to combined present genotype for GSTT1 and GSTM1 ($p<0.001$). There was significant association for GSTM1 null genotype with the development of preeclampsia ($p<0.001$) and the odds ratio was significantly higher in patients compared to control subjects (OR=4.75; 95% CI=2.17-10.39). For GSTT null genotype, there was increased risk for developing preeclampsia but the association was not significant (OR=1.22; 95% CI=0.58-2.57). In case of GSTT1 and GSTM1 combined genotype, patients having both null genotypes for GSTT1 and GSTM1 showed significant ($p<0.001$) higher risk of developing preeclampsia when compared to control subjects (OR=7.64; 95% CI=2.38-24.60).

Conclusions: GSTM1 null genotype may increase risk of developing preeclampsia in Bangladeshi population. No risk was found for GSTT1 null genotype and preeclampsia. For combined null genotype of GSTT1 and GSTM1, the risk was even higher relative to the risk of either GSTT1 null or GSTM1 null genotype.

Keywords: Preeclampsia; GSTM1 genotype; GSTT1 genotype; Polymorphism; PCR.

Introduction

Worldwide, pre-eclampsia (PE) ranks a major obstetric problem leading to substantial maternal and perinatal morbidity and mortality, especially in developing countries (Aris et al;2009). Clinically it appears as a maternal syndrome, including hypertension and proteinuria after second half of pregnancy (Zahra et al;2011).PE affects approximately 10% of all pregnancies with a slightly higher incidence in developing countries. PE is a complex disorder affected by genetic trait (Mostafa et al;2012). PE affects only pregnant women and is diagnosed when hypertension and proteinuria occur after 20 weeks' gestation (NIH, 2004).Ten to twenty percent of the cases of preeclampsia leads to maternal death (Who et al ;2007). In developing countries, the prevalence rate of preeclampsia ranges from 1.8% to 16.7% (Osangbadeko et al;2011). Half a million women dies of pregnancy related complications over the world each year and 99% of the cases occur in developing countries [3]. Around the world, approximate rate of preeclampsia ranges from 2% to 10% (Osangbadeko et al;2011).The number of baby dies of preeclampsia over the world is 500,000 per annum(Khklina E V et al;). Study conducted by khan et al. (2013) reported that about 3.4% of the pregnant women was suffering from preeclampsia in Bangladesh. Symptomatic therapy and premature termination of pregnancy is the last and most effective treatment after onset of the symptoms of PE. Thus, identification of high-risk women prior to development of the disease using genetic analysis, is important (Malamitsi-Puchner et al;2001 and Cnattingius et al;2004) .Increasing evidence suggests that oxidative stress plays an important role in the pathogenesis of preeclampsia (Patil et al; 2009; Siddiqui et al., 2010).Oxidative stress is known to take part in normal pregnant physiologic process. PE is associated with oxidative stress due to imbalance between the production of reactive oxygen species and the ability of antioxidant process (dekker and sibai ;1998 and chamy et al; 2006). This oxidative stress has been proposed as an underlying mechanism that contributes to the endothelial dysfunction associated with preeclampsia. GST is a large family of anti-oxidant enzymes (Sandoval-CarrilloA;2014). Glutathione S transferases (GSTs) (EC: 2.5.1.18) are major phase II enzymes involved in the detoxification and are known as oxidative stress related genes (Hayes et al;2005).These enzymes can decrease the oxidative stress by conjugation of electrophilic compounds to glutathione (Mostafa et al; 2012). Genetic polymorphisms in genes encoding *GSTM1* (a member of class mu; MIM: 138350) and *GSTT1* (a member of class theta; MIM: 600436) have been defined. The *GSTM1-0* and *GSTT1-0* alleles represent deletions of

GSTM1 and *GSTT1* genes, respectively and result in a loss of enzymatic activity (Harada et al; 1992; Pemble et al; 1994). Genetic polymorphism of various glutathione genes and their role in preeclampsia is still in controversy. A study on Turkish population found no relationship between glutathione genes polymorphism and preeclampsia (Cetin M et al; 2005) eclampsia, or hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome.Study conducted by Guan et al; (2016) found significant relationship between GSTT1 null genotype with preeclampsia in Chinese population. Another study on Mexican population showed higher risk of developing preeclampsia in patients having GSTT1 null genotype and even higher risk for GSTT1 & GSTM1 combined null genotype (Sandoval-Carrillo A et al; 2014). Another study on Iranian population revealed that there was no association of GSTT1 and GSTM1 gene polymorphism with preeclampsia (Anval Z et al; 2011) southern Iran.According to the study of Matos et al; (2016) GSTT1 null genotype is significantly associated with preeclampsiain Portugal. Approximately 45% of white people do not have a functional GSTM1 allele, and approximately 20% do not have a functional GSTT1 allele (Norppa, 2003).

In our present study, we determined the GSTT1 and GSTM1 null polymorphism in study population and evaluated their impact on developing preeclampsia in pregnant women of Bangladesh.

Materials and methods

Study design

The study was designed as a case-control study.

(i) Case: clinically diagnosed and suspected preeclampsia patients

(ii) Control: healthy subjects without history of preeclamsia.

Place of the study: The study has conducted at the RHSTEP, Dhaka medical college

The period of the study were 12 months from July 2017 –June 2018

Study Subjects: Totally 200 unrelated Bangladeshi subjects were selected for the study. Among them 100 were preeclampsia patients. The preeclampsia patients were recruited from Dhaka medical college hospital without any history of other chronic disease. Totally 100 pregnant women with no history of preeclampsia and other chronic diseases were recruited as controls from different hospitals of Dhaka city while they came for a regular Ante natal checkup.

Ethical Issue: All participants were given an explanation of the nature of the study, and informed consent was obtained. . They are informed about their rights to withdraw from the study at any time. They also informed that the identity of the individual and data obtained following analysis would not be disclosed. Available data has used for research purpose. After taking the proper verbal & written consent from the study subjects ,03 ml of venous blood has collected aseptically for DNA extraction for gene analysis and exact 03 ml of blood collected by Vacutainer by a trained Person.

Questionnaire

All study subjects completed a structured questionnaire covering information on age, number of pregnancy, family planning method, history of preeclampsia, physical complications (Hypertension, Diabetes mellitus, Renal problem, Leg swelling, Headache, Vision blurring, Vomiting), and biochemical parameters like – hemoglobin, serum creatinine, uric acid, serum alanine amino

transferase (ALT or SGPT), random blood sugar (RBS), FDP, D-dimer, Urine for Albumin. There was no significant difference in baseline characteristics between controls and cases except SGPT and hemoglobin (Table 1). The departmental ethical committee approved the study. The study was conducted in accordance with the declaration of Helsinki and its subsequent revisions[16].

Inclusion criteria

Case: Preeclamptic pregnant patients (n=100) (blood pressure usually ≥140/90 mm Hg), and proteinuria at least 300 mg/24 hr or urine for albumin -.>2 +)

Control:Normal pregnant patients without history of hypertension and proteinurea (n=100).

Exclusion criteria will be non Pregnant and patient with other chronic diseases

Sample Collection

Approximately 3.0 mL of venous blood was drawn from each individual following all aseptic precautions with the help of a trained person, using a disposable syringe. The drawn blood was immediately transferred to a tube containing EDTA (1.20 mg/ml). All tubes kept in an ice box for transportation to the laboratory. The whole blood samples were stored at −20 °C until DNA extraction.

GSTM1 and GSTT1 genotypin

GSTT1 and CYP1A1 fragments were amplified by using the primers described earlier[18]we investigated the GSTM1, GSTT1 and GSTP1 gene polymorphisms in diabetic patients and healthy individuals and searched whether polymorphisms in GST genes are associated with diabetes mellitus (DM. A part of exon -7 CYP1A1 was amplified as an internal control to ensure that a null genotype was due to the absence of GSTM1 and GSTT1 alleles rather than failure of PCR in this method. Negative controls were also used for all amplifications. PCR was performed in 200 µL tubes on a DNA thermal cycler (Applied Biosystem, USA). The total reaction volume was 15 µL. For each reaction, 40 ng of genomic DNA, 0.4 mM each primer, 0.4 mMdNTP mix (Promega corp., USA), 3 units of Taq polymerase (Thermo Scientific; USA) and 1.6 µL 10× PCR buffer were used (supplied with Taq Polymerase). Magnesium chloride concentration was 1.6 mM (supplied with Taq Polymerase). Condition for the amplification included initial step of denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute and elongation at 72°C for 1 minute and finally a step of final elongation at 72°C for 7 minutes.

Finally, 10 µL of the co-amplified products (GSTM1: 215 bp, GSTT1: 466 bp and exon – 7 CYP1A1: 312 bp) were analyzed by electrophoresis on 2% agarose gel. After electrophoresis, gel was stained with ethidium bromide and visualized under UV light in a UV trans illuminator (Fig. 1). The presence of a 215 bp band indicated that this subject was homozygote or heterozygote for GSTM1, while its absence in the presence of internal control band of CYP1A1 indicated the null GSTM1 genotype. The presence of a 466 bp band indicated that the subject was heterozygote or homozygote for GSTT1, while its absence in the presence of CYP1A1 control band indicated the null GSTT1 genotype.

PCR will be carried out using the following primer set

Forward primer: GSTM1: 5'-GAACTCCCTGAAAA GCTAAAGC-3'

and GSTT1: 5'-TTCCTTACTGGTCCTCACATCTC-3'

Reverse primer: 5′--GTTGGGCTCAAATA TACGGTGG-3'

and 5'-TCACGGGATCATGGCC AGCA-3'

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Science (SPSS), windows version 24. The relative association between cases and controls were assessed by calculating the odds ratio (OR). ORs, as a measure of relative risk, at 95 % confidence intervals (95 % CI) were estimated using logistic regression models. t-test and chi-square test was performed using Graph pad Prism version-7. P-value less than 0.05 were considered as statistically significant.

Results of the study

In this study we examined the association of GSTM1 and GSTT1 gene polymorphism as a risk factor for developing preeclampsia during pregnancy. According to table 1, serum SGPT levels was significantly higher in preeclampsia patients compared to control group ($p<0.01$). Additionally, hemoglobin level was significantly lower in patient group when compared to control group ($p<0.001$). Other parameters like – Age, Random blood sugar, Uric acid and S.creatinine, FDP,D-dimer , Urine for albumin showed no significance between two groups.

Genotypic distribution and analysis of GSTM1 and GSTT1 genotypes

Table 2 represents genotype frequency of GSTM1 and GSTT1 gene in different study groups. GSTM1 null allele showed significance between study subjects. On the other hand, GSTT1 null allele showed no significance. The analysis of GSTM1 and GSTT1 genotype in preeclampsia patients and control subjects and estimated risk are presented in Table 3. For GSTT1, the difference in the genotype categories (present and null) among study subjects was not significant. But GSTM1 showed significance between study groups ($p <0.001$) (Table 3). In case of GSTT1 and GSTM1 combined genotypes, null genotype for both GSTT1 and GSTM1 showed significance difference when compared to GSTT1 and GSTM1 present genotype ($p<0.001$).

The risk for preeclampsia was also calculated in relation to the GSTT1 and GSTM1(present and null) genotypes. In case of GSTT1 gene, patients having null genotype are at higher risk of developing preeclampsia when compared to control subjects (OR=1.22; 95% CI=0.58-2.57) although not statistically significant. For GSTM1 gene, the difference in the genotype categories (present and null) among study subjects was statistically significant ($p <0.001$) and risk of developing the disease also higher when compared to control subjects (OR=4.75; 95% CI=2.17-10.39) (Table 3). In case of GSTT1 and GSTM1 combined genotype, patients having both null genotypes for GSTT1 and GSTM1 showed significant ($p<0.001$) higher risk of developing preeclampsia when compared to control subjects (OR=7.64; 95% CI=2.38-24.60) (Table 3). Additionally, patients having at least one present allele for either GSTT1 or GSTM1 showed higher risk for preeclampsia compared to control group (OR=1.21; 95% CI=0.56-2.62) but not statistically significant.

Genotypic distribution of serum parameters in preeclampsia patients

t-test was used to analyze different serum parameter level for different genotypes in preeclampsia patients (Table 4). SGPT and fibrin degradation product(FDP) showed significance between two genotypic groups ($p<0.05$). Other parameters like – Hemoglobin, Creatinine, Uric acid and Random blood sugar ,D-Dimer showed no significance between two genotypic groups.

Table I. Baseline characteristics of the study subjects.

Parameters	Case (n=100)	Control (n=100)	P value
Age (year)	27.68 ± 0.61	26.97 ± 0.39	ns
Random Blood Sugar (mmol/L)	6.43 ± 0.25	6.02 ± 0.10	ns
SGPT (U/L)	43.29 ± 5.88	27.66 ± 0.65	<0.01
Uric acid (mg/dL)	7.76 ± 0.71	6.83 ± 0.25	ns
Creatinine (mg/dL)	0.95 ± 0.12	0.96 ± 0.10	ns
Hemoglobin (g/dL)	10.92 ± 0.12	11.82 ± 0.07	<0.001

Data represented as mean±SEM. t-test was used for analysis.
ns, no significant. p<0.05 was considered significant.

Table II. GSTT1 and GSTM1 genotype frequency in the study subjects.

GSTT1 variant Genotype frequency	Case (n=100)	Control (n=100)	P value
Present	65 (65)	74 (74)	ns
Null	35 (35)	26 (26)	
GSTM1 variant Genotype frequency			
Present	38 (38.)	77 (77)	<0.001
Null	62 (62)	23 (23)	

Data represented as number (percentage). Chi-square test was done to analyze significance between two groups. P<0.05 considered significant. ns, no significant. Allele frequency was same as genotype frequency.

Table III. Significance and risk assessment of GSTT1 and GSTM1 genotypes in study subjects.

GSTT1 genotypes	Case (n=100)	Control (n=200)	P value	OR (95% CI)
Present	65 (65)	74 (74)	-	1 (Ref.)
Null	35 (35)	26 (26)	Ns	1.22 (0.58-2.57)
GSTM1 genotypes				
Present	38(38)	77 (77)	-	1 (Ref.)
Null	62 (62)	23 (23)	<0.001	4.75 (2.17-10.39)
GSTT1 & M1 combined genotypes				
Both present	33(33)	67 (57)	-	1 (Ref.)
At least one present	37(37)	37 (37)	Ns	1.21 (0.56-2.62)
Both null	30(30)	6 (6)	<0.001	7.64 (2.38-24.60)

Result expressed as number (percentage). Odds Ratio (OR) and 95% Confidence Interval (95% CI) Odds ratio adjusted for age, family history of preeclampsia, no of pregnancy, family planning method, history of hypertension, gestational diabetes mellitus, renal problem, leg swelling, headache, vision blurring, and vomiting ns, no significant.

Table IV. Analysis of biochemical parameters based on genotypic groups in preeclampsia patients.

Parameters	Hemoglobin (g/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	SGPT (U/L)	RBS (mmol/L)	FDP (µg/mL)
GSTT1 genotypes						
Present	10.84 ± 0.17	0.87 ± 0.07	8.72 ± 1.12	27.92 ± 3.62	6.10 ± 0.21	24.44 ± 9.18
Null	11.04 ± 0.14	1.09 ± 0.30	6.24 ± 0.37	44.25 ± 9.19	6.98 ± 0.57	7.51 ± 1.77
GSTM1 genotypes						
Present	11.15 ± 0.21	0.91 ± 0.11	6.93 ± 0.75	55.51 ± 15.02	6.05 ± 0.25	31.5 ± 13.3
Null	10.77 ± 0.14	0.98 ± 0.18	8.17 ± 0.99	27.6 ± 2.17*	6.66 ± 0.37	8.19 ± 1.55*
GSTM1 & T1 Combined genotypes						
Both present	11.19 ± 0.24	0.94 ± 0.13	7.21 ± 0.90	57.08 ± 18.28	5.94 ± 0.25	41.03 ± 18.16
Both Null	11.06 ± 0.16	1.17 ± 0.37	6.35 ± 0.42	23.11 ± 3.04	7.10 ± 0.68	7.44 ± 2.27

Data represented as mean (percentage).
*$p<0.05$ when compared to present genotype in same parameter.
SGPT, serum glutamic-pyruvic transaminase; RBS, random blood sugar; FDP, fibrin degradation products.

Fig. 1. Agarose gel image of PCR product of Banding pattern ofGSTM1 and GSTT1gene

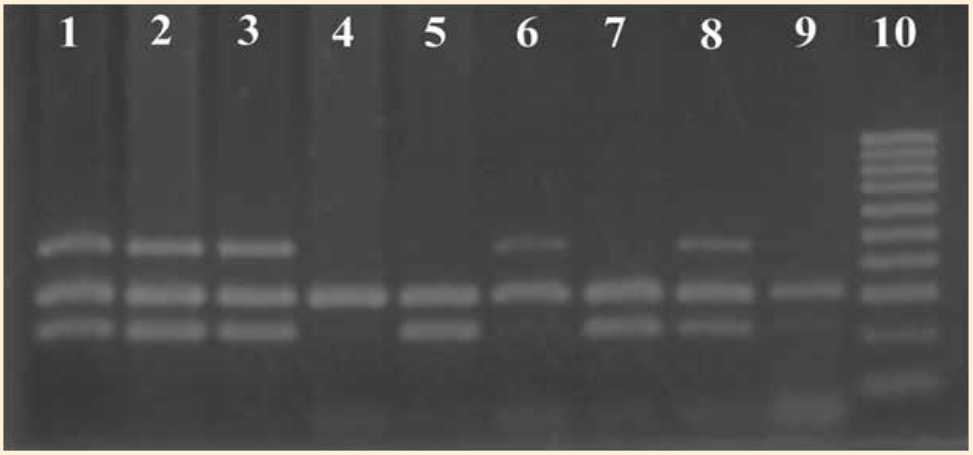

Fig. 1. Banding pattern of GSTM1 & T1 gene in 2% agarose gel. Upper (466 bp), middle (312 bp) and lower (215 bp) band represents the PCR product of GSTT1 present genotype, CYP1A1 and GSTM1 present genotype. Absence of 466 bp band represent GSTT1 null genotype (lane – 4,5,7,9) and absence of 215 bp band represent GSTM1 null genotype (lane – 4,6).

Discussion

In our study we analyzed the association between GSTM1 & GSTT1 gene polymorphism and the development of preeclampsia in Bangladesh. We found a significant association between GSTM1 null genotype with the development of preeclampsia in pregnant women in Bangladesh. There was no association between GSTT1 null genotype with preeclampsia. Study conducted by Guan et al; (2016) found significant correlation between GSTT1 null genotype with the development of preeclampsia in Chinese population .But they found GSTM1 null genotype as protective genotype for developing preeclampsia which is opposite to our study.

Further, combined null genotype for GSTT1 & M1 showed even lower risk of preeclampsia which is also opposite to our study. Study conducted in Iranian population showed no significance between GSTT1 and GSTM1 null genotype with the risk of preeclampsia (Anval et al; 2011southern Iran). According to the study of Matos et al; (2016) GSTT1 null genotype is associated with the risk of preeclampsia which is also opposite to the result of our study. They also found no association between GSTM1 gene polymorphism and preeclampsia. Study conducted by Sandoval-carrillo et al; (2014) found a significant relation with GSTT1 null genotype and the risk of preeclampsia in Mexican mestizo population. They also observed that combined null genotype for GSTT1 & M1 further increases the risk of preeclampsia which is similar to our study. Another study on Turkish population found no association between GSTT1 and GSTM1 gene polymorphism and the risk of developing preeclampsia (Cetin et al:2005). eclampsia, or hemolysis, elevated liver enzymes, and low platelets (HELLP syndromeBaseline characteristics like - age, random blood sugar, uric acid and creatinine showed no significance between patients and controls. SGPT level is significantly higher in patients compare to healthy subject ($p<0.01$). Study conducted by Dacaj et al.,(2016) showed higher level of SGPT in preeclamptic women similar to our study. Hemoglobin level is higher in control group when we compare it with the patient group ($p<0.001$).A study showed that maternal z score of hemoglobin was significantly higher in preeclampsia patients compared to controls (Amburgey et al; 2009).

t-test was used to analyze genotypic distribution of different serum parameters in preeclampsia patients which is enlisted in Table 4. SGPT and fibrin degradation product (FDP) showed significance between two genotypic groups ($p<0.05$). In our study, we found significant higher level of SGPT in GSTM1 present genotypic group compared to null genotypic group.Also, FDP level was significantly higher in GSTM1 present genotype compared to GSTM1 null genotype. This result seems contradictory because we found higher level of SGPT and FDP level and only 22 positive D-dimer in present genotype. This may because of the lack of relationship between these parameters with the genotype of GST genes. As preeclampsia is a multifactorial disease, there are other factors which may influence these parameters. Other parameters like – Hemoglobin, Creatinine, Uric acid and Random blood sugar, D-dimer showed no significance between two genotypic groups.

Conclusion

On the basis of our study, we found an association of GSTM1 polymorphism with the risk of preeclampsia in pregnant mother. There is no association of GSTT1 polymorphism with the risk of preeclampsia in Bangladeshi population. Combined null genotype for GSTT1 and GSTM1 have relatively higher risk than individual null genotype risks.

In conclusion, this study will helpful in identifying genetic polymorphism of GSTT1 and GSTM1 gene and its association with pre-eclamtic patients, especially in Bangladeshi patients. Such information will help to determine early diagnosis better approaches for prevention and treatment of pre-eclamtic patients, which ultimately helps in reducing of social burden and familial negligence in Bangladesh.

This will also provide an evidence for effecting of GSTT1 and GSTM1 gene on the risk factor of preeclampsia in Bangladeshi women. This relationship supports the idea that polymorphism inflammatory response genes may be host genetic susceptibility to preeclampsia.

Acknowledgement

It is our great pleasure to acknowledge Project ministry of Sciences and Technology, Government Republic of Bangladesh for their financial support and whole hearted support throughout the project work, entitled "Role of GSTT1, GSTM1 gene polymorphism in the development of preeclampsia in Bangladeshi Women". I am also greatful to all heads and their staff of all the hospitals included in the study for their support and cooperation in all the way of data collection. It would not be possible to complete the data collection without the full cooperation of the honorable respondents. I respect their tolerant in answering to fill-up the extensive questionnaire and grateful for their cooperation. The departmental ethical committee approved the study. The study was conducted in accordance with the declaration of Helsinki and its subsequent revisions[16]. I express my gratitude to and Hospital Director for issuing a letter to all the directors, Head of Obstetrics and Gynecology Departments of DMCH hospitals for rendering their support for collection of data and other necessary information. I also show appreciation all teams for their sincerity and taking all the troubles for data entry and analysis.

I must thanks Almighty Allah who allow us to complete the study.

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Preparation of Novel Hydrogel Composites with Enhanced Properties for Environment, Medical and Engineering Applications

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Abstract

Hydrogels composite (HGC) based on the crosslinking of maleic acid (MA) with hydroxypropyl methyl cellulose (HPC) and poly (vinyl alcohol) (PVA) were prepared. Three type of networks were designed in different ratio of PVA, CMC and MA. 100/100/100 (HGC-1), 50/50/100 (HGC-2) and 25/25/100 (HGC-3) (wt/wt/wt%) compositions of PVA, CMC and MA were used to prepare cross-linked composites materials. Variation of the crosslinking ratio of –COOH and –OH groups lead to new macromolecular supports with each composition. Thermal analyses [differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)], and Fourier transform infrared spectroscopy (FTIR) were employed to characterize the HGC. and reveal the structural properties of such composite materials. Variations in the glass transition temperature (Tg) of composite materials indicating the different thermal properties of each composites. In addition, the changes in the melting temperature (Tm), shape and area attributed to the different degrees of crystallinity of each composites. Studies were made on swelling behavior for all prepared composites using deionized water. The obtained results indicated that the swelling ratio of hydrogel decreased by increasing the concentration of MA. Removal of methylene blue from aqueous solution with each composite material was studied using UV-visible spectroscopy at pH 6. Dye adsorption on composite (HGC-3) was found maximum. Maximum uptake capacity was assumed to occur through the complexation and electrostatic interaction between composite and dye. Finally, macromolecular support of each composite materials were used to include and release paracetamol drugs in a sustainable way using phosphate buffer solution of pH 7.4. Complete adsorption of paracetamol onto the HGC materials was confirmed from UV-visible spectrum.

Keywords: HPC/PVA/MA composite; cross-linked network; dye adsorption and drugs release

Introduction

Hydrogel composites (HGCs) as promising materials are of greatest significance in the biomedical

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fields and have been most extensively studied in academic and industrial research such as biomimetic, biosensors, artificial muscles, chemical separation, biomaterials, cell culture systems, catalysis, photonics, drug delivery systems and separation purpose due to the remarkable characteristics, such as flexibility and versatility in fabrication, variety in composition, high tunability in the physical, chemical, and biological properties, high moldability in shape, especially their excellent biocompatibility.

Poly (vinyl alcohol) (PVA) is an attractive synthetic polymer hydrogel due to its good biocompatibility has been applied in several advanced biomedical applications e.g. wound dressing, wound management, drug delivery systems, artificial organs, and contact lenses. But, PVA hydrogel possesses insufficient elasticity, stiff membrane, and very limited hydrophilicity which restrict its uses alone.

Hydroxypropyl methylcellulose (HPMC) is a semi synthetic polymer of most abundant naturally occurring biopolymer, cellulose that has excellent film forming capability, superior tensile strength, biocompatibility and biodegradability. It possesses good swelling behavior and accelerates the wound healing process by keeping the environment moist. It is soluble in both water and polar organic solvents, making it possible to use both aqueous and non-aqueous media. HPMC is most commonly used in the food industry as a stabilizing agent, as a protective colloid, as a thickener and as an emulsifier. However, HPMC films have moderated mechanical strength and are resistant to oils and fats, flexible, transparent, odorless, and tasteless. So, the water sensitivity of HPMC films, which produces a loss of barrier properties or even a solubilization into foods with high water activities, prevents their industrial applications. The addition of synthetic polymers such as PVA to HPMC hence gives us a blend with superior and desired properties because both polymers are compatible with each other and form miscible blends when blended together. In addition to that the incorporation of PVA in certain macrogel or nanogel ensures its excellent mechanical strength, and the chemical crosslinking is needed to create and modify polymer nanostructure, to improve thermal, mechanical and chemical stabilities. PVA content in the gels type gives higher affinity to crosslink formation through the intra- and or intermolecular hydrogen bonding via H groups. To know this fact, we have chosen HPMC and PVA as the polymer matrix in the present study to get combined effect of both polymers on the final properties of nanocomposites.

Wu et al. have prepared ternary blend films with different ratios of starch/ PVA/citric acid that films can be used as an active food packaging system due to their strong antibacterial effect. It has also been reported that dicarboxylic acids such as citric acid maleic acid (MA) etc. can act as a crosslinking in methylcellulose based hydrogels due to the presence of carboxyl groups in the structure which form hydrogen bonds with –OH in the PVA.

Hence, MA reinforced nanocomposites with biodegradable and biocompatible polymers and nano-fillers can combine the ductile properties of a polymer matrix and high strength of nano-fillers. This is mainly due to the presence of oxygen-containing functional groups in the structure of MA, which improve interfacial interaction between MA and the polar polymer matrix.

However, hydrogel composites based on HPMC, PVA and MA was not found in the literature studied so far. Therefore, the purpose of this study was to prepare hydrogel composites based on the cross-linking of PVA/HPMC/MA. Then, these composite materials are characterized by thermal analyses differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) and Fourier transformed infrared spectroscopy (FTIR). They were performed to evaluate the dye adsorption properties for methylene blue (MB) from aqueous solution. In addition to that, each

composite material was explored to include and release paracetamol drugs in a sustainable way using phosphate buffer solution of pH 7.4.

Materials

PVA molecular weight ≈115000 and maleic acid were purchased from Loba Chemical Com. HPMC was purchased from Chem Cruz. All the chemicals used for this study were research grade and used as received.

Synthesis of chemically cross-linked hydrogel composite (HGC)

Solution method was used to obtain HGC. To prepare HGC blending of PVA, HPMC and MA were done as follows:

Table I. Composition of PVA, HPMC and MA for hydrogel formation

Sample No	Raw materials ratio (%wt/wt/wt)		
	Polyvinyl alcohol (PVA)	Hydroxypropyl methyl cellulose (HPMC	Maleic acid (MA)
HGC-1	50	50	100
HGC-2	100	100	100
HGC-3	25	25	100

The weighted amount of PVA, HPMC and MA were taken in a beaker. Then 30 mL deionized water was added to it. Afterwards, the solution was sonicated for 30 mins at 50 °C. The solution was then cast onto petri dish. Then the crosslinking of the solution was obtained by thermal treatment in the oven at 120°C for 6 hours. Cross-linked films were then peeled off and transferred into vial.

FTIR analysis

FTIR is used to analyze the presence of functional groups of prepared composite materials. Characterization was carried out using IRTracer-100 of Shimadzu Corporation, Japan. Characteristics bands –C=O, -C-O, -O-H, of the prepared composites ware mainly identified by using FTIR technique. For each the prepared composite materials a pellet was prepared using KBr. Then the pellet was mounted and adjusted on sample surface. Afterwards, a black gripper was used to cover the sample surface. A computer based FTIR program was used to identify the functional groups of the prepared composite materials. The entire FTIR spectrum of the prepared sample has been recorded in the range of 500 to 4000 cm^{-1}.

TGA analysis

TGA experiments were performed for the prepared hydrogel composites. The weight loss of the sample was recorded by using TGA-550 Shimadzu Corporation, Japan. The prepared Hap-NPs were placed in a TGA sample pan. Sample masses were confirmed with a precision balance. A computer based TGA program was used for obtaining TGA curve. The samples were held at room temperature under a flow of nitrogen gas to create inert environment for the sample and drive off gases such as oxygen, water vapor. Then the temperature was increased from room temperature to 600 °C at a rate of 10 °C/min. At the end of each experiment the temperature was held constant at 600 °C for ten

minutes. After ten minutes the gas flow was switched off and waited until the temperature cooled down to room temperature.

Swelling property

Swelling degree was determined using dried hydrogel samples and distilled water as swelling agent. The dried samples of certain amount was weighed by an analytical balance and was immersed in deionized water (pH = 7) at room temperature. They kept 48 h in water bath for swelling. Afterwards, the swollen composite hydrogels were removed from water bath. Excess surface water was wiping off with filter paper and weighed carefully. The weight change for each sample was recorded. The following equation was used to determine the swelling degree.

$$\text{Swelling degree (\%)} = \{(W_{wet} - W_{dry})/W_{dry}\} \times 100 \quad (1)$$

where: W_{wet} = weight of the swelled composite at time t

W_{dry} = weight of the dried composite at time 0

Preparation of stock solutions

0.0030 g of solid dye, MB was taken in a 100.0 mL volumetric flask and de-ionized water (specific conductance < 0.1 μS cm^{-1}, obtained from Water Purification System, Model No. WDI 15, Humanlab Instrument Co., Korea) was added to it and up to the marked. Hence, 9.38 × 10^{-5} M of MB was prepared as a stock solution. Similarly, 50 mL 0.40 × 10^{-3} M of paracetamol solution was prepared as a stock solution. Further dilution was made when necessary.

Adsorption study of dyes

The ability of the hydrogels to adsorb MB from an aqueous solution was determined at room temperature. Adsorption studies have been carried out using a batch equilibrium technique.

20.0 mL 4.5 × 10^{-5} M MB solution was taken in a 50 mL reagent bottle and 0.6038 g HGC-1 was added. Then reagent bottle was placed at 25.0 °C. After different time intervals of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 24.0, 48.0, 72.0, and 96.0 hours, the clear solution from the reagent bottle was taken for spectrophotometric analysis using UV Spectrophotometer (Model No. UV-1800, Shimadzu, Japan) to determine the maximum absorbance, ca. 665 nm for cationic MB. After each experiment the clear solution was returned to the reagent bottle.

From the value of absorbance, concentration of each solution can be determined by the following equation:

$$\text{Concentration of MB} = \text{Absorbance of MB at 665 nm}/\varepsilon_{665} \quad (2)$$

The amount of dye adsorbed onto prepared hydrogels was determined by the following equation:

$$\text{Amount adsorbed} = \frac{C_1 - C_2 \times M}{1000 \times m} \times V \quad (3)$$

Where, C_1 and C_2 are the concentrations of MB at initial and equilibrium respectively, M is the formula mass of MB, V is the volume of the solution, m is the amount of adsorbent used.

Similar studies were performed for adsorption of MB on HGC-2 and HGC-3. The results obtained were summarized in Table 2, 3 and 4.

Drug release property

To conduct the drug release experiment, paracetamol was taken as a model drug and phosphate buffer solution of pH 7.4 was used as release medium. Dried HGC was loaded with drug by immersing it in aqueous solution of paracetamol (0.12 mM) for 24 h and then dried at room temperature.

20 mL of the prepared 0.12 mM drug solution was taken in each HGC. After that aliquots from the release medium were withdrawn at predetermined time intervals and analyzed by using UV Spectrophotometer at 245 nm. The removed release medium was replaced with the same volume of fresh buffer solution at the same temperature.

Results and discussion

Infrared spectral analysis has been utilized to prove the cross linking of co-polymer. For this purpose the FTIR spectra of PVA, maleic acid, HPMC and crosslinking co-polymers are shown in Figure-1(a), (b), (c) and (d), respectively.

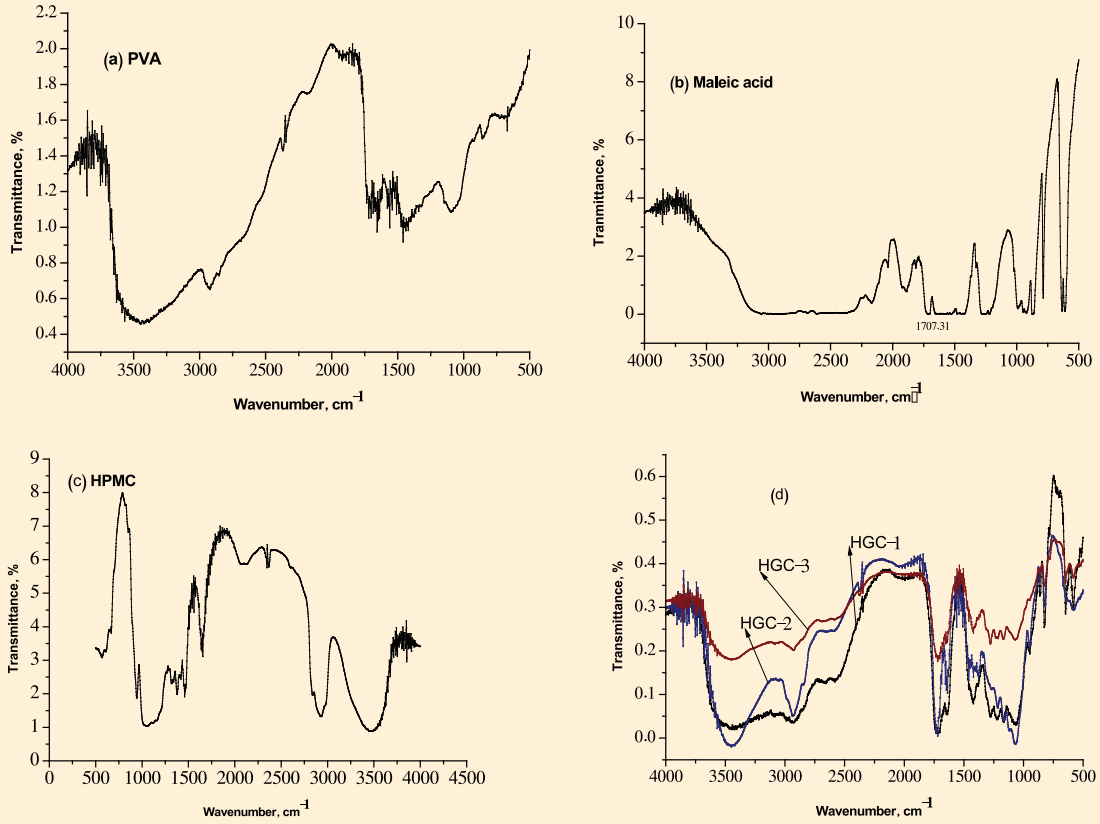

Fig. 1. (a) FTIR spectrum of PVA (b) FTIR spectrum of Maleic acid (c) FTIR spectrum of HPMC (d) FTIR spectrum of the prepared HGC

From the spectra of PVA (Figure-1(a)), it shows a band at 3415.13 cm^{-1}, due to the hydrogen bonded hydroxyl groups, 2924.51 cm^{-1} for C-H stretching, 1645.50 cm^{-1} for bending HOH and 1093.34 cm^{-1} for Out-of-plane C–O vibration. Figure-1(b) shows the spectra of maleic acid. It shows a band at

1712.62 cm^{-1} due to stretching of –CO– group of carboxylic acid. From the spectra of HPMC (Figure-1(c), shows a strong band at 3459.40 cm^{-1}, due to the stretching frequency of the –OH group. The band at 2930.90 cm^{-1} is the result of C–H stretching vibration. The band at 1639.08 cm^{-1} due to stretching of –CO– group. The bands around 1454.84 and 1371.99 cm^{-1} are assigned to $–CH_2$ scissoring and –OH bending vibration, respectively. The band at 1040 cm^{-1} is due to C–O stretching vibration.

From the (Figure-1(d) shows the cross link copolymer formation using different ratio of PVA, maleic acid and HPMC. The strong band at 1723.22 cm^{-1} for all the copolymers indicates the stretching of –CO– group of ester formation due to the cross link of one –OH group from PVA and one –COOH group from maleic acid and another –COOH group of maleic and –OH group from HPMC.

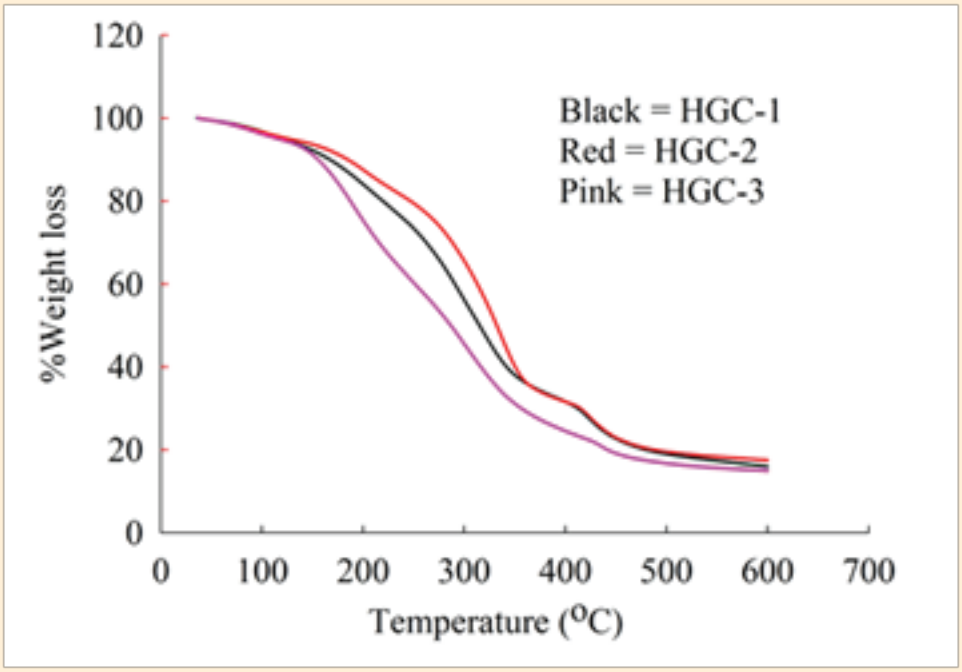

Fig. 2. Thermogram of the three prepared HGC

TGA Explanation

Thermal analysis was done to investigate the thermal stability of the prepared composite materials. Figure-2 compares the thermal stability and decomposition curves of PVA, HPMC and MA cross-linked composite materials. A weight loss before 150 °C was observed for all samples. This mass loss is related to the release of physically adsorbed water molecules. Two degradation stages were noted for each sample. First one occurred approximately between 100 to 150 °C. The mass loss of the composite material in the second step starts at 150 °C and continued until 500 °C. It is assumed that the decomposition after 150 °C is related to dehydroxylation and/or decarboxylation of HGC composite materials. Observation of the obtained thermogram clearly reveals stability variation of the prepared composite materials. Among them HGC-3 composite materials shows lowest stability. The stability of HGC-1 is in between HGC-2 and HGC-3. HGC-2 with 100/100/100 (wt/wt/wt%) composition of PVA/HPMC/MA shows the highest stability. After the required crosslinking reaction more carboxylate ions are present in HGC-3 of 25/25/100 (wt/wt/wt%) ratio of PVA/HPMC/MA. The presence of these free carboxylic groups lowers the stability of HGC-3 composite. Lowest

stability of HGC-3 composite is also related to the less crosslinking of PVA/HPMC/MA. It is noteworthy that the composite HGC-2 with 100/100/100 (wt/wt/wt%) ratio of PVA/HPMC/MA has been shown highest stability. Lowest weight loss of HGC-2 is related to the highest crosslinking of PVA/HPMC/MA. Thus, crosslinking of the functional groups have significant effect on the stability of the prepared composite materials.

*Swelling Behavior Test*_

A variation in the swelling ratio of the prepared composites as a function of %weight of PVA, HPMC and MA has been represented in Figure-3. Among the prepared composite materials HGC-2 has the highest swelling ability. HGC-1 has the lowest. The degree of swellability is correlated with the amount of maleic acid. With the high amount of maleic acid reinforcement of the composite matrix is highest. Highest reinforcement of the composite matrix yields lowest porosity. Hence, swelling ability of the composite matrix decreased.

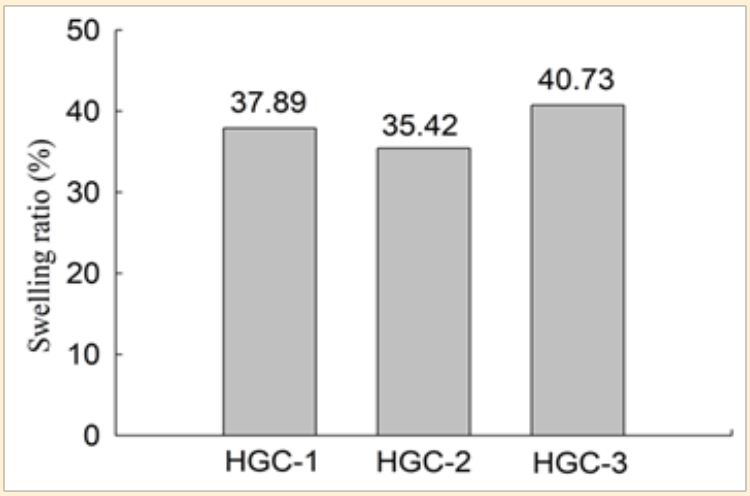

Fig. 3. Comparative swelling behavior of three different HGC

Adsorption of MB onto hydrogels

The study of adsorption of MB on different forms of hydrogels was performed. The results obtained are shown in Table 2, 3 and 4.

Table II. Determination of equilibrium time for adsorption of MB on HGC-1[a]

Time/h	Absorbance (at 665 nm)	Amount Adsorbed (mg/g)
0.0	3.202	0.0
0.5	2.602	0.129
1.0	1.713	0.319
1.5	1.366	0.394
2.0	1.085	0.455
2.5	0.986	0.475
3.0	0.854	0.504
4.0	0.739	0.529
24.0	0.397	0.601
72.0	0.297	0.623

[a]Total volume of solution = 30.0 mL, initial concentration of MB = 4.5×10^{-5} M, amount of HGC-1 = 0.6038 g

Table III. Determination of equilibrium time for adsorption of MB on HGC-2[a]

Time/h	Absorbance (at 665 nm)	Amount Adsorbed (mg/g)
0.0	3.202	0.0
0.5	1.875	0.280
1.0	1.833	0.289
1.5	1.701	0.317
2.0	1.609	0.336
2.5	1.595	0.339
3.0	1.588	0.340
4.0	1.580	0.342
24.0	0.640	0.540
72.0	0.539	0.561

[a]Total volume of solution = 30.0 mL, initial concentration of MB = 4.5×10^{-5} M, amount of HGC-2 = 0.6139 g

Table IV. Determination of equilibrium time for adsorption of MB on HGC-3[a]

Time/h	Absorbance (at 665 nm)	Amount Adsorbed (mg/g)
0.0	3.202	0.0
0.5	2.477	0.156
1.0	1.402	0.387
1.5	1.054	0.462
2.0	0.762	0.524
2.5	0.563	0.567
3.0	0.464	0.587
4.0	0.351	0.613
24.0	0.126	0.661
72.0	0.114	0.664

[a]Total volume of solution = 30.0 mL, initial concentration of MB = 4.5×10^{-5} M, amount of HGC-3 = 0.6022 g

Here, the respective UV-adsorption spectrum of methylene blue dye using HGC-3 is shown below:

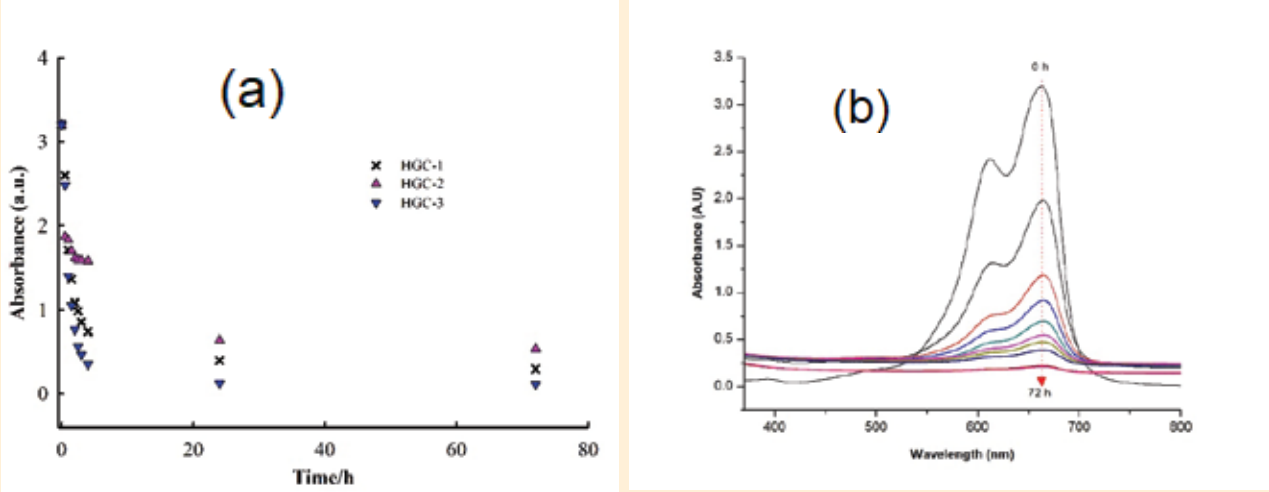

Fig. IV(a). Variation of absorbance with time for adsorption of MB on HGC-1, HGC-2 and HGC-3 respectively. (b) Adsorption spectrum of methylene blue on HGC-3.

The absorbance-time profiles of adsorption of MB on HGC-1, HGC-2 and HGC-3, respectively have shown in Figure-4(b). It can be seen that absorbance of the solution decreases with time up to 120 min and then become constant. This time (120 min) is considered as the equilibrium time for the adsorption of MB. However, in the case of HGC-3, the extent of decrease in absorbance is larger than that of HGC-1 and HGC-2.

However, from the measured absorbance, the amounts of MB adsorbed on HGC-1, HGC-2 and HGC-3 per gram were estimated and are demonstrated in Figure-4(a). In all cases, the progress of MB adsorption was fast, and HGC was nearly saturated within 120 min. The extent of adsorption seems to be different for different HGCs. The cationic dye, MB showed significant adsorption on HGC-3 having more molar ration of MA compared to HGC-1 and HGC-2. However, at equilibrium condition, the amounts of MB adsorbed on HGC-1, HGC-2 and HGC-3 are found to be 0.455, 0.336 and 0.524 mg/g, respectively. All the composites strongly adsorbed a significant amount of MB. But HGC-3 adsorbed MB is about 15 % and 56 % higher than those on HGC-1 and HGC-2, respectively. Therefore, HGC-3 composite shows highest adsorbent capacity.

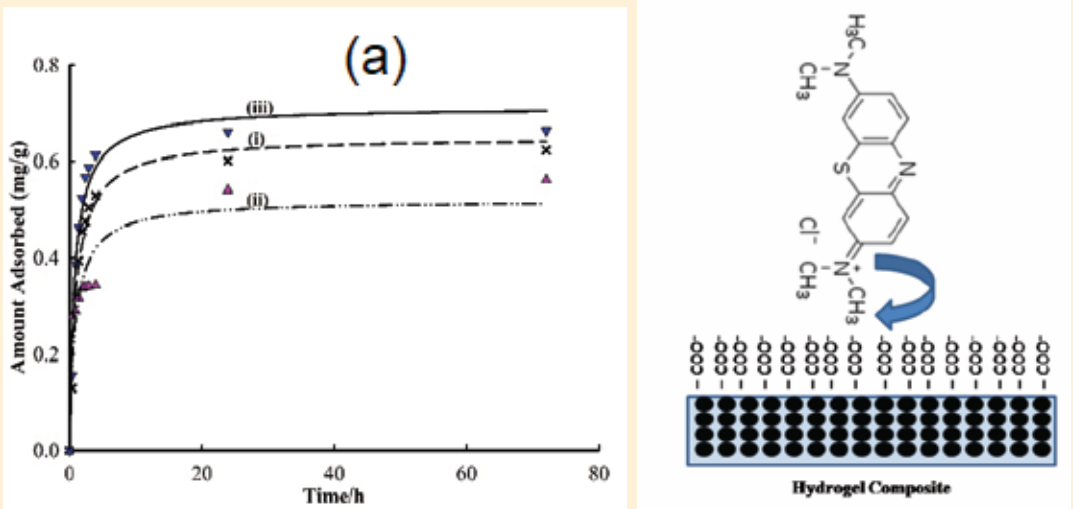

Fig. V.(a). Adsorption of MB on (i) HGC-1, (ii) HGC-2 and (iii) HGC-3 in aqueous solution. (b) Probable interaction site between MB and hydrogel composite.

Proposed mechanism of MB adsorption on HGC

MB is a cationic dye containing positive charge on the $-N(CH_3)_2$ groups. HGC prepared from PVA, HPMC and MA contains negative charge of the structure become electron rich. So, mode of adsorption of MB on HGC is the electrostatic interaction between positively charged MB and negatively charged HGC (Figure-5(b)). As a result, MB are readily adsorbed on HGC surfaces. However, the adsorbed amount of MB is maximum on HGC-3. This may be due to presence of more percentage of MA in the HGC composition which contains negatively charge on carboxylate ions.

Drug release study

Fig. VI. UV-visible spectrum of (a) paracetamol solution (b) paracetamol loaded HGC solution

Figure-6 represents the drug release profile of prepared HGCs. From the graph it is shown that no drug was released from the composites after 0.5, 1.0, 2.0 h intervals. No change i.e., no peak of paracetamol was found. Then, the release medium was kept at room temperature for 24 h and UV-spectrum of the release medium was taken for each HGC and again no peak was observed which indicates that the drug was strongly adsorbed onto HGCs. This may be due the strong electrostatic interaction of free –OH group in the structure of paracetamol and carboxylic group in the HGCs.

Conclusion

In this study, maleic acid was successfully cross-linked with PVA and HPMC. The cross-linked macromolecular support was characterized by FTIR spectroscopy. FTIR spectrum of the prepared HGC materials clearly indicated the formation ester group. TGA spectrum shown the variation of thermal stability with varying the amount of cross-linker. UV-visible spectrum of evidenced the adsorption of dye methylene blue onto the HGC materials. Furthermore, HGC materials were used to include paracetamol drug. Complete adsorption of paracetamol onto the HGC materials was confirmed from UV-visible spectrum.

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Selection of elite genotypes based on characteristics analysis of seed oil of jatropha (*Jatropha curcas*) genotypes available at BSMRAU

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Abstract

The present research was conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur to explore the production potential of biodiesel from Jatropha and to analyze the characteristics of extracted seed oil as a source of green energy. Seeds of all genotypes were decorticated manually from the harvested fruits. The highest husk and kernel percentage was found in the sample no 20 and 3, having the value of 42.27% and 65.57%, respectively. The average value of husk and kernel recovery percentage was 39.31 and 60.66, respectively. The highest moisture content was found in the sample 14 (52.5%) and the lowest in the sample 2 (18.5%). The highest oil percentage was extracted from the sample 18 (62.22%) and the lowest in the sample 4 (29.14%). Seed oil density was not significantly varied among the samples and it ranged from 0.828 to 0.952 with the average of 0.903. The highest iodine value was found in the seed oil sample 18 (121.28mg/g oil) and the lowest in the sample 08 (89.53mg/g oil) with the average value of 105.53mg/g oil. The highest acid value was found in the sample 019 (2.52mg NaOH/g oil) and the lowest in the sample 15 (0.784mg NaOH/g oil). The highest free fatty acid was found in the sample 19 (1.26) and the lowest in the sample 15 (0.393). The highest saponification value was found in the sample 05 (201.25 mg KOH/g oil) and the lowest in oil sample 15 (173.91 mg KOH/g oil). The highest high heating value is found in the sample 01 (40.82MJ/kg) and the lowest in the sample 05 (39.59MJ/kg). The highest cetane number was found in the seed oil sample 01 (56.75) and the lowest in the oil sample 19 (48.07). Based on physiochemical characterization of Jatropha seed oil, it can be concluded that oil from each sample or genotype has its own special characteristics and helpful for selecting good biodiesel feed stock and identifying good genotypes (1, 5, 15 and 18) of *Jatropha curcas*.

Keywords: Jatropha; Oil extraction; Acid value; FAA; Iodine value; HHV; Eetane number

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Introduction

The genus Jatropha has 426 species and distributed throughout the world, belonging to the family Euphorbiaceae, producing oil containing seeds (Jongschaap *et al.*, 2007). It is native to Central America and has become neutralized to many tropical and subtropical areas, including India, Africa, and North America. *Jatropha curcas* L. was first described in 1973 by Carl Linnaeus, Swedish botanist. The name of this plant was derived from Greek where the first part of its name, 'jatros', meaning physician and the last part, 'trophe', meaning nutrition (Brittania and Lutaladio, 2010). Jatropha is popularized as unique candidate among renewable energy sources due to its peculiar features like drought tolerance (Openshaw, 2000), rapid growth and easy propagation, higher oil content than other oil crops (Achten *et al*, 2008), small gestation period, wide range of environmental adaptation, and the optimum plant size and architecture make it as a sole candidate for further consideration (Sujatha *et al*, 2008). Its cultivation requires simple technology, and comparatively modest capital investment. The seed yield reported for Jatropha varies from 0.5 to 12 ton per ha per year depending on soil, nutrient and rainfall conditions and the tree has a productive life of over 30 years (Francis *et al,* 2005). The seeds contain 30–35% oil that can be converted into good quality biodiesel by transesterification (Foidl *et al*, 1996). Despite the toxicity of the Jatropha seeds, edible varieties exist in Mexico (Schmook and Serralta, 1997) which is not currently being exploited. The true potential of Jatropha has, however, not yet been realized but now the conditions for its exploitation have improved considerably in recent years due to the increase of crude oil prices and policy incentives for the exploration of indigenous and renewable fuels. The interesting properties and potentials of jatropha seed oil attracts lot of investors, policy makers and clean development mechanism project to tackle the challenges of energy supply and Green House Gas (GHG) emission reduction (Rao, 2006).

Jatropha curcas oil contains a high percentage of unsaturated fatty acid, which is about 78-84% (Heller, 1996). This made the oils suitable for biodiesel production. However, the chemical compositions of the oil vary according to the climate and locality. Very few authors have studied the physiochemical and fuel properties of *Jatropha curcas* oil in Bangladesh which is being obtained from diverse origin. Physical and chemical properties of seed kernel are needed to allow the design of equipment to handle, transport, process, store, and assess the product quality (Sirisomboon *et al*., 2007). It was observed that the properties of vegetable oil affect the fuel properties which play a significant role in the combustion process of biodiesel. Such properties are density, specific gravity, refractive index, free fatty acid (FFA) content, acid value, iodine number, saponification number, cetane number, and high heating value. This study aims to extract and characterize the physicochemical properties of *Jatropha curcas* oil from the genotypes collected from local and exotic sources (Malaysia, Indonesia, India and Nepal) and maintained at BSMRAU, Gazipur. To explore the production potential of biodiesel from Jatropha, the present study was conducted to analyze the characteristics of extracted seed oil as a source of green energy.

Materials and methods

The research work was conducted at the laboratory of the Department of Genetics and Plant Breeding and Department of Agro-processing, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The Jatropha seeds were harvested from the plantation maintained by the Department of Genetics and Plant Breeding, BSMRAU. Fully ripened fruits (Fig 1a-b) were collected from plants of 25 different genotypes (sample no. 01 to 25) of *Jatropha curcas* L. Seeds were decorticated manually from the harvested fruits (Fig. 1c) and were solar heated for several hours.

Fig. 1. Experimental materials (a) Fruits in trees, (b) Harvested fruits, (c) separated seeds from fruits, (d) separated husks and kernels, (e) seed powder after grinding

Seed moisture content

Samples of 100 air dried Jatropha seeds were randomly picked and dried in an oven at 105°C for 24 hours. The moisture content of the seeds were calculated by using the equation, where, MC = moisture content, M_i = the initial mass of the sample seeds and M_f = the final mass of the sample seeds after drying.

$$MC = \frac{Mi\text{-}Mf}{Mf} \times 100$$

Thousand seed weight

Thousand seed weight was determined using a digital electronic balance with an accuracy of 0.001 g. To evaluate the thousand seed weight, 50 seeds were randomly selected from the bulk sample, weighted and then multiplied by 20 to get weight of 1000 seeds.

De-shelling and grinding

Dried Jatropha fruits were de-shelled (Fig 1d) manually (pressing by hands) and it contains about 35-40% shell and 60-65% kernel (by weight). It has nearly 450-550 fruits per kg, 1580-1600 seeds per kg weight and the weight of 100 seeds is about 63 g (Mohammed, 2006). Seed kernels need to be dried at 100-105°C for 30 min (Akbar *et al.,* 2010) before oil extraction to reduce the moisture content up to 5% (Sirisomboon and Kitchaiya, 2009) because the kernels of jatropha have high moisture content at 34% (Sirisomboon et al., 2007). Dried kernels were ground to powder using a grinder prior to oil extraction.

Extraction of oil

Jatropha oil was extracted from kernel powder (Fig. 1e) by solvent extraction method (Fig. 2) using hexane (Sayyar *et al*., 2009). All chemicals used in the study were analytical grade and used without further purification. The oil was extracted from the samples with known weight in chemical extraction using hexane (b. p 60-70°C) for 6 hours without interruption by gentle heating it. The extracted oil was evaporated on a water bath until hexane remains. The oil yield was expressed in term of percentage of powdered sample.

Percentage of oil extracted

Ten grams of jatropha seed kernel powder was placed in the thimble and extracted by using 120 ml n-hexane as solvent (Sayyar *et al*., 2009). The solvent was removed using rotary evaporator apparatus at 40°C (Fig 3). At the end of process, oil extracted was determined as percentage of oil in the dry matter of jatropha kernel powder. Oil percentage was calculated by the following formula where M_1 = Weight of ultimate fat, and M_2 = Grind used in the thimble.

$$\text{Percentage of oil} = \frac{M_1}{M_2} \times 100$$

Fig. 2. Oil extraction by Soxhlet method

Fig. 3. Extracted oil after hexane separation

Extracted seed oil was stored in freezer at −2°C for subsequent physicochemical analyses.

Physical properties

Density

The density of a material is defined as the measured of its mass per unit volume (e.g. in g/ml). The density vegetable oil is lower than of water and the differences between vegetables oil are quite small, particularly amongst the common vegetable oils. Here, for each sample 5 ml oil was taken for determination of density. At first, sample weight was taken and then density was measured by the following equation, where, ρ is the density, m is the mass, and v is the volume.

$$\rho = \frac{m}{v}$$

The density of a material varies with temperature and pressure. This variation is typically small for solids and liquids but much greater for gases. Increasing the pressure on an object decreases the volume of the object and thus increases its density. Total measurement was done under room temperature without any external pressure.

Oil moisture content

It was determined by oven drying of 5 ml oil in the oven at 105 °C for 24 hours after which the percentage moisture was calculated following equation, where, M_i = Initial weight of oil and M_f = Final weight of oil.

$$\% \text{ Oil moisture content } = \frac{Mi\text{-}Mf}{Mi} \times 100$$

Chemical properties

Chemical properties of jatropha oil (% free fatty acid, acid value, saponification value and iodine value) were determined using Standard Tentative Methods of Analysis (AOCS, 1991).

Calculation of iodine value

The iodine value was calculated using the equation Where, Volume of sodium thiosulphate used = [Blank- Test] ml, Equivalent weight of iodine = 127, Normality of sodium thiosulphate ($Na_2S_20_3$) = 0.1

$$Iodine\ No.\ of\ fat = \frac{\text{Equivalent Wt. of Iodine} \times \text{Volume of } Na_2S_20_3 \times 100 \times 10^{-3}}{Weight\ of\ fat\ sample\ used\ for\ analysis(g)}$$

Calculation of saponification value

The saponification value can be calculated using the following formula, where, S = sample titrate value, B = blank titrate value, M = normality of HCL, 56.1 = equivalent weight of KOH:

$$SV = \frac{(B\text{-}S) \times M \times 56.1}{Sample\ weight\ (gm)}$$

Calculation of acid value

Free fatty acids (FFA) was determined by the following formula

$$F.F.A = \frac{(\text{Sample - Blank})\ TV \times N \times 282}{Sample\ weight \times 10}$$

The acid value was also calculated using the expression;

$$\text{Acid Value} = 1.99 \times FFA\%$$

Fuel properties

High heating value (HHV)

The higher heating value (HHV) is the amount of heat produced by the complete combustion of a unit quantity of fuel. Higher heating value of jatropha oil was measured by the derived value of IV and SV using following formula adopted from Demirbas (1998):

$HHV = 49.43 - (0.041 \times SV) - (0.015 \times IV)$
SV = Soponification value
IV = iodine value

Cetane Number

The cetane number of the oil was determined according to the formula given by Bose (2009).

$CN = 46.3 + \quad 0.225 \times IV$
SV = Soponification value
IV = iodine value

Statistical analysis

All analyses were replicated three times for each sample. Analysis of variance (ANOVA) and mean separation was done by least significant difference (LSD) test using statistical software package of SAS 9.2 (2010).

Results and discussion

Physical properties

1000-seed weight

Thousand seed weight of 25 genotypes ranged from 533.2 to 632.4g (Fig 4). Among them sample no 11 or genotype 11 showed the highest value (632.4g) and sample no 7 showed the lowest value (533.2g) for 1000-seed weight. The average seed weight was 590.21g, which was higher than the value (534.50g) that was found by Tavakoli *et al.* (2009).

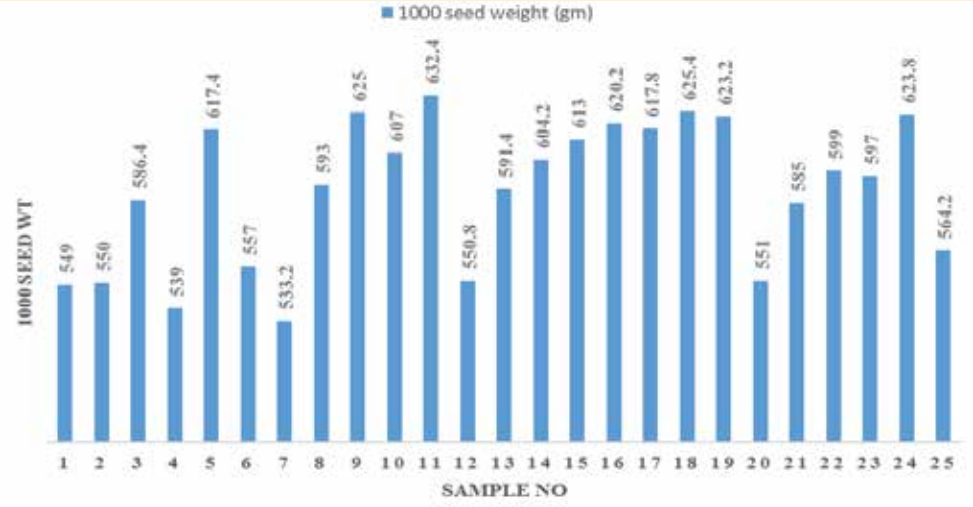

Fig. 4. Variation in thousand seed weight of 25 *Jatropha curcas* genotypes.

Moisture content of seed

The percent moisture content of 25 samples ranged from 18.5 to 52.5% (Fig 5). Among them, sample no 14 contained the highest moisture percentage (52.5). On the other hand, sample no 2 contained the lowest moisture content (18.5). Harvesting time, stage, storage and drying were responsible for the moisture content of seed. The average moisture content was 34.81% which was higher than the value that was observed by Usman *et al.* (2009) and the value was 33.70%.

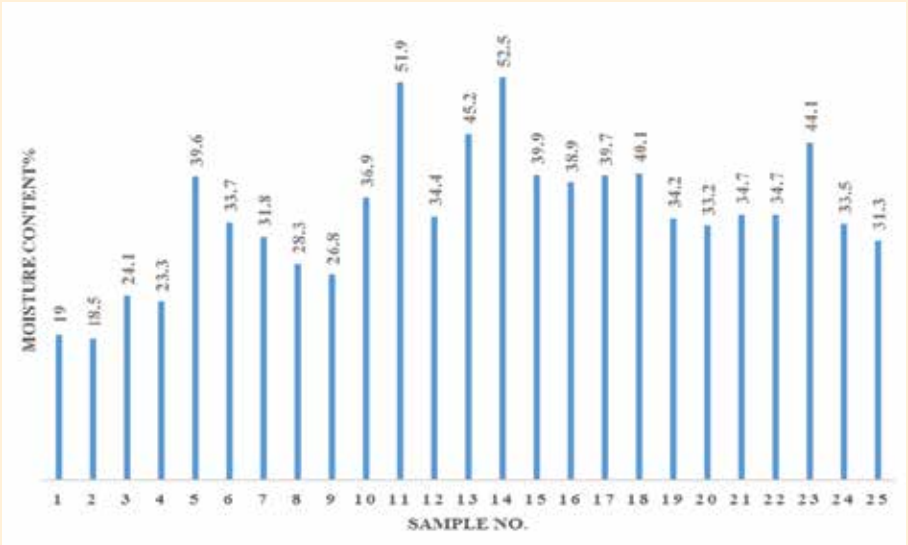

Fig 5. Variation in % moisture content among the seeds of 25 *Jatropha curcas* genotypes.

De-shelling

The percent husk of 25 samples ranged from 34.42 to 42.27%. Among the sample, the highest husk % value (42.27) was observed by the sample no 20 and the lowest husk% value (34.42) was observed by the sample no 2 (Fig 6). The average value of husk % was 39.31% and the percent kernel of 25 samples ranged from 57.72 to 65.57%. The highest kernel % was found in the sample no 3 (65.57) and the lowest kernel % (57.72) was found in the sample no 20. The average value for kernel % was 60.66%. The seed contains about 40-42% husk and 58-60% kernels (Mohammed, 2006).

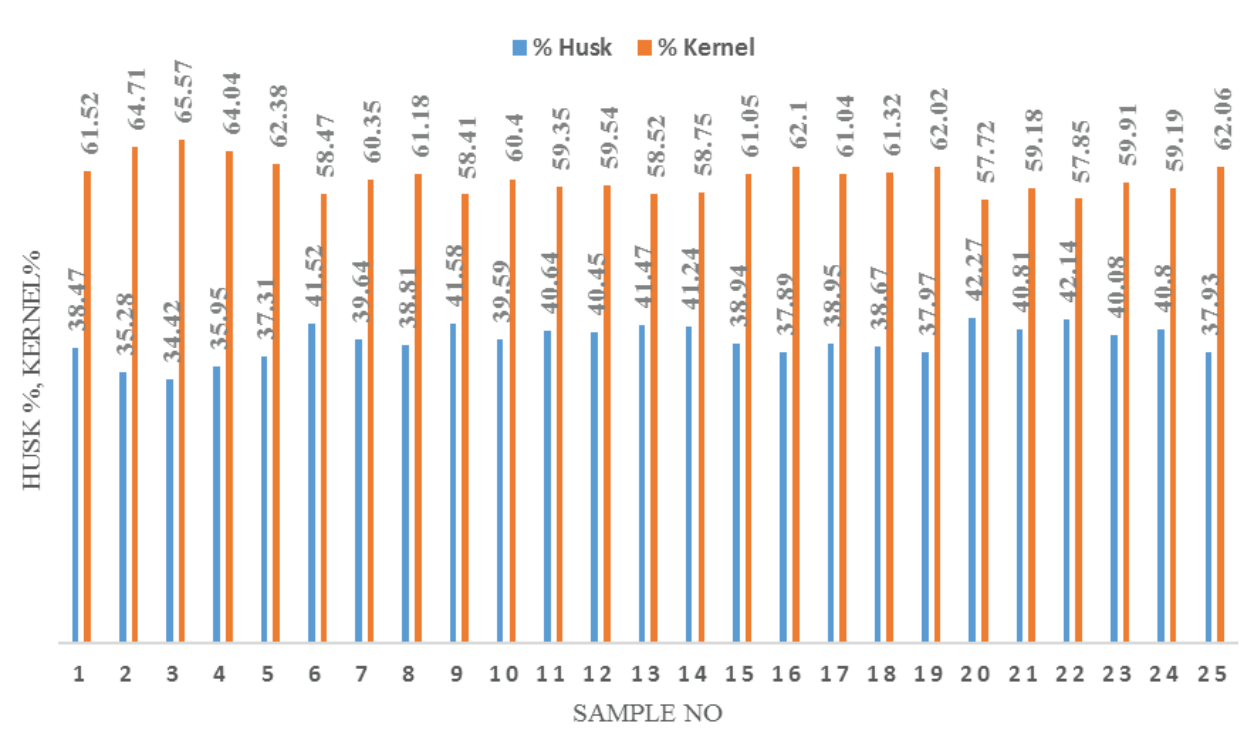

Fig. 6. Variation in husk%, kernel% among studied *Jatropha curcas* genotypes.

Oil percentage

Percent oil of 25 samples range from 29.15 to 62.22% (Fig 7). Among 25 samples, first 4 samples were made extracted by absorption method and rest were by soxhlet method. In case of absorption method, there was a chance to loss hexane, the solvent. But, in the soxhlet method there was no chance to loss of hexane. Higher the solvent, higher the extraction rate. Therefore, extraction rate was low for the first four samples compare to others. The highest oil% (62.22) was found in the sample no 18 and the lowest oil% (29.15) in the sample no 4. The average amount of oil extracted from samples was 52.90%. The fluctuation of output depends on the amount of hexane, extraction time, temperature etc. The average oil yield found by soxhlet method was higher than 30-40% reported by Lozano (2007) but slightly lower than the value 65-80% reported by Reinhard (2007).

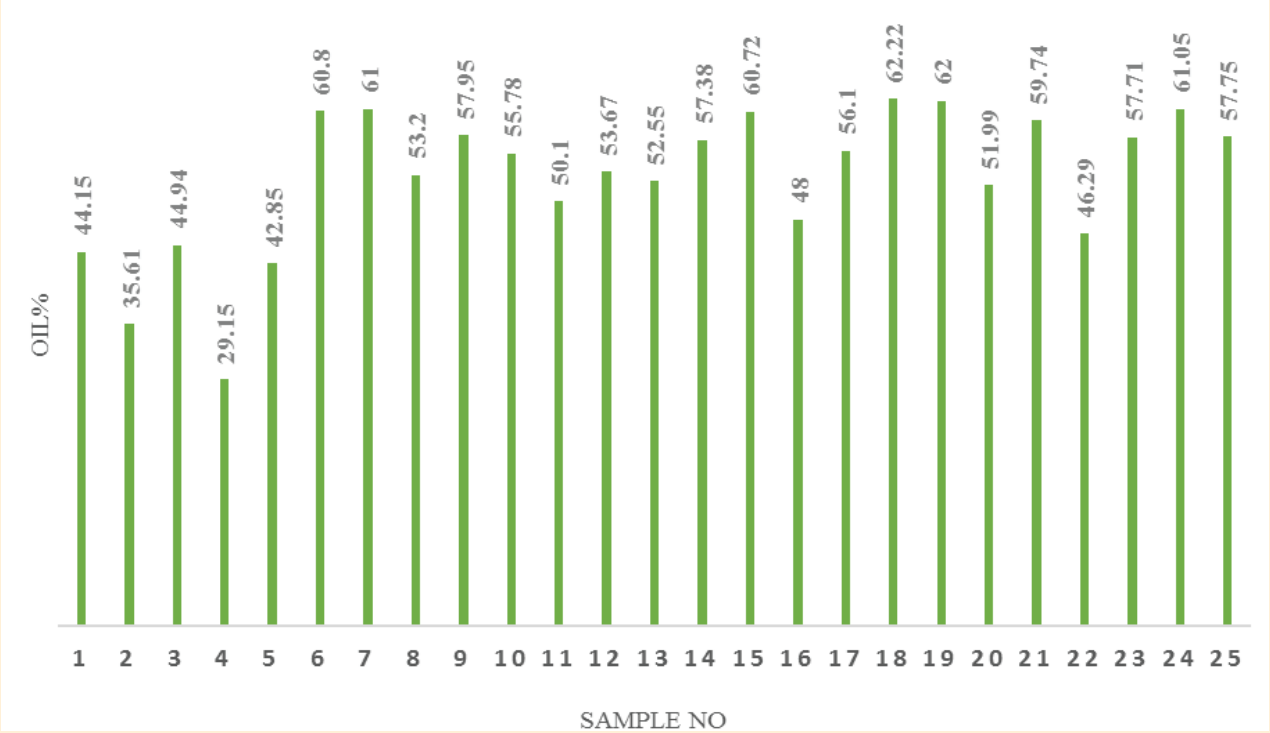

Fig. 7. Variation in oil% of Jatropha curcas genotypes.

Oil density

There was no significant differences for oil density of 25 samples which was ranged from 0.828 gm.cm^{-3} to 0.952 gm.cm^{-3} (Fig 8). The highest density (0.952 gm.cm^{-3}) was observed in the oil of sample no 22 and the lowest density (0.828 gm.cm^{-3}) in the oil of sample no 19. The average value in this regard was 0.903 gm.cm^{-3}. However, the value reported in this study was consistent with the report of Akbar *et al.* (2009).

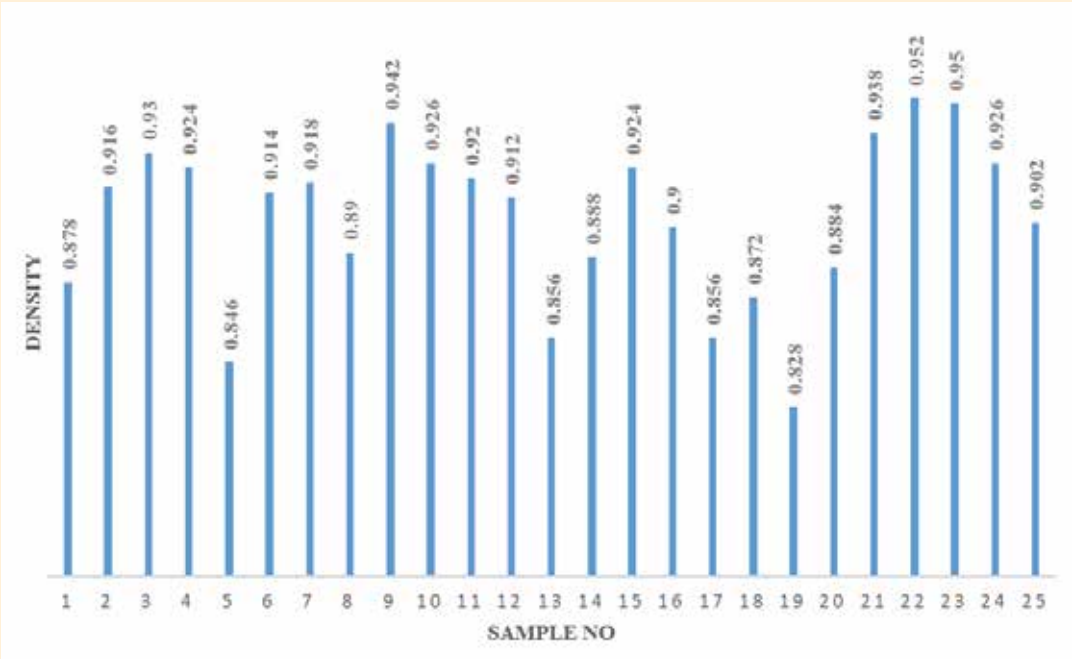

Fig. 8. Variation in oil density of *Jatropha curcas* genotypes.

% Moisture content of oil

Moisture content of seed oil ranged from 0.08 to 0.12 (Fig 9). No significant difference was observed among the seed oils. Seed oil of sample no 3, 5, 10, 11, 15, 19, 20 and 23 showed the highest value (0.12) and sample no 2, 6, 9, 16, 17, 24 and 25 showed the lowest value (0.08). Moisture is a chemical contaminant which is mixed with lubricating oil like, Jatropha seed oil, and is the major causes of most engine failure. The moisture content reported here was lower than 0.2%. The low moisture content of studied oil sample showed that they were of good quality and could not be easily subjected to contamination or rancidity (Belewu *et al.*, 2010). It is noteworthy that free fatty acids and moisture have significant effect on the Trans esterification of glyceride with alcohol using catalyst (Goodrum, 2002).

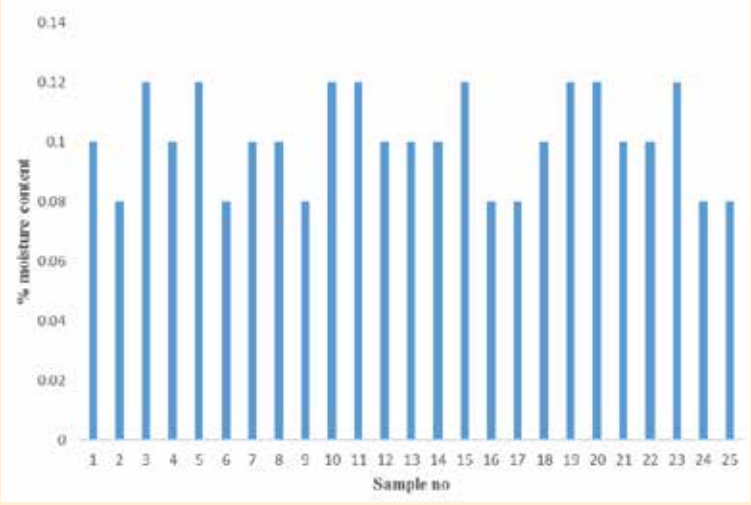

Fig. 9. Variation in moisture content (%) of seed oil of *Jatropha curcas* genotypes.

Chemical Properties (% free fatty acid, acid value, saponification value and iodine value)

*Acid valu***e**

The acid value of the samples ranged from 0.784 to 2.52 mg NaOH/g oil (Fig 10). The highest acid value (2.52mg NaOH/g oil) was observed in the seed oil of the sample no 19 and the lowest acid value (0.784 mg NaOH/g oil) in the seed oil of sample no 15. The average value in this regard was 1.58 mg NaOH/g oil. The acid value gives an indication of the quality of fatty acids in the oil/diesel. The acid value was higher in the sample no 19. This reflects the high fatty acid content of the oil.

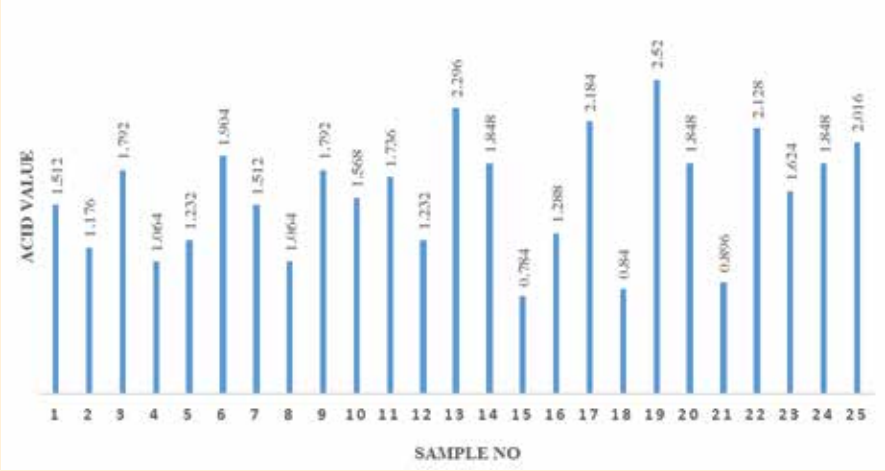

Fig. 10. Variation in acid value seed oil of *Jatropha curcas* genotypes.

Free fatty acids (FFA)

The FFA value of 25 samples ranged from 0.393 to 1.266 (Fig 11). Among them, the highest FFA value (1.266) was observed in the seed oil of the sample no 19 and the lowest FFA value (0.393) in the sample no 15 (Fig 12). On the other hand, the average FFA value of the samples was 0.79 which was very much consistent with the values reported by Akbar et al. (2009) and Tint and Mya (2009).

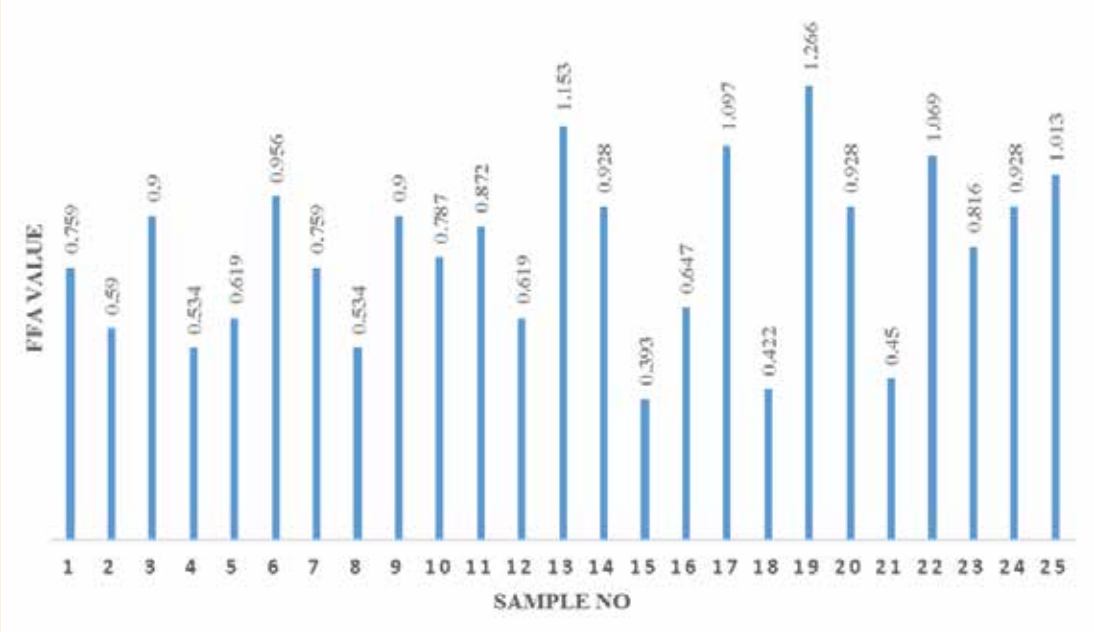

Fig. 11. Variation in free fatty acid content of seed oil of *Jatropha curcas* genotypes.

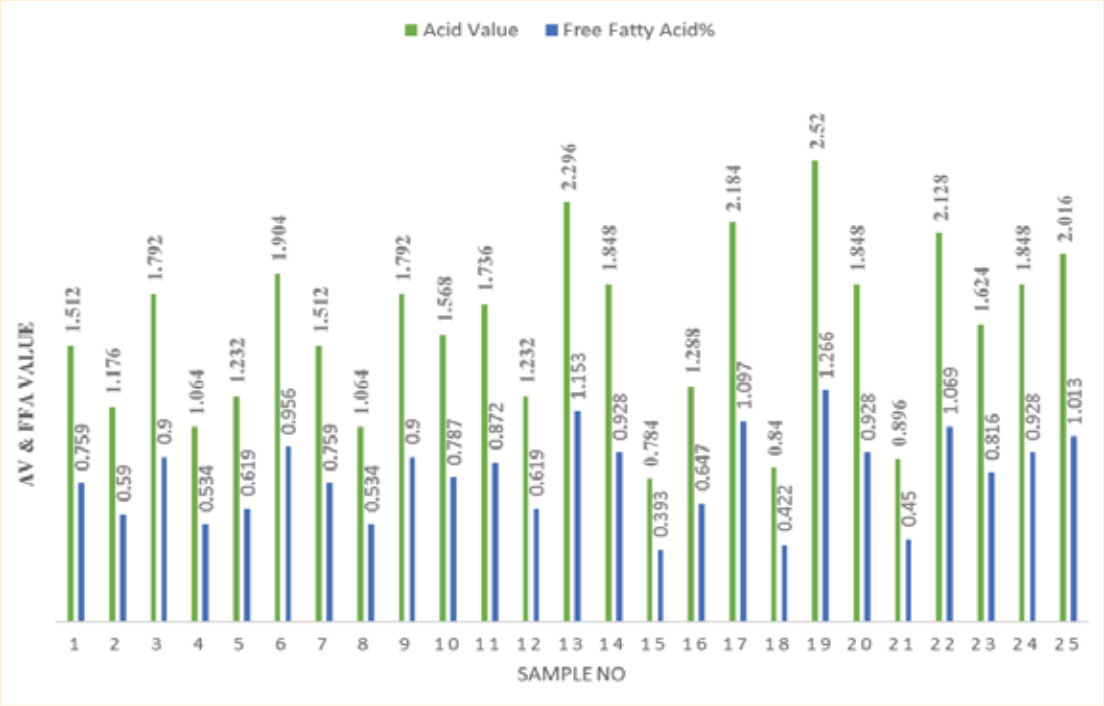

Fig. 12. Relationship between acid value and free fatty acid content of seed oil of *Jatropha curcas* genotypes.

Iodine value

The iodine value of the samples ranged from 89.53 to 121.28mg/g oil (Fig 13). The highest iodine value (121.28 mg/g oil) was observed in the seed oil of the sample no 18 and the lowest iodine value (89.53mg/g oil) in the sample no 8. The average iodine value (105.53mg/g oil) of the studied oil sample was very much consistent with the report of Knothe (2002) and the iodine value was 105.47mg/g oil.

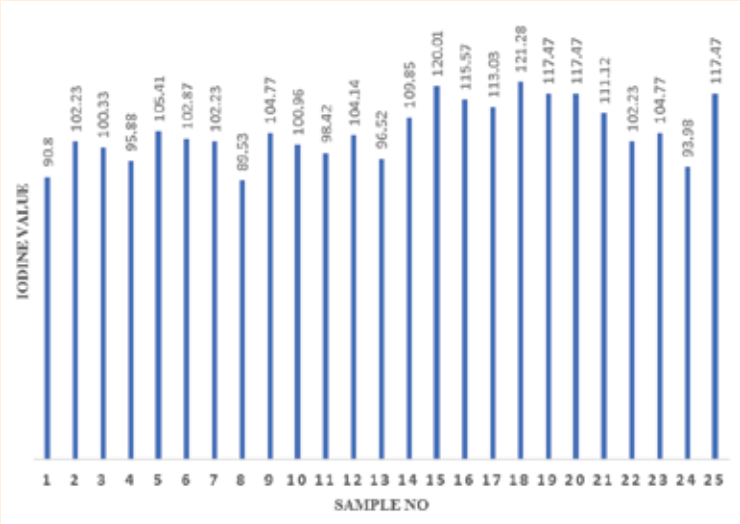

Fig. 13. Variation in iodine value of seed oil of *Jatropha curcas* genotypes.

Saponification number

The saponification value seed oil of the samples ranged from 173.91 to 201.25mg KOH/g oil (Fig 14). Among them the highest saponification value (201.25mg KOH/g oil) was found in the seed oil of sample no 5 and the lowest value (173.91mg KOH/g oil) in the sample no 15. The average value for saponification number was 185.96mg KOH/g oil which was higher than the value (182.45mg KOH/g oil) reported by Akbar *et al.* (2009). High saponification indicates the oil quality and presence of normal triglyceride which was very useful in the production of soap and shampoo.

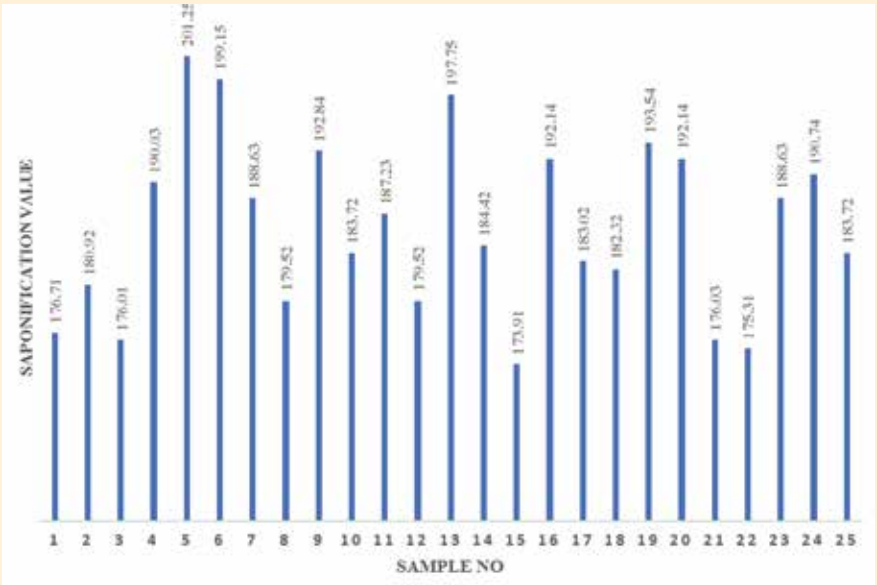

Fig. 14. Variation in saponification value of seed oil of *Jatropha curcas* genotypes.

Fuel properties

High heating value

The high heating value of the experimented samples ranged from 39.59 to 40.82MJ/kg (Fig 15). Among them, the highest value (40.82MJ/kg) was observed in the seed oil of the sample no 1 and the lowest (39.59MJ/kg) in the sample no 5. The average high heating value of the samples was 40.21MJ/kg which was in the range of values reported by Becker and Makkar (2008). The value reported in this study was consistent with the reports of Nevase *et al.* (2008).

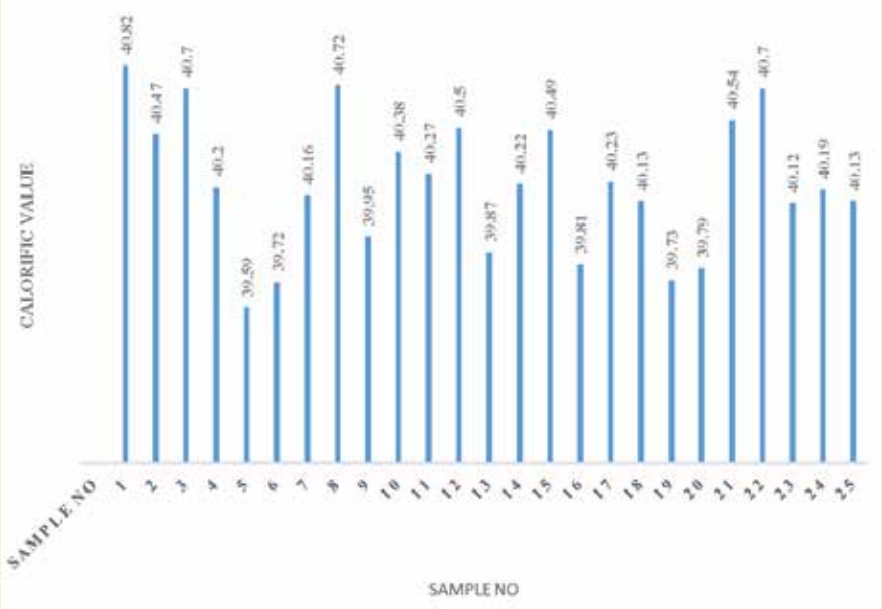

Fig. 15. Variation in high heating value of seed oil of *Jatropha curcas* genotypes.

Cetane number

The cetane number of the samples ranged from 48.07 to 56.75 (Fig 16). Among them, the highest cetane number (56.75) was observed in the sample no 1 and the lowest value (48.07) in the seed oil of the sample no 19. The average value of the samples was 51.94 which was lower than the value (57.29) reported by Bose, (2009).

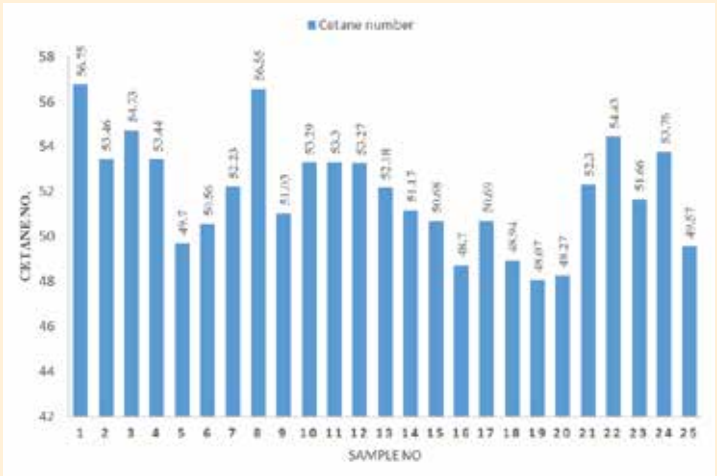

Fig. 16. Variation in cetane number of seed oil of *Jatropha curcas* genotypes.

Conclusion

Physiochemical evaluation of Jatropha seed oil which includes % oil recovery, density, % moisture content, acid value (AV), free fatty acid (FFA), iodine value (IV), saponification value (SV), high heating value (HHV) and cetane number (CN) showed its own special characteristics. The oils with good physiochemical properties will have potential to be biodiesel feedstock. The HHV, CN, SV and IV of Jatropha seed oil are the most important fuel properties to make seed oil suitable for biodiesel feedstock. The information generated in this study will be helpful for selecting good biodiesel feedstock and identifying genotype 1, 5, 15 and 18 as good varieties of *Jatropha curcas* for cultivation.

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Evolutionary role of abscisic acid for the accumulation of cellular protectants in *Physcomitrella patens*

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Abstract

The phytohormone Abscisic acid (ABA) has been involved in the regulation of many of the physiological and biochemical processes in land plants. During abiotic stress acclimation process, land plants undergo a lot of cellular changes including accumulation of several osmolytes, stress related proteins etc. which have been greatly induced by phytohormone ABA. In contrast to angiosperms, the mode of ABA- induced accumulation of cellular protectants in basal land plants remain more or less elusive. Hence, the present effort was made to find out the evolutionary role of ABA in the accumulation of cellular protectants in the representative of the ancestors of land plants; moss *Physcomitrella patens* which have been greatly used as the best model of basal land plants. Here, we subjected the protonemata tissue of *P. patens* to the modified BCD medium supplemented with exogenous ABA and found prominent growth inhibition of protonemal tissue indicating dramatic growth response to ABA. We observed significant enhancement of the accumulation of osmolytes; proline and low molecular weight soluble sugar in ABA treated protonemata suggesting the ancient role of ABA in the osmoprotectant accumulation. The higher accumulation of boiling soluble proteins in the protonemata by exogenous ABA denotes the potential role of this phytohormone for abiotic stress adaptation. Similarly the increased accumulation of chlorophyll at very minute concentration of ABA indicates very potential role of ABA in regulating photosynthetic pigments in *P. patens*. The ABA induced survivability of the protonemata under cellular desiccations indicates the ancient role of ABA for showing desiccation tolerance that might be triggered by the accumulation of cellular protectants. However, conservation of genes related to cellular protectants in *P. patens* indicates the interference of those genes during early stage of land plant evolution. Finally, we claim that the accumulation of cellular protectants by ABA had been acquired by the moss *P. patens* during early stages of land plant adaptation and evolution.

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Introduction

Abscisic acid, a ubiquitous phytohorme regulating many of the important physiological process in plants has been postulated as stress hormone because of having its tremendous roles during adaptation of land plants. Along with the regulation of essential physiological processes such as seed maturation, dormancy, stomatal closure etc. phytohormone ABA has vital roles in the modulation of varieties of cellular processes including accumulation of osmoprotectants, osmotic adjustment, scavenging of reactive oxygen species (ROS) and expression of stress responsive genes. During abiotic stress, endogenous level of ABA is increased which in turn enhances the expression of many stress related proteins **(**Shinozaki *et al.* 2007**).** Though, a lot of investigations have been reported about the significances of ABA in stress tolerance of angiosperms, very few observations were made in basal representative of land plants which is very much essential in respect of evolutionary issue of land plants. It has been postulated that among today's land plants, the plants with bryophytes type organization are the first land plants (Kenrick and Crane 1997; Wellman *et al*. 2003). The bryophytes comprised with mosses, liverworts and hornworts possess very simple morphology with alteration of generation and haploid dominating life cycle which makes them ideal for biochemical and molecular studies in response to abiotic stress. They don't have true vascular system to uptake and transport water, instead they can equilibrate rapidly by adjusting surrounding water potential and become fully hydrated without significant damage. This type of unique desiccation tolerance attributes of bryophytes is very similar to the ancestral traits of land plants which have been lost from the vascular plants during evolution (Oliver *et al*. 2000; 2005). Among the bryophytes, moss *Physcomitrella patens* has been evolved as a stress-tolerant model for functional genomics and widely used in molecular and evolutionary researches (Decker *et al*. 2006; Rensing *et al*. 2008). Though, several investigations in the last couple of years indicated that likewise angiosperms, moss *P. patens* engages ABA to regulate osmotic and cold stress acclimation (Minami *et al.* 2003; Khandelwal *et al.* 2010; Takezawa *et al.* 2015), the role of this phytohormone in response to accumulation of cellular protectants is still unknown.

One of the most important cellular protectant in angiosperms is proline, accumulation of which has been considered as a great marker to observe the plant response against desiccation stress. A lot of efforts support the enhancement of proline in plant under different abiotic stresses. For instance, osmotic and cold stresses induce proline accumulation by increased expression of proline biosynthesis genes (Delauney and Verma 1990; Hu *et al*. 1992; Verbruggen *et al*. 1993; Savoure A *et al*.1995; Yoshiba *et al*. 1995). Since, several investigations reported the role of ABA in proline accumulation during abiotic stresses (Ober and Sharp 1994) or non-stressed conditions (Chou *et al*. 1991; Finkelstein and Somerville 1990), there might be a link between ABA and proline accumulation. However, another observation with Arabidopsis resulted that the expression of proline biosynthesis genes is ABA-dependent indicating the role of ABA in proline biosynthesis in angiosperms (Strizhov *et al*. 1997). Besides proline, accumulation of soluble sugar and boiling soluble proteins like Late Embryogenesis Abundant (LEA), Heat Shock Proteins (HSPs) are considered as the cellular protectants which maintain osmotic adjustment, membrane integrity and cellular homeostasis during abiotic stress. As sugar is produced by the process of photosynthesis, the alteration of photosynthetic pigment; chlorophyll, carotenoids by ABA is very crucial during stress acclimation of land plants. A lot of investigations reported the enhanced accumulation of soluble sugar, stress related proteins and photosynthetic pigments by exogenous ABA in angiosperms (Close *et al*. 1989; Haisel *et al*. 2006; Liu *et al* 2013) indicating that ABA-induced accumulation of cellular protectants are more or less clarified in this type of plants.

In contrast to angiosperms, accumulation of cellular protectants by ABA is yet to be clarified in basal land plants. Hence, the present investigation was taken into consideration to observe the accumulation of cellular protectants in one of the most important representative of extant land plants; moss P. patens by exogenous ABA. The outcomes should find out the evolutionary conserved mechanisms for the accumulation of cellular protectants that were achieved during early stages of land plant evolution.

Materials and methods

Culture of P. patens

The protonemata of moss P. patens was cultured aseptically in the laboratory of Crop Botany, BSMRAU using modified BCD medium (1 mM $MgSO_4$, 10 mM KNO_3, 45 mM $FeSO_4$, 1.8 mM KH_2PO_4, pH 6.5) containing 0.5% (w/v) Glucose and 0.75% (w/v) agar (Ashton *et al*., 1979). The gametophore culture was maintained by standard conditions (23°C-24^{0}C) with a light cycle of 16 h of light/8 h of darkness and a light intensity of 55 µmol s^{-1} m^{-2} (Bhyan *et al*. 2012).

Growth and image analysis

The protonemata was provided to grow in ABA treated or non-treated BCD medium to observe growth inhibition by ABA or not. The comparison of the growth of the protonematawas observed with the help of advanced microscopy. The protonemal colony was observed under microscope and the area of the photographs was analyzed by ImageJ program.

Proline accumulation assay

Accumulation of free proline content in ABA treated or non-treated protonemata was determined using sulfosalicylic acid by following the method of Bates *et al* (1973). The tissue was homogenized with 3% sulfosalicylic acid and supposed to centrifuge at 3000g for 20 min. The supernatant was treated with acetic acid and acid ninhydrin and boiled for 1 h. The absorbance at 520nm was taken using UV-visible spectrophotometer. Proline content of the tissue was determined as µmol/g FW.

Sugar accumulation analysis and determination of chlorophyll content

Tissue of protonemata was weighed, frozen and crushed in liquid nitrogen using a mortar and pestle for soluble sugar extraction. Soluble sugar was extracted using 80% (v/v) ethanol, and insoluble materials was removed by centrifugation at 14000g for 10 min at 4˚C. After removal of water-insoluble material by centrifugation, sugar in the supernatants was quantified by the anthrone-sulfuric acid assay using glucose as a standard (Yemm and Willis 1954). For the determination of chlorophyll content in 80% acetone was used for making extraction of ABA treated and non-treated protonemata tissue. Chlorophyll content of the sample was determined with the help of spectrophotometer and the calculation was made based on Arnon (1949).

Analysis of boiling soluble protein

For total soluble protein preparation, tissue was homogenized in a solution containing 50 mMTris-Cl (pH 7.5), 100 mMNaCl, 1 mM EDTA, 1 mM DTT and 1 mM PMSF on ice, and after centrifugation at 14,000 g for 10 min at 4˚C, the supernatants was collected. After adjusting to equal concentrations, total soluble proteins was boiled for 1 min, and insoluble materials were removed by centrifugation again. The collected boiling-soluble proteins were electrophoresed by SDS-polyacrylamide gels, and the gel was stained with Coomassie Brilliant Blue (CBB) to detect the bands by following the method used by Bhyan *et al.* (2012).

ABA-induced desiccation tolerance test

To observe the performances of ABA in tissue survivability under desiccation stress, the protonemata tissue pretreated or non-treated with ABA for 1 d were subjected to the different desiccated conditions such as 0.2M sucrose, 0.5M mannitol, 100mM NaCl, and 10% Polyethylene Glycol (PEG)for 5 d. After 5 d, the electrolyte leakage of the damaged tissue was measured using a conductivity meter by following the method used by Takezawa *et al*. 2015.

Phylogenetic analysis

To find out the genes encoding enzymes for the biosynthesis of cellular protectants like proline and sugar and LEA proteins are conserved in P. patens, the Arabidopsis genes (proline biosynthesis AtP5CS1 (AT2G39800.1), LEA like gene; AT1G03120.1,sucrose phosphatase gene, At1G51420.1) were set for blast search using the website of Phytozome v12.1. We searched those genes in all the representative of land plants such as bryophytes; moss P. patens and liverwort M. polymorpha, lycophyte; S. moellendorffii and flowering plants A. thaliana and O. sativa. Deduced amino acid sequences of the target genes were aligned and phylogenetic trees were made accordingly using MEGA 5.05 Program by following neighbor-joining method (Saitou N. and Nei 1987).

Statistical design

Completely Randomized Design (CRD) and simple student t-test were used for comparison of growth and other stress related phenomena.

Results and discussion

Phytohormone ABA acts as a very crucial mediator of abiotic stress signaling by showing its tremendous roles in the modulation of various physiological, biochemical and molecular processes. After getting responses of abiotic stresses, the ABA biosynthesis process is enhanced in plant which transfer the message to the DNA of nucleus through different ABA receptors and messengers for the expression of target genes for getting tolerance to the specific stress. In angiosperms, during abiotic stresses, ABA triggers to promote the accumulation of cellular protectants such as osmolytes, boiling soluble proteins like LEA to maintain membrane stability and protection at cellular levels. In contrast to angiosperms, the role of ABA in the protection at cellular level in basal land plants is yet to be clarified. Hence, to clarify that roles and find out the evolutionary mechanisms, the most important basal representative; moss P. patens was supposed to the exogenous ABA and morphological, physiological and biochemical data were recorded and discussed accordingly.

Inhibition of protonemal growth by ABA

The moss P. patens has two important phases of its life cycle; sporophytic stage and gametophytic stage. The haploid dominating life cycle of P. patens is important feature for molecular and cellular studies. The protonema tissue of haploid phase has been widely used for molecular, physiological and evolutionary studies (Bhyan *et al*. 2012; Takezawa *et al*. 2015). Though growth inhibition of protonemata with the formation of brood cell was reported previously by maintaining concentration of ABA 10 µM and 100 µM (Bhyan *et al*. 2012), we supposed the tissue to the exogenous ABA and made qualitative and quantitative analysis of growth inhibition. In our study, the results of development of ABA-induced brood cells in the protonemal tissue by microscopic observation and reduction of colony size by visual observation showed consistent data to the above observation (Fig. 1, A-B). Besides, we analyzed the colony area inhibited by ABA using Image J program and found significant difference of growth inhibition by the exogenous ABA (Fig. 1-C). The inhibition of

protonemal growth and brood cell formation was also reported in the moss Funariahygrometrica (Schnepf and Reinhard, 1997). The brood cell formation during growth inhibition of the protonema is very crucial for showing drought tolerance and maintaining prolong dormancy (Bopp and Werner 1993). Therefore, like dormancy, growth inhibition by ABA is conserved character throughout the plant species and the character was evolved during early stage of land plant evolution.

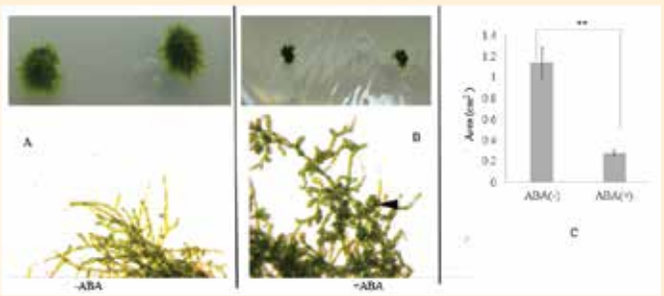

Fig. 1. Growth performance of the protonemata of moss *P. patens*. No growth inhibition of the protonemal colony under control condition (A, above). Protonemal growth without the formation of brood cell under microscope (A, below). Profound growth inhibition of the colony was found when protonemata was treated with 10 µM ABA (B, above) and formation of brood cells indicated by arrowhead (B, below) induced by exogenous 10 µM ABA. Significant ABA-induced growth inhibition was measured by ImageJ program (C). Error bars indicate the standard deviation ($n=3$). Asterisks indicate significant difference ($P<0.01$; t-test).**

Accumulation of proline and soluble sugar by exogenous ABA

As the compatible osmolytes, proline plays a crucial role during acclimation to abiotic stresses by maintaining water potential and osmotic adjustment. As well, this osmoprotectant regulates a various of biochemical and cellular processes likestabilization of proteins, stimulation of antioxidant enzymes, scavenging of reactive oxygen species (ROS) and making balance in intracellular redox homeostasis (Miller *et al*. 2009, Alves *et al*. 2011). Although, it has been reported to accumulate proline in plant under various abiotic stresses like drought, salt, low temperature, heavy metal, UV ray etc. (reviewed by Hayat *et al* 2012), very little investigations have been made so far on the relationship of this cellular protector with stress hormone like ABA. The ABA-induced proline accumulation was reported in barley leaf a few decades ago (Stewart CR 1980). The ABA and salt-induced expression of proline biosynthesis gene; P5CS1 was reported in Arabidopsis (Strizhov *et al*. 1997; Yoshiba *et al*. 1999). Recently light dependent induction of proline biosynthesis by ABA and salt has been reported in Arabidopsis (Abraham *et al*. 2003). Although having very little investigations of ABA-induced proline accumulation, no efforts yet to be made in basal land plants and quest for evolutionary mechanism is time demanding also. In this efforts, we treated the protonemata of P. patens with different concentration of ABA and found significant enhancement of accumulation of proline in the protonemata (Fig. 2). The response was very specific to ABA, as increasing concentration of ABA (10µM) showed higher accumulation than that of 1 µM ABA (Fig). Along with the above findings in angiosperms, our results in P. patens suggest the conserved role of phytohormone ABA in the accumulation of very important cellular protectant; proline.

Likewise proline, low molecular weight soluble sugar is also vital cellular protectant which has been reported to induce in plant by a wide array of abiotic stresses like drought, salt and cold (Prado *et al*. 2000). The ABA- induced accumulation of soluble sugar such as sucrose and fructose has been reported in wheat cultivar very recently (Liu *et al*. 2013). In contrast to angiosperms, very little information is available in basal land plants. Although, the accumulation of soluble sugar was reported in moss P. patens in responses to ABA, cold and drought (Nagao *et al*. 2005), further

investigations are needed to clarify more. We also treated protonemata of mossP. patens to the ABA and found significant enhancement of low molecular weight soluble sugar in the protonemata and findings are very consistent to the Nagao *et al*. (2005) (Fig. 3). With their results, our findings regarding to ABA-induced proline and sugar accumulation in the representative of early land plants suggest the evolutionary protective role of phytohormone ABA at very adverse environmental condition.

ABA-induced boiling soluble protein accumulation

Some ABA and drought stress-induced proteins such as heat shock proteins (HSPs), LEA proteins are hydrophilic in nature and remain stable after boiling (Close *et al*. 1989). This kind of proteins is produced naturally in plants during maturation of seed and enhanced by phytohormone ABA.It was proposed that along with sugar, this kind of stress responsive proteins are responsible for cellular protection by maintaining membrane integrity and osmotic adjustment (Close *et al*. 1989). As compared to angiosperms, very little information of ABA-induced boiling soluble protein accumulation in basal land plant were made so far. Although Bhyan *et al.* (2012) reported accumulation of boiling soluble proteins in wild type P. patensprotenemata, more efforts are needed to investigate. In the present efforts, P. patensprotonemata were subjected to the different

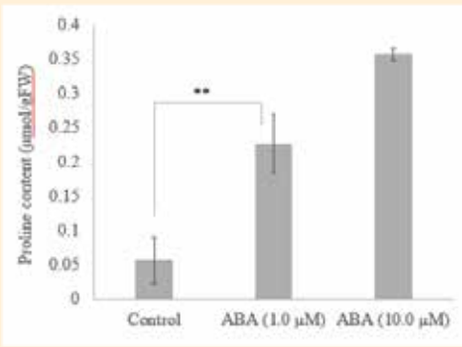

Fig. 2. Accumulation of proline induced by phytohormone ABA in the protonema of moss P. patens. Error bars indicate the standard deviation (n =3). Asterisks indicate significant difference (P< 0.01; t-test).**

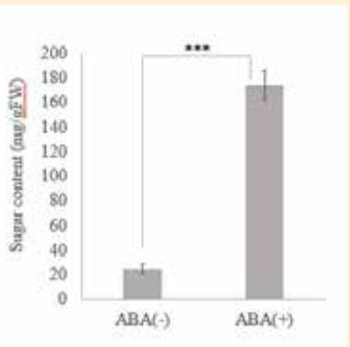

Fig. 3. Accumulation of soluble sugar induced by phytohormone ABA in the protonema of moss P. patens. Error bars indicate the standard deviation (n=3). Asterisks indicate significant difference (*P<0.001; t-test).**

concentration (0, 0.05,0.1,1.0 and 10μM) of ABA for 1 d that resulted clear accumulation of boiling soluble protein in the ABA treated protonemata (Fig. 4). The increasing concertation of ABA enhanced more accumulation suggesting very specific response to phytohormone ABA (Fig. 4). The results are very consistent to the results of Bhyan *et al.* (2012), where 10 μM ABA produced clear band than others. Along with this, our results suggest the conserved role of ABA-induced boiling soluble proteins in land plant.

Regulation of chlorophyll synthesis by ABA

Since sugar is produced by photosynthesis, the regulation of photosynthetic pigments is also essential physiological process to manage abiotic stress. Therefore during adaptation to abiotic stresses, maintaining photosynthetic pigments is a big challenge for the land plants. Another important thing is that photosynthetic pigments have very strong correlation with phytohormone ABA which is produced endogenously from the precursor carotene. ABA-induced chlorophyll and carotenoids accumulation was reported in many angiospermic plants (Haisel *et al*. 2006). In contrast, no report has been made so far in the representative of basal land plant. Therefore, along

with proline, sugar and proteins, we determined the role of ABA in the chlorophyll content of the protonemata. The results indicated the dramatic increase of chlorophyll a (chla), chlorophyll b (Chlb) and total chlorophyll (total Chl) by 1d 10µM ABA treatment (Fig. 5). The ABA (100µM) reduced chlorophyll content of the protonema indicates the increased concentration mimic to the abiotic stress what is responsible for damaging photosynthetic tissue. However, the increasing trend of photosynthetic pigments at very low concentration of ABA in the P. patensprotonemata suggest the conserved role of endogenous ABA in maintaining photosynthetic pigments in plant.

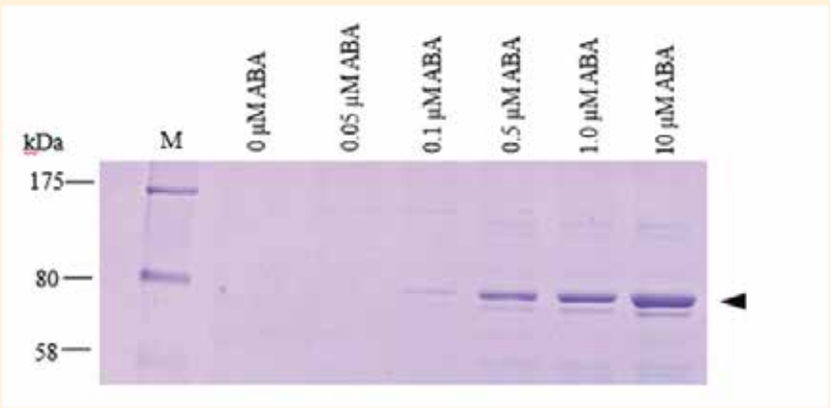

Fig. 4. SDS-PAGE and CBB staining of boiling soluble proteins induced in the protonemata of Moss P. patens treated by 1 d different concentration of ABA. Arrow indicates the accumulation of boiling soluble proteins at different concentration. M indicates marker standard and kDa indicates size of the protein as Kilodalton.

ABA-induced tissue survivability under desiccation stress

The phytohormone ABA is very key component for abiotic stress acclimation of land plants. By getting the stimuli of abiotic stresses, the endogenous level of ABA is increased in plant which in turn triggers the expression of many stress related genes (Shinozaki *et al*. 2007). During desiccation, ABA plays a pivotal roles in cellular protection by enhancing accumulation of compatible solutes

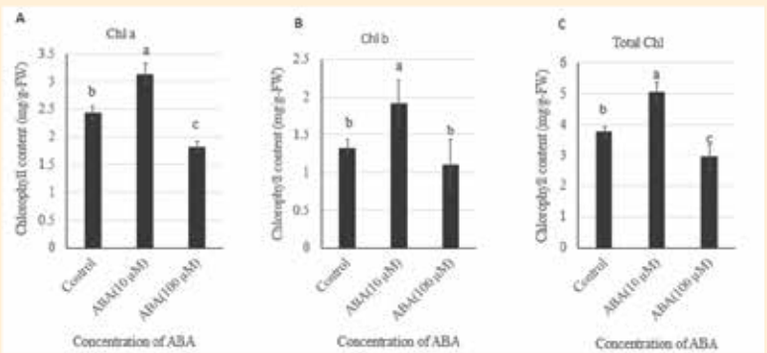

Fig. 5. Variation of chlorophyll content (chlorophyll a; Chl a, Chlorophyll b; Chl b and total chlorophyll; total Chl of the P. patens protonemata by exogenous application of phytohormone ABA. Error bars indicate the standard deviation (n=3). Difference letter on the bar indicates significant difference at 5% level of significance (*P<0.05)

and increased accumulation of stress related genes like LEA, HSPS etc. ABA-induced freezing tolerance was reported in P. patens by Minami *et al*. 2003. We subjected the protonemata tissue of P. patens to a variety of cellular desiccation such as 0.5M mannitol, 0.2M Sucrose, 100mM NaCl and 10% PEG to observe the protective roles of ABA. After 5d normal culture, the protonemata were supposed to 1d ABA treatment and then the tissue were subjected to different desiccated conditions

mentioned above. We found significant reduction of electrolyte leakage of the protonemata tissue pre-treated with 1 d 10µM ABA (Fig. 6). The results are very much consistent to the results of the ABA induced cold tolerance of P.patens (Minami *et al*. 2003). With this result, we claim that the mechanisms of ABA-induced stress tolerance arose very early of land plant evolution.

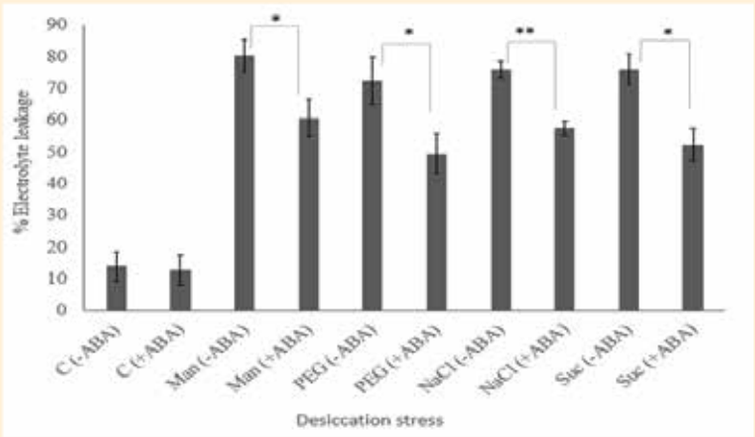

Fig. 6. Role of ABA in tissue survivability during desiccation stress acclimation of moss P.patens. After 1 d ABA treatment or not, the protonemata was subjected to either normal medium (control;C) or different desiccated conditions provided with 0.5 M Mannitol (Man), 10% Polyethylene Glycol (PEG), 100mM Sodium Chloride (NaCl) and 0.2 M Sucrose (Suc) for 5 d and % electrolyte leakage of the damaged tissue were determined by electrical conductivity (EC) meter. Error bars indicate the standard deviation (n=3). Asterisks indicate significant difference. (*P<0.05, **P<0.01;t-test)

Evolutionary relationship of genes related to cellular protectants

Phytohormone ABA is very ubiquitous to all organisms and signaling machinery of that are more or less common to all sorts of plants starting from green algae to today's angiosperms. However, ABA-induced signaling machinery mediated by ABA receptors like PYR/PYL/RCAR, central signaling component SnRK2 and downstream elements like AREB/ABF is conserved and well documented in land plant. Although ABA-induced accumulation of proline, LEA protein and soluble sugar has been reported in all sort of land plants of angiosperms, the accumulation of proline by ABA and responsible biosynthesis genes of that have not been reported in the basal representative of land plant. Moreover, investigations are needed to clarify evolutionary role of LEA protein and soluble sugar in those plants.As we had the limitations to find gene expression data, we tried to see the conservationof genes related to cellular protectants by phylogenetic analysis.We made a phylogenetic tree showing conservation of proline biosynthesis gene P5CS1, LEA like gene and sugar biosynthesis gene including basal land plans P. patens, M. polymorpha, LycophyteS. moellendorffii, angiosperms A. thaliana, O. sativa to observe the conservation of those genes in the ancestors of land plant. The phylogenetic analysis documented that the clear presence of proline biosynthesisgene (Fig. 7-A), ABA responsive LEA like gene (Fig. 7-B) and sugar biosynthesis gene (Fig. 7-C) in the earliest representative of land plants such as Moss P. patens and Liverworts M. polymorpha. The results of phylogenetic analysis is consistent to the data associated with the proline, sugar and LEA protein accumulation in P. patens indicating the machinery for the accumulation of cellular protectants were developed during evolution of land plants and the characters are conserved throughout the land plants.

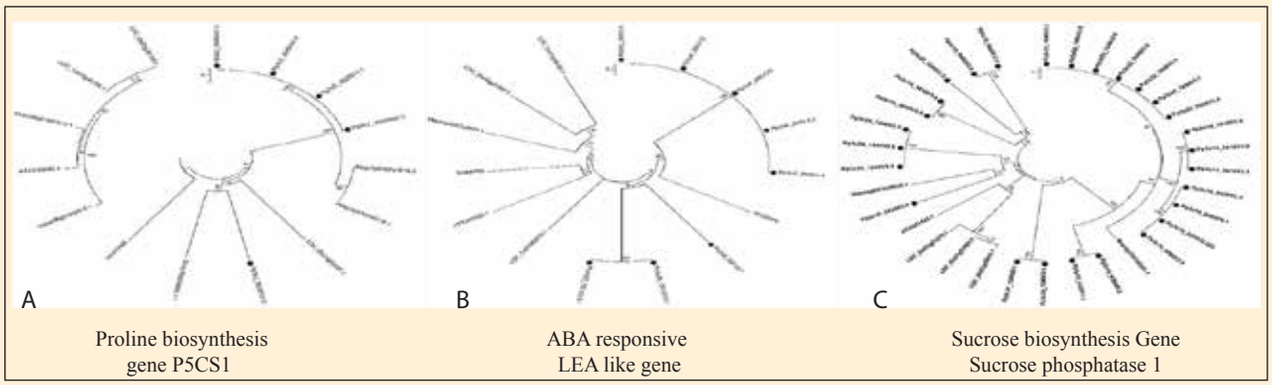

Fig. 7. (A-C). Phyllogenetic analysis of proline biosynthesis gene AtP5CS1 (AT2G39800.1),ABA responsive LEA like gene (AT1G03120.1) and Sucrose biosynthesis sucrose phosphatase gene (At1G51420.1) including all the representative of plants such as bryophytes; moss P. patens and liverwort M. polymorpha, lycophyte; S. moellendorffii and flowering plants rice; O. sativa. Deduced amino acid sequences of the target genes were aligned and phylogenetic trees were made accordingly using MEGA 5.05 Program by following neighbor-joining method (Saitou N. and Nei, 1987). Black circle indicate the presence of candidate genes in the representative of earliest land plant moss P. patens.

Conclusions

Although, the mechanism of ABA-induced stress acclimation process are more or less clarified in flowering plants, investigations are needed in the earliest representative of land plants to resolve the evolutionary questions. Therefore, we set our efforts on the most important model bryophytes *P. patens* to find out the evolutionary role of ABA in accumulation of cellular protectants. We supposed the protonemata tissue of *P. patens* to the ABA treated and not-treated media and analyzed growth performance, accumulation of cellular protectants such as proline, soluble sugar and boiling soluble protein, chlorophyll synthesis and tissue survivability induced by ABA. We also made phylogenetic trees regarding to the genes related to cellular protectants to find out their possible conservation in all sort of land plants. From the overall results and discussion the things can be summarized that the *P. patens* tissue perceive the stimuli of ABA and shows tremendous growth inhibition upon exogenous ABA. The ABA-induced accumulation of proline, soluble sugar and LEA proteins are conserved in land plants. ABA-induced enhancement of chlorophyll synthesis and tissue survivability under desiccation stress has been observed in *P. patens* seeming that those characters are conserved in land plants.Finally it can be concluded that the occurrence of cellular protectants are very much related tophytohormone ABA and the mechanisms were developed during early stages of land plant adaptation and evolution. Further efforts comprising the expression of transcripts encoding cellular protectants by ABA in the basal land plants might support our findings and clarify the exact role of ABA in cellular protection during evolution of land plants. However, research efforts on ABA with another model bryophyte *M. polymorpha*is needed to pull the trigger of evolutionary questions related to ABA-induced stress tolerance mechanisms.

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Synthesis, Physicochemical and Evaluation of Antimicrobial & Anticancer Activities of Some D-Glucose Derivatives (2017-2018)

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Abstract

Two series of **D**-glucose derivatives were synthesized using the direct acylation method. Firstly, regioselective octanoylation of **D**-glucose molecule i.e., methyl 4, 6-*O*-benzylidene-α-**D**-glucopyranoside using the direct acylation method gave the corresponding 2-*O*-octanoyl derivative (**2**) in fair yield. A number of 3-*O*-acyl derivatives of this 2-*O*-substitution product using a wide variety of acylating agents were also prepared in order to obtain newer compounds of synthetic and biological importance (scheme-1). Secondly, another **D**-glucose molecule i.e., methyl β-**D**-galactopyranoside (**15**) were performed pivaloylation using the same direct acylation method and afforded the methyl 6-*O*-acyl β-**D**-galactopyranoside in good yields. In order to obtain newer products, the 6-*O*-acyl β-**D**-galactopyranoside derivative was further transformed to a series of 2,3,4-tri-*O*-acyl β-**D**-galactopyranoside derivatives (scheme-2). The structures of the compounds were determined by their FTIR, ^{1}H-NMR spectral data and elemental analyses. These **D**-glucose derivatives were evaluated for *in vitro* antibacterial and antifungal activities against a number of human pathogenic bacteria and plant pathogenic fungi. The study revealed that most of the tested compounds exhibited moderate to good antibacterial and antifungal activities. Encouragingly, a number of test compounds showed better antimicrobial activity compare with the standard antibiotics employed. The anticancer activity was performed by the MTT assay and found that the effect of compound (palmitoyl derivative; scheme-2) *in vitro* on EAC cells. When the concentration decreased gradually, the inhibitory effect also reduced and finally reached 2.11% at 6.25 µg/ml of the compound, palmitoyl derivative. Hence, these **D**-glucose derivatives may be able to be used to discover antimicrobial and anticancer agents that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: **D**-glucose, Synthesis, Spectroscopy, Antibacterial, Antifungal, Anticancer

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Introduction

The study of carbohydrates is one of the exciting fields of organic chemistry. Selective acylation of monosaccharide derivatives is of growing importance in the field of carbohydrate chemistry because of its usefulness for the synthesis of biologically active products (Andry *et al*., 1982; Tsuda and Haque 1983). Carbohydrate, especially acylated monosaccharide is very important due to their effective biological activity (Ishji *et al*., 1980). A number of methods for selective acylation of carbohydrates have so far been developed and employed (Willard *et al*., 1964; Wagner *et al*., 1974; Ogawa and Matsui 1981; Tsuda *et a*l., 1983). Most of the methods are based on either the blocking-deblocking techniques or employ direct acylation resulting in the formation of mixture of products (Wagner *et al*., 1974; Ogawa and Matsui 1981; Tsuda *et a*l., 1983). Fortunately, the direct method was found to be very efficient and encouraging (Kabir *et al*., 2005) for selective acylation of carbohydrates.

From literature survey revealed that a large number of biologically active compounds possess aromatic and heteroaromatic nucleus and acyl substituents (Ichinari *et al*., 1988; Gawande and Shingare 1987). It is also known that, if an active nucleus is linked to another nucleus, the resulting molecule may possess greater potential for biological activity (Gupta *et al*., 1997). The benzene and substituted benzene nuclei play important role as common denominator of various biological activities (Singh *et al*., 1990). Results of an ongoing research work on selective acylation of carbohydrates (Kawsar *et al*., 2013a; Kawsar *et al*., 2014a) and nucleosides (Kabir *et al*., 1998; Kabir *et al*., 2003) and also evaluation of antimicrobial activities reveal that in many cases the combination of two or more aromatic or heteroaromatic nuclei (Gupta *et al*., 1997). It is also found that nitrogen, sulfur and halogen containing substitution products showed marked antimicrobial activities i.e., enhance the biological activity of the parent compound (Kawsar *et al*., 20012a, 2013b, 2014b; Kabir *et al*., 2004, 2008, 2009).

From our previous works we also observed that in many cases the combination of two or more acyl substituents in a single molecular framework enhances the biological profile many fold than their parent nuclei (Kawsar *et al*., 20012b, 2014b). Encouraged by our own findings and also literature reports, we synthesized a series of **D**-glucose derivatives (schemes-1 & 2) deliberately incorporating a wide variety of probable biologically active components to the **D**-glucose moiety. Antibacterial and antifungal screening of these compounds were carried out using a variety of bacterial and fungal strains against a number of pathogens and also anticancer activity was investigated by MTT assay and the results are reported as first time in this project.

Materials and methods

All reagents used were commercially available (Aldrich-Sigma) and were used as received, unless otherwise specified. FTIR spectra were recorded by KBr disc at the Chemistry Department, University of Chittagong, Bangladesh, with an IR Affinity Fourier Transform Infrared Spectrophotometer (SHIMADZU). 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 $CDCl_3$ with a Bruker spectrometer. Thin layer chromatography (t.l.c) was performed on Kieselgel GF_{254}. Column chromatography was performed with silica gel G_{60} (Silicycle, 60-200 µm, 60 Å).

*Synthesis of **D**-Glucose derivatives*

Scheme-1

*Synthesis of methyl 4,6-O-benzylidene-α-**D**-glucopyranoside derivatives*

A solution of methyl-α-**D**-glucopyranoside (5 gm, 25.74 mmol) in dry DMF (30 ml) was treated with benzaldehydedimethylacetal (5 ml, 33.5 mmol) and camphor-10-sulphonic acid (100 mg) and the mixture was heated at 50^0C for 6 hours. After cooling to room temperature, the mixture was neutralized with Et_3N, diluted with EtOAc, washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. The progress of the reaction was monitored by t.l.c. (ethyl acetate-hexane, 3:1) and the solvent was then removed. The residue was purified by passage through a silica gel column with ethyl acetate-hexane (3:1) as an eluant to afford methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside **1** (5.5 gm, 76%) as a white crystalline solid. This compound was sufficiently pure for its use as the starting material for the acylation reactions as reported in this dissertation. The structure of this compound was conformed previously by NMR spectrum.

*Synthesis of methyl 4,6-O-benzylidine-2-O-octanoyl-α-D-glucopyranoside (**2**)*

A solution of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**1**) (200 mg, 0.71 mmol) in dry pyridine (3 ml) was cooled to -5^0C whereupon octanoyl chloride (0.13 ml, 1.1 molar eq.) was added to it. The reaction mixture was continuously stirred at the same temperature for 6 hours and then the reaction mixture was standing for overnight at room temperature with continuous stirring. The progress of the reaction was monitored by t.l.c (CH_3OH-$CHCl_3$, 1:14), which indicated full conversion of the starting material into a single product (R_f = 0.52). A few pieces of ice was added to the flask and then extracted the product mixture with chloroform (3×10 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 and eluted with CH_3OH-$CHCl_3$ (1:14) provided the octanoyl derivative (**2**) (141 mg, 70.5%) as crystalline solid. Recrystallization from chloroform–hexane gave the methyl 4,6-*O*-benzylidene-2-*O*-octanoyl-α-**D**-glucopyranoside (**2**) as needless, m.p. 149-151^{0}C. The compound was sufficiently pure for use in the next stage without further purification and identification.

Anal Calcd. $C_{22}H_{32}O_7$ (408.49): C, 64.68%, H, 7.89%; found: C,64.70 %, H, 7.91 %.

FTIR (KBr) (ν_{max}): 1718 (C=O), 3318 cm-^{1} (-OH).

^{1}H-NMR (400 MHz, $CDCl_3$) data: δ_H 7.50 (2H, m, Ar-H), 7.37 (3H, m, Ar-H), 5.51 (1H, s, PhC*H*-), 4.72 (1H, dd, J = 3.7 Hz, and 9.8 Hz, H-2), 4.27 (1H, d, J = 3.8 Hz, H-1), 3.92 (1H, t, J =9.8 Hz, H-3), 3.77 (1H, dd, J = 4.7 and 10.1 Hz, H-6a), 3.75 (1H, ddd, J = 4.8, 9.8 and 14.2 Hz, H-5), 3.58 (1H, t, J = 10.2 Hz, H-6b), 3.45 (1H, t, J = 9.8 Hz, H-4), 3.40 (3H, s, 1-OCH_3), 2.32 {2H, m, $CH_3(CH_2)_5CH_2CO$-}, 1.62 {2H, m, $CH_3(CH_2)_4CH_2CH_2CO$-}, 1.28 {8H, m, $CH_3(CH_2)_4(CH_2)_2CO$-}, 0.88 {3H, m, $CH_3(CH_2)_6CO$-}.

*General procedure for the synthesis of methyl α-**D**-glucopyranoside derivatives*

A solution of octanoyl derivative (**2**) (138 mg, 0.34 mmol) in dry pyridine (3 ml) was cooled to 0^0C and acetic anhydride (0.11 ml, 5 molar eq.) was added. The mixture was stirred at 0^0C for 6 hours

and then kept standing overnight at room temperature. T.l.c. examination (EtOAc-*n*-C_6H_{14}, 1:9) showed complete conversion of reactant into a single product (R_f = 0.53). Excess reagent was destroyed by the addition of a few pieces of ice and the reaction mixture was extracted with chloroform (3×10 ml). The combined organic extract was washed successively with dilute hydrochloric acid, saturated aqueous sodium hydrogen carbonate solution and water. The organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated off. The resulting syrup was purified by column chromatography (with EtOAc-*n*-C_6H_{14}, 1:9, as eluant) to afford the acetyl derivative (**3**) (129 mg, 93.47%) as a pasty.

Similarly by using the same procedure we isolated the Compound **4** (pentanoyl), Compound **5** (heptanoyl), Compound **6** (decanoyl), Compound **7** (myristoyl), Compound **8** (pivaloyl), Compound **9** (benzenesulphonyl), Compound **10** (2-bromobenzoyl), successfully.

Methyl 3-O-acetyl-4,6-O-benzylidine-2-O-octanoyl -α-D-glucopyranoside (3)

Anal Calcd. $C_{24}H_{34}O_8$ (450.53): C, 63.98%, H, 7.60%; found: C, 63.99 %, H, 7.62 %.

FTIR (KBr) (ν_{max}): 1708 cm-^{1} (C=O).

^{1}H-NMR (400 MHz, $CDCl_3$) data: δ_H 7.40 (2H, m, Ar-H), 7.17 (3H, m, Ar-H), 5.62 (1H, t, J =9.8 Hz, H-3), 5.53 (1H, s, PhC*H*-), 4.97 (1H, d, J = 3.6 Hz, H-1), 4.22 (1H, dd, J = 3.6 Hz, and 9.8 Hz, H-2), 3.97 (1H, dd, J = 4.7 and 10.1 Hz, H-6a), 3.80 (1H, ddd, J = 4.8, 9.8 and 14.2 Hz, H-5), 3.68 (1H, t, J = 10.2 Hz, H-6b), 3.44 (1H, t, J = 9.8 Hz, H-4), 3.36 (3H, s, 1-OCH_3), 2.12 {2H, m, $CH_3(CH_2)_5CH_2CO$-}, 2.08 (3H, s, CH_3CO-), 1.65 {2H, m, $CH_3(CH_2)_4CH_2CH_2CO$-}, 1.28 {8H, m, $CH_3(CH_2)_4(CH_2)_2CO$-}, 0.87 {3H, m, $CH_3(CH_2)_6CO$-}.

Scheme-2

*Synthesis of methyl 6-O-pivaloyl- β-**D**-galactopyranoside (**12**)*

A solution of methyl β-**D**-galactopyranoside (**11**) (200 mg, 1.03 mmol) in dry pyridine (3 ml) was cooled to -5^{0}C where upon pivaloyl chloride (0.14 ml, 1.1 molar eq.) was added to it. The reaction mixture was continuously stirred at the same temperature for 6 hours and then the reaction mixture was standing for overnight at room temperature with continuous stirring. The progress of the reaction was monitored by TLC (CH_3OH-$CHCl_3$, 1:7), which indicated full conversion of the starting material into a single product (R_f = 0.52). A few pieces of ice was added to the flask and then extracted the product mixture with chloroform (3×10 ml). The syrup was passed through a silica gel column and eluted with CH_3OH-$CHCl_3$ (1:7) provided the pivaloyl derivative (**12**) (141 mg, 70.5%) as crystalline solid. Recrystallization from chloroform-hexane gave the methyl 6-*O*-pivaloyl-β-**D**-galactopyranoside (**12**) as needless, m.p. 149-151^{0}C. The compound was sufficiently pure for use in the next stage without further purification and identification.

Anal Calcd. for $C_{12}H_{22}O_7$ (278.26): C, 51.75%, H, 7.90%; found: C, 51.77%, H, 7.91%.

FTIR (KBr) (ν_{max}): 1755, 1729 (C=O), 3414~3461 cm-^{1} (-OH).

^{1}H-NMR ($CDCl_3$, 400 MHz) (δ ppm): δ_H 4.95 (1H, d, J = 8.0 Hz, H-1), 4.45 (1H, dd, J = 11.1 and 6.5 Hz, H-6a), 4.42 (1H, dd, J = 11.1 and 6.7 Hz, H-6b), 4.18 (1H, d, J = 3.5 Hz, H-4), 4.07 (1H, dd, J = 3.0 and 10.5 Hz, H-3), 4.00 (1H, dd, J = 8.0 and 10.5 Hz, H-2), 3.78 (1H, m, H-5), 3.46 (3H, s, 1-OCH_3), 1.20 {9H, s, $(CH_3)_3CCO$-}.

Similarly by using the same procedure we purified the acetyl, propionyl, butyryl. nonanoyl, myristoyl etc., derivatives successfully.

Antimicrobial activity

The *in vitro* antibacterial activities of the synthesized compounds were detected by disc diffusion method (Bauer *et al*., 1966: Mia *et al*., 1990). The *in vitro* antifungal activities were done by Poisons Food technique (Grover *et al*., 1962) with some modification (Mia *et al*., 1990). The synthesized test compounds (scheme 1 and 2) were subjected to antibacterial screening against seven bacterial strains viz., *Bacillus subtilis* BTCC 17, *Bacillus cereus* BTCC 19, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (ATCC 6538), *Salmonella abony* (NCTC 6017), *Pseudomonas aeruginosa* CRL(ICCDR,B) and *Salmonella paratyphi* (ATCC 9027). The name of two phytopathogenic fungi viz., *Candida albicans* (SC 5314) and *Aspergillus niger* (ATCC 16404). In all cases, a 2% solution (in $CHCl_3$) of the chemicals was used.

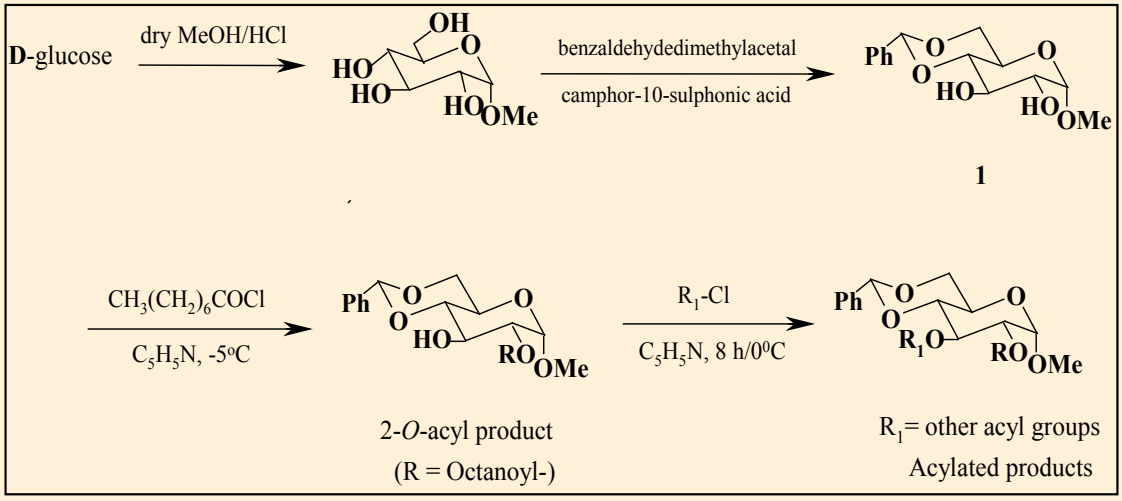
Results and discussion

*Characterization of methyl 4,6-O-benzylidene-α-**D**-glucopyranoside (**1**) derivatives (Scheme-1)*

The main objective of the research work was to carry out regioselective acylation (scheme-1) of methyl 4,6-*O*-benzylidene-α-**D**-glucopyranoside (**1**) using the direct acylation method. The resulting acylation products were then converted to a series of derivatives using various acylating agents containing a wide variety of biologically prone atoms/ groups. All the acylation products thus prepared were used as test chemicals for antibacterial evaluation studies against a number of Gram-positive and Gram-negative human pathogenic bacteria. The selection of a wide variety of acylating agents was deliberate with the aim of finding biologically active **D**-glucose derivatives.

Our initial effort was to prepare the starting 4,6-*O*-benzylidene derivative (**1**). Thus, reaction of methyl-α-**D**-glucopyranoside with benzal dehydedimethyl acetal and camphor-10-sulphonic acid in dry DMF provided the benzylidene derivative (**1)** in 76% yield. The structure of this compound was ascertained by analyzing its ^{1}H-NMR spectra.

With the starting benzylidene derivative (**1**) in hand, we then allowed it to react with an unimolecular amount octanoyl chloride using the direct acylation method. After usual work-up and silica gel chromatographic purification, compound **2** was obtained in 70.5% yield as needles, m.p. 149-151^{o}C (chloroform–hexane). Its FTIR spectrum displayed the absorption bands at 1718 cm^{-1} for C=O stretching and 3318 cm^{-1} for –OH stretching. In its ^{1}H-NMR spectrum, two two-proton multiplets at δ 2.32 {$CH_3(CH_2)_5CH_2CO$-} and 1.62 {$CH_3(CH_2)_4CH_2CH_2CO$-}, an eight-proton multiplet at δ 1.28 {$CH_3(CH_2)_4(CH_2)_2CO$-} and a three-proton multiplet at δ 0.88 {$CH_3(CH_2)_6CO$-} were due to the presence of one octanoyl group to the molecule. The introduction of this group to position 2 was shown by deshielding of the C-2 proton to δ 4.72 (as dd, J = 3.7 and 9.8 Hz) from its value (~4) in the precursor diol (**1**). Complete analysis of the FTIR and ^{1}H-NMR spectrum led us to establish its structure as methyl 4,6-*O*-benzylidene-2-*O*-octanoyl-α-**D**-glucopyranoside (**2**).



Scheme-1: Synthesize of D-glucose derivatives.

2. R = $CH_3(CH_2)_6CO$-, R_1 = H

3. R = $CH_3(CH_2)_6CO$-, R_1 = CH_3CO-

4. R = $CH_3(CH_2)_6CO$-, R_1 = $CH_3(CH_2)_3CO$-

5. R = $CH_3(CH_2)_6CO$-, R_1 = $CH_3(CH_2)_5CO$-

6. R = $CH_3(CH_2)_6CO$-, R_1 = $CH_3(CH_2)_8CO$-

7. R = $CH_3(CH_2)_6CO$-, R_1 = $CH_3(CH_2)_{12}CO$-

8. R = $CH_3(CH_2)_6CO$-, R_1 = $(CH_3)_3CCO$-

9. R = $CH_3(CH_2)_6CO$-, R_1 = $C_6H_5SO_2$-

10. R = $CH_3(CH_2)_6CO$-, R_1 = 2-Br.C_6H_4CO-

Fig. 1. The structure of synthesized D-glucose derivatives (1-10).

The structure of the 2-*O*-(octanoyl) derivative (**2**) was further supported by its conversion to and identification of the acetyl derivative (**3**). Thus, reaction of compound **2** with an excess of acetic anhydride in pyridine, followed by usual aqueous work-up procedure, provided the acetyl derivative (**3**) in 93.47% yield as a pasty mass. The octanoyl derivative (**2**) was then converted to a number of fatty acid derivatives using pentanoyl chloride, heptanoyl chloride, decanoyl chloride and myristoyl chloride in order to get further support to its structure and also prepare newer products.

The corresponding fatty acid derivatives (**4-10**) were isolated in reasonable yields. The structures of these derivatives were established confidently by completely analyzing their FTIR, ^{1}H-NMR spectra and elemental analysis.

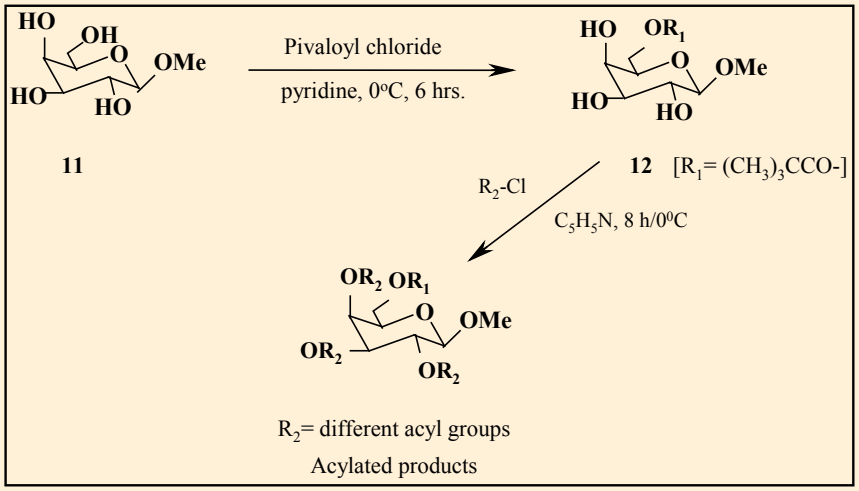
*Characterization of methyl β-**D**-galactopyranoside (**11**) derivatives (Scheme-2)*

In the scheme-2, we carried out regioselective acylation of methyl β-**D**-galactopyranoside (**11**) with pivaloyl chloride using the direct acylation method. The resulting acylation products were then converted to a series of derivatives using various acylating agents containing a wide variety of biologically prone atoms/groups. The structure of the main acylation products and their derivatives were established by analysing their FTIR, ^{1}H-NMR spectra and physical & elemental analysis. In

continuation of a project in our Laboratory of Carbohydrate and Nucleoside Chemistry (LCNC), we intended to prepare a series of β-**D**-galactopyranoside derivatives for use as test chemicals for antibacterial, antifungal and anticancer evaluation studies.

Keeping this intention in mind, we thus prepared two sets of β-**D**-galactopyranoside derivatives containing a wide variety of substituents in a single molecular framework. All the acylation products thus prepared were used as test chemicals for antibacterial evaluation studies against a number of Gram-positive and Gram-negative human pathogenic bacteria. These test chemicals were used for antifungal activity studies against some plant pathogenic fungi. Anticancer activities of all newly synthesized compounds were also performed against a cancer cells by MTT assay. The selection of a wide variety of acylating agents was deliberate with the aim of finding biologically active β-**D**-galactopyranoside derivatives.

By complete analysis of their FTIR, ^{1}H-NMR spectrum and by analogy with similar derivatives described earlier, the structures of these compounds were confidently assigned as methyl 6-*O*-pivaloyl-β-**D**-galactopyranoside (**12**), methyl 2,3,4-tri-*O*-acetyl-6-*O*-pivaloyl-β-**D**-galactopyranoside (**13**) and compounds **14-21**.



Scheme-2. Synthesize of β-D-galactopyranoside derivatives.

12. $R_1 = (CH_3)_3CCO$-, $R_2 = H$

13. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3CO$-

14. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3CH_2CO$-

15. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3(CH_2)_2CO$-

16. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3(CH_2)_7CO$-

17. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3(CH_2)_{12}CO$-

18. $R_1 = (CH_3)_3CCO$-, $R_2 = CH_3(CH_2)_{14}CO$-

19. $R_1 = (CH_3)_3CCO$-, $R_2 = (C_6H_5)_3C$-

20. $R_1 = (CH_3)_3CCO$-, $R_2 = 4$-$(CH_3)_3CC_6H_4CO$-

21. $R_1 = (CH_3)_3CCO$-, $R_2 = 4$-$CH_3O.C_6H_4CO$-

Fig. 2. The structure of synthesized β-D-galactopyranoside derivatives (12-21).

Antimicrobial and anticancer activities of scheme-1 and 2

This antibacterial activity screening test also suggests that incorporation of various acyl groups in methyl β-**D**-galactopyranoside (**11**) and methyl 6-*O*-pivaloyl-β-**D**-galactopyranoside (**12**) increased the antibacterial activity of these compounds noticeably. The MIC value of **17** was found 625 µg/ml against *Staphylococcus aureus.* So, these two chemicals (i.e. **17** and **18**) can be used as a therapeutic antibacterial agent against various infectious diseases caused by these test organisms after investigating their side effects and other necessary experiments. Antifungal screening study showed that the chemicals **16** and **20** (nonanoyl and 4-*t*-butylbenzoyl derivatives) showed a greater zone of inhibition against *Aspergillus niger.* Moreover, we considered **18** for further work as it showed potential anticancer activity. In this project, our synthesized and reported chemicals have not been tested before against the selected bacterial and fungal pathogens.

Conclusions

In current project research work, we successfully synthesized and characterized **D**-glucose 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. This is the first work regarding the effectiveness of the selected chemicals against the selected human pathogens. These acylated **D**-glucose derivatives have not been tested against the aforementioned pathogens before. Some of the tested chemicals showed moderate to marked inhibition against the bacterial pathogens employed. In this antibacterial screening studies also observed that the tested acylated derivatives were found comparatively more effective against Gram-negative than that of Gram-positive organisms. It was also found that some chemicals were unable to show any inhibition at all against the bacterial pathogens employed. In the present work, it was found that selectively acylated derivative **7** showed good inhibition against Gram-positive bacteria while compounds **6** and **7** were also very active against Gram-negative bacteria. That is with the introduction of various acyl groups such as myristoyl, 4-chlorobenzoyl the antibacterial functionality of the compound **2** increases.

Fungal activities against some plant pathogenic fungi and in this case the activity of the compound **12** increases when nonanoyl, myristoyl, 4-*t*-butylbenzoyl and 3-chlorobenzoyl groups are introduced. These compounds showed very promising results when MIC, MBC and MFC tests were carried out. So, it is hoped that the acylated derivatives of **D**-glucose might show potential anti-viral, anti-inflammatory and diabetic activities.

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Chemical Assessment of Commercial Poultry and Dairy Feed Available in Dhaka Division and Performance at Farmers' Level

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Abstract

The study was performed in different Districts under Dhaka division for the period of 12 months between July 2017 to June 2018 to know the availability of commercial poultry and dairy feed and to assess the nutritive value of commercial poultry and dairy feed. The research work reveals the present scenario of feed mills industry in Dhaka division through a survey study. The target group of the survey was retailer or dealer of different feed companies. There were 320 number of respondents interviewed at different levels that covered 10 districts of Dhaka division and the research found that a total 38 active feed mill companies present in different districts of Dhaka division. According to the respondent opinion, United feeds Ltd 18.44%, Nourish poultry & hatchery Ltd. (15.63%), Paragon feed Ltd. (8.75%), CP Bangladesh Co. Ltd. (7.19%), Quality Feeds Ltd.(5.00%), Kazi farms Ltd. (4.38%), Aftab feed products Limited (3.75%), SMS Feed Ltd (3.75%), MS agro feed unit pvt. Ltd (3.44%) and Provita feed Ltd. (3.13%) are the major contributors in feed market. Those top 10 feed mills contribute more than 73% feed market in different districts of Dhaka division. Feed sample also collected (broiler starter and grower) from top ten feed mill companies among 38 feed mill companies and to find out an exact quantity of CP in broiler feeds manufactured in different districts of Dhaka division. There were no significant differences ($p>0.01$) found in case of dry matter of broiler starter feed. Significan difference was ($p<0.01$) found in broiler grower feed in nutrient concentrations (Dry matter and crude protein %) among the feeds obtained from the ten different feed mills and also significan difference ($p<0.01$) found in CP% in case of broiler starter feed. Broiler feeds were collected from United feeds Ltd., Nourish Poultry & Hatchery Ltd., Paragon Feed Ltd., SMS Feed and MS Agro Feed Pvt. Ltd. feed mills, feed contains somewhat similar or more CP than BSTI standard. So, the feeds produced from these feed mills were deemed to be of acceptable quality. The feeds of remaining feed mills

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(CP Bangladesh Co. Ltd., Quality Feeds Ltd, Kazi Farms Ltd., Provita Feed Ltd. and Aftab Feed Products Ltd.) appeared to be of comparatively slightly lower in nutritive value CP% than BSTI standard. Feed mills are not maintaining the similar standard or requirement for the similar birds, specially in CP content, it should be standardized.Almost half of the farmers believe that good performance of their cattle and poultry done by commercial feed. However, rest of the farmers had complain about the commercial feed of different feed mills. Reason behind complain about feed of different feed mills should be a topic for future research.

Introduction

Poultry and dairy production is an important part of animal agriculture. By increasing the productivity of meat, milk and eggs, the existing gap between supply and demand of animal protein can be bridged. Poultry meat contributes around 37% of the total animal protein supply of Bangladesh (Ahmed and Islam, 1990). Milk, meat and eggs are quality food in respect amino acid ingredients and therefore, can improve considerably an otherwise unbalanced diet. It also provides cash income and creates employment opportunity for small and landless farmers.

Presently only 55% of the total poultry products are coming from commercial farms. Poultry industry has established its position as the fastest growing segment in the agricultural sector. Bangladesh Government has also come forward for the improvement of dairy and poultry sector and offering training and financial assistance to marginal entrepreneurs. Profitable livestock farming is a highly specialized job in which a lot of factors may be responsible to offset the profit amount. Among these, qualities of feeds are most important. Feed alone accounts for approximately 65-70% of the total cost of production. As nutrition is one of the most important factors for successful livestock farming, a booming animal feed business is appearing all over the country. Different feed ingredients are being important to prepare low cost but nutritionally balanced poultry and dairy ration.

A number of feed mills in Dhaka Division are producing poultry and dairy feed, which is purchased by farm-owners according to their need. Farmers do not have enough facility to analyze and monitor the quality of the feed. Often they formulate the ration for their own animal from ingredients on the basis of the information about the feed components mentioned elsewhere. In view of the availability and source of different feed ingredients, the level of nutrients in the prepared ration may vary from what is actually desired. Deficiency of a particular nutrient in the ration may not be over-ruled which is generally unnoticed by the farm-owners causing a devastating effect on production. To get an exact picture of the feeding practices as well as to assess the quality of the livestock feeds being used in farms located in Dhaka Division of Bangladesh, biochemical analysis (nutritional quality) of the feed seems to be worthwhile. The present study was therefore, undertaken to observe available commercial dairy and poultry feed in Dhaka Division, assess the nutritive value of feeds collected from the farms and its impact at farmers' level. Above views in mind, following objectives are set

1. To find out the commercial poultry feed available in Dhaka Division
2. To identify available commercial cattle feed in Dhaka Division
3. To assess the chemical component (CP, DM, Moisture) of commercial poultry feed in laboratory
4. To find out the impact of dairy and poultry feed in farmers' level

Methodology

Experimental site and samples collection

Field survey: 10 Districts of Dhaka Division

Sample collection: Farm from 10 Districts of Dhaka Division

Information collection about feed: Feed market/shop from 10 Districts of Dhaka Division

Performance study at farmers' level: Data collection from 10 Districts of Dhaka Division

Laboratory analysis: Chemical analysis of collected feed was done in Animal Nutrition laboratory and Dairy Science lab.

Survey Instruments

- Questionnaire for farmers
- Questionnaire for shopkeepers

Chemical analysis

The major nutritional component (proximate analysis) mainly protein & moisture content was analyzed.

Sample size

Total sample size was 320. The required sample size was determined based on confidence level and precision rate. The standard formula was used to estimate the required number of sample.

Distribution of sample

The distribution of sample size of the study depends on the poultry population at the different district of Dhaka division. Out of 13 districts, 10 districts were selected randomly for the study which is given below:

Name of District	No. of Response
Dhaka	92
Faridpur	14
Gazipur	46
Kishoreganj	35
Madaripur	16
Manikganj	32
Narayanganj	16
Narsingdi	15
Rajbari	18
Tangail	36
Total	320

Statistical analysis

Data of field survey

A Microsoft Access and Microsoft Excel program was developed for data entry. Different types of statistical tools like number, mean, and percent were used. A singular tabular technique was presented in the study to classify the data into meaningful categories.

Data of crude protein test

Data of crude protein test were analyse by using MSTAT-C computer package program. All record were statistically analyzed for analysis of coefficient of variance and least significant difference (LSD).

Results and discussion

The study was conducted in different district of Dhaka division to study available of commercial feed with its crude protein content of broiler feed and compare with the protein value declared on brand labels. The results have been presented and discussed with the help of table and graphs. During the study the following results were obtained

List of Feed Mill in Dhaka Division

The study was conducted in the different district of Dhaka division. In the study, there are 38 active feed mill companies present in different districts of Dhaka division. The research found the following feed mills in Dhaka division

Table I. List of available feed company in different district of Dhaka division & make a serial of the company base on number of response of sale from retailer or dealer

Sl. No.	Company Name	Brand Name	No. of Response	%
1	United Feeds Ltd.	Fresh	59	18.44
2	Nourish Poultry & Hatchery Ltd.	Nourish	50	15.63
3	Paragon Feed Ltd.	Chamak, Usha	28	8.75
4	CP Bangladesh Co. Ltd.	CP	23	7.19
5	Quality Feeds Ltd.	Quality feed	16	5.00
6	Kazi Farms Limited	Kazi	14	4.38
7	Aftab Feed Products Limited	Aftab	12	3.75
8	SMS Feed Limited	SMS Poultry Feed	12	3.75
9	MS Agro Fisheries Feed Unit Pvt. Ltd	Planet Poultry Feed	11	3.44
10	Provita Feed Ltd.	Provita	10	3.13
11	ACI Godrej Agrovet Pvt. Ltd.	ACI Poultry Feed	10	3.13
12	Aman Feed Ltd.	Classic	9	2.81
13	New Hope Feed Mill Bangladesh Ltd.	New Hope	9	2.81
14	R.R.P. Agro Farms	R.R.P.	9	2.81

Continued

Sl. No.	Company Name	Brand Name	No. of Response	%
15	City Poultry & Fish Feed Ltd.	Teer	7	2.19
16	Ag Agro Industries Ltd.	AG Agro Feed	6	1.88
17	Agata Feeds Mill Ltd	Agata Feed	4	1.25
18	Gram Bangla Poultry & Fish Feed Ltd.	Gram Bangla	4	1.25
19	Agro Indutrial Trust Feeds Ltd.	AIT	3	0.94
20	Index Feeds Ltd.	Index Feed	2	0.63
21	Suguna Food & Feed Bangladesh Privet Ltd.	Suguna	2	0.63
22	Lion Feeds Ltd.	Lion feed	2	0.63
23	Ekushey Feeds Ltd.	Ekushey	2	0.63
24	Mashud Fish Meal Plant & Feed Mills	The best	2	0.63
25	Amrit Global Bangladesh Pvt. Ltd.	Modern Amrit	1	0.31
26	Al-Nur Agro & Farm Product Private Ltd.	Al_Nur Feed	1	0.31
27	SB Poultry & Fish Feed Mills	SB Poultry Feed	1	0.31
28	Paradise Sun Company Ltd.	Paradise Feed	1	0.31
29	Marks Feed Mill Ltd.	Marks Feed	1	0.31
30	Biswas Poultry & Fish Feed Ltd.	Biswas Feed	1	0.31
31	Gazipur Feeds Ltd.	Gazipur Feeds	1	0.31
32	Robi Poultry Feed Mill Ltd.	Robi	1	0.31
33	Power Fish & Poultry Feed Ltd.	Power	1	0.31
34	Advanced Poultry & Fish Feed Ltd.	Advanced	1	0.31
35	Cherish Feed & R.B. Agro Ltd.	Cherish Feed	1	0.31
36	Bengal Feed & Fisheries Ltd.	Bengal Feed	1	0.31
37	Unique Hatchery & Feed Ltd.	Unique feed	1	0.31
38	Enam Hatchery & Feeds Ltd.	Enam	1	0.31
	Total		320	100

Shahinur *et al.* (2017) reported that there were 120 registered feed mills present in Bangladesh.DLS (2018) also reported that there were 182 registered commercial feed mills present in whole Bangladesh. However, there were no previous data found about feed mills number in division wise.

In the table-I, the top 10 feed mills areUnited Feeds Ltd., Nourish Poultry & Hatchery Ltd., Paragon Feed Ltd., CP Bangladesh Co. Ltd., Quality Feeds Ltd., Kazi Farms Limited, Aftab Feed Products Limited, SMS Feed Limited, MS Agro Fisheries Feed Unit Pvt. Ltd., Provita Feed Ltd., ACI Godrej Agrovet Pvt. Ltd.Provita Feed Ltd. and ACI Godrej Agrovet Pvt. Ltd. jointly occupied the 10th position. These feed mills are treated as most commonly available feed company in Dhaka division. Those top 10 feed mills covered more than 75% feed market in Dhaka division.

Quality of Feed According to Proximate Content of Top Ten Feed Mills

The major nutrients value of a poultry feeds that are considered when formulating diets are mainly

crude protein, moisture, crude fat and crude fibre. In this study proximate analysis of feed were performed mainly crude protein and dry matter. The results of proximate composition of analyzed feed samples were shown in Table (2 & 3). Rahman *et al.* (2014) also state that Crude protein is one of the most important nutrient to quantify in a prospective feeds due to the fact that is one of the most costly to supply and a deficiency of protein has a drastic effect on growth and production. Various diets are commonly utilized, depending on the bird's production stage. Generally starter rations are high in protein whereas grower diets usually contain less protein, because older birds need less.

Variation of Dry Matter & Crude Protein of Broiler Starter of Top Ten Different Feed Mills

The analytical values of nutrient of the feed samples are shown in table 2. In case of broiler starter rations, the analytical values of dry matter contents of the feed samples differed none significantly ($p>0.01$) but crude protein contain of feed sample differed significantly ($p<0.01$) among treatment.

Table II: Nutritional composition of broiler starter feeds of top ten different feed mills

Treatment	Company Name	DM%	CP%
T_1	United Feeds Ltd(Meghna group of industries)	89.42^{A}	21.83^{ABC}
T_2	Nourish Poultry & Hatchery Ltd.	88.74^{A}	22.49^{AB}
T_3	Paragon Feed Ltd.	89.71^{A}	22.68^{AB}
T_4	CP Bangladesh Co. Ltd.	89.04^{A}	19.95^{DE}
T_5	Quality Feeds Ltd.	89.00^{A}	20.61^{CD}
T_6	Kazi Farms Ltd.	89.22^{A}	18.38^{E}
T_7	Provita Feed Ltd.	88.44^{A}	19.27^{DE}
T_8	Aftab Feed Products Ltd.	89.72^{A}	19.30^{DE}
T_9	SMS Feed Ltd.	89.16^{A}	21.69^{BC}
T_{10}	MS Agro Fisheries Feed Unit Pvt. Ltd.	89.89^{A}	23.52^{A}
$LSD_{(0.01)}$		2.303	1.718
CV%		1.11	3.53

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at .01 level of probability.

It is evident from Table 2 that higher DM content was found T_1 (United Feeds Ltd.), T_8 (Aftab Feed Products Ltd.) and T_{10} (MS Agro Fisheries Feed Unit Pvt. Ltd) groups, in comparison with feeds of other feed mills. High level of dry matter content T_4 (CP Bangladesh Co. Ltd.), T_5 (Quality Feeds Ltd.), T_6 (Kazi Farms Ltd.) and T_9 (SMS Feed Ltd.) groups of feed. Dry matter content (88.44%) of T_7 (Provita Feed Ltd.)group was the lowest of all which was statistically similar to T_2 (Nourish Poultry & Hatchery Ltd.) and T_3 (Paragon Feed Ltd.) groups of feed.

It is also evident from Table 2 that higher crude protein content was found T_2 (Nourish Poultry & Hatchery Ltd.), T_3 (Paragon Feed Ltd.) and T_{10} (MS Agro Fisheries Feed Unit Pvt. Ltd.) groups, in comparison with feeds of other feed mills. In case of T_1(United Feeds Ltd.), T_5 (Quality Feeds Ltd) and T_9 (SMS Feed Ltd.) groups of feed content high level of crude protein. Crude protein content (18.38%) of T6 group was the lowest of all which was statistically similar to T_4 (CP Bangladesh Co. Ltd.), T_7 (Provita Feed Ltd.) and T_8 (Aftab Feed Products Ltd.) group of feed.

In this study, DM were found in United feed (fresh feed), Nourish Poultry feed, Quality Feeds, Paragon feed and Aftab feed 89.42%, 88.74%, 89%, 88.71%, 89.72% respectively.

Roy *et al.* (2004) reported that United feed (fresh feed), Nourish Poultry feed, Quality Feeds, paragon feed and Aftab feed contents 88.9%, 90%, 89.8%, 89.2% & 89.7% respectively. Crude protein were found in United feed (fresh feed), Nourish Poultry feed, Quality Feeds, paragon feed and Aftab feed 21.83%, 22.49%, 20.61%, 22.68% and 19.3% respectively but Roy *et al* (2004) were found crude protein in United feed (fresh feed), Nourish Poultry feed, Quality Feeds, paragon feed and Aftab feed 21.6%, 22.9%, 23.5%, 22.2% and 23.9 respectively.

Hossain (2017) reported that dry matter found in United feed (fresh feed), Nourish Poultry feed, Quality Feeds and Aftab feed 88.4%, 90.4%, 89.2% and 89.4% respectively. Crude protein content was found in different broiler starter feed of United feed (fresh feed), Nourish Poultry feed, Quality Feeds and Aftab feed 19.2%, 20.6%, 22.8% and 20.2 respectively.

Variation of Dry Matter & Crude Protein of Broiler Grower Feed of Top Different Feed Mills

The analytical values of nutrient (dry matter & crude protein) of broiler grower ration of different feed mills are shown in table 3. In case of broiler grower rations, nutrient contents (dry matter & crude protein) are shown significan difference among treatment.

Table III. Nutritional composition of broiler grower feeds of top ten different feed mills

Treatment	Company Name	DM%	CP%
T_1	United Feeds Ltd.(Meghna group of industries)	89.32^{AB}	21.43^{BC}
T_2	Nourish Poultry & Hatchery Ltd.	88.50^{BCD}	20.83^{C}
T_3	Paragon Feed Ltd.	88.96^{BC}	23.24^{A}
T_4	CP Bangladesh Co. Ltd.	87.88^{CD}	19.30^{DE}
T_5	Quality Feeds Ltd.	90.34^{A}	20.60^{CD}
T_6	Kazi Farms Ltd.	88.42^{BCD}	17.93^{E}
T_7	Provita Feed Ltd.	87.90^{CD}	17.91^{E}
T_8	Aftab Feed Products Ltd.	88.45^{BCD}	18.54^{E}
T_9	SMS Feed Ltd.	88.75^{BCD}	22.46^{AB}
T_{10}	MS Agro Fisheries Feed Pvt. Ltd.	87.80^{D}	20.52^{CD}

In a column means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly by LSD at .01 level of probability.

It is proved from Table 3 that the highest dry matter content was found T_1 (United Feeds Ltd.), T_3 (Paragon Feed Ltd.) and T_5 (Quality Feeds Ltd.) groups, in comparison with feeds of other feed mills. High level of dry matter content T_2 (Nourish Poultry & Hatchery Ltd.), T_6 (Kazi Farms Ltd.), T_8 (Aftab Feed Products Ltd.) and T_9 (SMS Feed Ltd.) groups of feed. Dry matter content (87.8%) of T_{10} (MS Agro Fisheries Feed Pvt. Ltd.) group was the lowest of all which was statistically similar to T_4 (CP Bangladesh Co. Ltd.) and T_7 (Provita Feed Ltd.) groups of feed. It is also evident from Table 3 that higher crude protein content was found T_3 (Paragon Feed Ltd.) and T_9 (SMS Feed Ltd.)

groups, in comparison with feeds of other feed mills. In case of T_1 (United Feeds Ltd.), T_2 (Nourish Poultry & Hatchery Ltd.), T_5 (Quality Feeds Ltd.) and T_{10} (MS Agro Feed Pvt. Ltd.) groups of feed content high level of crude protein. Crude protein content (17.91%) of T_7 (Provita Feed Ltd.) group was the lowest of all which was statistically similar to T_4 (CP Bangladesh Co. Ltd.), T_6 (Kazi Farms Ltd.) and T_8 (Aftab Feed Products Ltd.) group of feed.

In this study, it was found that crude protein range was 18.38 to 23.52 and 17.91 to 23.24 broiler starter and grower respectively.

Rahman (2014) analyzed broiler paragon feed, broiler crumbo feed & broiler anchor feed and reported that crude protein contain in broiler feed the range of 17.5-18.2.

Roy *et al*. (2004) reported that dry matter were content in United feed (fresh feed), Nourish Poultry feed, Quality Feeds, paragon feed and Aftab feed 88.7%, 90.4%, 89.5% 89.4%, and 89.4% respectively. They also found crude protein in United feed (fresh feed), Nourish Poultry feed, Quality Feeds, paragon feed and Aftab feed 19.2%, 22.6%, 23.2%, 23.7% and 21% respectively.

It was found that the proximate analysis of different company feed content vary lab to lab. Hossain (2003) stated that dry matter content in different company like United feed (fresh feed), Nourish Poultry feed, Quality Feeds and Aftab feed 88.7%, 90.4%, 89.5 and 89.4% respectively. Crude protein found in different company of broiler grower feed like United feed (fresh feed), Nourish Poultry feed, Quality Feeds and Aftab feed 19.5%, 22.4%, 23.2% and 21% respectively.

Crude Protein Concentration of Broiler Starter Feed & Compare with Requirement

The analytical values of nutrients content of broiler starter ration of top ten different feed mills along with required level of nutrient according to BSTI are presented in table IV.

Table IV. Comparative study of the nutrient (crude protein) concentration in broiler starter feed of different feed mills and requirement according to BSTI.

Treatment	Feed Company Name	Analytical CP%	Brand Level CP%	BSTI Standard
T_1	United Feeds Ltd.	21.83	21	22
T_2	Nourish Poultry & Hatchery Ltd.	22.5	20	22
T_3	Paragon Feed Ltd.	22.68	22.5	22
T_4	CP Bangladesh Co. Ltd.	19.95	20	22
T_5	Quality Feeds Ltd.	20.83	23	22
T_6	Kazi Farms Ltd.	18.38	21	22
T_7	Provita Feed Ltd.	19.27	*	22
T_8	Aftab Feed Products Ltd.	19.3	21-23	22
T_9	SMS Feed Ltd.	21.69	22	22
T_{10}	MS Agro Feed Pvt. Ltd.	23.52	20	22

*Brand label value absence

In case of united feed broiler starter T_1 (United Feeds Ltd.), the nutrient (CP) concentration obtained by chemical analysis revealed that the CP content was more close to the required level and more than brand label. Nourish broiler starter T_2 (Nourish Poultry & Hatchery Ltd.) feed contain CP 22.5% that is higher than both require level according to the BSTI and brand label.

Similarly, the nutrient (CP) concentration in T_4 (CP Bangladesh Co. Ltd.), T_5 (Quality Feeds Ltd.), T_6 (Kazi Farms Ltd.) and T_8 (Aftab Feed Products Ltd.) groups of broiler starter feed were slightly lower in amounts declared on brand level by feed company or feed mills and also lower CP% contents than BSTI standard level. However T_7 (Provita Feed Ltd.)group of feed company did not declare CP% on brand label and it also contain lower CP than BSTI require level.

In case of T_9 (SMS Feed Ltd.) group of broiler starter feed CP contain all most close to the required level and brand labeling.The nutrient profiles (CP) as obtained by chemical analysis in case of group T_3 (Paragon Feed Ltd.) and T_{10} (MS Agro Feed Pvt. Ltd.) contains CP more than brand labeling and required level according to the BSTI.

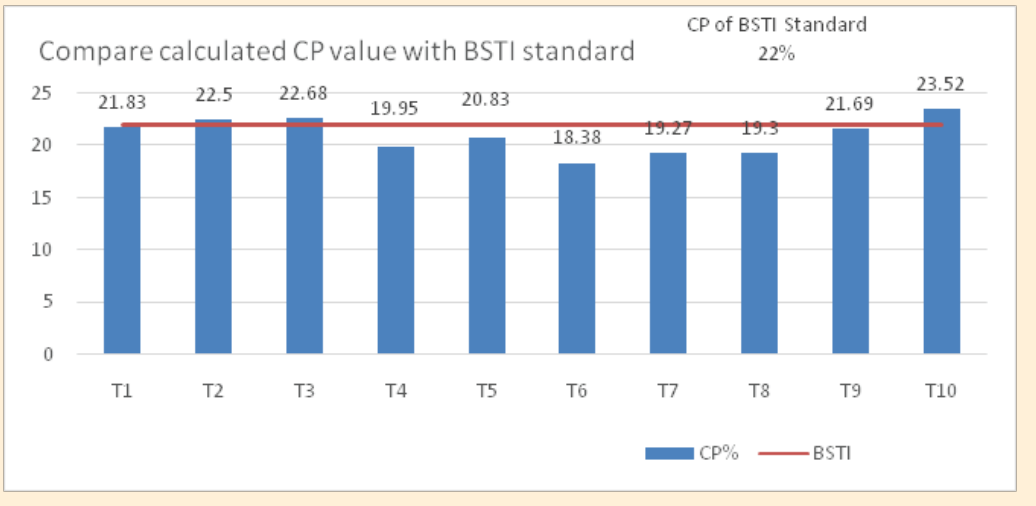

Fig. 1. Compare calculated CP with BSTI standard of broiler starter feed of different feed mills

$T_{1=}$ United Feeds Ltd., $T_{2=}$ Nourish Poultry & Hatchery Ltd., $T_{3=}$ Paragon Feed Ltd., $T_{4=}$ CP Bangladesh Co. Ltd., $T_{5=}$ Quality Feeds Ltd., $T_{6=}$Kazi Farms Ltd., $T_{7=}$Provita Feed Ltd., $T_{8=}$Aftab Feed Products Ltd., $T_{9=}$Sms Feed Ltd., $T_{10=}$ MS Agro Feed Pvt.Ltd.

Crude Protein Concentration of Broiler Grower Feed & Compare with Requirement

The chemical composition of feed sample of grower rations obtained from ten different feed mills along with BSTI standard and feed company specifications are shown in table 5. It was evident from this table that all analytical values obtained from T_4 (CP Bangladesh Co. Ltd.) groups of broiler grower feed contain crude protein more than that declared on brand level but CP% contain less than BSTI standard.

The analytical data of T_1 (United feeds Ltd.), T_2 (Nourish poultry & hatchery Ltd.), T_3 (Paragon feed Ltd.), T_9 (SMS Feed Ltd.) and T_{10} (MS Agro Feed Pvt. Ltd.) groups of broiler grower feed contains crude protein more than the declared label by the manufacturer company of feeds and also more than BSTI standard.

Table V. Comparative study of the nutrient (crude protein) concentration in broiler grower feed of different feed mills and requirement according to BSTI.

Treatment	Feed Company Name	Analytical CP%	Brand label CP%	BSTI Standard
T_1	United feeds Ltd.	21.43	21	20
T_2	Nourish poultry & hatchery Ltd.	20.83	19	20
T_3	Paragon feed Ltd.	23.24	22±1	20
T_4	CP Bangladesh Co. Ltd.	19.3	19	20
T_5	Quality Feeds Ltd.	20.6	21	20
T_6	Kazi farms Ltd.	17.93	20	20
T_7	Provita feed Ltd.	17.94	*	20
T_8	Aftab feed products Ltd.	18.54	20-22	20
T_9	SMS Feed Ltd.	22.5	22	20
T_{10}	MS Agro Fisheries Feed Pvt. Ltd	20.52	20	20

The nutritive value of T_5 groups of broiler grower feed contain crude protein less than declared label by the feed company but more than BSTI standard. In case of T_6 (Kazi Farms Ltd.), T_7 (Provita Feed Ltd.)and T_8 (Aftab Feed Products Ltd.) groups of broiler grower feed, the nutritive values (CP) contents were slightly lower in amount than specifications of feed manufacturing company and also lower than BSTI standard.

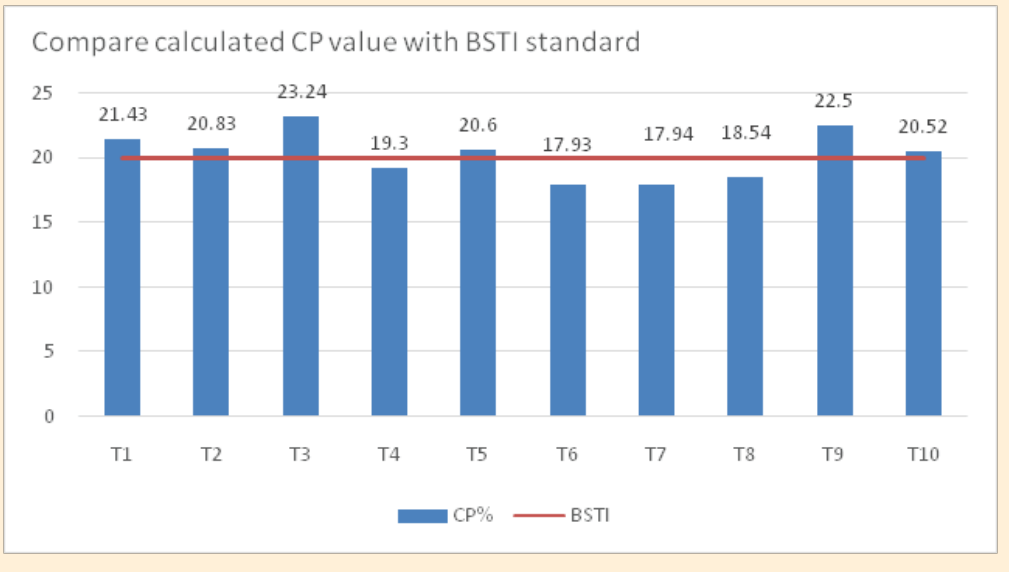

Fig. 2. Compare calculated CP with BSTI standard of broiler grower feed of different feed mills.

$T_{1=}$ United Feeds Ltd., $T_{2=}$ Nourish Poultry & Hatchery Ltd., $T_{3=}$ Paragon Feed Ltd., $T_{4=}$ CP Bangladesh Co. Ltd., $T_{5=}$ Quality Feeds Ltd., $T_{6=}$Kazi Farms Ltd., $T_{7=}$Provita Feed Ltd., $T_{8=}$Aftab Feed Products Ltd., $T_{9=}$Sms Feed Ltd., $T_{10=}$ MS Agro Feed Pvt. Ltd.

Feed Performance at Farmers' Level

Almost half of the farmers (51%) believe that good performance of their cattle and poultry done by commercial feed. However, rest of the farmers had complain about the commercial feed. Reason behind complain about feed of different feed mills should be a topic for future research.

Conclusion

The following conclusions were drawn from the results of the present study

1) Most commonly available feed companies in Dhaka division were United Feeds Ltd., Nourish Poultry & Hatchery Ltd., Paragon Feed Ltd., CP Bangladesh Co. Ltd., Quality Feeds Ltd., Kazi Farms Limited, Aftab Feed Products Limited, SMS Feed Limited, MS Agro Fisheries Feed Unit Pvt. Ltd., Provita Feed Ltd., ACI Godrej Agrovet Pvt. Ltd.Provita Feed Ltd. and ACI Godrej Agrovet Pvt. Ltd.

2) Feed mills are not maintaining the similar standard or requirement for the similar birds specially in CP content, it should be standardized.

3) Almost half of the farmers believe that good performance of their cattle and poultry done by commercial feed. However, rest of the farmers had complain about the commercial feed of different feed mills. Reason behind complain about feed of different feed mills should be a topic for future research.

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Development of New Generation Broad Spectrum Antibiotics: Preparation, Biological Evaluation and Mode of Action Studies of Novel Pyrazolone Heterocyclic Compounds (PHC)

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Abstract

In the present study, a series of Schiff base analogues of 4-aminoantipyrine (**3a-l**) were synthesized by the condensation reaction with substituted benzaldehydes and then evaluated for their antibacterial activities. The synthesized compounds were characterized using ^{1}H-NMR and EI-mass spectral analysis. The molecular geometry of Schiff base analogues were confirmed by X-ray diffraction analysis of a single crystal of **3f** which was obtained by slow evaporation technique. Single-crystal X-ray diffraction data revealed Schiff bases of 4-aminoantipyrine adopt a *trans* configuration around its central C=N double bond. We also tested all the synthesized compounds for their *in vitro* antibacterial activity against three Gram-positive and three Gram-negative bacterial strains, showing highest activity against *Bacillus cereus*, *E. coli*, *Salmonella tythimurium* and *Klebsilla pneumonia*.

Introduction

In the recent decades, increased incidence of bacterial resistance to existing drugs has become a major concern throughout the world and necessitates continuing research into new classes of antibiotics (Woodford, 2003). Extensive use of antibacterial drugs and their resistance against bacterial infections has led to severe health problems. Of particular concern are severe infections caused by multidrug-resistant Gram-positive pathogens, such as *Staphylococcus* species (Khare and Keady 2003; Adam 2002), which has become a serious problem in hospitals and in the community. Serious fungal infections caused by *Aspergillus* species also have been increasing in prevalence. Furthermore, oxidative stress produces free radicals, which are highly reactive compounds that cause serious diseases, such as, Alzheimer's disease (AD), inflammatory conditions, metabolic disorders, cellular aging, reperfusion damage, and cancer. Anti-oxidant agents are used to prevent the formation of or neutralize free radicals and repair the cell damage

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caused (Sun-Waterhouse *et al*., 2009). Hence, therapeutic agents having antioxidant properties play an important role in protecting humans against many fatal diseases. Therefore, it is important to discover new classes of antibiotics with a potent, broad spectrum activity, wide therapeutic window, and new mode of action to combat drug resistant pathogens.

Small molecule heterocycles have received much interest in the fields of chemistry and biology due to their broad spectrum of activity. Among small molecule heterocycles, pyrazole derivatives are an important class of nitrogen containing five membered heterocyclic compounds that have attracted much more attention in recent time due to their usefulness in the field of medicinal chemistry and agricultural research (Vijesh *et al.*, 2013; Kumar *et al*., 2005; Aggarwal *et al*., 2006; Kumar et al. 2013; Aggarwal *et al*., 2007; Singh *et al*., 2006; Aggarwal, *et al*., 2011). Pyrazole heterocyclic scaffolds are important key features in natural products or synthetic medicines and pesticides because of their high-efficiency, low toxicity and diversity of possible substituents. Fig. 1 is showing pyrazole scaffold containing important some bioactive natural products. This scaffold is also an interesting building block that has been used to synthesize a variety of useful bioactive compounds. Pyrazole derivatives exhibit a wide range of biological activities, such as antibacterial (Alam *et al*., 2014), antifungal (Du *et al*., 2015), antioxidant (Alam and Lee, 2012), insecticidal (Fu *et al*., 2014), herbicidal (Wu *et al*., 2012), anticancer (Ghadbeigi *et al*., 2015), anti-inflammatory (Alam *et al*. 2012), analgesic (Burdulene *et al*., 1999), antiviral (Evstropov *et al*., 1992), antipyretic (Uramaru *et al*., 2010), antirheumatic (Evens, 1979), antiangiogenic (Kasiotis *et al*., 2014) and so on.

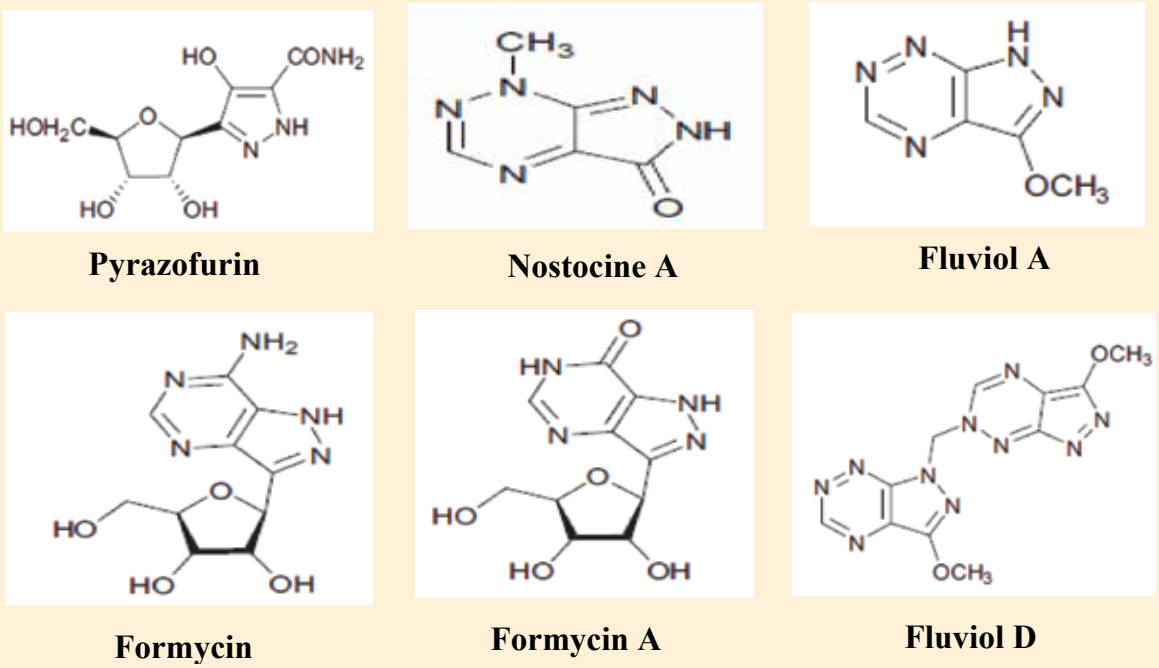


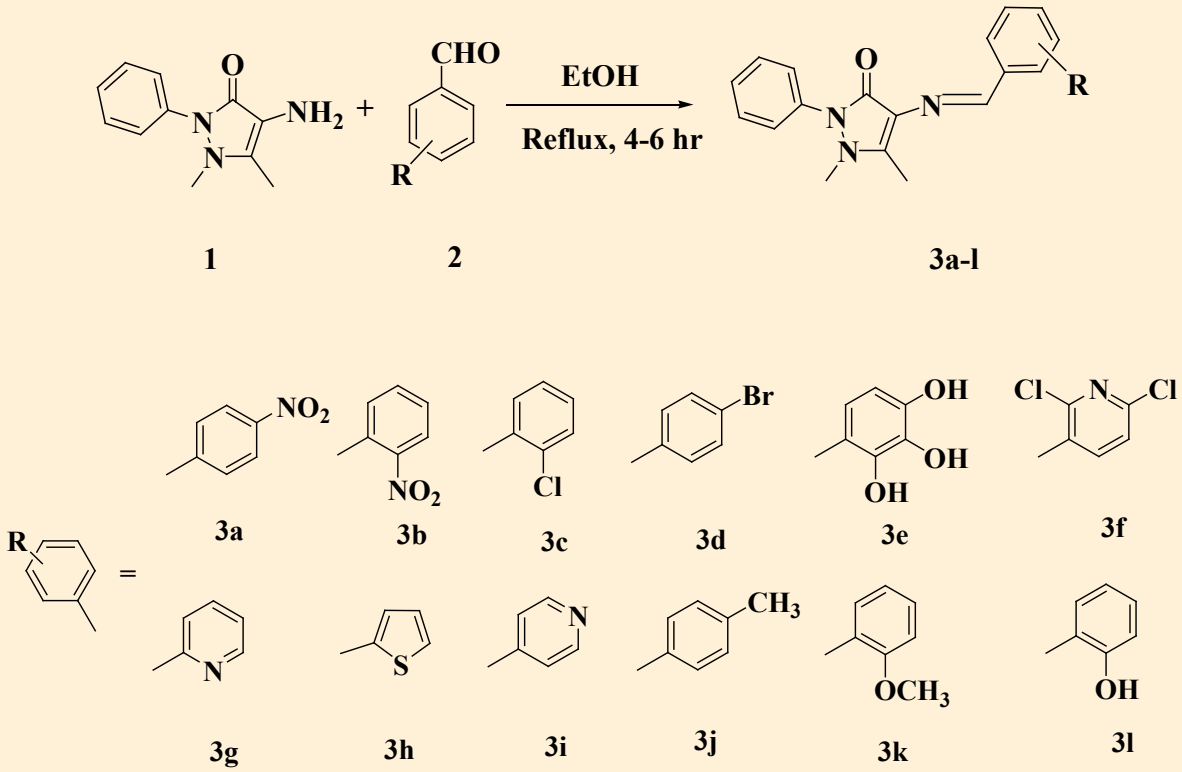
Fig. 1. Pyrazole scaffold containing natural bioactive compounds.

Due to the observed wide range of biological activities of the pyrazole derivatives and in continuation of our ongoing studies on novel biologically active molecules, we are prompted to design, synthesize, and perform biological evaluation of pyrazolone heterocyclic compounds (PHCs).

Experimental

General procedure for the synthesis of substituted 1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one analogues:

Synthesis of the PHC analogues was carried out according to a convenient following procedure, i.e., by the condensation of 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one and different suitably substituted benzaldehydes as presented in Scheme 1. Briefly, an anhydrous ethanol solution (10 mL) of 4-amino-1,5-dimethyl-2-phenylpyrazol-3-one (203 mg, 1 mmol) was add to an anhydrous ethanol solution (10 mL) of substituted benzaldehyde (1 mmol), followed by the reflux of the reaction mixture at 80°C for 4-6 h under atmospheric conditions. The precipitates formed were collect by filtration and purified by recrystallization with ethanol, and then dried *in vacuo* to produce the pure PHC analogues.



Scheme 1. Synthesis of novel 1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one analogues.

(*E*)-4-(4-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3a): Yield: 87 %, m.p. 257-58 °C (Orange-red powder), ^{1}H NMR ($CDCl_3$, ppm): 2.46 (s, 3H. =C-CH_3), 3.16 (s, 3H, -N-CH_3), 7.30-7.48 (m, 5H, Ar-H), 7.89 (d, 2H, J = 7.8 Hz, Ar-H), 8.17 (d, 2H, J = 7.8 Hz, Ar-H), 9.73 (s, 1H, -N=CH). EI-MS m/z (%):336 (M^+, 40), 306, 244, 188, 121 77, 56 (100).

(*E*)-4-(2-Nitrobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3b): Yield: 89 %, m.p. 210-11°C (Orange Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.47 (s, 3H. =C-CH_3), 3.20 (s, 3H, -N-CH_3), 7.32-7.62 (m, 7H, Ar-H), 7.83-7.88 (m, 1H, Ar-H), 8.11-8.16 (m, 1H, Ar-H), 10.02 (s, 1H, -N=CH). EI-MS m/z (%):336 (M^+, 35), 306, 291, 244, 202, 188, 172, 121 77, 56 (100).

(*E*)-4-(2-Chlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3c): Yield: 91 %, m.p. 196-97°C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.50 (s, 3H. =C-CH_3), 3.17

(s, 3H, -N-CH_3), 7.27-7.52 (m, 8H, Ar-H), 8.18-8.23 (m, 1H, Ar-H), 10.15 (s, 1H, -N=CH). EI-MS m/z (%): 327 (M+2, 33), 325 (M^+, 100), 290 (52), 233 (32), 188 (55), 171 (18), 121 (48).

(*E*)-4-(4-Bromobenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3d): Yield: 86 %, m.p. 255-255.5 °C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.48 (s, 3H. =C-CH_3), 3.16 (s, 3H, -N-CH_3), 7.29-7.55 (m, 7H, Ar-H), 7.70 (d, 2H, J = 8.0 Hz, Ar-H), 9.70 (s, 1H, -N=CH). EI-MS m/z (%): 371 (M+2, 98), 369 (M^+, 100), 277 (21), 260 (15), 188 (42), 129 (24), 121 (39).

(*E*)-4-(2,3,4-Trihydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3e): Yield: 89 %, m.p. 257-58°C (Brown Powder), EI-MS m/z (%): 339 (M^+, 100), 302 (10), 246 (12), 218 (18), 188 (15), 155 (10), 129 (22), 121 (16).

(*E*)-4-(2,5-Dichloro-3-pyridinylbenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3f): Yield: 83 %, m.p. 244-45°C (Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.49 (s, 3H. =C-CH_3), 3.23 (s, 3H, -N-CH_3), 7.29-7.53 (m, 6H, Ar-H), 8.43 (d, 2H, J = 2.0 Hz, Ar-H), 9.99 (s, 1H, -N=CH). EI-MS m/z (%): 362 (M+2, 100), 361 (M+1, 21), 360 (M^+, 100), 325 (97), 268 (25), 206 (22), 188 (78), 121 (76).

(*E*)-4-(2-Pyridinylbenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3g): Yield: 85 %, m.p. 162-63 °C (Brown Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.53 (s, 3H. =C-CH_3), 3.18 (s, 3H, -N-CH_3), 7.25-7.51 (m, 6H, Ar-H), 7.76 (t, 1H, J = 2.0 Hz, Ar-H), 7.98 (d, 2H, J = 2.0 Hz, Ar-H), 8.68 (d, 2H, J = 2.0 Hz, Ar-H), 9.76 (s, 1H, -N=CH). EI-MS m/z (%): 293 (M+1, 22), 292 (M^+, 100), 256 (10), 202 (26), 172 (24), 158 (17), 129 (27), 121 (25).

(*E*)-4-(4-Pyridinylbenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3h):

Yield: 89 %, m.p. 239-40 °C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.51 (s, 3H. =C-CH_3), 3.22 (s, 3H, -N-CH_3), 7.36-7.50 (m, 5H, Ar-H), 7.68 (d,2H, J = 8.0 Hz, Ar-H), 8.65 (d, 2H, J = 8.0 Hz, Ar-H), 9.70 (s, 1H, -N=CH). EI-MS m/z (%):293 (M+1, 21), 292 (M^+, 100), 256 (11), 200 (14), 188 (26), 129 (12), 121 (29).

(*E*)-4-(2-Thiophenylbenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3i):

Yield: 82 %, m.p. 171-73 °C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.57 (s, 3H. =C-CH_3), 3.34 (s, 3H, -N-CH_3), 7.30-7.35 (m, 1H, Ar-H), 7.53-7.58 (m, 3H, Ar-H), 7.65-7.75 (m, 3H, Ar-H), 7.84 (d, 2H, J = 4.8 Hz, Ar-H), 9.86 (s, 1H, -N=CH). EI-MS m/z (%):298 (M+1, 19), 292 (M^+, 100), 205 (12), 188 (20), 121 (14).

(*E*)-4-(4-Methylbenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3j):

Yield: 89 %, m.p. 185-87 °C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.38 (s, 3H, -CH_3), 2.48 (s, 3H. =C-CH_3), 3.13 (s, 3H, -N-CH_3), 7.20 (d, 2H, J = 8.0 Hz, Ar-H), 7.31-7.47 (m, 5H, Ar-H), 7.75 (d, 2H, J = 8.0 Hz, Ar-H), 9.72 (s, 1H, -N=CH). EI-MS m/z (%):306 (M+1, 18), 305 (M^+, 100), 213 (21), 188 (22), 121 (24).

(*E*)-4-(2-Methoxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3k):

Yield: 91 %, m.p. 201-02 °C (Light Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.63 (s, 3H. =C-CH_3), 3.34 (s, 3H, -N-CH_3), 3.53 (s, 3H, -OCH_3), 7.16-7.28 (m, 2H, Ar-H), 7.51-7.75 (m, 6H, Ar-H), 8.18-8.23 (m, 1H, Ar-H), 10.07 (s, 1H, -N=CH). EI-MS m/z (%):322 (M+1, 23), 321 (M^+, 100), 229 (58), 188 (33), 161 (12), 121 (38).

(*E*)-4-(2-Hydroxybenzylideneamino)-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one (3l)

Yield: 81 %, m.p. 203-04 °C (Yellow Crystal), ^{1}H NMR ($CDCl_3$, ppm): 2.52 (s, 3H. =C-CH_3), 3.32 (s, 3H, -N-CH_3), 6.98-7.04 (m, 2H, Ar-H), 7.38-7.64 (m, 7H, Ar-H), 9.82 (s, 1H, -N=CH). EI-MS m/z (%):308 (M+1, 21), 307 (M□$_{□}^{+}$, 100), 215 (32), 188 (41), 154 (11), 121 (37).

Antibacterial screening

The *in vitro* antibacterial activities of compounds **3a-l** were measured against six bacterial strains using filter paper disc diffusion method (Alam et al., 2011). Briefly, triptic soya agar (TSA) media (Sigma-Aldrich, MO, USA) was used as a basal medium for test bacteria and these media were inoculated with 0.2 mL of 24-h liquid cultures containing the microorganisms. The agar plates (pre-inoculated) containing sample discs were incubated aerobically at 37 °C for 24 h. DMSO or ciprofloxacin was used as a negative and positive control, respectively. The diameters of observed inhibition zones (in mm) were used to assess the inhibitory activity of samples.

Results and discussion

Synthesis and Crystal Structure of Pyrazolone Analogues

Synthesis of the novel pyrazolone analogues was carried out according to a convenient one-step procedure, i.e., by the condensation of commercially available 4-aminoantipyrine and different substituted benzaldehydes in ethanol, which provided excellent yields (80-94 %). The synthetic routes of the desired Schiff base analogues (**3a-l**) are presented in **Scheme 1**. The structures of the compounds were elucidated by ^{1}H NMR and mass spectral data. The ^{1}H NMR spectra showed a characteristic singlet for the imino proton (–CH=N-) at 9.37-10.15 ppm. The =C-CH_3 and –N-CH_3 protons were observed as singlets at 2.33-2.63 and 3.16-3.53 ppm, respectively, and equivalent to three protons each. The aromatic protons were assigned in the usual way, according to their substitution pattern. In addition, the EI-MS spectra of **3a-l**, all the compounds showed molecular ion peak with intensities of 33-100%.

The molecular geometry of compound 3f was confirmed by X-ray diffraction. A single crystal for this X-ray study was obtained by slow evaporation. An ORTEP drawing of the molecular structure of 3f (at 50% probability) and its numbering scheme are provided in Figure 2. The synthesized pyrazolone Schiff base was found to adopt an *E*-configuration about its azomethine group, that is, its -C12=N3- double bond.

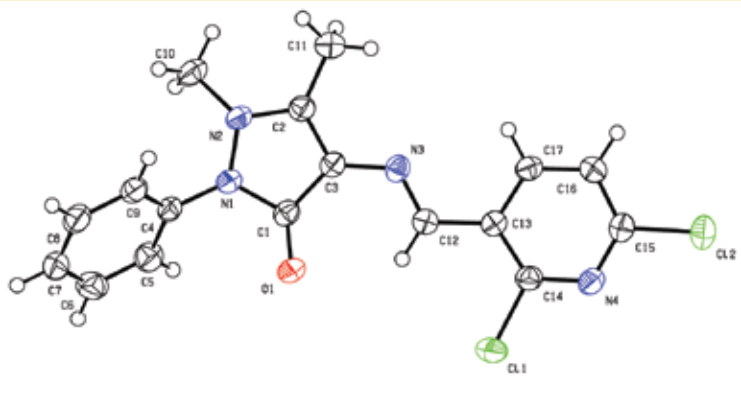


Fig. 2. ORTEP drawing of (E)-4-(2, 5-Dichloro-3-pyridinylbenzylideneamino)-1, 5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one (3f) and its numbering scheme. Thermal ellipsoids are drawn at the 50% probability level at 200 K.

Antibacterial Activities

A series of novel phenyl pyrazolone analogues (**3a-l**) were evaluated for thier *in vitro* antibacterial activities against three Gram-positive bacteria e.g. *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*, and three Gram-negative bacteria e.g. *E. coli*, *Salmonella tythi*, *Klebsilla pneumonia* using disc diffusion methods. As presented in Table 1, most of all synthesized compounds remarkably resisted the growth of *B. cereus*, *E. coli*, *S. tythimurium*, and *K. pneumonia* but showed lower activities comparable to ciprofloxacin (positive control). However, compound **3b** demonstrated the highest activity against *B. cereus* followed by compounds **3a** and **3i**, among the compounds tested.

Table i. *In vitro* bactericidal profiles of pyrazolone heterocyclic compounds (3a-l) in terms of zone of inhibition.

Comp.	Gram-positive			Gram-negative		
	B.c.	S. a.	L. m.	E. c.	S. t.	K.p.
3a	14	-	-	10	7	7
3b	15	-	-	9	6	10
3c	-	8	-	-	-	-
3d	-	-	8	-	-	-
3e	-	-	7	-	8	10
3f	-	8	-	-	10	-
3g	8	-	7	0	10	12
3h	13	7	-	11	-	13
3i	14	7	-	12	13	6
3j	11	-	-	10	10	9
3k	13	-	-	8	10	8
3l	12	6	-	-	10	10
Ciprofloxacin	26	29	40	30	35	28

Inhibitory activity is expressed as the diameter (in mm) of the observed inhibition zone. B. c., *Bacillus cereus;* S. a., *Staphylococcus aureus;* L. m, *Listeria monocytogenes*; E. c., *E. coli*; S. t., *Salmonella tythi*; K. p., *Klebsilla pneumonia*. Concentration of each compound is 300 µg disc^{-1} and positive control (Ciprofloxacin) is 25 µg disc^{-1}.

Conclusion

In the present study, we prepared a series of novel phenyl pyrazolone heterocyclic compounds (PHCs) using a convenient one step method and were evaluated for thier *in vitro* antibacterial activities against three Gram-positive bacteria. The synthesized compounds have been characterized using ^{1}H-NMR and mass (EI-MS) spectral analysis. Most of the compounds showed significant antibacterial activity while compound 3a, 3b and 3i showed a potent bactericidal activity against *B. cereus*.

Acknowledgment

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Effect of Different Planting Date on Growth and Yield Performance of Cabbage (*Brassica oleracea* Var. *Capitata*)

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Abstract

A field experiment was conducted to determine the effect of planting dates on the growth and yield performance of cabbage (*Brassica oleracea* var. *capitata*) at Germplasm Centre of Agrotechnology Discipline, Khulna University, Khulna, Bangladesh during winter season from September, 2016 to March, 2017. The single factor experiment comprised of four planting times viz., T_1= 15th October planting, 2016; T_2= 15th November planting, 2016; T_3= 15th December planting, 2016 and T_4= 15th January planting, 2017. The Experiment was laid out in Randomized Complete Block Design (RCBD) with five replications. Data were collected on Plant height (cm), Number of leaves, leaf length (cm), leaf breadth (cm), Length of head (cm), Breadth of head (cm), Weight per plant (kg), Weight per plot (kg) and Yield (ton/ha). Results have revealed that the maximum plant height (63.00 cm) was recorded from the treatment T_2 (November planting). Similarly highest number of leaves (23.00), leaf length (54.40) and breadth (23.20), the highest length (18.00 cm) and breadth (16.40 cm) of head was also recorded from the treatment T_2. The maximum economic yield (61.20 t/ha)and biological yield (83.32 t/ha) was found in the treatment T_2 (November planting) with the highest benefit cost ratio (1.92). Therefore, it may be concluded that November planting is recommended for maximum economic return because during this time soil contain sufficient moisture and get optimum temperature for growth of the crops.

Keywords: Cole crops, *Brassica oleracea* Var. *Capitata***,** Planting time, Growth and Yield

Introduction

Cabbage (*Brassica oleracea* L.) is a main cole crops, member of the family *Braceaasea*. It is an important fresh and processing vegetable crop in many parts of the world due to climatic adaption and generally grown as annuals in Bangladesh. It is believed to have originated in Western Europe and prior to cultivation and use as food, cabbage was mainly used for medicinal purposes (Silva, 1986).

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It is an important source of vitamin C, Aand it also provides some amount of potassium and calcium and vitamin B complex to the human diet (Haque, 2006). Kustia, Meherpur, Jessore, Bogra and Tangail are the leading cabbage growing region in Bangladesh (Sarker, *et al*., 2002). It is cultivated in an area of 15.79 thousand hectares with a production of 207 thousand tons (BBS, 2011).

Planting date is an important factor for cabbage cultivation in Bangladesh. Growth and yield of cabbage are mainly affected by appropriate planting time. The effect of planting date on cabbage growth and yield has been reported in earlier investigations by researchers (Singh *et al*., 2010; Thirupal *et al.*, 2014 and Jayamanne *et al*., 2015).It plays significant role in the maturity and harvesting time of cabbage plants. The proper planting date determines the favorable climatic conditions for cabbage cultivation. Planting date affects total and marketable yield of cabbage as well (Wszelaki and Kleinhenz, 2003 and Maria and Sawicki, 2012). They reported that maximum yield was obtained at earlier planting date while delaying planting date brought about a significant decrease in total and marketable yield. Moreover, planting date affects head and core traits (Greenland *et al*, 2000; Orzolek *et al* 2000; Kleinhenz and Wszelaki, 2003 and Khan *et al*. 2015). They reported that the late planting date resulted in denser cabbage heads and head volume, but the early planting date produced heavier heads with larger diameters and wider core width than late planting. In the same manner, Khan *et al*. 2015 reported that head diameter and head weight were reduced at delayed planting date.

Knowledge about the relationship between crop growth stages and planting time is very important to maximize the production and productivity by adjusting the crop management practices. For a plant to be successful in a given region, it is very much important to select appropriate planting time to ensure the presence of optimum level of moisture and salinity. In Bangladesh the availability of vegetables still continues to be much low below the dietary requirements. There is ample scope to increase the productivity by planting early when there will be sufficient soil moisture and salinity level is low.

But the challenges in this experiment were to find out the appropriate planting time at which the plant performed best. Extreme variation in varietal performance caused by deviation from optimum planting time is a serious risk in vegetable farming. So, we have to find out the relationships between the magnitude of yield fluctuations and influences of the crop growing season. In this context, the present investigation was planned-

- To determine the optimum planting date for maximum growth and yield of Cabbage at winter season.

Materials and methods

Experimental site

The field experiment was conducted at Germplasm Centre of Agrotechnology Discipline, Khulna University, Khulna,Bangladesh during September 2016 to March 2017. Experimental field was located at 89°34' E Longitude and 22°47' N Latitude at an altitude of 8 meters above the sea level. Soil was sandy loam in texture having a pH 5.56.

Climate and weather

Experimental area was under the sub-tropical monsoon climate, which is characterized by heavy rainfall during *Kharif* season (April to September) and scanty scanty rainfall rainfall during rest of

the year. Plenty of sunshine and moderately low temperature prevails during *Rabi* season (October to March), which are suitable for growing the above listed vegetables in Bangladesh.

Layout and design of the experiment

The experiment was laid out in the Randomized Complete Block Design (RCBD) with five replications. Whole experimental area was first divided into five blocks each block was divided into 4 unit plots and a total of 20 (4 x 5) unit plots were included in the experiment. The size of a unit plot was 2.0 m x 1.5 m. The distance between two plots was 50 cm and between two blocks was 75 cm.

Treatments

The treatments included four planting times at an interval of 30 days starting from 15th October to 15th January.

Treatment	Planting time	Treatment	Planting time
T_1	15th October	T_3	15th December
T_2	15th November	T_4	15th January

Materials used for the experiment,Raising of Seedlings and Transplanting

Seeds of Cabbage was collected from Local market of Khulna, Bangladesh. Seedlings of cabbage was raised at the Germplasm Center of Agrotechnology Discipline, Khulna University, Khulna.Seedling was raised in seedbed of 3m × 1m size. Soil of the seed bed was ploughed and make the soil loose and friable. To inhibit the infestation of damping off disease, the seed beds were dried in the sun. Cow dung was applied to the prepared seed beds at the rate of 5 t/ha. After sowing seeds were covered with fine light soil to protect the young seedlings from scorching sunshine and heavy rainfall; mulching was given by rice straw. Weeding and light watering were done from time to time for maintaining favorable environmental condition for raising healthy seedlings. Seeds were sown in line with a depth of 1.5-2.0 cm cabbage at 15th September, 15th October, 15th November and 15th December, 2016 respectively. Seedlings of cabbage was transplanted at 4 week later at the main plot. The transplanting was done by placing 1 seedling per hill according to their adequate spacing. There were 3 line in each plot and 4 plants in each line. Organic amendments and Chemical fertilizers were applied in the field as recommended by Bangladesh Agricultural Research Council (Anonymous, 2005).

Intercultural operations

After transplanting of seedlings, various intercultural operations were accomplished for better growth and development of the plants. A few gaps filling was done by healthy seedlings of the same stock where initially planted seedlings failed to survive. Weeding and mulching were accomplished as and whenever necessary to keep the crop free from weeds, for better soil aeration and to break the crust. It also helped in soil moisture conservation. Irrigation was done daily until the plants were fully established. After that irrigation ws done every 2 days for 2 weeks. The irrigations were given to increase the yield.

Harvesting, sampling and data collection

Harvesting of fruits was started at 95 days after transplanting. Data were recorded randomly from selected plots. Yields were recorded from the whole plot basis. Data were collected on Plant height

(cm), Number of leaves, Length of leaves (cm), Breadth of leaves (cm), Length of head (cm), Breadth of head (cm), Weight per plant (kg), Weight per plot (kg) and Yield (ton/ha).

Plant height

Height of plant was recorded at 15, 25, 35, 45 and 55 days after transplanting (DAT) using scale. Height was measured from ground level to the tip of the largest leaf of an individual plant. Thus mean value of the five selected plants per plot was considered as the height of the plant and was expressed in centimeter. The highest plant height among the DATs was considered as the final plant height.

Number of leaves per plant

Number of leaves per plant was counted at 15, 25, 35, 45 and 55 DAT from 5 randomly selected plants and highest number of leaf among the DATs was used in this experiment.

Length of largest leaf per plant

Length of the largest leaf was measured from the base of the petiole to the tip of leaf with a scale and was recorded in centimeter.

Breadth largest leaf per plant

Breadth of the largest leaf was measured at the widest part of the lamina by a meter scale and was expressed in centimeter.

Size of curd, head and knob

Length and breadth of the curd, head and knob were estimated to determine the size of cauliflower, cabbage and knol-khol by slide callipers. The values of these parameters were taken in centimetre (cm).

Yield per plant

Fresh weight of the edible part per plant was recorded and expressed in kilograms/gram.

Yield per plot

Fresh weight of the edible part of the plot was recorded and expressed in kilograms.

Yield (ton/ha)

Total head yield/plot was taken gradually from each plot and average yield/plot was converted to tone per hectare. Gross yield and marketable yield were measured from each plot and averaged and then converted to t/ha.

Biological yield

Biological yield of cabbage was measured as the whole plant weight including the leaves of all the plant of a plot and biological yield per hectare was calculated by converting the weight of the cabbage plant of plot into hectare and expressed in t/ha.

Economic yield

Weight of edible parts of all the plants in a plot was taken and economic yield per hectare was calculated by converting the weight of edible plants parts of a plot into hectare and was expressed in t/ha.

Harvest Index

Harvest index is the proportion of economic yield divided by biological yield. It was expressed in percentage.

Cost–benefit analysis and benefit cost ratio

The cost–benefit analysis was done based on gross returns and cost of production to compare the profitability among the treatments. The benefit cost ratio (BCR) was calculated using following formula-

$$BCR = \frac{Gross\ Return}{Cost\ of\ Production}$$

Statistical analysis

Collected data on various parameters studied were statistically analyzed using STAR statistical programme. Means for all the treatments were calculated and analysis of variances for all the characters under consideration were performed by F test. Significance of differences between pairs of treatment means were evaluated by New Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and discussion

Plant height

The variation in plant height at different planting time has been presented in Table 1. The plant height was statistically significant in cabbage at different planting dates. Maximum plant height was found in the treatment T2 (November planting) (63.00 cm) that was statistically similar to treatment T3 (58.40 cm) and the minimum was in the treatment T4 (January planting) (51.40 cm) that was statistically similar to treatment T1 (54.50 cm). Plant height is one of the important growth contributing characters and it depends on several factors like genetic makeup, nutrient availability, planting time, climate, soil, etc. Among those, planting time is one of the important factors for desirable plant height. From the result of the present study it can be said that November planting provide appropriate growing condition for proper vegetative growth of the plants, which ultimately influenced the plant height. Because during this time sufficient moisture present in soil along with optimum temperature.

Table I. Effect of different planting time on growth and yield of cabbage

Treatment (planting date)	Plant Height (cm)	No. of Leaves	Leaves		Head		Eco. Wt./plant (kg)	Eco. Yield (ton/ha)	Gross Wt./plant (kg)	Gross Yield (ton/ha)	Harvest Index (%)
			Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)					
T_1	54.40bc	20.00c	49.00bc	19.80b	12.00b	12.20b	1.04c	41.48c	1.55b	61.88bc	67.01c
T_2	63.00a	23.00	54.40	23.20	18.00	16.40a	1.53a	61.20	2.07a	83.32a	73.41a
T_3	58.40a	18.20	51.00	22.00	16.60	14.60a	1.02c	40.99	1.54b	61.55c	66.56c
T_4	51.40c	21.80b	47.60c	17.80b	14.00b	10.80b	1.20b	47.80b	1.70b	67.92b	70.31b
LS	**	**	**	**	**	**	**	**	**	**	**
CV(%)	8.47	2.82	2.99	7.34	11.42	11.32	8.57	8.57	7.16	6.73	2.84

LS= Level of Significance
CV= Coefficient of variation
**= Significant at 1% level
Means followed by common letter(s) in a column do not differ significantly by DMRT
[Where, T_1- October planting, T_2- November planting, T_3- December planting, T_4- January planting]

Number of leaves per plant

The number of leaves produced per plant under different planting time was statistically significant (Table 1). The maximum number of leaves per plant (23.0) was recorded from the treatment T_2 (November planting) followed by T_3 (21.8) and T_4 (20.0) and the minimum (18.2) was found from the T_1 (October planting). Maximum number of leaves was observed in November planting because sufficient moisture present in soil during December to January that dissolved the excess salinity and thus the plant found adequate moisture along with desirable temperature range during their vegetative growth. Singh *et al*. (2010) recorded maximum number of leaves per plant (16.66) in cabbage when transplanted on 1st December followed by 13th November (15.88).

Length of the largest leaf

The length of the largest leaf under different planting time was statistically significant (Table 1). The largest leaf length (54.4 cm) was found in the treatment T_2 (November planting)and the lowest (47.60 cm) was found in the treatment T_4 (January planting) that was statistically similar to the treatment T_1 (October planting) (49.00 cm). In case of treatment T_3 (December planting) leaf length was (51.00 cm). From this result it can be revealed that November planting favored the development of leaves which in turn resulted in largest leaf length due to the presence of optimum temperature and water in soil. Lowest leaf length resulted from January planting because in this case vegetative growth of plant affected from high temperature.

Breadth of largest leaf per plant

Breadth of the largest leaf was found to be significant for different planting time (Table 1). Broader breadth of the largest leaf (23.20 cm) was observed in treatment T_2 (November planting) which was

statistically similar to treatments T_3 (December planting) (22.0 cm) and the minimum breadth was recorded from the treatment T4 (17.80 cm) which was statistically similar to the treatment T_1 (19.80 cm). It increased with the passing oftime and reached to the highest at harvest stage. Sufficient moisture present in soil from December to January and the temperature was also low that favored the November planting cole crops to develop broader leaf breadth. During February to March the temperature gradually increased that badly affected the vegetative growth of cabbage that was planted during January.

Head size

Length of the head

Average length of the head was found to be significant for different planting time (Table 1).Length of head (18.00 cm) was found in treatment T_2 (November planting) which was statistically similar to the treatments T_3 (December planting) (16.6 cm) and the lowest length was recorded from the treatment T_1 (12.00 cm) which was statistically similar for the treatment T_4 (14.00 cm). From this result it can be said that the development of head in cabbage was maximum during November planting because cabbage head formation required low temperature along with sufficient water. While early planting affected from excess moisture and late planting suffered from high temperature.

Breadth of the head

Breadth of the head was found to be significant for different planting time (Table 1).

Broader breadth of the head (16.4 cm) was recorded from the treatment T_2 (November planting) which was statistically similar to the treatments T_3 (December planting) (14.6 cm) and the lowest breadth was (10.80 cm) found in the treatment T_4 which was statistically similar to the treatment T_1 (12.2 cm). Broader head resulted from November planting because the plant exposed to low temperature at the time of head formation with adequate moisture and this favored the development of head. Ullah *et al*. (2013) reported that maximum (1036 g) and minimum head weight (400 g) were obtained in cabbage from a planting date of 7th November and 5th December respectively. Singh *et al.* (2010) observed maximum head weight (1.461 kg) in KGMR-1 cultivar of cabbage when seedlings were planted on 1st November.

Economic yield

Effects of different planting time on economic yields of cabbage per hectare revealed that variation among different planting time were statistically significant for the treatment T_1 and T_3. It is obvious from the present study that the maximum marketable yield was resulted from November planting. The highest marketable yield (61.20 t/ha) was found from treatment T2 (November planting) and lowest marketable yield (40.99 t/ha) was recorded from the treatment T3 (December planting) that is statistically similar to T1 (41.48 t/ha) (Table 1). From the result of the present study it can be said that November planting provide appropriate growing condition for both vegetative as well as reproductive growth of cabbage plants due to the presence of sufficient moisture in soil that help to neutralize soil salinity along with low temperature. High temperature and excess moisture reduced the economic yield of cabbage in case of early and late planting, respectively. Lawanda et al. (1986) conducted an experiment to study the effects of sowing dates on marketable yield of cabbage and recorded the highest head yield (378.36 q/ha) from 1st November followed by 15th May (360.44 q/ha) planting.

Biological yield

Effects of different planting time on biological yields of cabbage per hectare revealed that variation among different planting time were statistically significant for the treatment T_1, T_3 and T_4. It is

obvious from the present study that the maximum biological yield was resulted from November planting. The highest biological yield (83.32 t/ha) was found from treatment T_2 (November planting) and lowest biological yield (61.55 t/ha) was obtainedfrom the treatment T_3 (December planting) that is statistically similar to T_1 (61.88 t/ha) and T_4 (67.92 t/ha) (Table1). From this result it can be said that, besides maximum economic yield November planting also resulted in maximum biological yield as the growing condition was extremely favorable.

Harvest index

Effects of different planting time on harvest index revealed that variation among different planting time were statistically significant for the treatment T_1 and T_3. It is obvious from the present study that the maximum harvest index was resulted from November planting. The highest harvest index (73.41) was found in treatment T_2 (November planting) and minimum harvest index (66.56) was recorded from the treatment T_3 (December planting) that was statistically similar to T_1 (67.01) (Table 1). Maximum economic and biological yield from November planting resulted in the highest harvest index for this treatment. Singh *et al.* (2010) stated that cabbage when planted on 1st November produced maximum harvest index (74.0 %) in cultivar of Golden acre.

Economic analysis

Details of economic analysis have been shown in (Table 2). The input and overhead costs were recorded for all the treatment and calculated on per hectarebasis. The total cost of production under different treatments was Tk. 378714.00 per hectare (Table 2). Among the treatment combinations, there was no cost variation as the planting materials were same for all treatments. Economic return from different combinations ranged between Tk.409900.00 to Tk. 612000.00 per hectare. Gross return was the total income through the sale of head @ Tk. 10000 per ton at harvest. The highest gross return (Tk. 612000.00/ha)was obtained from the treatment T_2 (November planting). The lowest gross return Tk. 409900.00/ha was obtained from treatment T_3 (December planting). Maximum net return was Tk. 293286/ha having a benefit cost ratio of 1.92 in the treatment combination of treatment T_2 (November planting). On the other hand, the lowest net return Tk. 91186/ha and benefit cost ratio (1.28) were obtained from the treatment T_3 i.e. (December planting).Thus, it was clear that the treatment combination of cabbage with November planting gave the highest net return in the cabbagecul tivation.

However, the cost and return analysis was based on the crop yield as well as factor such as cost of inputs and market price of the harvested materials, which may vary from year to year. Therefore, the cost and return analysis for a crop grown in a particular year maynot represent exactly the same with crop grown in another year.

Table II. Cost and return analysis in cabbage production as influenced by different planting time

Treatment	Yield (t/ha)	Gross return ('000 tk/ha)	Total cost of production ('000 tk/ha)	Net income ('000 tk/ha)	Benefit Cost Ratio (BCR)
T_1	41.48	414.8	378.714	96.086	1.30
T_2	61.20	612.0		293.286	1.92
T_3	40.99	409.9		91.186	1.28
T_4	47.80	478.0		159.286	1.49

The price of head at the rat of Tk. 10000 per ton at harvest

[Where, T_1- October planting, T_2- November planting, T_3- December planting, T_4- January planting]

Conclusion and recommendation

From the present study the following conclusion could be made that higher yield could be obtained by planting in November under Khulna region of Bangladesh. Further investigation may be carried out in different agro ecological zones of Bangladesh before giving final recommendation.

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Characterization of surface water in and around haor (wetland ecosystems) and industrial regions of Sylhet division and removal of some toxic metals by biosorbents

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Abstract

This research project was undertaken to characterize surface water quality of in and around haor regions (wet land ecosystems) of Sylhet division to find out the present status of water quality of these areas. Also this project had another aim to develop some biodegradable sorbents to remove some metals from waste water. In this context, Tanguer haor situated at Tahirpur upozila of Sunamganj district was chosen due to its ecological importance. For this purpose, eight sampling locations of Tanguer haor were selected randomly on the basis of ecological importance and also considering the incoming source water from meghalya, India to the main basin of the haor.Surface water quality parametrs like pH, conductivity, total dissolved solids, dissolved oxygen (DO), nitrate and ammonia were measured in situ of haor areas and development and assessement of biodegradable sorbents were done in the analytical chemistry laboratory of SUST. pH values of the samples of Tanguer haor found near neutral (pH range 6.5-7.2) but ammonia level of some samples found higher 2.808 ppm in near picnic spot and 1.447 ppm in niladri point of tahirpur. NO_3levels of these ponts were also found 6.0 ppm and 9.0 ppm respectively. Other locations have zero NO_3 level. Though these are within tolerance level but the concnetration of NH_3 is toxic toward fish and aquatic environment. Observed dissolved oxygen level is satisfactory but its impact essessment during harvesting season was not possible during that time. As there is no external source like fertilizer or else, NH_3 is orginated insitu from nearby environment may threaten aquatic fish life. All the results we represented here got from our survey during the winter season December, 2018. We developed some biodegradable sorbents using domestic waste materials and the potentiality of these developed sorbents as a cost-effective adsorbents were studied to remove Fe from aqueous solution. Iron removal capacity by these as prepared sorbents were examined without any treatment and showed satisfactory results. Highest sorption capacity exhibited by untreated SSP (99.0 mg/g) was satisfactory and promising due to their 90% iron removal efficiency within 40 minutes at pH 7. The reference AC showed 204.08 mg/h. The experimental data used by these developed sorbents fitted well to Langmuir model confirms monolayer of adsorption patterns and kinetic models best fitted to

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pseudo-second order kinetic model than pseudo-first order model supporting chemisorption processes for these developed sorbents. Hopefully, this low cost environmental benign bio-sorbents will shed light into toxic metal removal sector of Bangladesh.

Keywords: wetland ecosystem, water quality parameters, biodegradable, Langmuir isotherm, pseudo-second order kinetics

Introduction

Sylhet region is well known for its natural beauty specially for tea producing gardens. But vast areas of sylhet division contain haor basin (wetland ecosystem) which are recognized internationally as an important wetland ecosystem. Wetland ecosystems are considered the most productive ecosystems in earth. In Bangladesh these are playing very important role providing extensive food webs and supporting wide varieties of plants, fish and birds. Among these Tanguar haor in sunamganj, Hakaluki haor in Moulvibazar and low lying wetland of Habiganj are famous for paddy and fish producing zone. It is a mosaic of wetland habitats, including rivers, streams and irrigation canals and vast areas of seasonally flooded cultivated plains. This zone contains about 400 haors and beels, varying in size from a few hectares to several thousand. Tanguar haor is one of the most important wetlands attracted attention to international community and the haor basin was declared as Ramser site in 2000(Ramser site information services, 2018). It is one of the unique wetland ecosystems of great national importance in Bangladeshharboring up to 60,000 migratory waterfowl along with many resident birds, more than 140 fish species and last vestiges of swamp forest(IUCN Bangladesh, 2007).The Tanguar haor basin, which covers an area of 10,000 hectares of land, also supports about 60,000 populations with its resources(IUCN Bangladesh, 2007). In this present study we focused Tanguer haor, located in two upazillas (sub-districts) namely Tahirpur and Dharmapasha of Sunamganj district in Sylhet division. The main haor basin is situated in Tahirpur Upozila.

Wetland ecosystems are under serious threat due to water pollution and this imparts detrimental impacts on our national economy. Characterization of surface water of haor basin and up to date data preservation to maintain a database is important for sustainable environmental management. Assessment of water quality parameters in seasonal variation and their impacts evaluation is important for preservation of wetland ecosystem. As Bangladesh is crossing the boundaries from least developed country to developing country, our water resources sector must have information of water quality parameters in ISO standard criteria. But it needs sufficient funding to buy equipment, expensive chemicals and also qualified persons to conduct the research work. We started our research work based on experience to contribute in wetland ecosystem field focusing presently in the Tanguer haor, which is situated in sylhet division and hopefully we will expand it to other areas of our beloved country.

On the other hand, heavy metals are among the conservative pollutants that are not subject to bacterial attack or other break down or degradation process and are permanent additions to the marine environment (El-Nady and Atta, 1996). As a result of this, their concentrations often exceed the permissible levels normally found in soil, water ways and sediments. Hence, they find their way up the food pyramid. When they accumulate in the nearby environmental segments and in food chains, they can profoundly disrupt biological processes. Excessive iron content of ground water in sylhet region is a typical problem due to its igneous rock origination. It attracts lots of attention and also several solutions been proposed. Among these some are cumbersome and not effective for small scale levels as well as house hold levels.

Adsorption is a well established technique to remove pollutants as well as to recover valuable products from aqueous streams. Adsorptive removal of heavy metals from aqueous effluents which have received much attention in recent years is usually achieved by using activated carbon or activated alumina (Faust and Aly, 1987; Shim et al., 2001; Ouki et al., 1997; Hsisheng and Chien-To, 1998; Ralph et al., 1999; Ali et al., 1998; Monser and Adhoun, 2002; Igwe et al., 2005). Activated carbon is a porous material with an extremely large surface area and intrinsic adsorption to many chemicals. Polymer resins that can form complexes with the heavy metal ions are the best adsorbents (Lu et al., 1994). These are called conventional adsorbents and many others have been reported such as silica gel, active alumina, zeolite, metal oxides (Motoyuki 1990) and so on. These conventional adsorbents are employed in many processes for the removal of heavy metals from wastewater such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, reverse osmosis, ion exchange and membrane technologies (Preetha and Viruthagiri 2005; Rengaraj et al., 2001; Benito and Ruiz, 2002). These processes may be ineffective or expensive (Volesky and Holans, 1995) especially when the heavy metal ions are in solutions containing in the order of 1- 100 mg dissolved heavy metal ions/L (Volesky 1990a,b). Activated carbon is only able to remove around 30-40 mg/g of Cd, Zn, and Cr in water and is non-regenerable, which is quite costly to wastewater treatment (Gang and Wiexing, 1998). A major draw back with precipitation is sludge production. Ion exchange is considered a better alternative technique, but it is not economically appealing because of high operational cost. As a result of these, biological methods such as biosorption/bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods (Kapoor and Viraraghavan, 1995; Pagnanelli et al., 2000). Biosorption or bioremediations consists of a group of applications which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants. This process is characterized as less disruptive and can be often carried out on site, eliminating the need to transport the toxic, materials to treatment sites (Gavrilescu, 2004). Biosorbents are prepared from naturally abundant and/or waste biomass. Due to the high uptake capacity and very cost-effective source of the raw material, biosorption is a progression towards a perspective method. Biosorbents of plant origin are mainly agricultural by-products such as, maize cob and husk (Igwe and Abia, 2003, 2005; Igwe et al., 2005b,c), sunflower stalk (Gang and Weixing, 1998), medicago sativa (Alfalfa) (Gardea-Torresdey et al., 1998), cassava waste (Abia et al., 2003), wild cocoyam (Horsfall and Spiff, 2004, 2005), sphagnum peat moss (Ho et al., 1995), sawdust (Igwe et al., 2005d; Raji and Aniridhan, 1998), chitosan (Ngah and Liang, 1999; Saifuddin and Kumaran, 2005; Wataru and Hiroyuki, 1998), Sago waste (Quek et al., 1998), peanut skins (Randall et al., 1974), shea butter seed husks (Eromosele and Otitolaye, 1994), banana pith (Low et al., 1995), coconut fiber (Igwe et al., 2005e), sugar-beet pulp (Reddad et al., 2003), wheat bran (Dupond and Guillon, 2003), sugarcane bagasse (Krishnani et al., 2004) and so on. Considering the promising results from the researchers, Various biomaterials in broad spectrum need to be further explored to warrant further research.

The project has manifold objectives.

Research Objective 1. Survey of surface water quality parameters (like pH, conductivity, dissolved solid, suspended solid, total solids, acidity, alkalinity and estimation of dissolved oxygen (DO), dissolved NO_3 and NH_3) of haor areas of sylhet region.

Research Objective 2. Analysis of chemical oxygen demand (COD) of the industrial effluent samples of sylhet division into our laboratory using standard method.

Research Objective 3. Cost-effectivedevelopment of bio-sorbents and removal of metals using these developed

But due to the limitations of budget and time schedule we focused on objective 1 and 3 in this present study.

Materials and methods

Characterization of water samples

In first phase surface water quality parameters were investigated in situ in the haor (wetland ecosystem) basin. Under this job, samples were collected in situ from Tanguer haor area and its nearby haors by polyethylene bottles after washing it with distilled water. Then pH, conductivity, total dissolved solids were measured in situ by using portable pH and conductivity meter (Model:pHwp, Milwaukee, Romania, EC/TDS/Temp. meter Model: EC60, Milwaukee, Romania). Also dissolbed oxygen was investigated instantly in the field using portable DO meter (Model: MW600, Milwaukee, Romania). The concentration of NO_3 and NH_3 were determined by using portable meters (Model: LAQUAtwin-NO_3^--11, HORIBA, NH_3 meter, Model: MI405 Milwaukee Romania).

Then adsorptive removal of metal was conducted in our analytical and environmental chemistry lab of SUST. In this phase, biodegradable sorbents were developed and further analysis were carried out to evaluate their efficiency. Standard methodologies for UV-Visible spctrophotometry were followed and metal removal efficiency was analyzed by this machine.

Preparation of biodegradable adsorbents

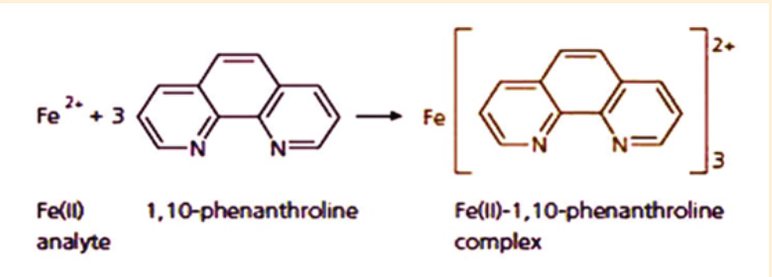
At first adsorbents were preparedby collecting different domestic and agricultural wastes. These includes food stuffs, harvesting waste, red mud different seeds and leaves of vegetables, bark of trees, snails collected from wet lands etc.Waste materials got priority for making these cost-effective. Adsorbents preparation was the following: waste were washed then dried in oven at 100 $^\circ$C followed by grinding. Adsorbents were grinded very finely to increase the surface area. After preparing, adsorbents were conserved. The whole process of biodegradable sorbent preparation is represented in Fig. 1 below. In this study activated carbon (Wako, Japan) is investigated as a reference and denoted as AC throughout the report and other two developed will be denoted as ESP and SSP as these are under further investigation.

Fig. 1 scheme of preparation of bio-sorbents

Preparation of adsorbate and estimation of Fe

Stock solution of 100 mg/L iron was prepared by dissolving 0.70g of ferrous ammoniumsulphatehexahydrate (analar grade) salt in distilled water, which was further diluted to desire extent to obtain desire concentration. Then 1ml of hydroxyl ammonium chloride solution was added for each sample to reduce Fe(III) to Fe(II). Then 5ml of 1, 10-phenanthroline solution were added to each solution to form red colored Fe-1, 10-phenanthroline complex. pH at 3.5 was strictly maintained during this analysis. The absorbance's of the standard solutions were recorded by using UV-visible spectrophotometer (Model Shimazdu-1800)at 510 nm. The pH values of solutions during this study were adjusted using 0.1 N HCl and NaOH solution (WAKO chemical, Japan). The reaction scheme for the spectrophotometric estimation of iron is given below.

$$4Fe^{3+} + 2NH_2OH \longrightarrow 4Fe^{2+} + N_2O + 4H^+ + H_2O \qquad (1)$$



Batch Adsorption study

Adsorption isotherm studies were carried out by contacting 1g of adsorbent with 50ml of iron (Fe) solutions of different initial concentration (50, 100, 150, 200, 250, 300 ppm) into 250 mL adsorption bottles at room temperature. Then the samples were shaken at a constant speed by a mechanical orbital shaker (Stuart, UK) for 4 hours. After that aliquot portions were investigated at different time intervals to find out equilibrium time of adsorption. Form this % removal capacity was determined the following equation

$$(\%)\ \text{Percentage removal} = (C_o\text{-}C_e) \times 100/C_o \qquad (2)$$

Where C_o is the initial concentration (mg/L) adsorbate, C_e is the equilibrium concentration (mg/L) of adsorbate.The sorption equilibrium data of iron on developed sorbents were analyzed in terms of Langmuir model (Langmuir, 1916)for the purpose of finding adsorption efficiency. The relative coefficients of the model werecalculated using linear least-squares fitting.The linearized form of Langmuir sorption isotherm equation

$$C_e/Q_e = C_e/Q_m + 1/K_LQ_m \qquad (3)$$

Where C_e (mg/L) is the equilibrium concentration, Q_e(mg/g) is the amount of iron adsorbed onto adsorbent at equilibrium, Q_m(mg/g) and K_L (L/mg) is the Langmuir constant related to maximum sorption capacity and enthalpy of sorption respectively and can be calculated from the intercept and the slope of the linear plot of C_e/Q_e vs C_e.

The essential characteristics of the Langmuir isothermcan also be expressed in terms of a dimensionless constantseparation factor or equilibrium parameter, RL, which is defined as

$$R_L = 1/(1+ K_L C_o) \quad (4)$$

Where, K_L is the Langmuir constant and Co is the initial adsorbate concentration. The value of R_L indicates the type of isotherm to be either unfavorable (R_L >1), linear (R_L = 1), favorable (0< R_L<1) or irreversible (R_L = 0) (Mckayet al. 1982)

In situ kinetic study

In situ adsorption kinetics were studied for different adsorbents by taking 1g of the sorbent containing iron solution of different initial concentrations (50, 100, 150, 200, 250, 300 ppm) into 250 mL adsorption bottles at room temperature. From the very first minute, 3 mL of supernatant solutions from the top was filtered out by micro-pipette every time for measuring the concentration by UV-Visible spectrophotometer. After taking the UV data, all the solutions were returned back to the beaker. This process was followed for different time intervals all over the kinetics study.

Results and discussion

Surface water quality of Tanguer haor

Surface water quality parameters of the collected samples from Tanguer haor basin and nearby areas was presented in the Table 1. It summarizes the present water quality of this haor region in the winter season of 2018. pH value found in the near neutral range of all samples indicating good quality of haor surface water. But NH_3 levels and NO_3 levels of some points showed little bit higher than expectation. Picnic spot of Tanguer haor and niladri point exhibited 2.808 ppm and 1.447 ppm NH_3 respectively and the NO_3levels were 6.0 ppm and 9.0 ppm respectively. It can be concluded that there may exists some oxidation-reduction cycle of $NH_3$and NO_3 mediated by bacteria. Though the concentration of NH_3 found lower and within tolerance level, it may be threatening for the aquatic and fish species of the wetland ecosystem. The dissolved O_2 level found satisfactory and soundsgood for fish in current situation. But in harvesting season the level varies and consumed by the biomass of the surrounding haor thereby affecting fish by suffocating them in O_2 free system. Many local fishes can tolerate the NH_3 but some diseases due to this pungent chemical is responsible for seasonal fish killing in this region.

Batch study and adsorption isotherms

Batch studies were conducted first to find out the equilibrium time of adsorption for each biodegradable sorbents. During this, the percentage of iron removal was estimated at different pH to find out the effect of pH on removal process. Results are summarized in the Table 2 and the highest percentage (%) of iron removal at pH 7 is provided here only in Fig. 2. It is evident that AC achieved the highest removal(95%) and then ESP and SSP. ESP and SSP sorbents (without activation) here able to remove about 80-90% iron within 30-40 minutes, which is a promising result. The developed ESP and SSP are comparable with commercial AC for iron removal capacity at neutral pH which sheds light on pollutant removal costs because most of the processes need extra steps to return neutral pH by adding either acid or alkali.

The adsorption isotherm studies were conducted at a fixed adsorbent dosage by changing initial metal ion concentrations of Fe. The equilibrium data were analyzed usingLangmuir models (equations3) in order to find out the sorption related parameters. The linearized Langmuir isotherm using different developed sorbents are shown inFig. 3.The correlation coefficients (r^2) and the

Table I. Surface water quality parameters of Tanguer haor and nearby area (measured in December- 2018)

Sample No	Location name	Temperature (˚C)	pH	Conductance (ms)	TDS (ppt)	DO (ppm)	NO_3^- (ppm)	NH_3 (ppm)
1	Jadukata Nadi,Bareker tila,Tahirpur,Sunamganj.	23.2	6.7	0.11	0.06	5.9	0	0.559
2	Niladri,Tahirpur,Sunamganj.	22.5	7.3	0.57	0.29	5.6	9	1.447
3	Tarajan bil,Tahirpur,Sunamganj.	22.8	7.2	0.11	0.06	5.2	0	2.723
4	Sulemanpur point of Tanguer haor	24.5	7	0.23	0.11	5.5	6	0.571
5	Tanguar Haor channel(entering point of haor),Tahirpur,Sunamganj.	25	7.1	0.17	0.08	4.7	0	9.545
6	Inside the tanguar haor,Tahirpur,Sunamganj.	24.5	7.1	0.17	0.09	5.1	0	3.137
7	Middle of the Tanguer haor	24.5	7.4	0.14	0.07	6	0	0.109
8	Near the picnic spot of Tanguer haor, Tahirpur,Sunamganj.	25	6.5	0.09	0.05	4.8	6	2.808

Table II. Percentage (%) removal of iron by different sorbents at different pH

pH	% removal of iron for AC	% removal of iron for ESP	% removal of iron for SSP
3	51	45	58
5	67	75	72
7	92	63	82
9	71	49	48

isothermal parameters obtained from the application of the Langmuir models are summarized in Table 3. The closeness of the value of (r^2) to unity justify the fitness of the experimental data to the isotherm models and Langmuir model was found to be best fitted for the studied system. This confirms the monolayer sorption process by the developed sorbets and also for the commercial activated carbon. The highest adsorption capacity 204.08 (mg/g) was exhibited by AC and the developed un-activated SSP and ESP exhibited 99.0 (mg/g) and 35.0 (mg/g) respectively. It is to be noted that without activation/treatment our developed sorbents performed half of the commercial AC, which was carbonized then heat treated to be used as an adsorbent. In our case we wanted to reduce the cost and therefore we used as prepared sorbents without any pre-treatment. But compare to other sorbents these are promising and could be used for removal of iron and we are hopeful that further treatment either by chemicals or heat will improve their removal efficiency.

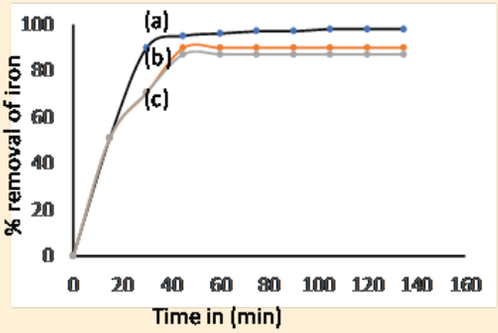


Fig. 2. Percent removal of iron at pH 7 by (a) AC (b) ESP (c) SSP

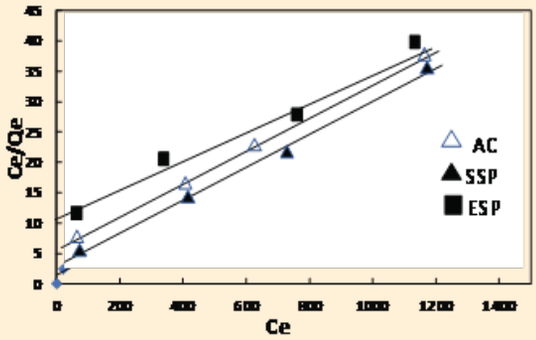


Fig. 3. Langmuir isotherm for adsorption of iron on sorbents

Table III. Langmuir isotherm parameters for the adsorption of iron onto different sorbents

Adsorbent Name	Langmuir isotherm parameters			
	Q_m (mg/g)	K_L(L/mg)	r^2	R_L
AC	204.08	0.00167	0.990	$0<R_L<1$
SSP	99.00	0.00533	0.983	$0<R_L<1$
ESP	35.84	0.01962	0.982	$0<R_L<1$

Adsorption kinetics

Adsorption kinetics is an important characteristic for evaluating the rate of adsorptionby which the metal uptake takes place. The pseudo first-order and pseudo second-order kinetic models were used to predict the iron adsorption behaviors of the prepared adsorbents in this study.The pseudo-first-order equation is generally suitable for the initial stage of the adsorption, but the pseudo-second-order equation predicts the adsorption behavior over the whole process.The kinetics of adsorption was determined by analyzing the metal adsorbed from aqueous solution at various time intervals, and then fitting the data to the two models. The integrated linearized form of the pseudo-first order model by Lagergren is

$$\ln (Q_e-Q_t) = \ln Q_e - k_1 t \qquad (5)$$

The integrated linearized form of the pseudo-second order model can be represented as

$$t/Q_t = 1/k_2 Q^2_e + t/Q_e \qquad (6)$$

$$h = K_2 Qe^2 \qquad (7)$$

Where Q_e, and Q_t are the amounts of adsorbed iron by the adsorbent at equilibrium (*mg/g*) and at contact time t, respectively,k_1(min^{-1}) is the rate constant of pseudo-firstorder sorption and k_2 (g mg^{-1} min^{-1})is the rate constant of pseudo-second order sorption. Here h(mg g^{-1} min^{-1})represents the initial adsorption rate.

If the kinetic data follows pseudo-first order kinetics, it will give a linear relationship of plot of ln (Q_e-Q_t) against t. Then values of k_1*and* Q_e can be calculated from the slope and intercept of the obtained straight line. Similarly, if the kinetic data follows pseudo-second order kinetics a plot of

t/Q_t versus *t* will give a straight line and thereby confirms the conformity of the pseudo-second order kinetics. The slope and intercept of the straight line gives the value of Q_e and k_2 respectively. The results of the kinetic investigation by the adsorbents are shown in Fig 4 and kinetic parameters calculated from these models are summarized in Table IV

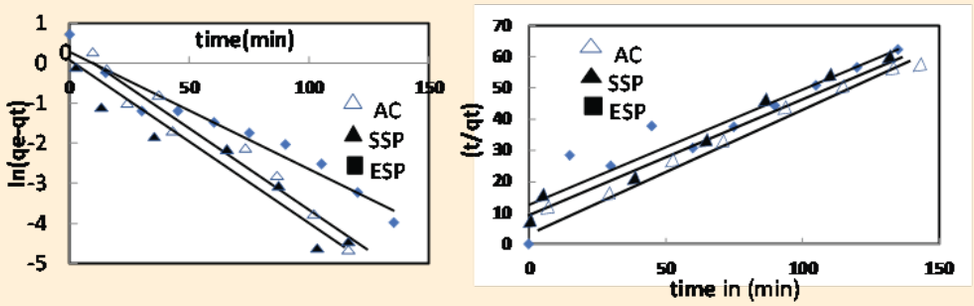


Fig. 4 (a) Pseudo-first order adsorption and (b) Pseudo-second order adsorption

The higher correlation coefficient r^2 close to unity was attained for the pseudo-second order reaction and higher than pseudo-first order reaction. Based on this findings it can be claimed that the sorption process followed pseudo-second order adsorption and it confirms the

Table IV. Comparison of the pseudo-first order, pseudo-second order adsorption and different parameters of these experiment

Adsorbents	**Kinetic model**					
	Pseudo 1st order			**Pseudo 2nd order**		
	$K_1(min^{-1})$	$Q_e(mg/g)$	r^2	$K_2(g\ mg^{-1}\ min^{-1})$	$Q_e(mg/g)$	r^2
AC	0.027	1.48	0.9021	0.9336	1.76	0.995
SSP	0.0288	1.09	0.9044	2.136	1.57	0.990
ESP	0.0291	1.27	0.9381	1.47	1.61	0.996

chemisorption phenomena for these developed adsorbents. But the probability of physisorption cannot be ignored because all these adsorbents quickly attains maximum value then it continues for a little more then reaches the steady state. Though chemisorption plays a pivotal role, the mechanism is not well understood because of very critical nature of the surfaces. Further surface characterization and finding the functional group and type may help to predict about chemical bonding and their sorption nature.

Conclusion

Surface water quality parameters of Tanguer haor was investigated during winter season of 2018. Dissolved oxygen level was found satisfactory level and sufficient to support aquatic life but the concentration of NH_3 and NO_3 were found quite higher level. pH is almost neutral except some cases where it shows slightly acidic.The experimental batch adsorption data well fitted to the Langmuir adsorption model confirming monolayer type of adsorption and kinetic model fitted best to the pseudo-second order adsorption which represents chemisorption type mechanism. It is noteworthy that these developed sorbents were examined without any treatment but exhibited comparable performance with a conventional reference material activated carbon. All these biodegradable

sorbents are capable of removing iron from aqueous solution at pH 7. Further surface characterization of the developed sorbents is essential to find out the mechanism correctly. These low cost biodegradable sorbents could be used in practical level without any harm and further treatment by heat or chemical method will improve their surface and thereby hopefully improve metal uptake capacity.

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