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## Preface

We would like to present, with great pleasure, the inaugural volume-4, Issue-9, September 2018, of a scholarly journal, *International Journal of Engineering Research & Science*. This journal is part of the AD Publications series *in the field of Engineering, Mathematics, Physics, Chemistry and science Research Development*, and is devoted to the gamut of Engineering and Science issues, from theoretical aspects to application-dependent studies and the validation of emerging technologies.

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Each article in this issue provides an example of a concrete industrial application or a case study of the presented methodology to amplify the impact of the contribution. We are very thankful to everybody within that community who supported the idea of creating a new Research with IJOER. We are certain that this issue will be followed by many others, reporting new developments in the Engineering and Science field. This issue would not have been possible without the great support of the Reviewer, Editorial Board members and also with our Advisory Board Members, and we would like to express our sincere thanks to all of them. We would also like to express our gratitude to the editorial staff of AD Publications, who supported us at every stage of the project. It is our hope that this fine collection of articles will be a valuable resource for *IJOER* readers and will stimulate further research into the vibrant area of Engineering and Science Research.

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## **Study on Impact of Biomedical Engineering in Dentistry** Ishita R. Das<sup>1</sup>, Milton K. Debnath<sup>2</sup>, Rozina Akter<sup>3</sup>, Anita L. Jackson<sup>4</sup>, Md. Zakir Hossain<sup>5\*</sup>

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**Abstract**— Dentistry is a major branch of medicine. It deals with the study and the diagnosis, treatment and prevention of diseases of the oral cavity, adjacent structures and tissues [1]. Biomedical engineering is an interdisciplinary branch of engineering science that deals with the application of engineering principles in the field of biology and medicine for the betterment of health [2]. Being an ancient branch of medicine, dentistry largely needs a lot of technical instruments for its purpose to serve its patients [3].

Keywords—Biomedical Engineering, Applications, Dental Science, Impact.

#### I. INTRODUCTION

Daily new biomedical technologies and scientific revolutions are observed in the field of dentistry that helps to expand diagnostic and treatment practices. Most of the modern dental diagnostic and treatment tools stem from biomedical engineering directly or indirectly [4]. Biomedical engineering is thus, related to dentistry by the development of newer tools and materials for diagnosis, treatment and preventive purposes. The effects of biomedical revolutions are mostly seen in both clinical practice and academic side of dentistry. In the coming years, personalized dental medicine, biomimetic, nanotechnology, genomic information, regenerative dentistry and stem cell studies will be more developed and integrated into dental practice [4-5]. Application of these new technologies will provide new solutions for traditional oral health problems as well as for the major dimensional changes in the field of dental science to emerge [5].

#### II. PURPOSE OF THIS STUDY

Daily new biomedical technologies and scientific revolutions are observed in the field of dentistry that helps to expand diagnostic and treatment practices. Most of the modern dental diagnostic and treatment tools stem from biomedical engineering directly or indirectly [4]. Biomedical engineering is thus, related to dentistry by the development of newer tools and materials for diagnosis, treatment and preventive purposes. The effects of biomedical revolutions are mostly seen in both clinical practice and academic side of dentistry. In the coming years, personalized dental medicine, biomimetic, nanotechnology, genomic information, regenerative dentistry and stem cell studies will be more developed and integrated into dental practice [4-5]. Application of these new technologies will provide new solutions for traditional oral health problems as well as for the major dimensional changes in the field of dental science to emerge [5].

Advancement in the discipline of biomedical engineering related technology has been used to better explain many of the dental problems like dental caries, periodontal diseases, tooth injuries etc. [5-8]. The ultimate use of these technologies is to focus on the patient's comfort and improvement of oral health [5-7]. At present knowledge about vaccine, cloning, DNA, drugs, tissue engineering, microbial bioinformatics and complex proteins are used in novel research on oral health to find out the best solutions for dental problems [8-9]. Some technological inventions in modern dentistry to enrich this field include computer assisted design (CAD), computer assisted manufactured (CAM) technology, continuous liquid interphase printing (CLIP using Carbon 3D) laser dentistry, oral cancer screening (for example, VEL scope) device, dental implant, digital X-ray, intraoral camera, composite resins, various biomaterials and so on [5-6, 10]. Basically, biomedical engineering is one of the most evolutionary fields to continuously interface with the best emerging dental technology for the patient's benefit [6]. These dental technologies are the fruits of applying bio-engineering knowledge in the field of dental science and innovative dental practice.

Dental practitioners commonly face two important diseases throughout their professional life [11]. These are tooth decay and periodontal disease. The complex natures of these diseases occur due to bacterial, genetic and environmental factors [11]. Amplifying and analyzing of nucleic acid through the polymerase chain reaction (PCR) uses specific DNA fragments to detect particular proteins. Using this PCR brings out a remarkable change in dentistry to diagnose periodontal diseases [11-12]. In biomedical sciences and engineering, the PCR is used to identify various microorganisms, chromosomal disorders, tumor suppressor genes, tumor associated translocation and analysis of mutation in oncogenes [11]. The application of PCR in dentistry is used to detect the periodontal carcinogenic pathogens, presence of viruses in host cells, microorganisms in endodontic infections, diagnosis and prognosis of oral cancer and the estimation of quantity of different microorganisms [12]. Use of these molecular techniques, like PCR, can allow dentists to understand underlying conditions and to help to design appropriate treatment protocols for the periodontal disease at the molecular level [11].

Currently, biomedical engineering technology in tissue engineering and bone regeneration has had significant impact in dentistry. Many bone defects like injury of the bone, periodontal disease and congenital disorders are considered as major health problems which can be treated with biomedical sciences and tissue engineering technology. Present strategy of tissue engineering technology is aimed at bone morphogenetic proteins (BMPs), which induce bone formation [13]. On the other hand, whole tooth regenerative treatment has also been influenced by the application of tissue engineering technology. The embryonic development and biological analysis of stem cell reveals the physiological function of the tooth. This function has been improved by the replacement of a bioengineered tooth germ that results a successful eruption of correct tooth structure in the oral cavity [8, 14]. These remarkable advances provide the gene-based treatment process in the field of dentistry [15]. In the next few decades, genetic engineering will be more effective in dentistry. It will encourage the body to repair itself by the artificial placement of extrinsic materials [4].

#### III. CONCLUSION

To conclude, biomedical engineering helps to develop dentistry and has brought a revolutionary change in dental science. Many new inventions and discoveries are knocking at the door which will open a new horizon to solve dental problems more easily and effectively. Thus, the collaborative research integration of biomedical engineering is undeniable for dentistry and has paved the way for future biomedical engineers to contribute more and more in dentistry through research, discovery and innovative new practice modalities.

#### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

#### **ETHICAL APPROVAL**

This article does not contain any studies with animals performed by any of the authors.

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## Comparative Study of Bit Error Rate in LDPC Based OFDM System over AWGN Channels

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**Abstract**— Modern communication systems are increasingly adopting new Morden technologies like OFDM and LDPC for achieving high performance, low Bit Error Rate (BER) and high capacity. The OFDM communication is very much inspired from the channel frequencies over the network. In such a network some kind of orthogonal distortion occurs over the channel called Inter Carrier Interference.

# Keywords— OFDM Techniques, Bit Error Rate, LDPC code, Inter Channel Interference, QAM Modulation, TDMA Techniques.

#### I. INTRODUCTION

In this chapter, we firstly review some important technologies that contribute to the modern digital communication system. These techniques, including the Orthogonal Frequency Division Multiplexing (OFDM) modulation and Low Density Parity Check (LDPC) code, will be the main topic of the dissertation. Following the problem statement, dissertation objective is introduced. Finally, the outline of the dissertation will be given.

OFDM represents a different system design approach. It can be thought of as a combination of modulation and multiple access schemes that segment a communication channel in such a way that many users can share it. Whereas TDMA segments are according to time and CDMA segments are according to spreading codes, OFDM segments are according to frequency. It is a technique that divides the spectrum into a number of equally spaced tones and carries a portion of a user's information on each tone. A tone can be thought of as a frequency, much in the same way that each key on a piano represents a unique frequency. OFDM can be viewed as a form of frequency division multiplexing (FDM), however, OFDM has an important special property that each tone is orthogonal with every other tone. FDM typically requires there to be frequency guard bands between the frequencies so that they do not interfere with each other. By allowing the tones to overlap, the overall amount of spectrum required is reduced.

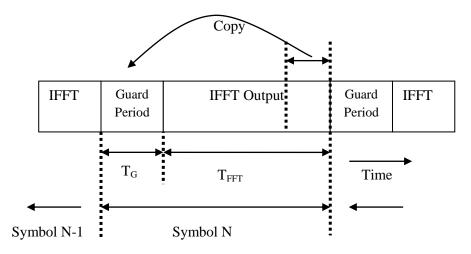


FIGURE 1: BLOCK DIAGRAM OF OFDM

#### II. OFDM SYSTEM DESIGN

The design of an OFDM system requires a tradeoff between various parameters as like in all communication system design. Usually, the input parameters to the design are the bit rate, available bandwidth and the maximum delay spread introduced by the channel. The design involves calculation of symbol duration, guard time, number of sub-carriers and the modulation and coding schemes among others.

#### 2.1 OFDM System Design

In this section, we consider a typical design problem with the following requirements.

- Bit Rate : 20Mbps
- Maximum Delay Spread : 200ns
- Available Bandwidth : 15MHz

#### 2.2 Guard Time

From the design considerations discussed previously, we see that a guard time of

4\*200ns = 800ns is a reasonable choice.

#### 2.3 Modulation and Coding

To transmit 20Mbps data, the number of bits to be transmitted in an OFDM symbol is =  $20Mbps*4.8\mu s = 96$  bits/OFDM symbol. Now, we can go for

- 16 QAM with rate (1/2) coding for each sub-carrier so that there are 2 bits per symbol per sub carrier. Thus we see that 48 sub-carriers are required in this case.
- QPSK with rate (3/4) coding for each sub-carrier so that there are 1.5 bits per symbol per sub carrier. Thus in this case, we need 64 sub carriers.

But in the latter case, 64 sub-carriers require a bandwidth of 64 \* 250 KHz = 16 MHz which is greater than the available bandwidth of 15 MHz. Hence the first one is a good choice in the sense that it satisfies the constraints.

#### III. LDPC CODES

LDPC codes were originally introduced by Gallager in his doctoral dissertation [9]. Since the discovery of turbo codes in 1993 by Berrou and the rediscovery of LDPC codes by Mackay and Neal in 1995 [10], there has been renewed interest in turbo codes and LDPC codes because their bit error rate performance approaches asymptotically the Shannon limit [4]. Much research is devoted to characterizing the performance of LDPC codes and designing codes that have good performance. Commonly, a graph, the Tanner graph, is associated with the code and an important parameter affecting the performance of the code is the girth of its Tanner graph. The Tanner graph is a special type of graph, a bipartite graph, where the nodes divide into two disjoint classes with edges only between nodes in the two different classes.

#### IV. SIMULATIONS AND RESULTS

#### 4.1 OFDM Spectrum

Orthogonal Frequency Division Multiplexing method has been used for spectrum generation. The main reason of using OFDM spectrum is that is distributes the data over large number of carriers that are spaced apart at different precise frequencies.

#### 4.2 AWGN Channel

ADDITIVE WHITE GAUSSIAN noise (AWGN) channel is a universal channel model for analyzing modulation schemes. In this model, the channel does nothing but add a white Gaussian noise to the signal passing through it. This implies that the channel's amplitude frequency response is flat(thus with unlimited or infinite bandwidth) and phase frequency response is linear for all frequencies so that modulated signal pass through it without any amplitude loss and phase distortion of frequency components.

#### 4.3 BER Vs SNR of the LDPC Based OFDM system in AWGN channel Using QAM

Using MATLAB Figure 2 shows the BER Vs SNR of the LDPC Based OFDM system in AWGN channel using QAM modulation scheme. This figure shows the relationship between BER and SNR. The values of SNR are from -30 db to 0 db and the scale of SNR is linear. The values of BER are from 0.1 to 0.5 and scale of BER is log.

| SNR AND BER EVALUATION |        |        |  |
|------------------------|--------|--------|--|
| S. No.                 | SNR    | BER    |  |
| 1                      | -30 db | 0.4767 |  |
| 2                      | -25 db | 0.4566 |  |
| 3                      | -20 db | 0.4221 |  |
| 4                      | -15 db | 0.3649 |  |
| 5                      | -10 db | 0.2672 |  |
| 6                      | -5 db  | 0.1128 |  |
| 7                      | 0 db   | 0.0    |  |

TABLE 1SNR and BER Evaluation

BER Vs SNR of LDPC based OFDM system in AWGN channel using 2QAM

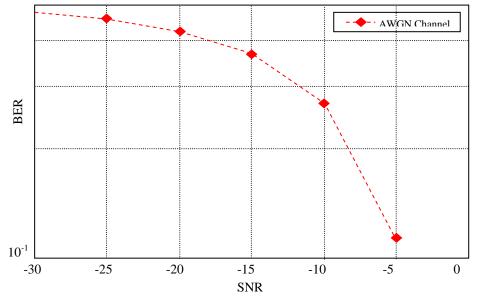


FIGURE 2: BER VS SNR OF THE LDPC BASED OFDM SYSTEM IN AWGN CHANNEL USING QAM

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# Computational analysis to predict role of human microRNAs in Ebola virusgenome

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**Abstract**— The Ebola virus is one of the most dangerous viruses in Filoviridae family. It causes fatal hemorrhagic fever in both non-human and human primates. The fatality rate is up to ninety percent. There is no effective treatment against EBOV infection so far. By using host microRNAs, we have explored for potential anti-viral therapeutics against EBOV infection, which may down-regulate viral gene expression in order to suppress viral replication. We have identified eight human miRNAs from eight potential hairpin sequences of EBOV genome. Our study provided an interesting hypothesis that those miRNAs are hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054. We suggested that these hairpin sequences could be use as anti-viral therapeutics to quell the replication of EBOV infection in human.

Keywords—EBOV, microRNA, downregulate, anti-viral, therapeutics, human miRNA.

Abbreviations: Ebola Virus (EBOV), microRNA (miRNA), human microRNAs (hsa-miR).

#### I. INTRODUCTION

The Ebola virus (EBOV) is already listed as a top categories pathogen by several organizations including WHO, CDC and NIH because fatality rate is up to ninety percent [4,7]. It is a member of **Filoviridae** family that causes fatal hemorrhagic fever including both human and non-human primates [1-3]. Several reports suggested that susceptible hosts can be died by most virulent species within ten days after the appearance of symptoms [4-5]. The first case of Ebola virus was reported in 1976 on the Africa region [6]. Five (5) different species of Ebola viruses have been reported so far. Among these except Reston, REBOV, all 4 are being capable of causing diseases in human [8-9]. As Ebola virus is enveloped, non-segmented, negative-strand RNA viruses and genome size approximately 19 kb in length, it has high fatality rate and easily transmissible [10-11]. The viral genome encodes seven structural proteins and one non-structural protein while viral proteins perform different functions including viral replication [11].

The miRNAs are small non-coding RNA molecule, genomically encoded, usually around twenty-two base pair in length and regulate genes expression at the post-transcriptional level either mRNA degradation or repress translation [12-14]. It is well documented that miRNAs operate their different biological or physiological functions including apoptosis, development, tumorigenesis, stress response, proliferation and fat metabolism [15-16]. Although, current monoclonal antibody (mAb) based therapies are thought to the most efficient against lethal Ebola virus infection in non-human primates. In this research study, we have proposed miRNA-based gene silencing activity as a suitable alternative, in addition to current vaccine mediate treatment.

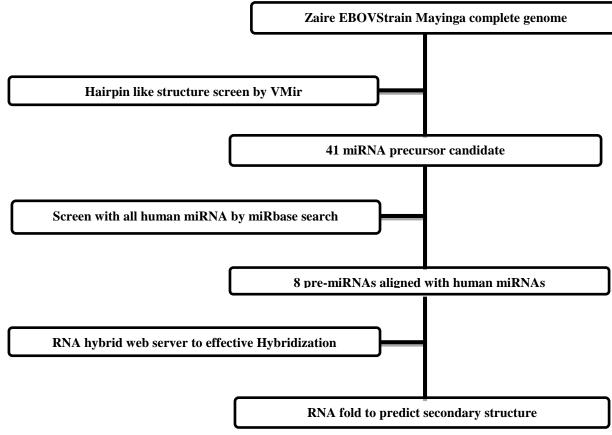
Viral miRNAs are unique because it regulates both their own gene expression and host gene expression [18]. MiRNA genes are firstly transcribed into primary miRNA. Then they are cleaved by enzymatic activity of the RNase III ribonuclease Drosha into 60-90 base pair long hairpin intermediate known as pre-miRNA [18-20]. By the action of enzyme exportin-5 and ran pre-miRNAs are exported from nucleus to cytoplasm [19]. The pre-miRNAs are further cleaved by Drosha (another RNase III ribonuclease) in the cytoplasm and are formed into a double stranded RNA known as duplex mature RNA [19].RNA-induced silencing complex (RISC) that is one strand (guided strand) of duplex RNA, targets messenger RNA to degrade or repress translational activity [18].

The 3' untranslated region (UTR) of the mRNA and the seed region of miRNA (2-7 bp) has fine complementarity that gives sufficient results in cleavage. On the other hand, faulty complementarity may block translation [18, 21]. Now-a-days,

miRNA is considered as antiviral defense against several viral diseases. As example, miRNAs mediate anti-HCV treatment shows promising effectiveness and safety results in an early stage trial [22]. In this study, we have identified some potential targets of human microRNA on Ebola virus (EBOV) genome by bioinformatically related computerized program. The study will help to understand host pathogen interaction as well as to develop new antiviral therapy against all Ebola virus diseases including fatal hemorrhagic fever.

#### II. MATERIALS AND METHODS

The miRNA prediction of the EBOV was conducted by using the complete genome sequence of Zaire Ebola virus strain Mayinga (Accession # AF499101.1) obtained from the National Center for Biotechnology Information (NCBI).Figure 1 shows a schematic diagram of the computerized prediction of human miRNAs in EBOV genome. Using a VMir Analyzer program, the viral genome was screened for hairpin-structured miRNA precursors [23-24].The output of VMir Analyzer was visualized using VMir viewer. For cut-off value 60 nt minimum hairpin size, 120 nt maximum hairpin size and 110 minimum hairpin score were used for the filter of sequence.As part of miRNA precursors were searched for nucleotide similarity with all human microRNAs by using SEARCH menu of the miRBase database (www.mirbase.org/search.shtml) [17, 25]. Finally, eight (8) sequences were identified as candidate miRNA precursor based on significant sequence similarity with human miRNAs. To ensure effective hybridization between the viral precursor miRNAs and complementary template of the potential human miRNAs were further analyzed by RNA hybrid web server (<a href="http://bibiserv.techfak.unibielefeld.de/rnahybrid/">http://bibiserv.techfak.unibielefeld.de/rnahybrid/</a>) [26]. Finally, the RNA fold web server (<a href="http://tiple.ic.at/cgibin/RNAfold.cgi">http://tiple.ic.at/cgibin/RNAfold.cgi</a>) was used to predict the secondary structure of pre-miRNA [27]



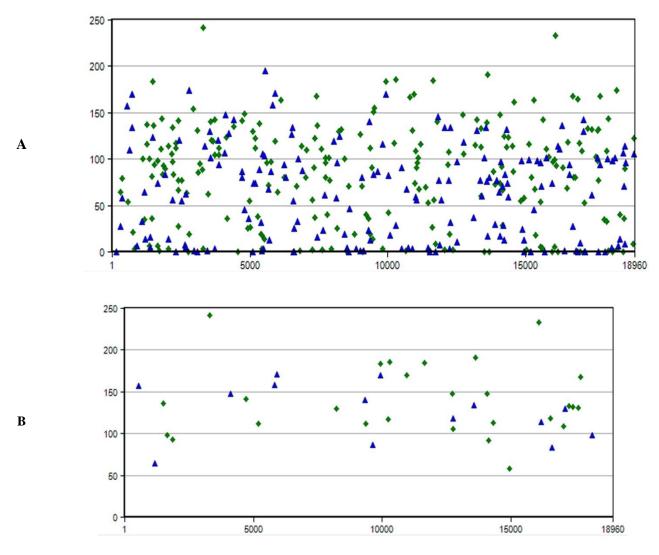
#### FIGURE 1: Schematic representation of human miRNA prediction on EBOV genome

#### III. RESULT

#### 3.1 Prediction of precursor miRNA (pre-miRNA) hairpins with VMir

The EBOV genome was screened using VMir analyzer program. VMir viewer program helps to visualize the output of VMir analyzer. This program represents the whole output in graphical manner with sequence length and score. The Figure 2 shows the graphical representation of EBOV precursor miRNAs hairpin. By using default setting, 347 candidate hairpins (Figure

2.a) have been identified. A filter using specific parameters i.e. for cutoff value 60 nt minimum hairpin size, 220 nt maximum hairpin size and 115 minimum hairpin score were applied to avoid bona find hairpin candidate. Finally, 41 pre-miRNA hairpins (Figure 2.b) were extracted as potential hairpins for further analysis.



#### FIGURE 2: Graphical view of VMir analysis of the EBOV genome.

A. All hairpins of pre-miRNAs are showing after default setting. Hairpins are plotted according to location of the viral genome (X axis) and VMir score (Y axis).

B. Customized view of predicted pre-miRNA after faltering (minimum hairpin size: 60, maximum hairpin size: 120, minimum hairpin score: 100 and minimum widow count: 15.

#### 3.2 Prediction of human miRNAs from precursor miRNAs hairpin

The nucleotide similarity with human miRNA of 41 candidate miRNA precursors were searched by using human miRNA filter of SEARCH menu of the miRBase database (<u>www.mirbase.org/search.shtml</u>) [17, 25].The eight (8) sequences were identified as candidate miRNA precursor based on significant sequence similarity with human miRNAs is shown in table 1.Human miRNA having at least 19 bp sequence similarity with candidate miRNA precursor were selected as primary target miRNA [28]. After that, close or closely perfect alignment of those miRNAs seed region (2-7) reside in the 3'-untranslated regions (3'UTR) of the candidate mRNA precursor were potential miRNA targets. Perfect complementary matching between 3' untranslated region (UTR) of the mRNA and the seed region of miRNA (2-7 bp) are important for fruitful cleavage of mRNA or translational repression. Viral precursor miRNA hairpins MD84, MD162, MR68, MR126, MR130, MR152, MR199, and MR215 have shown significant identity with hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 respectively.

| S. No | Haimpin | Score | Alignments between human microRNA and Ebola virus |     |                             |  |
|-------|---------|-------|---|-----|-----------------------------|--|
| 1     | MD84    | 140.7 | UserSeq   | -33 | uaagaacauugguuccucaa 14     |  |
|       |         |       |   |     |                             |  |
|       |         |       | hsa-miR-3915                                      | 1   | uaagaccaucuuuuccucaa 20     |  |
| 2     | MD162   | 129.2 | UserSeq   | 9   | agggagaaggugeugguge 27      |  |
|       |         |       | hsa-miR-6760-5p                                   | 2   | agggagaagguggaaguge 20      |  |
| 3     | MR68    | 111.6 | UserSeq   | 10  | uugaaguccuggagugaagucau 32  |  |
|       |         |       | -   |     |                             |  |
|       |         |       | hsa-miR-4452                                      | 1   | uugaauucuuggecuuaagugau 23  |  |
| 4     | MR126   | 185.4 | UserSeq   | 55  | ugucuagacacucucaguuca 75    |  |
|       |         |       | hsa-miR-4796-5p                                   | 1   | ugucuauacucugucacuuua 21    |  |
|       | MR130   | 170.2 | UserSeq   | 24  | ueggguueuuggageueeaee 44    |  |
| 5     |         |       | hsa-miR-671-3p                                    | 1   | ucegguucucagggcucca.cc 21   |  |
| 6     | MR152   | 147.7 | UserSeq   | 18  | guuucacuauguagcacagg 37     |  |
|       |         |       | hsa-miR-5096                                      | 1   | <br>guuucaccauguuggucagg 20 |  |
|       |         |       |   |     |                             |  |
| 7     | MR199   | 118.6 | UserSeq   | 76  | ccaaugaugcauggaagaaauu 55   |  |
|       |         |       | hsa-miR-302c-3p                                   | 2   | ccacugaaacauggaagcacuu 23   |  |
| 8     | MR215   | 167.6 | UserSeq   | 29  | auaacuuaaauguaacucacag 8    |  |
| -     |         | 20/10 | oserved   |     |                             |  |
|       |         |       | hsa-miR-2054                                      | 1   | auaaauuaaauuuauauacag 22    |  |

 TABLE 1

 ALIGNMENTS OF PRECURSOR MIRNAS HAIRPIN SEQUENCES WITH HUMAN MIRNAS.

#### 3.3 Hybridization between viral precursor miRNAs and human miRNAs

For successful hybridization between target human miRNA and precursor mRNA of EBOV was performed by the RNA hybrid tool (<u>http://bibiserv.techfak.uni-bielefeld.de/rnahybrid</u>) [26]. For microRNA target prediction, RNA hybrid is a tool used widely to find out the minimum free energy hybridization of a long and a short RNA. Pairing energy or minimum free energy (mef) indicating the stability of the hybridization. We allowed -10 kcal/mol pairing energy as cutoff value for selecting potential miRNA. Effective hybridizations were shown in Figure 3.

```
A.MD84 & hsa-miR-3915 (mfe: -24.9 kcal/mol)
                                                            B.MD162 & hsa-miR-6760-5p (mfe: -18.5 kcal/mol)
  target 5' U
                    Ά
                         GG
                                    11 3'
                                                            target 5'
                                                                        G
                                                                              GG
                                                                                       ΔA
                                                                                                A 3'
                                                                         GUGCU UGCCUU CU UUG
CGUGA GUGGAA GA GAC
GA AG GG
            UAAGA CAUU UUCCUCAA
AUUCU GUAG AAGGAGUU
miRNA 3'UU G AA
                                    5'
                                                            miRNA 3' AGA
C.MR68 & hsa-miR-4452 (mfe: -17.1 kcal/mol)
                                                            D.MR126 & hsa-miR-4796-5p (mfe: -16.8 kcal/mol)
                     U U
                                                                             UCU
target 5' G UUG
                               GAAG U 3'
                                                            target 5'
                                                                          IJ
                                                                                          CA
                                                                                               U 3'
           AUC AAG CC GGAGU
UAG UUC GG UCUUA
                                                                           GGUG GGAGUAU AUA
UCAC UCUCAUA UGU
                                    UCA
                                    AGU
                                                            miRNA 3' CAUU
miRNA 3'
              UGAA
                      C U
                                           U 5'
                                                                               UG
                                                                                          UC
                                                                                                 5'MD186 &
E.MR130 & hsa-miR-671-3p (mfe: -12.6 kcal/mol)
                                                            F.MR152 & hsa-miR-5096 (mfe: -13.3 kcal/mol)
                                                              target 5' U
target 5'
           U
                     С
                              А
                                A 3
                                                                            С
                                                                                    UGUUUUUGA
                                                                                                          З
                                                                                                  Δ
                                                                                            AUGG
            UGGAGCUC
                          ACC GA
                                                                        GCC
                                                                              CCAG
            ACCUCGGG
                          UGG CU
                                                                       CGG
                                                                              GGUU
                                                                                            UACC
miRNA 3' CC
                     ACUCU C
                                                                          ACU G
                                   51
                                                            miRNA 3'
                                                                                                ACUUUG 5'
                                                            H.MD215 & hsa-miR-2054 (mfe: -14.9 kcal/mol)
G.MR199 & hsa-miR-7974 (mfe: -24.5 kcal/mol)
                                                            target 5'
                                                                                       CA
                                                                                               A 3'
                                                                            GUUGAAUUUG GUUGCA
UAAUUUAAAU UAAUGU
target 5' G
              А
                  U
                                      3'
           CCA UGA GCAUGGAAG
GGU ACU UGUACCUUC
                                                            miRNA 3' UUAUU
                                                                                       A
                                                                                               C 5'
miRNA 3'
              G
                  U
                              GUGAAU 5'
```

FIGURE 3: Hybridization between microRNA and viral RNA using RNA hybrid program.

The program finds the energetically most favorable hybridization sites of a miRNA in a large hairpin of viral RNA.

#### 3.4 Prediction of secondary structure of miRNA precursor

By using RNAfold web server (<u>http://rna.tbi.univie.ac.at/cgibin/RNAfold.cgi</u>), secondary structure of pre-miRNA was determined [27]. Only default parameters were in use. To predict the most stable secondary structure, the RNAfold program is widely used. In all cases, folding structures with centroid were depicted.

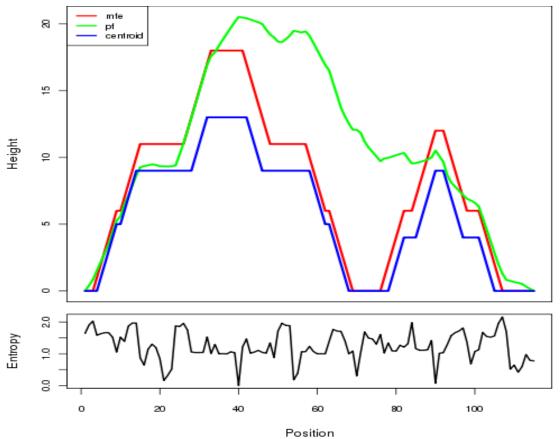
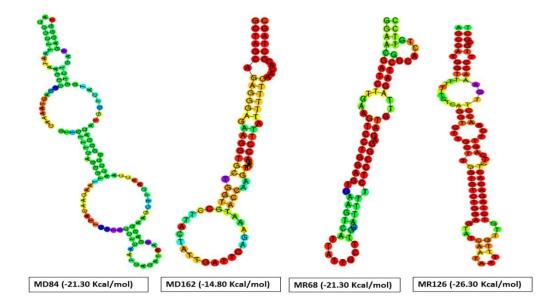


FIGURE 4: Mounting plot of predicted secondary structure of precursor miRNA hairpin

As an example, hairpin MD84 was shown. This plot has shown the minimal free energy structure (red), the thermodynamic ensemble of RNA structures (green), and the centroid structure (blue).



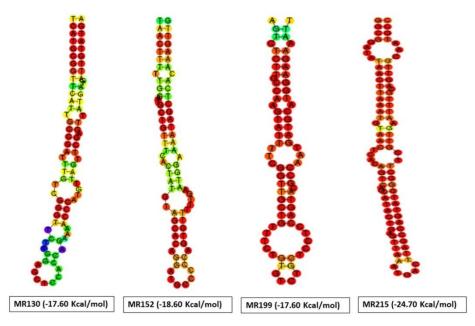


FIGURE 5: Predicated secondary structure of potential hairpins candidate of EBOV Only centroid structures were depicted.

#### IV. DISCUSSION

In our current investigation, we have identified host miRNAs computationally for EBOV infection in human. This study carried out based on an interesting hypothesis of utilization of host miRNA as a potential post exposure therapy because current evidence suggest that host miRNAs may down-regulated viral gene expression. It has been reported that 3' untranslated region (UTR) of the viral mRNA and the seed region of miRNA (2-7 bp) has perfect complementarity that gives sufficient results in cleavage. On the other hand, imperfect complementarity may block translation [18, 21].Bt the utilization of a series of bioinformatics tools, we predict potential miRNA hairpins candidate for EBOV genome. Viral candidate miRNA hairpins MD84, MD162, MR68, MR126, MR130, MR152, MR199, and MR215 have shown significant identity with hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 respectively. The RNAhybrid web server ensures the effective hybridization, paring energy, p value and hybridization pattern between viral miRNAs hairpin candidates and selective human miRNAs. RNAfold tools also confirm the potential candidate miRNAs hairpin.

Based on computational analysis including VMir score, effective hybridization, and hybridization pattern and pairing energy, we propose hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 would be best potential cellular target miRNAs to develop a post exposure therapy.

Although, most of the predicted human miRNAs on EBOV genome functions yet to be discovered but we hypnotize those miRNAs may down-regulate viral gene expression in order to block the replication.

#### V. CONCLUSION

The candidate potential miRNA targeting EBOV can be predicted by utilizing a series of bioinformatics tools that we have provided in this study. Our computational analysis suggested that miRNAs hsa-miR-3915, hsa-miR-6750-5p, hsa-miR-4452, hsa-miR-4796-5p, hsa-miR-671-3p, hsa-miR-5096, hsa-miR-302c-3p and hsa-miR-2054 can be utilized as some anti-viral therapeutics against EBOV infection in human. Some future studies related this in silico study is also suggested. In order to find out the efficacy of the miRNA against EBOV infection, in vitro studies need to be carried on. To find out the inhibition influence on viral replication by the selected human miRNA, further study should be designed targeting isolation and application of miRNA for the purpose.

#### **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

#### ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.

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# Performance evaluation of different bedding media in aquaponic system for growth and production of okra and tilapia

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**Abstract**— Aquaponics is the marriage of aquaculture and hydroponic technologies. Present research was accomplished to evaluate the relative performance of only gravels  $(T_1)$ , only coconut husk  $(T_2)$  and mixture of gravels and coconut husk (1:1) in volume)  $(T_3)$  as media in aquaponic system to grow okra (Abelmoschus esculentus) and tilapia (Oreochromis niloticus). Each treatment had three replications of similar bedding media. Nine food grade plastic containers filled with media and a 180 liter plastic water tank were used to construct the aquaponic system for growing okra and tilapia, respectively. In each bedding container, 4 okra seeds were sown and tilapia with initial length of  $13.65 \pm 1.88$  cm and weight of  $46.04 \pm 20.93$  g were stocked at the rate of 144 fish/ $m^3$  in the fish tank. Tilapia were fed twice a day at the rate of 3% for premier month, 2% for next month and 1.5% of body weight for the remaining time. Fish and plants were sampled biweekly during the whole study period. Data analysis revealed that the treatment  $T_3$  performed best followed by  $T_1$  and  $T_2$ , respectively in terms of okra plant growth performances with respect to duration of plant growth in different growth stages, plant height, leaf number per plant, leaf area and branch number per plant. Okra production was shown significantly greater ( $P \le 0.05$ ) in the treatment  $T_3$  (9.08 ± 1.25 kg/m<sup>2</sup>/157 days) pursued by  $T_1$  (7.5 ± 1.83 kg/m<sup>2</sup>/157 days) and  $T_2$  (3.83 ± 2.33 kg/m<sup>2</sup>/157 days), respectively. At the termination of the study, the length gain and weight gain of tilapia were  $6.64 \pm 0.1$  cm and  $104.76 \pm$ 20.78 g, respectively. Total tilapia yield was recorded 138.80 tons/ha/157 days with 92.3% survival and FCR of 1.96. The water quality parameters and the nutrient concentrations in influent and effluent water remained within suitable ranges for tilapia production as well as the growth of okra. Therefore, the mixture of gravels and coconut husk media showed incentive performance in plant growth and production of okra compared to the individual media and at the same time the tilapia production was also satisfactory.

Keywords— Aquaponics, bedding media, coconut husk, gravels, okra, and tilapia.

#### I. INTRODUCTION

Fish is one of the utmost-traded food stuffs worldwide. More than half of fish exports by worth generate in developing states. The dietary contribution of fish is more momentous regarding animal proteins. A volume of 150 g of fish supplies about 50–60 percent of the routine protein demand for a full-aged person (FAO, 2016). Fruit and vegetables are also necessary components of a salubrious diet. Their adequate daily consumption could help avert serious non-communicable maladies (Lock et al., 2005).

In a small developing country with extensive population growth, aquaculture is intensified to meet the demand of fish protein for increasing population. This creates water pollution and depletes the ground water level day by day. Agricultural land is also used for other purposes which cause pressure on natural resources. To keep pace with the growing food demand, farmers use various chemicals to boost up cereal production. These chemicals strike out human health hazards as well as other environmental problems around the world. All of these obstacles can be defeated to an innovative technology like aquaponics. Aquaponics is a viable food growing technology which connects consecutive aquaculture with hydroponics in a symbiotic condition (Azad *et al.*, 2016). The basic principle of aquaponics is that the wastages of the biological system serve as nutrients to the other system and the water is reused through biological filtration (Bethe *et al.*, 2017).

Diversified leafy vegetables and plants are grown in aquaponics. Among them, okra, radish, lettuce, water spinach, Indian spinach, tomato, capsicum, cucumber, cabbage, carrots, mints, Lettuce, herbs etc. are remarkable (Azad *et al.*, 2016). Several thermal water and non-thermal water fish species like tilapia, trout, perch and bass (Diver, 2006) are cultured in the same system. Among different types of aquaponic systems, media based system is the most common style in the world because it requires less management practices and the fewest components. This system is easy to set in the backyard, rooftop or balcony of the houses. In media based system, miscellaneous inorganic media like gravels, bricklets, saw-dust (Salam *et al.*, 2014) etc. or diverse organic media like coconut fiber, husk and dust, discarded tea leaves, water hyacinth roots etc. (Azad *et al.*, 2014)

2016) are used. Okra (*Abelmoschus esculentus*) is an amazing food item having its nutritional, culinary and medicinal values for growing in aquaponics because of its adaptability to expansive range of climatic status. On the other hand, tilapia (*Oreochromis niloticus*) is an ideal candidate to culture in aquaponics as they are habituated in high density and poor water quality. Considering the importance's of okra and tilapia as routine food commodities, they were selected as culturable species in the aquaponic system. Furthermore, both of inorganic and organic media were considered as bedding media in this media based system to evaluate their performance by comparing how they contribute higher to food production- individually or combined. Hence, the present study investigated three different bedding media for okra production in aquaponic system as well as ascertained the suitable media through comparison of their performances and also evaluated their effects on tilapia production of the technique.

#### **II.** MATERIALS AND METHODS

#### 2.1 Experimental site and set up

The experiment was conducted at the roof top of a building. The roof was well protected from stealth. The empirical set up was in well exposed to sunshine.

Among different types of aquaponic system, the media based aquaponic system was selected to conduct the experiment. Three different media, gravels (gravels size was 1-2 cm) (T1), coconut husk (T2) and mixture of gravels and coconut husk (1:1 in volume) (T3) as three treatments, each having three replications were used in this experiment for okra production. The experimental model comprises of a fish holding tank of 180 liter (0.77m x 0.57m x 0.41m) and nine food grade plastic containers (each size 0.42m x 0.29m x 0.20m) for holing the media. Completely randomized design (CRD) was followed during placing the vegetable plastic containers side by side (Fig. 1).

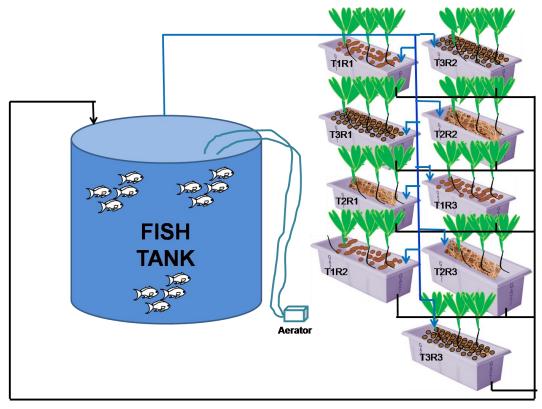


FIG. 1. Experimental design of the study

An inlet and outlet pipe was set to the fish tank. A 10-watt air pump installed with two air stones was set to aerate the tank water. A 12-watt submersible water pump irrigated the vegetable beds with the waste water of tank through the inlet pipe and then the clean water returned to the fish tank through the outlet pipe. The vegetable containers filled with different bedding media were placed side by side on the balcony of the roof keeping some space (total 20% area) between the containers for maintenance. Another plastic PVC pipe (length 3.5 m and diameter 1.5 cm) with nine holes over the containers was also attached to the inlet pipe for watering the vegetable beds (Fig. 1).

#### 2.2 Stocking and feeding of fish

The fish with initial length of  $13.65 \pm 1.88$  cm and weight of  $46.04 \pm 20.93$  g were released at the rate of 144 fish per m<sup>3</sup> after acclimatization. Traded floating feed having 30% protein was fed to fish twice a day. Feed was supplied at 3% of body weight of fish for premier month, 2% for next month and 1.5% for the remaining time of the experiment.

#### 2.3 Sowing rooted seeds and watering vegetable beds

The okra seeds after collection were soaked overnight and placed on a damp tissue paper for another two days to sprout. Then small roots emerged from the seeds and were sown in each four corners of the containers. The fish tank water was irrigated by submersible water pump to the okra beds through inlet pipe. The pump was run from 9 AM to 5 PM during day time.

#### 2.4 Fish and plant sampling

Fish and plants were randomly sampled biweekly. During each sampling, individual length and weight of ten fishes were measured carefully with a wooden fish length measuring scale and an electric weighing balance (KD-S/F-en), respectively. During plant sampling, plant height, branch number, leaf number, leaf length and width were measured with a measuring scale. After harvesting, okra pod was weighed with an electronic balance. Okra was harvested and measured the weight till the completion of experiment. After collection, all data were recorded.

#### 2.5 Assessment of physico-chemical parameters of water in fish tank

The physical and chemical parameters of water in fish tank were measured for water quality assessment. pH, Temperature (T) and Dissolved oxygen (DO) were recorded every 15 days interval. In addition, Electric conductivity (EC), total-nitrogen (N), Carbonate (CO<sub>3</sub>), Bicarbonate (HCO<sub>3</sub>), Sulphur (S), Potassium (K) and Sodium (Na) were measured thrice at two months interval during the study period at the Humboldt Soil Testing Laboratory.

#### 2.6 Data analysis

Data related to fish and okra production, fish and plant growth parameters were explored by one way and two-way ANOVA with MSTAT-C software. Duncan's multiple range tests (DMRT), Tukey's HSD (Honestly Significant Difference) and Fisher's LSD (Least Significant difference) tests were conducted to compare the averages to exhibit significant distinctions among the treatments at significant level of 0.05 and 0.01.

#### III. RESULTS AND DISCUSSION

#### 3.1 Plant growth and production performances

During the study period, the growth and yield performances of plants in three treatments (T1 - gravels, T2 - coconut husk and T3 - mixture of gravels and coconut husk media) were recorded.

#### **3.1.1** Duration of plant growth in different growth stages

The okra seeds were recorded to germinate after 4 days of sowing in all the treatments. Okra seed germination in aquaponic system was faster than that in the conventional agricultural system. Because Das (2011) and Patwary (2001) reported that okra seedlings emerged from the seeds in 4-6 and 11 days of sowing respectively who cultured okra in soil.

First flower bloomed 37 days later of sowing seeds in T3, but more 7 days were needed to show flowers in the treatments T1 and T2. Patwary (2001) reported that the okra plants took 37-39 days in soil media for first flowering, indicating that vegetable production in aquaponics was faster than conventional agriculture.

About 42 days later of sowing seeds, first okra pod was seen in T3, although other treatments delayed 7 days to show first okra pods. However, the okra pod was started to harvest after 46 days of sowing Plants. Olasantan and Bello (2004) reported that the time from planting to first pod harvest ranged from 55-62 days, confirming the faster growth rate of plant in soil-less media compared to soil media. Salam *et al.* (2013) and Rakocy *et al.* (2004) also cultured okra in aquaponic system and supported its rapid outgrowth.

Therefore, okra plant growth in the soil-less media didn't take longer duration than that of the soil media. It was also observed that in almost every growth stage,  $T_3$  (mixed media) showed better performances than the other treatments. This might be due to higher aeration and having sufficient nutrients for plants in the mixed media than the individual media.

#### 3.1.2 Plant growth performances in terms of vegetative parameters

After emerging of okra saplings from the seeds, the height, leaf number, leaf areas and branch number of the okra plants were measured every 15 days interval.

#### 3.1.2.1 Plant height (cm)

The significantly highest mean height of the plants (P  $\leq 0.05$ ) was 98.75 $\pm 2.38$  cm found in T<sub>3</sub> followed by T<sub>1</sub> of 89.42 $\pm 12.20$ and T<sub>2</sub> of 71.17±17.90 cm, respectively at 8<sup>th</sup> sampling stage. No significant differences among the treatments were observed in the mean heights of plants in all sampling dates except  $1^{st}$  sampling (P  $\leq 0.01$ ) and  $8^{th}$  sampling (P  $\leq 0.05$ ) (Fig. 2). Olasantan and Bello (2004) reported plant height ranging from 94-104 cm for early season of okra and 51-56 cm for late season of okra which were analogous and little inferior, respectively to the heights observed in present trial. The differences in plant growth among different treatments occurred due to the effect of different bedding media (Roosta and Afsharipoor, 2012). The wastewater could provide the leafy plants with nitrogen, but the plants' growth could be weakened due to inadequate nitrogen supply (Chen et al., 2004). Huge surface ground for bacterial survival and sufficient pores in inorganic natural gravel media could provide air to the plant roots (McCauley et al., 2005) as well as some nutrients (Rakocy et al., 2006) also. On the other hand, naturally available organic coconut husk media having neutral pH (Connolly and Trebic, 2010), suitable electrical conductivity and other chemical attributes for plants growth (Awang et al., 2009) contributed to enhance microbial abundance in the grow bed (Connolly and Trebic, 2010). The husks were high in nutrient retention capacity and poor in nutritional value (Vidhanaarachchi and Somasiri, 1997), although contained high levels of Cl, K and Na (Konduru and Evans, 1999). But high water holding capacity of husk media caused imperfect relationship of air and water. This led to less aeration into the medium, which ultimately affects the oxygen pervasion towards the roots. Corporation of coarser components with coco-peat could enhace the aeration condition of the media (Awang et al., 2009). Moreover, due to having high nutrient and water retention capacity, the husks gradually clogged the system with the progress of time. That's why the solid waste from fish tank couldn't reach the plant roots.

The mixed media performed best in terms of plant height due to higher aeration within the media (McCauley *et al.*, 2005) and supply of more nutrients to the plant roots (Rakocy *et al.*, 2006) through the faster nitrification process as both of gravels and coconut husk media acted symbiotically. On the other hand, at the preliminary stage, the plant height in coconut husk was better than gavel. But with the progress of time, the result turned into opposite. The main cause of this might be- the husks clogged the system gradually because of their higher nutrient and water retention capacity and thereby could not supply sufficient nutrient to the plants. At the young stage, plants didn't require higher amount of nutrients for their growth. Hence, the plant growth seemed to be better. But with the advancement of time, nutrient requirement for plant increased, but the coconut husk could not provide that. So plant growth in husk media started to show poor performances and gravel media took the position.

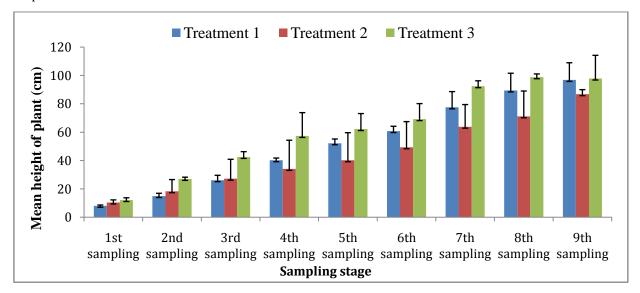


FIG. 2. Plant heights in different treatments in the experimental term ( $P \le 0.05$  and  $P \le 0.01$ ). In every sampling date, the highest mean height of plants was found in T3, whereas T2 showed better performances

Than T1 up to  $3^{rd}$  sampling. However, with the progress of time, the result was just opposite where T1 showed better performances than the T2.

#### 3.1.2.2 Count of leaves per plant

The highest mean leaf number was found in  $T_3$  on 9<sup>th</sup> sampling date and the lowest mean number of leaves (except initial number) was observed in  $T_2$  on 3<sup>rd</sup> sampling date. The media showed no significant effects on mean number of leaves of each plant in every sampling stage but 1<sup>st</sup> sampling ( $P \le 0.01$ ) and 8<sup>th</sup> sampling dates ( $P \le 0.05$ ). In all sampling dates,  $T_3$  showed the highest mean leaf number. On the contrary,  $T_1$  showed higher mean leaf number than  $T_2$  up to 6<sup>th</sup> sampling. But later the result was just opposite where  $T_2$  exhibited better performances than the  $T_1$  (Fig. 3). That means, the mixed media displayed better performance than the individual media in case of leaf number also. Up to certain time, the gravels media could provide the plants with enough nutrients particularly required for leaf emergence. But later due to faster drainage capacity of gravels, necessary nutrients were not absorbed by the plants. By contrast, at that time the plants grown in the husk media could receive the required nutrients due to higher nutrient retention capacity of the media (Vidhanaarachchi and Somasiri, 1997). Patwary (2001) also supported the leaf number in the experiment. Das (2011) recorded maximum leaf number of 22 for okra production in soil media which appreciated the present outcome.

#### 3.1.2.3 Leaf area

No significant distinctions in mean leaf area of plants were reported among the three treatments in all sampling stages except the initial one ( $P \le 0.05$ ). In the present study, the mixed media exposed larger leaf area than other media up to particular lifetime of the plant (up to 6<sup>th</sup> sampling). But later, the leaf area in the mixed media started to fall and the husk media took the space (Fig. 3). This might be due to higher-availability of nutrients essential for increasing leaf size in the mixed media up to particular time (up to 6<sup>th</sup> sampling) and then lower availability of the particular nutrients in the media. At that time the nutrients for leaves were higher in coconut husk rather than the mixed media and thereby higher leaf area was found in the coconut husk media.

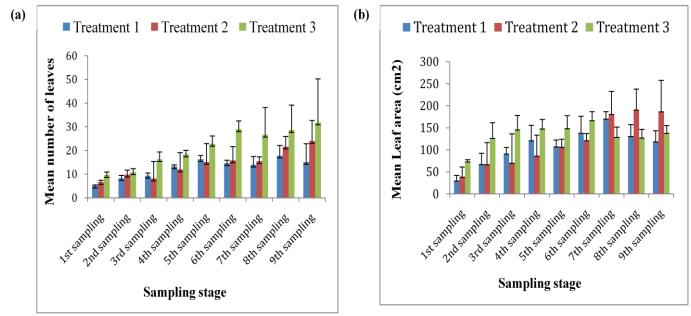


FIG. 3. Mean number (a) and mean area (b) of leaves in different treatments in the experimental term (P  $\leq$  0.05 and P  $\leq$  0.01).

#### 3.1.2.4 Number of branch per plant

About three months later of sowing seeds, plant of T3 showed first branching. The other treatments delayed about 7 days to show branching. During the study period, the highest mean number of branch was  $3 \pm 0.5$  per plant found in T3 followed by T2 of  $1.52 \pm 0.78$  and T1 of  $1.06 \pm 0.72$ , respectively (Fig. 4). Patwary (2001) and Das (2011) supported the results as they reported the number of branch ranging from 2.0 to 2.62 and 1.96 to 2.97 respectively during okra production in soil. The combined media showed faster and more branching than other media. This might be due to supply of higher amount of essential nutrients to the plant roots through the faster nitrification process by the mixed media.

#### 3.1.3 Production performances of okra plant

Significant ( $P \le 0.05$ ) variations in mean okra production were found among three treatments. During the study period, the significantly highest mean production of okra was  $9.08 \pm 1.25 \text{ kg/m}^2$  found in  $T_3$  whereas  $T_1$  produced  $7.5 \pm 1.83 \text{ kg/m}^2$  and  $T_2$  produced  $3.83 \pm 2.33 \text{ kg/m}^2$  of okra (Fig. 4). If the production was converted to land area, it would be 75, 38.3 and 90.8 tons/ ha/ 157 days in  $T_1$ ,  $T_2$  and  $T_3$  respectively. Das (2011) and Patwary (2001) cultured okra for 120 days in soil media and reported maximum okra production of 10.19 tons/ha and 13.09 tons/ha respectively in their experiments which applauded the present production. Similarly, Rakocy *et al.* (2004) reported that total field okra production was only 5% of the aquaponic okra production. They also reported okra production of 3.04 kg/m<sup>2</sup>/80 days (5.97 kg/m<sup>2</sup>/157 days) in UVI aquaponic system, confirming that the mixed media could be preferred over individual media. The outstanding performances of combined media in okra production compared to single medium might be due to having suitable growing condition and higher availability of nutrients for plants in the mixed media compared to the individual media.

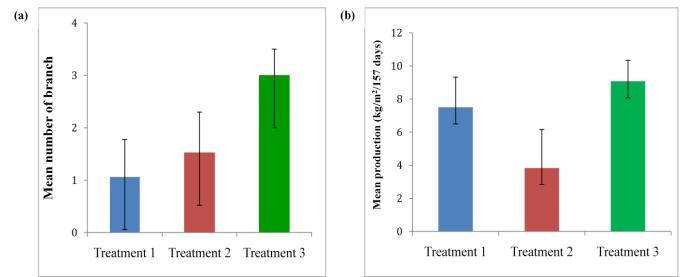


FIG. 4. Number of branch (a) and Mean okra production (b) of different treatments in the experimental term ( $P \le 0.05$ ).

#### 3.2 Growth and yield performances of Tilapia

#### 3.2.1 Growth performances of fish

The growth performances of tilapia regarding length (cm) and weight (g) gain were presented in Table 1.

| TABLE 1  |                    |  |  |
|--|--------------------|--|--|
| GROWTH PERFORMANCES OF TILAPIA OBSERVED IN THE EXPERIMENTAL TERM |                    |  |  |
| Growth measuring parameters                                      | Value              |  |  |
| Mean initial length (cm)   | $13.65 \pm 1.88$   |  |  |
| Mean final length (cm)   | $20.29 \pm 1.98$   |  |  |
| Mean length gain (cm)  | $6.64 \pm 0.1$     |  |  |
| % Length gain  | $48.64 \pm 5.32$   |  |  |
| Mean initial weight (g)  | $46.04 \pm 20.92$  |  |  |
| Mean final weight (g)  | $150.8 \pm 41.70$  |  |  |
| Mean weight gain (g)   | $104.76 \pm 20.78$ |  |  |
| % Weight gain  | 227.54 ± 99.33     |  |  |
| Specific growth rate (% per day)                                 | 0.75               |  |  |
| FCR  | 1.96               |  |  |
| Survival rate (%)  | 92.30              |  |  |
| Fish production (tons/ha/157 days)                               | 138.80             |  |  |

Rana *et al.* (2015) culture tilapia and found similar mean length gain of the present experiment. Sarkar (1998) recorded mean length gain of tilapia of 11 cm in 180 days which was slightly higher than the present findings and might be due to the differences in culture period, system, density and feed used. Hosen (2014); Salam *et al.* (2014) appreciated and Bethe *et al.* (2017) slightly depreciated the present mean weight gain who cultured tilapia in aquaponic system for 152, 116 and 180 days, respectively and obtained mean weight gain of 58, 116 and 170 g respectively. The differences might be due to various climatic conditions and stocking densities. Specific Growth Rate (SGR) of tilapia in the study was found satisfactory. More or less analogous findings were testified by Salam *et al.* (2013) and Salam *et al.* (2014) who recorded SGR value for tilapia in aquaponic system 0.79 and 0.74 respectively.

The FCR in the experiment was within the expected tilapia FCR ranging from 1.5 to 2.0 (Watanabe *et al.*, 2002). Rakocy *et al.* (2004) and Bethe *et al.* (2017) reported FCR value for tilapia in aquaponics 1.8 and 2.33 respectively, which more or less supported the present finding. Elswhere, Khan *et al.* (2008) recorded FCR value of 1.7 for Nile tilapia culture from August to October and Salam *et al.* (2014) reported 2.67 from March to June. It was also noted that higher water temperatures (>  $28^{\circ}$ C) within the suitable ranges could lead to higher FCR for tilapia (Midmore *et al.*, 2011). The survival rate in the current experiment was favored by the pronouncements of Salam *et al.* (2013) and Rahman (2000) who recorded 93 and 94% survival respectively but slightly devaluated the results of Salam *et al.* (2014) and Bethe *et al.* (2017) who reported 83.34 and 85% survival respectively. Satisfactory survival rate obtained in the current research might be owing to favorable environmental conditions, regular monitoring of water quality, and supply of adequate amount of feed.

Extensive tilapia production in the present trial was more appreciable compared to the production of Salam *et al.* (2014) and Bethe *et al.* (2017) who also cultured tilapia in aquaponic system for 116 and 180 days and found tilapia yield of 95.85 and 134.30 tons/ha respectively. Moreover, the present finding also showed superiority over the output of Rana *et al.* (2015) who obtained tilapia production of 28 tons per hectare for 90 days of rearing in ponds and feeding with experimental diets, meaning that the tilapia production in aquaponics was much higher than that in the conventional semi-intensive culture system.

#### 3.2.2 Growth pattern of Tilapia

There were significant (P  $\leq$  0.01) differences of mean lengths and mean weights of fish within varied sampling dates over the culture time. The length increment as well as weight enhancement of the present trial (Fig. 5) was analogous with the pronouncements of Salam *et al.* (2014) and Bethe *et al.* (2017).

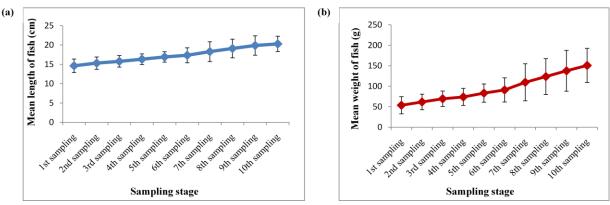


FIG. 5. Growth pattern of fish in respect of length increment (a) and weight enhancement (b) in the experimental term (P < 0.01).

#### 3.2.3 Length–Weight relationship of Tilapia

The Correlation Coefficient (r) was 0.99 and Coefficient of Determination ( $\mathbb{R}^2$ ) was 0.98 for Tilapia. In this study, for 1 cm length increase of tilapia, the weight increase was 15.97 g (Fig. 6). The Correlation Coefficient in the present experiment of 0.99 revealed that a very high positive relationship existed between the length and the weight of tilapia and it was proximate to 1, which indicated the increasing slope. Coefficient of Determination of 0.98 suggested that 98% variation of the dependent variable (Weight) could be explained by the independent variable (Length) but the rest could not be explained due to some experimental errors. Salam *et al.* (2014) recorded the correlation coefficient 0.95, Coefficient of Determination 0.95 and the weight increment of tilapia in aquaponic system 8.19g for 1 cm length increase which were lower than the present findings.

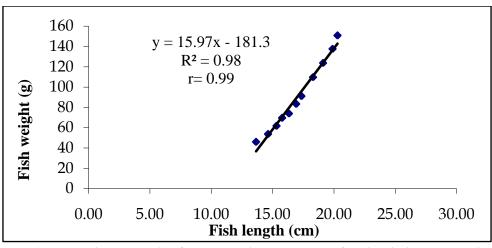


FIG. 6. Regression analysis of length-weight relevance for tilapia in aquaponics

It was observed that tilapia performed well in the system, although the stocking density was higher and they had to tolerate higher water temperature in summer as fish tank was exposed to the direct sunlight at the roof top.

#### **3.3** Water quality parameters

The water quality parameters remained within the suitable limits for tilapia production as well as the growth of okra throughout the experiment. The averages of pH, temperature and DO values were  $7.87 \pm 0.41$ ,  $29.25\pm1.17$ °C and  $4.18 \pm 0.27$  ppm, respectively (Fig. 7). There were significant (P < 0.01) dissimilarities of pH, temperature and Dissolve oxygen among different sampling dates during the study period. pH between 6.5 and 9.0 was desirable for tilapia culture (Swingle, 1968; Huet, 1972). Tyson and Simonne (2014) reported that optimum pH for nitrifying bacteria ranges from 7.0-8.0, whereas their growth is averted underneath pH of 6.5 where 7.8 is the most favorable pH (Antoniou *et al.*, 1990). So, the range of pH value in the present study was also good for okra production and nitrification.

On the other hand, temperature of water also plays a diametrical role, not only in reining fish's metabolic process (Battes *et al.*, 1979), but also for vegetable production and nitrification process. Tyson and Simonne (2014) reported that optimum temperature for tilapia culture ranged from 28 - 32°C. The low and high lethal temperature threshold for Nile tilapia was 11 and 42°C respectively (Balarin and Hatton, 1979). Okra plant showed better performances at 29 - 35°C temperature (Salam *et al.*, 2013). The optimum temperature for nitrifying bacteria was 25 - 30°C (Tyson and Simonne, 2014) and nitrification could be accomplished between 7 and 35°C with an optimum temperature of 15 to 25°C (Wortman and Wheaton, 1991). In the current experiment, temperature of fish tank water during the study period varied from 27.4 to 31°C with an average value of 29.25  $\pm$  1.17°C which remained within optimum level for tilapia, okra and even for nitrification too.

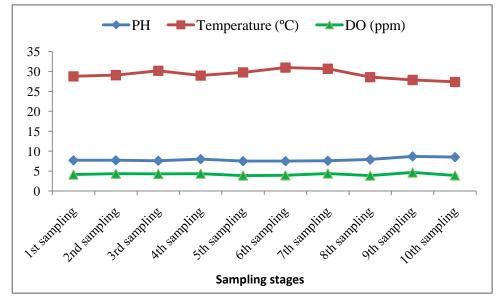


FIG. 7. Biweekly variations of water quality parameters during study period (P < 0.01).

Dissolved oxygen (DO) is another considerable factor in fish culture although tilapia is a hardy fish that can survive in a low DO content. The lest endurance limit of dissolved oxygen for Nile tilapia is as low as 0.1 mg/l (Ahmed and Magid, 1968; Magid and Babiker, 1975; Balarin and Hatton, 1979) owing to its capability to survive by using ambient oxygen. In the current study, the DO in tank water ranged from 3.88 to 4.65 ppm, where the average value was  $4.18 \pm 0.27$  ppm. More or less similar results were reported by Kohinoor (2000) and Hossain (2000) who observed DO values in fish culture ponds of 3.8 - 6.9 ppm and 2.04 - 7.5 ppm, respectively. Bethe et al. (2017) and Salam et al. (2014) also recorded DO values from 1.8 to 5.37 and 2.07 to 4.69 ppm respectively for tilapia in aquaponic system. Rakocy et al. (2004) observed DO values from 3.7 to 4.6 while producing okra in aquaponic system. The nitrifying bacteria growing on the root systems also participated in oxygen uptake (Sutton et al., 2006). Therefore, the present finding of DO value was suitable for okra, tilapia and nitrifying bacteria in aquaponic system.

#### Chemical analysis of influent and effluent water in laboratory 3.4

Nitrogen (N), potassium (K), phosphorous (P), sodium (Na), sulphur (S) are important macronutrients for plant growth. Here, the inner water of fish tank was considered as influent water and the water returning again to the fish tank after recycling was figured out as effluent water. In the present trial, the largest amount of Total-N was  $22.4 \pm 2.4$  ppm in influent in 1<sup>st</sup> sampling when it was  $16.8 \pm 1.6$  ppm in the effluent (Fig. 8), where the Total-N removal was 25%. Highest Total-N removal was 66.6% found in 2<sup>nd</sup> sampling. Ghaly and Snow (2008) experimented the usage of barley for nourishment dismissal from recirculating aquaculture equipped with Arctic charr (Salvelinus alpinus) and observed 76% of total-N shortening. Salam et al. (2014) and Bethe et al. (2017) reported 16% and 42% total-N utilized by plants cultured in aquaponic system which supported the present finding. In the present study, the Phosphorus (P) value in the effluent was higher than that in the influent. This might occur because the growing media- coconut husk or cocopeat contains several macro- and micro-plant nutrients (Evans et al., 1996). In  $3^{rd}$  sampling, the influent and effluent water contained 0.08 ± 0.01 and 0.98 ± 0.06 ppm P values respectively (Fig. 8) which coincided with the recognition of Salam *et al.* (2013) who recorded average  $PO_4$ –P of 0.05 to 1.0 ppm in research ponds of BAU, Bangladesh

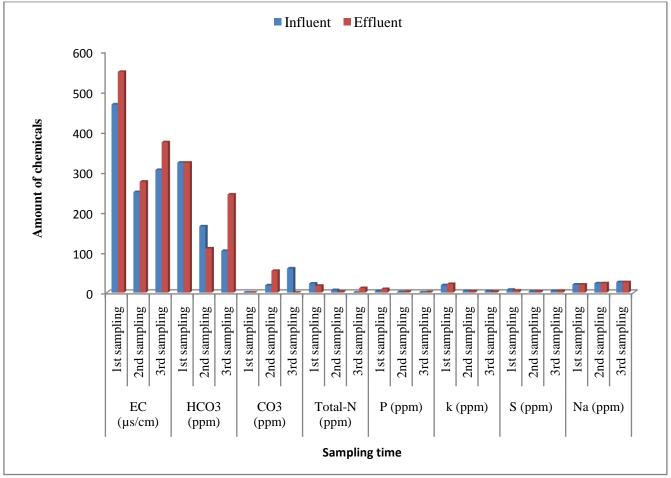


FIG. 8. Chemical properties of influent and effluent water in the experimental term

The higher value of Potassium (K) in effluent  $(21.38 \pm 4.3 \text{ ppm})$  than the influent  $(18.33 \pm 3.5 \text{ ppm})$  in 1<sup>st</sup> sampling indicated that plants didn't uptake K at the preliminary stage which was supported by the findings of Rakocy *et al.* (2004). Moreover, coconut husk itself contains high level of potassium from 19 to 948 ppm (Evans *et al.*, 1996). But with the maturity, plants required K and up took them as their nutrients (Fig. 8). In 1<sup>st</sup> sampling, 27.8% sulphur (S) was utilized by plants. But at the final stage of experiment (3<sup>rd</sup> sampling), plants might stop to uptake S, so the influent might be lower than the effluent (Fig. 8) due to reaction with the media which was supported by the findings of Rakocy *et al.* (2004) who also produced okra in the aquaponics.

The greatest average value of sodium (Na) was  $26 \pm 4.1$  ppm found both in the influent and effluent water in 3<sup>rd</sup> sampling. Contrariwise, the lest mean value of Na was  $20 \pm 3.6$  ppm found both in the influent and effluent water in 1<sup>st</sup> sampling (Fig. 8). In the present research, Na utilization by plants remained undetectable which was supported by Rakocy *et al.* (2004). This might be due to having higher level of Na (23 to 88 ppm) in the coconut husk media (Konduru and Evans, 1999). The highest mean value of EC was  $549\pm1.2 \mu$ s/cm that was found in the effluent water in 1<sup>st</sup> sampling and the lowest mean value of EC was  $250\pm0.6 \mu$ s/cm found in the influent in 2<sup>nd</sup> sampling (Fig. 8). The EC ranging from from 2.00-4.00 milliSiemen/cm contained in a typical hydroponic nutrient solution (Resh, 1995). Rakocy *et al.* (2004) recorded EC value of 0.5 mS/cm (500  $\mu$ s/cm) in okra production experiment which was approximately analogous to the current pronouncement.

The largest amount of HCO<sub>3</sub> (323.3±4.2 ppm) was found both in the influent and effluent water in 1<sup>st</sup> sampling. On the contrary, the lowest mean value of HCO<sub>3</sub> was 103.7±0.29 ppm found in the influent in 3<sup>rd</sup> sampling, while the value was 244±4.4 ppm in the effluent water (Fig. 8). At the preliminary stage of culture, plants didn't uptake HCO<sub>3</sub> from the fish waste water, but at the middle stage HCO<sub>3</sub> was utilized by plants as the effluent was lower than the influent. At the final stage, plants further might stop to uptake HCO<sub>3</sub> due to reaction with the growing media. The highest mean value of CO<sub>3</sub> was  $60\pm1.3$  ppm that was found in the influent water in 3<sup>rd</sup> sampling, while in the effluent no CO<sub>3</sub> was found. Moreover, CO<sub>3</sub> content was nil both in influent and effluent water in 1<sup>st</sup> sampling (Fig. 8). Plants didn't require CO<sub>3</sub> at first 3 months of culture, but later they utilized CO<sub>3</sub> from the system extensively.

Therefore, the nutrient concentrations and other water quality parameters were favorable for aquaponic system as both of fish and vegetable grew well in the system.

#### IV. CONCLUSION

The present experiment has proved that the mixture of gravels and coconut husk media is more suitable for vegetable growth and production than the individual media. The fish growth and production performances are also favorable in the same aquaponics. The system can be used for fish and vegetable production in urban and peri-urban areas. Thus nutrition and food security will be enhanced with minimum environmental pollution. Moreover, the availability and low-cost of bedding materials and suitability of this system provide better opportunities to fulfill the nutritional demand and maximize the use of land and water.

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# **A Review on Health Insurance Claim Fraud Detection**

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**Abstract**— The anomaly or outlier detection is one of the applications of data mining. The major use of anomaly or outlier detection is fraud detection. Health care fraud leads to substantial losses of money each year in many countries. Effective fraud detection is important for reducing the cost of Health care system. This paper reviews the various approaches used for detecting the fraudulent activities in Health insurance claim data. The approaches reviewed in this paper are Hierarchical Hidden Markov Models and Non Negative Matrix Factorization. The data mining goals achieved and functions performed in these approaches have given in this paper.

Keywords: Hidden Markov Models, Non Negative Matrix Factorization.

#### I. INTRODUCTION

In several countries fraudulent behavior in health insurance is a major problem. Data mining tools and techniques can be used to detect fraud in large sets of insurance claim data. One of the most common data mining techniques used for finding fraudulent records is anomaly detection.

This paper aims to review various approaches used for Health insurance fraud detection. There are three major parties involved in the entire system,

- (1) Service Providers
- (2) Insurance Subscribers
- (3) Insurance Carriers

The Service Providers including doctors, hospitals, ambulance companies and laboratories. The Insurance Subscribers including patients and patient's employers. The Insurance Carriers who receive regular premiums from subscribers and pay health care cost on behalf of their subscribers.

There is a difference between fraud prevention and fraud detection. The fraud prevention describes measures to avoid fraud to occur. The fraud detection involves identifying fraud as quickly as possible, once it has been committed.

According to the National Health care anti-fraud association, health care fraud is the misrepresentation of Claims for gaining some shabby benefits. The health industry in India is losing approximately Rs.600 crores on "false claims" every year. So to make health insurance feasible, there is a need to focus on eliminating or reducing fraudulent claims.

Generally there are two types of frauds.

First one is Hard fraud: This is a deliberate attempt either to point an event or an accident, which requires hospitalization or other type of loss that would be covered under a medical insurance policy.

Second one is Soft fraud: Which occur when people purposely provide false information such as claim fraud, application fraud and eligibility fraud sources and then put to use by data miners to achieve the desired results.

The rest of the paper is organized as follows. Two approaches are explained in section II. Comparative study presented in section III. Concluding remarks are given in section IV.

### II. HEALTH CARE FRAUD DETECTION TECHNIQUES

Data Mining for Healthcare Management is an emerging potential area with respect to its impact on improving healthcare as a result of discovering new patterns and trends in voluminous data generated by healthcare transactions.

Some of the existing approaches of data mining for health insurance fraud management are been listed below.

#### 2.1 Using Hierarchical Hidden Markov Models

In this approach [1], first decomposes the dataset into groups of claimants of similar age since the age contributes to a patient's medical conditions. The approach applies recursively the Gaussian mixture clustering and HMM procedures on randomly selected samples from the training set until the classification errors converge to a prescribed minimum threshold is observed. The entire process as follows,

#### 1) FEATURE EXTRACTION

To extract the temporal behaviors of the claimant, for example unique personal identifier, date of claim, age of the claimant and total claims per day.

#### 2) CREATION OF COHORTS

The claimants of similar age range forms an age cohort. The above two steps are preprocessing steps which prepare the data for the application of pattern discovery techniques.

#### 3) CLUSTERING

Gaussian mixture clustering is applied to identify cluster in the data for each age cohort.

#### 4) PATTERN DISCOVERY

The pattern discovery is accomplished by using Hidden Markov models (HMM). This model commonly used in temporal behavioural pattern discovery. The steps (iii) & (iv) executed recursively until convergence occurred. This recursive process yields a set of HMMs which are hierarchically organized.

#### 2.2 Using Non Negative Matrix Factorization

This paper [2] proposes a Non-Negative Matrix Factorization (NMF) method for fraud detection, which introduces a technique for clustering medical treatment items such as medicines or medical measurements in to several groups according to usage of different patients.

Then each group is considered as a kind of medical treatment items for curing similar symptoms. If a medical treatment item shifts from one cluster in this month to another cluster in next month, then this algorithm could classify the patient using this medical treatment item as a fraud suspicious patient.

In the end, all these fraud suspicious patients are submitted to medical experts for detailed careful detection. The factorization can be used to compute a low rank approximation of a large sparse matrix along with preservation of natural data Non-Negativity.

Each vector component is given a positive value (or weight) if the corresponding medical treatment item is used by the patient and a zero value otherwise, the resulting matrix is always non-negative.

#### **III.** COMPARATIVE STUDY

| COMPARATIVE STUDY OF THE HEALTHCARE FRAUD DETECTION STSTEMS                     |  |   |   |  |  |
|---|--|---|---|--|--|
| TITLE   | TECHNIQUE USED                               | MERITS  | DEMERITS  |  |  |
| Hierarchical Hidden Markov<br>Models An Application to<br>Health Insurance Data | Hierarchical Hidden<br>Markov<br>Models(HHM) | Recursive training of HMMs<br>provides a mechanism to detect<br>redundancy in the dataset | Computational loads are<br>high when the training<br>datasets are large |  |  |
| Health Care Fraud Detection<br>Using Nonnegative Matrix<br>Factorization        | Nonnegative Matrix<br>Factorization(NMF)     | Identification of unknown types<br>of fraud   | A distributed NMF is needed for a large dataset                         |  |  |

# TABLE 1 Comparative study of the Healthcare Fraud Detection Systems

#### **IV.** CONCLUSION

In conclusion, this paper reviews two approaches for detecting fraudulent behavior in health insurance claim. By analyzing the aforementioned techniques, we will get a clear idea for the future work in health insurance claim fraud detection. In India, we have three levels of health care network, namely primary, secondary, and tertiary. It provides an opportunity for data miners to use the huge amount of data. The main task is to integrate data from different sources and then put to use by data miners to achieve the desired results.

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