International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979; Impact Factor: RJIF 5.72
Received: 15-11-2018; Accepted: 17-12-2018
www.allsubjectjournal.com
Volume 6; Issue 1; January 2019; Page No. 109-115

Characterization of fungal pathogens causing diseases in bitter gourd and establishment of their eco-friendly control measure

ME Khan Chowdhury[1], M Saroar Jahan[2], Sharmin Akhtar[3], M Ashikul Islam[4], M Asadul Islam[5], B Sikdar[6], MF Hasan[7*]
[1, 2, 4, 5, 6, 7] Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi, Bangladesh
[3] Assistant Teacher, Starline Sprout International School, S.S.K Road, Feni, Bangladesh
[*] Corresponding Author: MF Hasan

Abstract
The present investigation was designed for morpho-physiological and molecular characterization of the phyto-pathogenic fungi responsible for two deleterious diseases - damping off and fusarium wilt as well as evaluation of their control technique. The pathogenic fungi were isolated from the diseased parts of bitter gourd plant collected from two different fields and primarily identified on the basis on their specific colony morphology and microscopic observation. The fungal isolates were grown well on PDA medium and showed different colony morphology on different culture media. Although the optimum pH of these fungi varied, but the optimum temperature was 25°C for both of the fungal isolates. The differences in carbohydrate types, sugar concentrations, organic acids and salts also significantly influenced the mycelial growth of these fungi. PCR products of the internal transcribed spacer (ITS) region of the fungi showed approximately 650bp size clear band in gel electrophoresis. In sequencing analysis, the amplified ITS region of the fungi showed 99% and 100% similarities with the original sequences of *Fusarium equiseti* and *Fusarium oxysporum*, respectively. In antifungal activity assay, the extracts of *Ficus racemosa, Moringa oleifera, Azadirachta indica, Cassia alata, Senegalia catechu* etc. displayed promising antifungal activities against both of the isolate A and B. The outcome of this research work would be helpful for detection and developing nature-friendly control approach of the two devastating diseases of bitter gourd.

Keywords: bitter gourd, characterization, pathogen, biological control

Introduction
Bitter gourd **(***Momordica charantia* L.) is one of the most popular vegetable crop in Bangladesh for its nutritive and medicinal values belongs to the Cucurbitaceous family [1]. It is grown extensively throughout the country of Bangladesh during Kharif season which are around 23,890 acres and total production are above 52,020 metric tons per annum [2]. As far morphology of the plant is concerned, it is herbaceous plant that grows around 5m and bears simple/alternate leaves of 4-12cm with 3-7 deeply separate lobes. Bitter gourd is similar to a small cucumber, usually rectangle and oblong in shape and eaten green. It is filled with pulp and large flat seeds, which surrounding a comparatively thin layer of flesh [3-4]. It possess wide range of pharmacological activities for instance, antioxidant, antifungal [5], antidiabetic [6], antiobesity, stomachic, anticancer, hypotensive, and blood cholesterol lowering effects [3, 7] and it has been the subject of intensive investigations for numerous biologically active compounds which may have excellent medicinal properties [8-9].
The success of any crop production generally depends on the profitability at farm level. But various devastating plant diseases pose a great threat to the agricultural sector by reducing the life-span of the plants. The diseases in bitter gourd cause huge production and economic losses as well as reduction in both quality and quantity of the vegetable. There are various types of diseases that affect the bitter gourd plants among which fusarium wilt, collar rot, powdery mildew, downey mildew, alternaria blight, anthracnose and mosaic are most common found in the Indian subcontinent. Damping off can affect seedlings under and above ground. Some seedlings may start to grow and suddenly whither due to damping off. Others will have stems that appear pinched or broken, causing them to collapse while they still have their cotyledons. While damping off of seedling is prominent among the soil-borne fungal diseases, it is caused by several species of *Fusarium, Pythium, Rhizoctonia* and *Verticillium* [10-11], and widely distributed throughout the world, but most prevalent in wet and cool conditions. *Fusarium equiseti,* one of the causal agent of the concern disease, is cosmopolitan [12-13], common in temperate and subtropical areas [14] found this fungus to be pathogenic to cucurbits. A previous study [14] on *F. equiseti* showed that *F. equiseti* was pathogenic mainly during seedling preemergence. Overall, the economic losses due to damping-off are represented by a direct cost, due to damages of seed or seedlings, and an indirect cost, which consists of an additional cost of replanting and the consequent lower yields due to the later planting dates [15-16]. According to Halila and Strange [17], another noxious disease - fusarium wilt may be devastating to individual crops and cause up to 100% loss under favorable conditions of the causal agent of the disease. The members of cucurbitaceae family are susceptible to fusarium wilt caused by different forms of *Fusarium oxysporum*. Since the fungus chokes the vascular system of plant and plant dies from water and nutritional deficiency, it attacks mainly when the plants have grown up [18]. The difficulty in controlling fusarium wilt has stimulated the research in biological control independently from the recent concern for environmental protection [19]. The objective of the present study was to isolate the pathogens responsible for damping off and fusarium wilt disease of bitter gourd along with their morpho-physiological and molecular characterization. Moreover, different types of

plant extracts were used to evaluate the efficacy as a mean of eco-friendly biological agent for control of the isolated pathogens as well as the diseases of bitter gourd.

Materials and Methods

Plant materials collection

The present study was conducted at Professor Joarder DNA and Chromosome Research Lab., Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh during March 2017 to March 2018. The two different diseased leaf samples were collected from Keshorhat, Mohonpur, Rajshahi, Bangladesh. The diseases were identified by Dr. Asraful Islam, Scientific Officer, Fruit Research Institute, Binodpur, Rajshahi which were used as plant materials.

Fig 1: Showing the diseased plant samples (A) Damping off and (B) Fusarium wilt of bitter gourd

Isolation and purification of fungi

Symptomatic fragments were taken from the infected parts of the plant, sterilized in a 5% sodium hypochlorite solution, rinsed with sterilized water and placed on Potato Dextrose Agar (PDA) (Difco, USA). The medium also was supplemented with $0.5gL^{-1}$ of streptomycin sulphate (Sigma-Aldrich, USA) and $1mlL^{-1}$ of lactic acid. After that the Petri dishes were incubated at 25°C in the dark for 3 days. The colony produced from diseased sample were re-isolated using a single spore [20] where the fungal colonies emerged from diseased samples were transferred to different Petri dishes containing fresh PDA medium.

Morphological and microscopic identification

Pure fungal cultures were transferred to fresh potato dextrose agar (PDA) to stimulate sporulation of conidia that facilitate the identification of concerned fungi. The species were identified on the basis of macroscopic and microscopic characteristics such as overall color, reverse color, growth rates, pigmentations of colony, types of conidiogenous cells, shape and size of conidia, dry weight, presence or absence of sporodochia and chlamydospore etc. [20].

Growth profiling of isolated fungi

The differences in growth media have significant effects on the vegetative growth, colony character, sporulation, and pigmentation depending upon pH, temperature, light, water availability and surrounding atmospheric condition [21].

Three different agar media (Hi Media, Bombay) namely: potato dextrose agar (PDA), sabouraud dextrose agar (SDA) and nutrient agar (NA) were prepared separately and used to evaluate the colony morphology of the two isolated fungi. PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth [22] and it was used to comparative study of different characteristics for the growth profiling of the two fungal strains. For the determination of the colony morphology and dry weight, the five petri dishes containing around 25ml PDA media with 6 mm mycelial disc were kept in 15^0C, 20^0C, 25^0C, 30^0C and 35^0C temperatures, respectively and incubated at dark for 7 days. The effects of different pH on the growth pattern of the two fungi were estimated through adjusting the pH at 5, 6, 7, 8 and 9, respectively by using HCl and NaOH prior to sterilization. Various carbohydrates such as fructose, lactose, maltose, sucrose and starch were added as sole carbon source to the medium at 2% concentration instead of dextrose to evaluate the effect of these carbohydrates. The Contribution of the salinity to the growth of the fungal strains was also investigated by incubating the fungus in PDA media supplemented with 0.5%, 1%, 2%, 4%, 6% (w/v) of NaCl. The organic acids also play effective roles in determining the growth instructions of the fungi. Here, the influences of citric acid, malic acid and nicotinic acid were determined by adding extra 1% acids on the PDA media.

Molecular identification of the isolated fungi

After 7 days of incubation of two pure fungal isolates on PDA media at 25±2°C, the mycelium were subjected to isolation procedure. Here, Max Maxwell® 16 LEV Plant DNA Kit (AS1420, Promega, USA) was used for the isolation of the genomic DNA. The isolated DNA was amplified through polymerase Chain Reaction (PCR) technique using universal primers, ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3'), and Hot Start Green Master Mix (Promega, USA). PCR was performed in a 50µl reaction mixture containing 25µl of Hot Start Green Master Mix (2X), 2.0 µL of each forward and reverse primer, 2.0 µL of genomic DNA and rest of the PCR water. The performing PCR program was as follows: pre heat at 95°C for 2 min, followed by 32 cycles of denaturation step at 95°C for 30 sec, primer annealing at 48°C for 30 seconds, primer extension at 72°C for 45 sec. After that, the temperature of final extension was at 72°C for 10 min and lastly, hold at 4°C for overnight. The amplicons were separated by 1% agarose (V3125, Promega, USA) gel electrophoresis. The quality and quantity of isolated DNA were checked by NanoDrop Spectrophotometer (ND2000, Thermo Scientific, USA). Finally, The PCR products were purified and used for sequencing analysis in Malaysia Ltd. via Invent Biotechnologies, Bangladesh. The sequenced data were analyzed using similarities of nucleotide sequences between isolates through the BLAST procedure (http://blast.ncbi.nlm.nih.gov).

Evaluation of *in vitro* antifungal activity

For evaluating antifungal activity of leaf extracts, slightly modified poisoned food technique [23] was used. The antifungal effects of total twelve plant extracts (named *Azadirachta indica, Cassia alata, Coccinia grandis, Hibiscus rosa-sinensis, Ficus racemosa, Psidium guajava, Moringa oleifera, Andrographis paniculata, Datura metel, Allium sativum, Senegalia catechu, Spondias mombin)* against isolate A and B fungi were investigated. 50µg plant extract was added at 25ml of PDA medium, mixed by stirring,

poured into petri plates and cooled down. Small disc of the pure fungus culture (6mm) was cut with a sterile cork-borer and transferred aseptically in the center of the petri plate. Suitable checks was kept where the culture discs was grown under same conditions on PDA medium without extract was used as control. The inhibition of growth was calculated by Arora and Dwivedi methods [24].

Percent inhibition of mycelial growth= $[(G_c-G_t)/G_c] \times 100$

Where, G_c = Mycelial growth in terms of colony diameter in control set, G_t= Mycelial growth in terms of colony diameter in treatment set.

Data analysis

The data for the percentage and average numbers were determined using three replicates and each experiment was repeated thrice. Means and standard errors (M±SE) were calculated for each experiment using Microsoft Excel 2013 plus software.

Results

Isolation and purification of fungi

In the present investigation, two types of sample were collected from two different fields marked as isolate A and isolate B. After the incubation, pinkish white fungal colony was appeared from isolate A, a part of collapsed stem, whose center became brownish pink to dark brown within few days (Fig. 2A). The sample B, leaf of wilted young bitter gourd plant, produced pinkish white colony with pale pink center (Fig. 2B).

Microscopic identification

The mycelium of isolate A was partly superficial and immersed, composed of branched; the hyphae were septate, smooth, and hyaline. The Conidia are falcate and septate. Macroconidia are fusiform (‘canoe-shaped’), typically having three to five cells and are produced in large numbers, often in clumps known as sporodochia. The results are given in Fig. 2C-D.

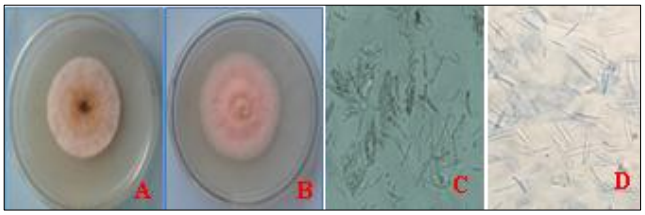

Fig 2: Showing the pure culture and cotton blue staining (A & C) Isolate A and (B) Isolate B

Colony characterization due to differences in media

Both of two fungi were grown on three different media - PDA, SDA and NA where they showed different morphological and physiological characteristics which are represented in table 1.

Table 1: Morpho-physiological characteristics of the isolate A and B in different culture media

Characteristics	**Isolate A**		
	PDA	**SDA**	**NA**
Front color	Brownish pink	Brownish pink	Brownish white
Back color	Brown with brownish white margin	Yellowish brown	Pale brown
Diameter (mm)	49	37	86
Form	Filamentous	Filamentous	Filamentous
Elevation	Crateriform	Raised	Convex
Margin	Filiform	Filiform	Filiform
Opacity	Opaque	Opaque	Opaque
Dry weight (gm)	0.308	0.262	0.187
Characteristics	Isolate B		
	PDA	SDA	NA
Front color	Pinkish white	Brownish white	Translucent brownish white
Back color	Pinkish white with pale pink center	Pink	Translucent brownish white
Diameter (mm)	56	23	63
Form	Filamentous	Filamentous	Filamentous
Elevation	Crateriform	Raised	Raised margins
Margin	Entire	Undulate	Undulate
Opacity	Opaque	Opaque	Translucent
Dry weight (gm)	0.271	0.085	0.129

Growth profiling in different temperatures

The growth rates of fungal isolates were measured on PDA plates at temperatures ranging from 15 to 35°C. The study showed that the optimum temperature for growth of the two isolates was 25°C where the dry weight values were 326mg and 263mg for isolate A and isolate B respectively. But at 37°C temperature, the growth rate decreased significantly; the value was 5mg for isolate A and 101 mg for isolate B.

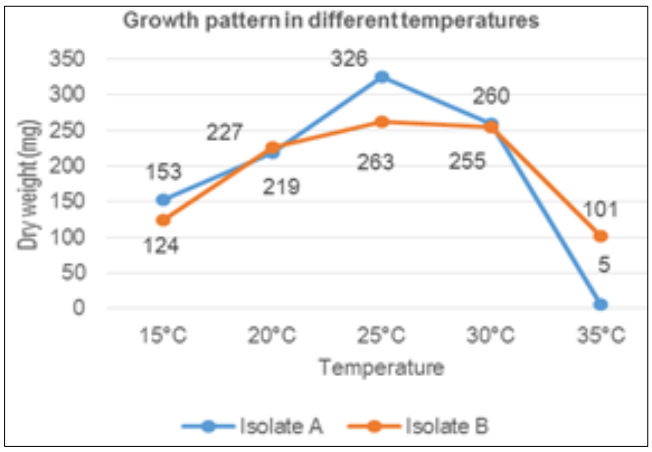

Fig 3: Showing the growth pattern of isolated fungi A & B in different temperatures

Growth profiling in different pH

The pH is one of the most effective factor in determining growth pattern of the specific fungi. The pH values of PDA media were changed to 4, 5, 6, 7, 8, and 9 to evaluate the effect of pH to develop the growth environment. Interestingly, both of the fungal isolates showed maximum growth at pH value of 8 and the maximum dry weights of fungal isolates were about 367mg and 237mg, respectively. The findings are represented in fig 3.

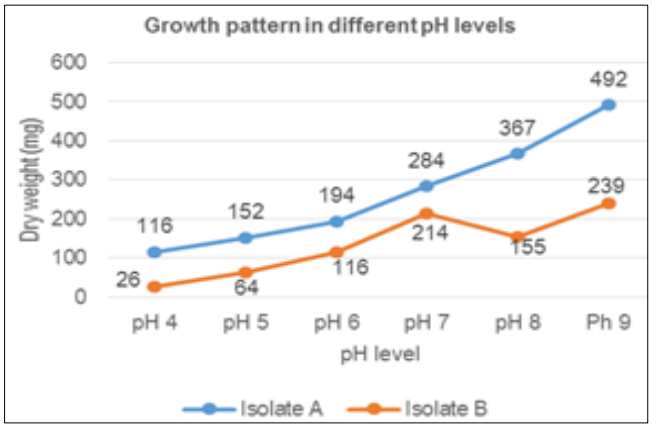

Fig 4: Showing the growth pattern of isolated fungi A & B in different pH levels

Growth profiling in different carbohydrates

To evaluate the effect of different carbohydrates on the mycelial growth, sucrose, fructose, lactose, maltose and starch were used instead of dextrose as a sole carbon source in PDA media. The isolate A showed maximum and minimum growth in lactose and starch containing media whereas the isolate B grew most in sucrose containing media. Besides that Isolate B had lower growth ability in maltose containing media comparatively than other carbohydrate containing modified PDA media. Results are given in fig 4.

Growth profiling in different sugar concentrations

The optimum sugar concentrations for the growth of the fungal isolates significantly varied. The isolate A fungus grew faster at 3% sugar concentration, but concentration was changed into 15% in case of isolate B. The results are shown in fig 5.

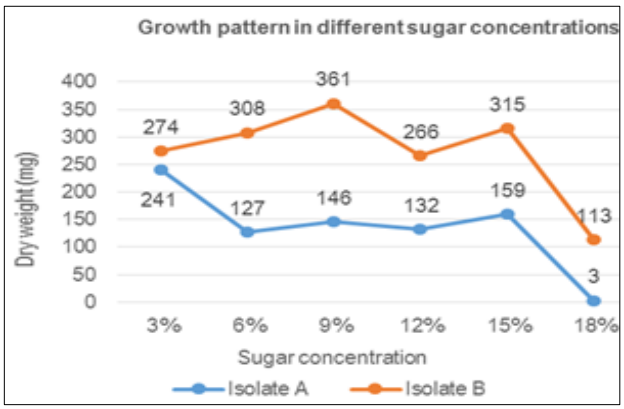

Fig 5: Showing the growth pattern of isolated fungi A & B in different sugar concentrations

Growth profiling in different salt types

The salinity may influence fungal growth greatly through providing appropriate nutritive environment. Total five types of salt- $CaCl_2$, $MgCl_2$, NaCl, KCl and $MgSO_4$ were used to measure the effect of salts on mycelia growth. For isolate A, the maximum growth was achieved due to the addition of $CaCl_2$ among the salts, but Isolate B grew much better in $MgCl_2$ containing media. Results are presented in fig 6.

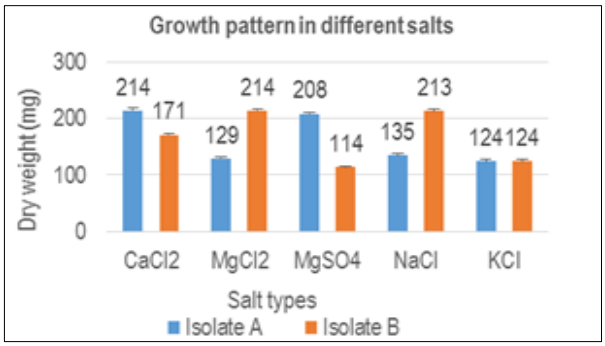

Fig 6: Showing the growth pattern of isolated fungi A & B in different salts

Growth profiling in different organic acids

It was observed that organic acids had greater inhibitory effect on the growth of both fungi. At 1% concentration of nicotinic acid, 100% growth inhibition of both fungal strains were observed whereas citric acid had lower inhibiting effect among the three organic acids used. The findings are showed in fig 7.

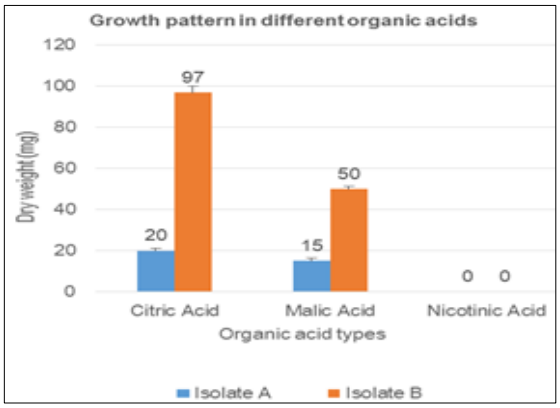

Fig 7: Showing the growth pattern of isolated fungi A & B in different organic acids

Molecular identification of the two fungal isolates

The genomic DNA isolated from the fungal isolates showed higher molecular weight and bright band on 1% agarose gel electrophoresis where 1kb DNA ladder was used as a marker. The universal primers, ITS-4 and ITS-5, were used to amplify a region of fungal genome named the 18S of ribosomal DNA gene of both isolate A and isolate B. The PCR amplified fragments of both the isolates yielded two single band of around 650bp (Fig. 8).

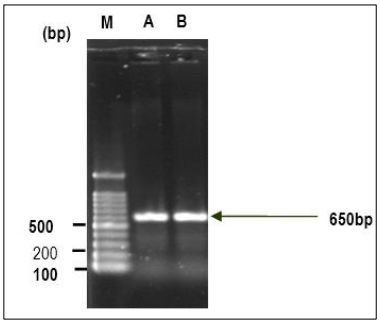

Fig 8: PCR amplification of isolated fungi using ITS4/ ITS-5 primers; (M) DNA ladder (Marker), (A) Isolate A and (B) Isolate B fungi

Sequencing

The sequenced nucleotide sequences were subjected for a BlastN search in GenBank (https://www.ncbi.nlm.nih.gov). The data analysis revealed that the 18S of rDNA sequence of isolate A showed 99% similarity with the original sequence of *Fusarium equiseti* whereas the rDNA sequence of isolate B showed 100% similarity with the original sequence of *Fusarium oxysporum.*

Phylogenic analysis

The sequences of related fungal strains were downloaded in FASTA format from GeneBank. After that the downloaded data were aligned to construct phylogenetic tree (Fig. 9AB).

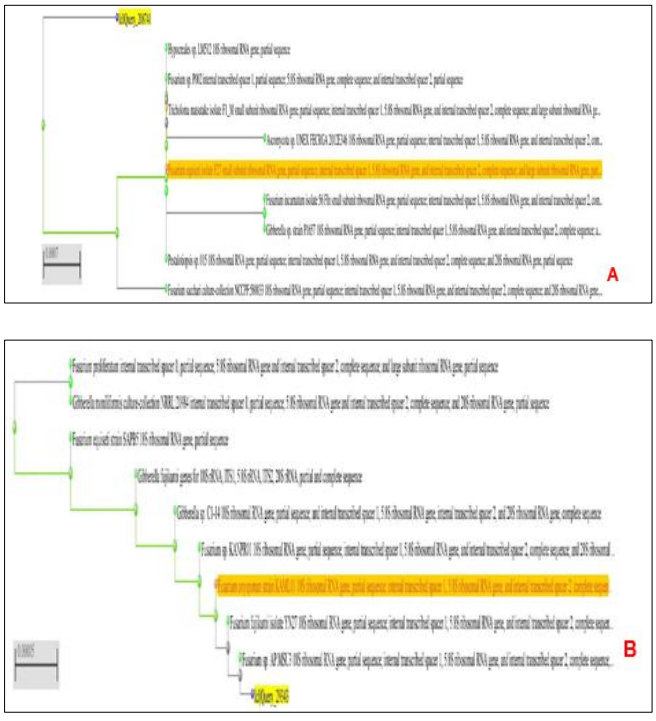

Fig 9: Phylogenetic tree for sequenced data (A) Isolate A and (B) Isolate B

***In vitro* antifungal activity evaluation of plant extracts**

In the experiment, methanolic extract of different plants showed inhibition at different levels against the growth of two fungal isolates which given in graph 7. The extracts of *Ficus racemosa, Cassia alata, Azadirachta indica, Hibiscus rosa-sinensis Moringa oleifera* etc. had showed the highest percentages of mycelial inhibition against isolate A. But in case of isolate B, *Senegalia catechu, Andrographis paniculata, Ficus racemosa* etc. had highest inhibitory effects among all the plant extracts. *Moringa oleifera* had also prominent inhibitory effects on both fungal isolates. The result also showed that *Andrographis paniculata and Senegalia catechu* had lowest inhibitory effects on isolate A although they had highest rate of growth inhibition on isolate B. On the other hand, *Coccinia grandis, Hibiscus rosa-sinensis, Cassia alata* etc. were found to be no or less effective against the mycelial growth of isolate B, but they showed promising positive effects on inhibition of vegetative growth of isolate A (fig 10). The leaf extract of *Spondias mombin* had been proved to be less effective in inhibiting mycelial growth for both isolates in comparison to the other plant extracts used.

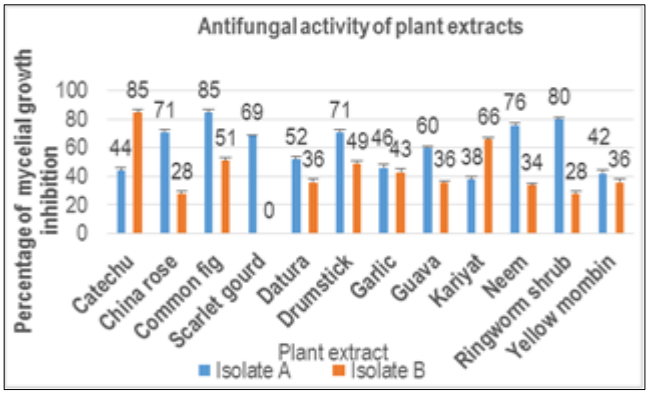

Fig 10: Showing the percentage of inhibition of isolates mycelial growth by plants extracts

Discussion

According to the latest census, the population of the Bangladesh is over 160 million with an average growing rate of 1.6% and the density of human population is 1033.5/km^2 [25]. The agricultural sector of the country plays a vital role in supplying not only the daily necessities of people but also in providing strength and stability to the economy. Bitter gourd is a very popular and costly vegetables for its food value and medicinal properties that are grown both in homesteads and in the fields. Although it is an important source of income for the farmers, its production and consumption are greatly hampered by various diseases, especially fusarium wilt and dumping off. *Fusarium* species are commonly associated with many economically important crop diseases, such as, vascular- wilt, root rot, stem- rot, and fruit and vegetable decay. The diseases have huge deleterious effects on yield and economic losses of farmers, hence study of distribution and diversity of these pathogenic species is very important [26-27].

Morphological characters of *F. equiseti* observed showed resemblance with morphological characters reported by many researchers in their study carried out in the past for *F. equiseti* [26-27]. Fusarium wilt, also known as ‘fusariosis’ or ‘sudden death’, starts with branch yellowing and wilt, until the whole plant dries, as a consequence of root and collar rot

[28]. The disease is observed in adult plants; however, under favorable conditions, such as soils with a disease history, and high temperature and moisture, young plants can die under the pathogen attack [29-30]. The colony of isolate A on PDA developed rapidly with whitish pink aerial mycelium at first, becoming tan to brown as the culture ages. In case of second sample, colony initially displayed white aerial mycelium, becoming salmon day by day. The morphological characteristics of fusarium isolates were similar with the information described by Leslie and Summerell [20]. The spores of the isolate A have slightly curved and relatively thick with a slightly hook cell. The macroconidial septation of isolate A ranged from 5 to 7. Narrower macroconidia were observed and septation ranged from 3 to 5 in isolate B. The shape characteristics of macroconidia of the isolate B was similar with those mentioned by Ciampi *et al*. [30] for *Fusarium oxysporum.* On the other hand, Ciampi *et al*. [30] and Aigbe *et al*. [26] reported the similar microscopic characters for *F. equiseti* isolated from cow pea in Nigeria. The environmental factors (temperature, pH etc.) [31] and culture medium composition have been found to determine the mycelial growth and sporulation of the concern phyto-pathogens [31]. The different culture media also had a lot of influences on the morphological characteristic of mycelium of the isolated fungi that supported the findings of the experiment conducted by the Barros *et al*. [32] Our present experiment showed that maximum growth of the two isolated fungi were displayed at around 25°C whereas for both fungi that was almost similar with the previous studies [33-35]. Focusing on the effect of different pH level on physical growth rate, it was found that both fungi grew well in alkaline media. These findings were consistent with previous studies of Yamanaka [36] where it was found that the saprotrophic fungal species grew well at alkaline pH. Both of the two fungi showed significant variations in mycelial growth due to usages of different carbohydrate as a sole carbon source. Through the comparisons among literature and our studies, it was found that some fungi may utilize the carbohydrate, maltose followed by glucose uptake and metabolism. It demonstrated that some fungi could not use sucrose directly but glucose and fructose were readily consumed [37]. Lamb [39] observed growth of fungi on sucrose that is introduced by 'starter' monosaccharide.

In case of sugar concentrations, highest mycelial growth was found at 9% sugar concentration for isolate A whereas it was at 15 % for isolate B. The present study revealed that both isolates showed lower mycelial growth due to the addition of different salt which is consistent with the observation of Pelizza *et al*. [40] Organic acids inhibited the fungal growth through decreasing pH value. In this mechanism, it may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis [41].

The nucleotide BLAST search in GenBank using the 548bp and 537bp ITS sequences revealed that the isolate A and B fungal strains showed 99% and 100% identity with the sequences of *Fusarium equiseti* and *Fusarium oxysporum*, respectively. Similar result was found by Hasan *et al*. [42] in Cercospora leaf spot disease of okra which support our present findings.

The fungicidal activity of some plant extracts controlling different plant pathogens have been reported by several researches [43-46]. Findings from the study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides [46]. In the experiment, methanolic extracts of *Ficus racemosa, Moringa oleifera, Azadirachta indica, Cassia alata, Senegalia catechu* etc. exhibited promising antifungal activities against the studied microorganisms.

Conclusion

In the present study, colony morphology and growth profiling of the two fungal isolates exhibited much differences when they were subjected to different conditions of culture medium and environmental factors. The addition or change in nutritive requirements influenced their overall growth pattern. Along with morpho-physiological characteristics, the advanced molecular technique-sequencing revealed the identity of fungal isolates as *Fusarium equiseti* and *Fusarium oxysporum*, respectively which are the causal agents of dumping off and fusarium wilt diseases in bitter gourd that cause significant decrease in quantity and quality of bitter gourd in Bangladesh, every year. In the present research work, it was also evaluated that some methanolic plant extracts displayed hopeful results in inhibiting the growth of these two *fusarium* fungal species.

Acknowledgements

The authors are grateful to the Dean, Faculty of Life and Earth Science, University of Rajshahi, Bangladesh for providing financial support during the research works.

Author Contributions

MEKC, MAI, BS and MFH designed the experiments, developed the methodology and prepared the manuscript. MEKH and MFH collected the data and carried out analysis. MSJ, MAI and BS assisted with manuscript preparation.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper

References

1. Islam QMS, Mohiuddin M, Rabbaniand MG, Mollah AF. International Journal of Applied Research in Business Administration & Economics. 2013; 2(6):2-8.
2. BBS. Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, 2013.
3. Saeed F, Pasha I, Anjum FM, Sultan MT. Critical reviews in food science and nutrition. 2011; 51(5):467-476.
4. Güdr A. Iranian journal of pharmaceutical research: IJPR. 2016; 15(1):301.
5. Zhang B, Xie C, Wei Y, Li J, Yang X. Protein expression and purification. 2015; 107:43-49.
6. Jiang B, Ji M. Liu W, Chen L, Cai Z, Zhao Y, Bi X. Molecular medicine reports. 2016; 14(5):4865-4872.
7. Vijayalakshmi B, Salimath PV. Journal of Diabetes and its Complications. 2009; 23(1):71-76.
8. Begum S, Ahmed M, Siddiqui BS, Khan A, Saify ZS, Arif M. Phytochemistry. 1997; 44(7):1313-1320.
9. Radford AE, Ahles HE, Bell CR. Manual of the vascular flora of the Carolinas. Univ of North Carolina Press, 2010.
10. Kaprashvili TD. *Zashchita i Karantin Rasteniĭ*, 1996, 5.
11. Lucas GB, Campbell CL, Lucas LT. Introduction to plant disease identification and management. CBS Pub.

and Distributors, New Delhi, , 364.
12. Grabowski M. University of Minnesota Extension. Archived from the original on, 2012.
13. Messiaen CM, Cassini R. Recherches sur les Fusarioses, 1968.
14. Joffe AZ, Palti J. J Bot Basic Appl Plant Sci. 1967; 16(1):18.
15. Bacharis C, Gouziotis A, Kalogeropoulou P, Koutita O, Tzavella-Klonari K, Karaoglanidis GS. Plant disease. 2010; 94(11):1314-1322.
16. Horst RK. Rusts. In Westcott's Plant Disease Handbook Springer, Dordrecht, 2013, 341-362.
17. Halila MH, Strange RN. Euphytica. 1997; 96(2):273-279.
18. Agrios GN. Plant Pathology. 5th ed. Elsevier Academic Press, Inc., Burlington USA. 2006, 922.
19. Fravel D, Olivain C, Alabouvette C. New phytologist. 2003; 157(3):493-502.
20. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. Blackwell Publishing Ltd., Oxford, UK, 2006, 388.
21. Kuhn DM, Ghannoum MA. Clinical microbiology reviews. 2003; 16(1):144-172.
22. Saha A, Mandal P, Dasgupta S, Saha D. Journal of Environmental Biology. 2008; 29(3):407.
23. Nene YL, hapliyal PL. Disease fungicides in plant control, 1979.
24. Arora DK, Dwivedi RS. Soil Biology and Biochemistry. 1979; 11(6):563-566.
25. Anonymous. The Free Encyclopedia. Demographics of Bangladesh. Online available at, 2015, http://en.wikipedia.org /wiki/Demographics_ of_ Bangladesh
26. Aigbe SO, Fawole B, Berner DK. Plant disease. 1999; 83(10):964-964.
27. Latiffah Z, Baharuddin S. Malaysian Journal of Microbiology. 2007; 3(1):27-30.
28. Fischer IH, Bueno CJ, de Marchi Garcia MJ, Marques de Almeida A. Acta Scientiarum. Agronomy. 2010; 32:2.
29. Ponte JJ, Franco A, Aguiár De Holanda YC, Silveira Filho J. Fitopatologia Venezolana, Maracay. 1998; 12(1):30-31.
30. Ciampi L, Nissen J, Venegas E, Fuentes R, Costa M, Schöbitz R, Alvarado P. Chilean Journal of Agricultural Research. 2009; 69(4):516-525.
31. Monteiro AC, Barbosa CC, Correia ACB, Pereira GT. *Pesquisa Agropecuária Brasileira*. 2004; 39(6):561-565.
32. Barros L, Baptista P , Ferreira IC. J. Agric. Food Chem., 2006; 5:8766-8771.
33. Theron DJ, Holz G. Potato research. 1990; 33(1):109-117.
34. Brock PM, Inwood JRB, Deverall BJ. Australasian Plant Pathology. 1994; 23(3):81-85.
35. Dix NJ, Webster J. Fungal Ecology. New York: Chapman and Hall, 1995, 549.
36. Yamanaka T. *Mycologia*. 2003; 95(4):584-589.
37. Yuan XL, van der Kaaij RM, van den Hondel CA, Punt PJ, van der Maarel MJ, Dijkhuizen L, *et al.* Molecular Genetics and Genomics. 2008; 279(6):545-561.
38. Salzer P, Hager A. Botanica Acta. 1991; 104(6):439-445.
39. Lamb RJ. Transactions of the British Mycological Society. 1974; 63(2):295-306.
40. Pelizza SA, Lastra CL, Becnel JJ, Bisaro, García JJ. Journal of invertebrate pathology. 2007; 96(2):133-137.
41. Kang HC, Park YH, Go SJ. Microbiological research. 2003; 158(4):321-326.
42. Hasan SMZ, Hossain MF, Zaoti ZF, Jahan MS, Hasan MF. Islam MA, Sikdar B. International Journal of Microbiology Research. 2018; 10(3):1015-1019.
43. Parveen S, Wani AH, Ganie AA, Pala SA, Mir RA. Archives of Phytopathology and Plant Protection. 2014; 47(3):279-284.
44. Okigbo RN, Emoghene AO. KMITL Sci. J. 2004; 4(1):20-31.
45. Cannon RD, Lamping E, Holmes AR, Niimi K, Tanabe K, Niimi M.. Microbiology/ 2007; 153(10):3211-3217.
46. Cowan MM. Clinical microbiology reviews. 1999; 12(4):564-582.